EISSN 2459-1459

Formerly Marmara University Institute of Health Sciences (MUSBED)

CLINICAL AND EXPERIMENTAL HEALTH SCIENCES

Volume 15 | Issue 1 | March 2025 | www.dergipark.gov.tr/marusbed

Clinical and Experimental Health Sciences

VOLUME 15 • ISSUE 1 • MARCH • 2025

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VOLUME 15 • ISSUE 1 • MARCH • 2025

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Clinical and Experimental Health Sciences

VOLUME 15 • ISSUE 1 • MARCH • 2025

ABOUT

Clinical and Experimental Health Sciences (Clin Exp Health Sci) is an international, scientific, open-access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles.

The journal was published under the name MUSBED until April 22, 2016.

The target audience of the journal includes specialists and medical professionals working in all disciplines of health sciences. The journal is the official publication of Marmara University, Institute of Health Sciences and it is published four times a year in March, June, September, and December. The journal publishes content in English. The journal publishes clinical and experimental research articles, review articles, case reports, letters to the editor, and editorial comments prepared in accordance with ethical guidelines in all disciplines of health sciences.

Aim & Scope

The aim of the journal is to contribute to the literature by publishing clinical and experimental research articles, case reports, letters to the editor, and and editorial comments prepared in accordance with the ethical guidelines in all disciplines of health sciences. The target audience of the journal includes specialists and medical professionals working in all disciplines of health sciences.

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Indexing

TR Dizin

Emerging Sources Citation Index (E-SCI)

ProQuest

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Clinical and Experimental Health Sciences

Cone-Beam CT Evaluation of Intracranial Physiological Calcifications by Age and Gender

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Received: November 9, 2022 **Accepted:** March 4, 2025

ABSTRACT

Objective: To assess the prevalence of physiological intracranial calcifications detected in cone-beam computed tomography (CBCT) images in a group of Turkish population by age and gender.

Methods: Full head CBCT images of 1000 patients (535 men, 465 women) with age range of 6-91 years were retrospectively analyzed. The presence of habenular, pineal gland, coroid plexus, petroclinoid ligament, interclinoid ligament, carotico-clinoid ligament, falx cerebri, tentorium cerebelli and basal ganglia calcifications were investigated by age groups and gender. Mann Whitney U test was used to calculate the calcification frequency by mean age and χ2 test was used for gender.

Results: CBCT examination of 1000 cases aged between 6 and 91 were evaluated in six groups respectively; ages under 19 (13.3%), age 20-29 (14.8%), age 30-39 (11.9%), age 40-49 (19.3%), age 50-59 (20.9%) ages over 60 (19.8%). Habenular calcification was the most common calcification with a rate of 69%. Only petroclinoid ligament calcification was significantly higher in men ($p < .001$). Statistically significant relationship was found between age groups and calcifications of habenular, pineal gland, choroid plexus, petroclinoid ligament, interclinoid ligament, caroticoclinoid ligament ($p < .001$).

Conclusion: Habenular calcification was the most common type of intracranial calcification in all age groups. As the probability of calcification increases with aging, an increase in the association of calcifications was observed. Physiological intracranial calcifications may be an incidental finding frequently encountered in CBCT examinations.

Keywords: Cone-beam computed tomography, intracranial calcifications, habenular, pineal gland, choroid plexus

1. INTRODUCTION

Intracranial calcifications may be physiological or pathological as a result of mineral or metal deposition in blood vessels, glands, cortices, or other structures in the brain (1). Physiological ones are generally not accompanied by the disease (2). Degenerative alterations and aging are considered to be related to physiological calcifications, but the reason is not fully understood (3). Various imaging techniques can be used to diagnose intracranial calcifications, but over the past decade, cone beam computed tomography (CBCT) has become one of the most preferred visualization systems for evaluating the anatomical structure of the head and neck region (4). CBCT provides images with high diagnostic values with short scanning time and low radiation dose (5, 6) . The most common locations for intracranial physiological calcifications are the pineal gland, habenular commissure, choroid plexus, falx, basal ganglia, and vessel walls (4). The pineal gland, a neuroendocrine organ resembling a small pine cone, regulates circadian rhythm and sleep by secreting the hormone melatonin (7). When the size of pineal gland calcification is over 14 mm, the possibility of a pathological lesion (pinealoma, teratoma) increases (4). The habenular commissure is a pair of small nuclei that are anatomically associated to the epitalamus and pineal gland (8). Choroid plexus calcification is mostly seen in the atrial parts of the lateral ventricles. It is rare in the third or fourth ventricle and in patients under 9 years of age (2). Choroid plexus calcification is usually related to frontal cortex, parietal-temporal, and cerebellum atrophies and is one of the neuroimaging findings of cognitive impairment in schizophrenia (9). Areas with thicker dura mater, such as the falx and petroclinoid ligaments, often tend to calcification (2). The petroclinoid ligament is the dura mater folds that extend between the petrosal part of the temporal bone the anterior and posterior clinoid process (10). Petroclinoid ligament is associated with the trigeminal, abducens, and oculomotor nerves. its calcification or ossification can cause trigeminal neuralgia (11). The clinoid process, the attachment sites for the dura mater, is located on the

https://doi.org/*10.33808/clinexphealthsci.1201994*

sphenoid bone. The anterior and posterior clinoid processes are interconnected by interclinoid ligament. The medial and anterior clinoid processes are connected to each other by caroticoclinoid ligament. These ligaments can sometimes ossify. Although the ossification of the interclinoid ligament is underestimated, cadaver studies have shown that the existence of the ossified interclinoid ligament makes it difficult to remove the anterior clinoid process, especially in the existence of aneurysm, and increases the risks (12, 13). Ossification of the caroticoclinoid ligament may also be associated with clinical problems such as pressure on the internal carotid artery (14). Falx calcifications usually have a characteristic appearance as dense and flat plaques and in the midline of the cerebrum (15). It has been reported that 70-80% of basal ganglia calcifications are related with hypoparathyroidism (16).

There are limited number of studies on intracranial calcifications in the literatüre (17-20). As far as we know, only the relationships between pineal gland, habenular, and choroid plexus calcifications were evaluated. The aim of this study was to investigate the frequency of intracranial calcifications according to six age groups and gender on CBCT images of a group of Turkish population and to examine the relationships between the following parameters: pineal gland, habenular, choroid plexus, petroclinoid ligament, interclinoid ligament, caroticoclinoid ligament, falx cerebri, basal ganglia, tentorium cerebelli.

2. MATERIALS AND METHODS

The current study was approved by the Ethics Committee of the Gaziantep University (Approval No: 2020/387). In this study, all facial CBCT images of the patients who applied to Gaziantep University Dentomaxillofacial Radiology Department for various reasons were retrospectively analyzed. Patients with congenital disorders, bone disease, history of trauma, surgery, tumor or malignancy, and images containing artifacts were excluded from the study. Full head CBCT images of 1000 patients (535 men, 465 women) with age range of 6-91 years were retrospectively analyzed with Planmeca ProMax 3D Mid (Helsinki, Finland) device and Planmeca Romexis version 3.2.0 software (Helsinki, Finland). Images with a field of view (FOV) range of 16x16 cm, voxel resolution of 0.4 mm3 and a slice thickness of 1 mm were used. All CBCT scans were performed according to a standard screening protocol. The images were examined in multiplanar (axial, sagittal and coronal) sections by two dentomaxillofacial radiologists, one is (MED) and the other with 9 years of experience (EDY). The hyperdense area seen in the relevant area examined in the images was accepted as calcification. Pineal gland, habenular, choroid plexus, petroclinoid ligament (Figure 1), interclinoid ligament, caroticoclinoid ligament, falx cerebri (Figure 2), basal ganglia and tentorium cerebelli (Figure 3) calcifications were investigated on the images. All these parameters were assessed according to age and gender, it was also investigated whether there was a relationship between the parameters.

Figure 1. *Axial CBCT images show (a) habenular calcification (short arrow), pineal gland calcification (arrow), (b) choroid plexus calcification (arrowheads), (c) petroclinoid ligament calcification (arrowheads).*

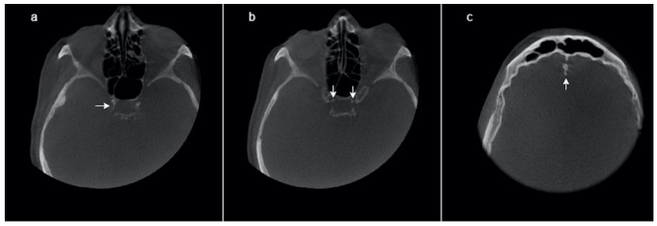

Figure 2. *Axial CBCT images demonstrate (a) interclinoid ligament calcification (arrow), (b) caroticoclinoid ligament calcification (arrows), (c) falx cerebri calcification (arrow).*

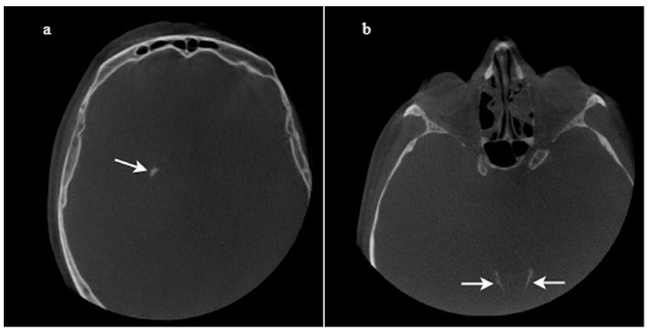

Figure 3. *Axial CBCT images indicate (a) basal ganglia calcification (arrow), (b) tentorium cerebelli calcification (arrows).*

When there was disagreement among observers, consensus was reached through discussion. For the reliability of the intraobserver calibration and assessments, the images were reviewed by the same observers two weeks after the initial assessment.

Statistical analysis of the study was performed with SPSS program version 20.0 (Armonk, NY, IBM). In the analysis of the data, Mann Whitney U test was used to calculate the frequency of calcification by mean age. Relationship between the age groups and calcifications and the prevalence difference between men and women were analyzed using the $\chi 2$ test. The grade of importance was set at $p<.05$.

3. RESULTS

The coefficient of intra and inter-observer reliability for all the assessments was found to be excellent (0.91 and 0.88, respectively). CBCT examination of 1000 cases aged between

6 and 91 were evaluated in six groups respectively; ages under 19 (13.3%), age 20-29 (14.8%), age 30-39 (11.9%), age 40-49 (19.3%), age 50-59 (20.9%) ages over 60 (19.8%). The cases consisted of 535 (53.5%) men and 465 (46.5%) women with a mean age of 42.9 years. Intracranial calcification was observed in 826 (82.6%) of 1000 CBCT images. It was detected in 82.5% of men and 82.7% of women. The prevalence of at least one calcification is shown in Table 1.

Table 1. *The prevalance of at least one intracranial calcification*

		Gender		Total N (%)
		Female	Male	
Intracranial Calcification	Absent N (%)	81 (17.3)	93 (17.5)	174 (17.4)
	Present N (%)	388 (82.7)	438 (82.5)	826 (82.6)
Total N (%)		469 (100)	531 (100)	1000 (100)

Habenular calcification was the most common calcification with a rate of 69%. Other calcifications were as follows, respectively: pineal gland 53.2%, choroid plexus 44.6%, petroclinoid ligament 10.9%, interclinoid ligament 8.2%, caroticoclinoid ligament 8.8%, falx cerebri 0.5%, tentorium cerebelli 0.2%, basal ganglia 0.2%. The distribution of the calcifications by gender is demonstrated in Table 2. When examined by gender, only the petroclinoid ligament was found to be significantly more common in males than females (p <.001).

Table 2. *Distribution of the calcifications according to gender*

Calcifications		Gender		p
		Female	Male	
		N (%)	N (%)	
Habenular	Absent	156 (33.3)	154 (29.0)	.146
	Present	313 (67.7)	337 (71.0)	
Pineal gland	Absent	232 (49.5)	236 (44.4)	.112
	Present	237 (50.5)	295 (55.9)	
Choroid plexus	Absent	251 (53.5)	303 (57.1)	.261
	Present	218 (46.5)	228 (42.9)	
Petroclinoid ligament	Absent	440 (93.8)	451 (84.9)	< .001*
	Present	29 (6.2)	80 (15.1)	
Interclinoid ligament	Absent	427 (91.0)	491 (92.5)	.413
	Present	42 (9.0)	40 (7.5)	
Caroticoclinoid ligament	Absent	428 (91.3)	484 (91.1)	.951
	Present	41 (8.7)	47 (8.9)	
Falx cerebri	Absent	465 (99.1)	530 (99.8)	.192
	Present	4 (0.9)	1 (0.2)	
Tentorium cerebelli	Absent	468 (99.8)	530 (99.8)	.000
	Present	1 (0.2)	1 (0.2)	
Basal ganglia	Absent	467 (99.6)	531 (100)	.220
	Present	2 (0.4)	0 (0.0)	

**p< .05*

The distribution of presence and absence of calcifications by mean age is indicated in Table 3. A statistically significant difference was found between the presence and absence of habenular, pineal gland, choroid plexus, petroclinoid ligament, interclinoid ligament, caroticoclinoid ligament calcifications, according to the mean age (p<.001). The presence of these calcifications was significant in advanced age. There was no significant difference according to mean age for falx cerebri, tentorium cerebelli, basal ganglia calcifications.

T***able 3.*** *Distribution of the calcifications by mean age*

Calcifications		N (%)	Mean ± SD	p
Habenular	Absent	310 (31)	34.96 ± 18.46	< .001*
	Present	690 (69)	46.53 ± 16.59	
Pineal gland	Absent	468 (46.8)	37.13 ± 18.35	< .001*
	Present	532 (53.2)	47.91 ± 16.14	
Choroid plexus	Absent	554 (55.4)	37.13 ± 18.33	< .001*
	Present	446 (44.6)	50.17 ± 14.66	
Petroclinoid ligament	Absent	891 (89.1)	41.99 ± 18.22	< .001*
	Present	109 (10.9)	50.72 ± 13.81	
Interclinoid ligament	Absent	918 (91.8)	41.22 ± 17.42	< .001*
	Present	82 (8.2)	62.23 ± 12.25	
Caroticoclinoid ligament	Absent	912 (91.2)	40.93 ± 17.36	< .001*
	Present	88 (8.8)	63.85 ± 8.75	
Falx cerebri	Absent	995 (99.5)	42.9 ± 18	.262
	Present	5 (0.5)	51.4 ± 16.68	
Tentorium cerebelli	Absent	998 (99.8)	43 ± 17.97	.035
	Present	2 (0.2)	15 ± 4.24	
Basal ganglia	Absent	998 (99.8)	42.91 ± 17.97	.316
	Present	2 (0.2)	60.5 ± 28.99	

**p< .05*

The distribution of the calcifications by age groups is given in Table 4. A statistically significant relationship was found between age groups and calcifications of habenular, pineal gland, choroid plexus, petroclinoid ligament, interclinoid ligament, caroticoclinoid ligament (p<.001). While habenular calcification is not observed in 66.2% of individuals aged 19 and under, it is seen in 82.3% of individuals aged 60 and above. While pineal gland calcification is not detected in 82% of individuals aged 19 and under, it is seen in 69.7% of individuals aged 60 and over. In addition, choroid plexus, petroclinoid ligament, interclinoid ligament, caroticoclinoid ligament calcifications were also detected more frequently in advanced ages.

The binary relationships of calcifications with each other are indicated in Table 5. In the absence of petroclinoid ligament calcification, the absence of caroticoclinoid ligament calcification was 82.2% (p= .001) and the absence of interclinoid ligament calcification was 86.6% (p<.001). In the absence of caroticoclinoid ligament calcification, the presence of habenular and pineal gland calcification was 61.8% and 46.5%, respectively (p<.05). In the absence of interclinoid ligament calcification, the presence of habenular and pineal gland calcification was 62% and 47.8%, respectively (p<.05). In the absence of petroclinoid ligament calcification, the presence of habenular and choroid plexus calcification was 60.5% and 38.6%, respectively (p<.05).

Table 4. *Distribution of the calcifications by age groups*

		Habenular	Pineal gland	Choroid plexus	Petroclinoid ligament	Interclinoid ligament	Caroticoclinoid ligament
Age groups		**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
< 19	**Presence**	45 (33.8)	24 (18.0)	8 (6.0)	1 (0.8)	2 (1.5)	0 (0.0)
	Absence	88 (66.2)	109 (82.0)	125 (94.0)	132 (99.2)	131 (98.5)	133 (100)
	Total	133 (100)	133 (100)	133 (100)	133 (100)	133 (100)	133 (100)
20-29	**Presence**	92 (62.2)	66 (44.6)	39 (26.4)	12 (8.1)	0 (0.0)	0 (0.0)
	Absence	56 (37.8)	82 (55.4)	109 (73.6)	136 (91.9)	148 (100)	148 (100)
	Total	148 (100)	148 (100)	148 (100)	148 (100)	148 (100)	148 (100)
30-39	**Presence**	88 (73.9)	66 (55.5)	54 (45.4)	7 (5.9)	3 (2.5)	0 (0.0)
	Absence	31 (26.1)	53 (44.5)	65 (54.6)	112 (94.1)	116 (97.5)	119 (100)
	Total	119 (100)	119 (100)	119 (100)	119 (100)	119 (100)	119 (100)
40-49	**Presence**	137 (71.0)	112 (58.0)	98 (50.8)	29 (15.0)	4 (2.1)	6 (3.1)
	Absence	56 (29.0)	81 (42.0)	95 (49.2)	164 (85.0)	189 (97.9)	187 (96.9)
	Total	193 (100)	193 (100)	193 (100)	193 (100)	193 (100)	193 (100)
50-59	**Presence**	165 (78.9)	126 (60.3)	124 (59.3)	29 (13.9)	20 (9.6)	22 (10.5)
	Absence	44 (21.1)	83 (39.7)	85 (40.7)	180 (86.1)	189 (90.4)	187 (89.5)
	Total	209 (100)	209 (100)	209 (100)	209 (100)	209 (100)	209 (100)
> 60	**Presence**	163 (82.3)	138 (69.7)	123 (62.1)	31 (15.7)	53 (26.8)	60 (30.3)
	Absence	35 (17.7)	60 (30.3)	75 (37.9)	167 (84.3)	145 (73.2)	138 (69.7)
	Total	198 (100)	198 (100)	198 (100)	198 (100)	198 (100)	198 (100)
	p	< .001	< .001	< .001	< .001	< .001	< .001

**p< .05*

Table 5. *The relationships of calcifications with each other*

Calcifications		Caroticoclinoid ligament			Interclinoid ligament			Petroclinoid ligament			Choroid plexus			Pineal gland		
		Absent N (%)	Present N (%)	p	Absent N (%)	Present N (%)	p	Absent N (%)	Present N (%)	p	Absent N (%)	Present N (%)	p	Absent N (%)	Present N (%)	p
Habenular	**Absent**	294 (29.4)	16 (1.6)	.006*	298 (29.8)	12 (1.2)	.001*	286 (28.6)	24 (2.4)	.032*	220 (22.0)	90 (9.0)	.001*	259 (25.9)	51 (5.1)	.001*
	Present	618 (61.8)	72 (7.2)		620 (62.0)	70 (7.0)		605 (60.5)	85 (8.5)		334 (33.4)	356 (35.6)		209 (20.9)	481 (48.1)	
Pineal	**Absent**	447 (44.7)	21 (2.1)	< .001*	440 (44.0)	28 (2.8)	.017*	426 (42.6)	42 (4.2)	.067	302 (30.2)	166 (16.6)	.001*	-	-	-
	Present	465 (46.5)	67 (6.7)		478 (47.8)	54 (5.4)		465 (46.5)	67 (6.7)		252 (25.2)	280 (28.0)		-	-	
Choroid plexus	**Absent**	524 (52.4)	30 (3.0)	< .001*	521 (52.1)	33 (3.3)	.004*	505 (50.5)	49 (4.9)	.020*	-	-	-	-	-	-
	Present	388 (38.8)	58 (5.8)		397 (39.7)	49 (4.9)		386 (38.6)	60 (6.0)		-	-		-	-	
Petroclinoid ligament	**Absent**	822 (82.2)	69 (6.9)	.001*	828 (82.8)	63 (6.3)	.001*	-	-	-	-	-	-	-	-	-
	Present	90 (9.0)	19 (1.9)		90 (9.0)	19 (1.9)		-	-		-	-		-	-	
Interclinoid ligament	**Absent**	866 (86.6)	52 (5.2)	< .001*	-	-	-	-	-	-	-	-	-	-	-	-
	Present	46 (4.6)	36 (3.6)		-	-		-	-		-	-		-	-	

**p< .05*

4. DISCUSSION

There are simply two studies in the literature examining intracranial calcifications with CBCT (17, 18). In these, only the relationships between pineal gland, habenular and choroid plexus calcifications were evaluated. As far as we know, this is the merely study in which the prevalence of nine physiological intracranial calcifications detected on CBCT images was investigated according to age and gender. According to our findings, the most common calcification was habenular calcification in all age groups. In respect to gender, only petroclinoid ligament calcification was found to be significantly more common in males than females.

CBCT is widely used in dental diagnosis and treatment planning before and after surgical procedures, and can also be used in the evaluation of intracranial calcifications as it allows imaging of bone and calcified structures. The

detectability of calcification on computed tomography (CT) can be influenced by a number of factors and levels, such as slice thickness and window width (18). CBCT has made it possible to obtain clearer images by allowing the target structure to be examined in all directions of space by taking different cross-sectional images to reveal anatomical variations, high resolution, detail reflection capacity, clarity in hard tissue imaging, low metal artifact, ease of use and relatively low radiation dose (21). Intracranial calcifications are often an incidental finding on a wide FOV CBCT scan. Therefore, clinicians can contribute to early diagnosis and prevention. Since most dental implant patients are middle-aged or older, the possibility of examining neurodegenerative calcifications in these patients is ideal (22). Detection of soft tissue calcifications is generally made according to their anatomical location, distribution and morphological features in radiographic images. It has been reported that the increase in the size of some physiological calcifications may be pathological calcification or pathological lesion (4, 23). To distinguish whether the calcification is physiological or pathological, if it is larger than 1 cm and seen in children under 9 years of age, it may suggest that it is pathological. They may not have symmetrical borders compared to irregular physiologic calcifications or may be located in different areas than those where physiologic calcifications are commonly seen. (15,17, 24).

Physiological intracranial calcifications are generally asymptomatic and sometimes symptomatic depending on their location and extent (25). Intracranial calcifications and ossifications may cause pressure on anatomic neighborhoods or complications in the surgery of that area (26). Jassim et al. (25) observed intracranial calcification in 58.6% of the cases. Sedghizadeh et al. (17) reported that 35.2% of the cases had intracranial calcification. In the study of Bayrak et al. (18), the prevalence of intracranial physiological calcifications was found to be 33.1%. Unlike previous studies, this rate was 82.6% in our study.

Pineal gland calcification is observed as a well-circumscribed radiopaque mass in the midline plane on radiography. The size of these calcifications ranges from approximately 1 mm to 7 mm, with an average diameter of 4 mm. Calcifications of 1 cm or greater may indicate the presence of neoplasm (15, 17). In the literature, the pineal gland calcification rates on CT were as follows; 73%, 71.6%, 71%, 68.5%, 68%, 67.7%, 66.1%, 46.2% (1, 6, 19-20, 25, 27-29). In studies conducted in CBCT, these rates were as follows; 80% (pineal / habenular region), 64.5%, 58.8%, 19.2%, 12.92% (17, 22, 23, 30, 31). In the present study, this rate was 53.2%. It can be thought that the reason why the result we obtained is different from other studies is the high number of scanned images, the wide age range and ethnic origin.

The habenular commissure is situated in the upper layer of the stem of the pineal gland (32, 33). While the role of the habenular commissure is unknown, the habenular nuclei play a significant role in influencing how the brain reaction to diverse stimuli such as fear, ache, award, stress, and sleep (34). Habenular commissure is usually reported to be calcified on skull radiographs and CT imaging (35). In previous studies, the prevalence of this calcification was 80%, 35.2%, 20.1%, 19.2%, 6.4% (1, 17, 19-20, 25). Only one of them was a CBCT study, and in that study, the habenular and pineal regions were examined together (17). In our study, habenular and pineal were assessed separately and habenular calcification was found in 69%. The reason why this ratio is different from other studies may be due to population differences. There are very few studies examining choroid plexus calcification with CBCT. In these studies, choroid plexus calcification was detected in 12%, 2.4%, 1.7% (17, 18, 26). In studies performed with CT, choroid plexus calcification was observed in 81.6%, 70.2%, 69.3%, 66.2%, 56.82% and 53.6% (1, 19, 20, 22-25, 28, 29). The ratio in our study was higher than studies with CBCT and lower than studies with CT. We think that the different frequencies in the studies are due to the mineralization mechanism, genetic factors, hormonal values and population differences.

In studies performed with CBCT, petroclinoid ligament calcification was reported in 33.4%, 8% and 2.7% (17, 18, 26). In a study with CT, this rate was found to be 18.3% (36). In our study, this rate was observed as 10.9%. Unlike other studies, it was determined more in men than in women. This difference may be due to the geographical region and ethnic origin of the people whose data were used in the study.

Interclinoid and caroticoclinoid ligament calcification might induce dysfunction of left eye muscles because of possible compression of the carotid artery or oculomotor nerve (37). Only one CBCT study was found in literature and the prevalence was 4.88% (18). In study of Cederberg et al. (25) evaluating the prevalence of interclinoid ligament calcification on lateral cephalometric radiographs, it was found to be 8%. Erturk et al. (37) detected interclinoid calcification at a rate of 8.18% in their cadaver study and 7.89% in Ozdogmuş et al. (12)'s autopsy study. In the current study, interclinoid ligament calcification was found to be 8.2%, which is consistent with previous studies. There is only one study in the literature examining caroticoclinoid ligament ossification or calcification with CBCT. Bayrak et al. (18) reported 3.83% caroticoclinoid ossification in their study. In our study, this rate was 8.8%. The reason for this difference may be the larger number of images examined and the wide age range.

Dorenbeck et al. (38) reported that tertiary hyperparathyroidism and chronic renal failure may cause tentorium cerebelli and dural calcification. Dural (falx cerebri) calcification was found to be 12.5%, 11.2% and 6.6% in studies performed on CT (1, 19, 25). Tentorium cerebelli calcification was observed in 7.3% of CT examinations conducted by Daghighi et al (1). In previous CT researches, basal ganglia calcification was detected as 14.4%, 1.3%, 0.8% (1, 19, 25). The reason why our study is different from other studies may be the soft tissue imaging difference between CBCT and CT. As far as we can see, there are not enough studies in the literature regarding the co-occurrence of pineal gland, choroid plexus, habenular, interclinoid ligament, caroticoclinoid ligament, petroclinoid ligament,

falx cerebri, tentorium cerebelli calcifications. Orcan et al. (20) stated that the calcifications of pineal gland and choroid plexus were coexistence in 51.9% of cases, pineal gland and habenular commissure in 28.7%, and choroid plexus and habenular commissure calcifications in 28.4% of cases. We think that the reason why associations with interclinoid and caroticoclinoid ligaments are less common is that these calcifications occur at a later age than others. Different the previous study, the most common coexistence of habenular and pineal calcifications was seen in our study.

In the study by Daghighi et al. (1), pineal calcification was the most prevalent calcification between individuals aged 15 to 54 years, whereas choroid plexus was the most prevalent calcification in individuals aged 55 to 85 years. Habenular calcification ranked third in all age groups. In our study, the most common calcification in all age groups was habenular calcification. Calcification of the choroid plexus and pineal gland increased in cases over 50 years of age. Petroclinoid ligament calcification increased over 40 years of age, while interclinoid and caroticoclinoid calcifications were observed more frequently over 60 years of age. Unlike other calcifications, caroticoclinoid ligament calcification was not observed before the age of 40. The reason for this difference may be the population discrepancy and the large number of individuals we included in the study.

The limitation of this study was that it was a retrospective study, so systemic diseases of the patients, hormone levels, blood calcium and iron levels and the drugs they used were not known.

Though the reasons of intracranial calcifications are not fully known, factors such as age, gender, race, geographical region, nutrition and lifestyle might influence their formation (39, 40). Intracranial calcifications and ossifications may cause pressure on anatomic neighborhoods or complications in the surgery of that area. Therefore, the presence of calcifications that can be easily visualized with CBCT in regional surgery should be carefully examined. Due to the larger number of data examined, it is considered that the results we obtained will be a solid data source in this region's population. In future studies, multicenter studies can be planned in different populations by increasing the sample size, and it may be useful to investigate these calcifications with the use of magnetic resonance imaging.

5. CONCLUSION

In this study, the most common type of intracranial calcification in all age groups was habenular, followed by the pineal gland and choroid plexus on CBCT images. Petroclinoid ligament calcification was significantly more common in males than females. The probability of calcification increased with aging, and a tendency to increase was detected in the association of calcifications. Among all calcifications, habenular and pineal gland calcifications were most common observed together. Physiological intracranial calcifications may be an incidental finding frequently encountered in CBCT examinations.

Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This retrospective study was approved by Ethical Committee of Gaziantep University (Protocol No:2020/387).*
Peer-review: *Externally peer-reviewed.*
Author Contribution
Research idea: EDY
Design of the study: EDY, MED
Acquisition of data for the study: MED
Analysis of data for the study: EDY, MED
Interpretation of data for the study: EDY, MED
Drafting the manuscript: EDY, MED
Revising it critically for important intellectual content: EDY, MED
Final approval of the version to be published: EDY, MED

REFERANSLAR

[1] Daghighi M, Rezaei V, Zarrintan S, Pourfathi H. Intracranial physiological calcifications in adults on computed tomography in Tabriz, Iran. Folia Morphol. 2007;66(2):115–119.

[2] Kieffer SA, Gold LH. Intracranial physiologic calcifications. Semin Roentgenol. 1974;9(2):151–162. https://doi.org/10.1016/0037-198x(74)90030-3

[3] Dahnert WF. Radiology review manual. North American Edition, 2017.

[4] William S. Radiology Review Manual. Philadelphia, PA: Lippincott Williams & Wilkins, 2003;5:230.

[5] Scarfe WC, Farman AG, Sukovic P. Clinical applications of cone-beam computed tomography in dental practice. J Can Dent Assoc. 2006;72(1):75–80.

[6] Kwak R, Takeuchi F, Ito S, Kadoya S. Intracranial physiological calcification on computed tomography (Part 1): Calcification of the pineal region. No To Shinkei 1988;40(6):569–574.

[7] Acer N, Ilıca AT, Turgut AT, Ozçelık O, Yıldırım B, Turgut M. Comparison of the three methods for the estimation of pineal gland volume using magnetic resonance imaging. Scientific World Journal 2012:123412. https://doi.org/10.1100/2012/123412

[8] Hikosaka O, Sesack SR, Lecourtier L, Shepard PD. Habenula: crossroad between the basal ganglia and the limbic system. J Neurosci. 2008;28(46):11825–11829. https://doi.org/10.1523/JNEUROSCI.3463-08.2008

[9] Marinescu I, Udristoiu I, Marinescu D. Choroid plexus calcification: clinical, neuroimaging and histopathological correlations in schizophrenia. Rom J Morphol Embryol. 2013;54(2):365–369.

[10] Lang J. Wurzburg Skull Base and Related Structures. Atlas of Clinical Anatomy. J. Lang.– Stuttgart, 1995.

[11] Wysiadecki G, Haladaj R, Polguj M, Zytkowski A. Bilateral extensive ossification of the posterior petroclinoid ligament: an anatomical case report and literature review. J Neurol Surg A Cent Eur Neurosurg. 2019;80(02):122–126. https://doi.org/10.1055/s-0038-1666782

[12] Ozdogmus O, Saka E, Tulay C, Gurdal E, Uzun I, Cavdar S. Ossification of interclinoid ligament and its clinical significance. Neuroanatomy 2003;2(1):25–27.

[13] Inoue T, Rhoton Jr AL, Theele D, Barry ME. Surgical approaches to the cavernous sinus: A microsurgical study. Neurosurgery

1990;26(6):903–932. https://doi.org/10.1097/00006123-199006000-00001

[14] Shaikh SI, Ukey RK, Kawale DN, Diwan CV. Study of carotico-clinoid foramen in dry human skulls of Aurangabad district. Int J Basic Med Sci. 2012;3:148–154.

[15] Deepak S, Jayakumar B. Extensive intracranial calcification. J Assoc Physicians India 2005;53:948.

[16] Lowenthal A. Calcification of the striopallidodentate system. Handb Clin Neurol. 1968;6:703–725.

[17] Sedghizadeh P, Nguyen M, Enciso R. Intracranial physiological calcifications evaluated with cone beam CT. Dentomaxillofac Radiol. 2012;41(8):675–678. https://doi.org/10.1259/dmfr/33077422

[18] Bayrak S, Bulut DG, Çakmak ES, Orhan K. Cone Beam Computed Tomographic Evaluation of Intracranial Physiologic Calcifications. J Craniofac Surg. 2019;30(2):510–513. https://doi.org/10.1097/SCS.0000000000004918

[19] Yalcin A, Ceylan M, Bayraktutan OF, Sonkaya AR, Yuce I. Age and gender related prevalence of intracranial calcifications in CT imaging; data from 12,000 healthy subjects. J Chem Neuroanat. 2016;78:20–24. https://doi.org/10.1016/j.jchemneu.2016.07.008

[20] Orcan CG, Nas OF, Cavusoglu IG, Alan O, Kılıç H, Uyguc A, et al. The incidence and co-existence of physiological pineal gland, choroid plexus and habenular commissure calcifications detected in cranial computed tomography. Med Bull Sisli Etfal Hosp. 2010;44(1):22–26.

[21] White SC, Pharoah MJ. Oral radiology-E-Book: Principles and interpretation: Elsevier Health Sciences, 2014.

[22] Mutalik S, Tadinada A. Prevalence of pineal gland calcification as an incidental finding in patients referred for implant dental therapy. Imaging Sci Dent. 2017;47(3):175–180. https://doi.org/10.5624/isd.2017.47.3.175

[23] Ozdede M, Kayadugun A, Ucok O, Altunkaynak B, Peker I. The assessment of maxillofacial soft tissue and intracranial calcifications via cone-beam computed tomography. Curr Med Imaging 2018;14(5):798–806. https://doi.org/10.2174/1573405613666170428160219

[24] Whitehead MT, Oh C, Raju A, Choudhri AF. Physiologic pineal region, choroid plexus, and dural calcifications in the first decade of life. AJNR Am J Neuroradiol. 2015;36(3), 575-580. https://doi.org/10.3174/ajnr.A4153

[25] Jassim MH, George NT, Jawad MM. Radiographic Anatomical Study of Intracranial Calcifications in Patients underwent Computerized Tomography Imaging. Int J Pharm Sci Med. 2019;4(2):1–13.

[26] Cederberg RA, Benson BW, Nunn M, English JD. Calcification of the interclinoid and petroclinoid ligaments of sella turcica: a radiographic study of the prevalence. Orthod Craniofac Res. 2003;6(4):227–232. https://doi.org/10.1034/j.1600-0544.2003.00243.x

[27] Turgut AT, Karakas HM, Ozsunar Y, ALtın L, Ceken K, Alıcıoglu B, et al. Age-related changes in the incidence of pineal gland calcification in Turkey: A prospective multicenter CT study. Pathophysiology 2008;15(1):41–48. https://doi.org/10.1016/j.pathophys.2008.02.001

[28] Al-Ameri LT, Al-Zuhairi EA, Al-Shirwani HM. Prevalence of Pineal Gland and Choroid Plexus Calcification Among Iraqi Patients Attending CT Scan Units. Int J Morphol. 2021;39(1):244–251.

[29] Uduma UF, Pius F, Mathieu M. Computed tomographic pattern of physiological intracranial calcifications in a city in central Africa. Glob J Health Sci. 2012;4(1):184–191. https://doi.org/10.5539/gjhs.v4n1p184

[30] Dief S, Veitz-Keenan A, Amintavakoli N, McGowan R. A systematic review on incidental findings in cone beam computed tomography (CBCT) scans. Dentomaxillofac Radiol. 2019;48(7):20180396. https://doi.org/10.1259/dmfr.20180396

[31] Bhuiyan PS, Rajgopal L, Shyamkishore K. Inderbir Singh's Textbook of Human Neuroanatomy: (Fundamental & Clinical). JP Medical Ltd. 2017.

[32] Newton HB, Handbook of neuro-oncology neuroimaging. Academic Press, 2016.

[33] Velasquez K, Molfese D, Salas R. The role of the habenula in drug addiction. Front Hum Neurosci. 2014;8:174. https://doi.org/10.3389/fnhum.2014.00174

[34] Orrison WW. Atlas of brain function. Georg Thieme Verlag, Stuttgart, 2008.

[35] Touska P, Hasso S, Oztek A, Chinaka F, Connor SE. Skull base ligamentous mineralisation: evaluation using computed tomography and a review of the clinical relevance. Insights Imaging 2019;10(1):1–17. https://doi.org/10.1186/s13244-019-0740-8

[36] Skrzat J, Szewczyk R, Walocha J. The ossified interclinoid ligament. Folia Morphol. 2006; 65(3):242–245.

[37] Erturk M, Kayalıoglu G, Govsa F. Anatomy of the clinoidal region with special emphasis on the caroticoclinoid foramen and interclinoid osseous bridge in a recent Turkish population. Neurosurg Rev. 2004;27(1):22–26. https://doi.org/10.1007/s10143-003-0265-x

[38] Dorenbeck U, Leingärtner T, Bretschneider T, Krämer B, Feuerbachet S. Tentorial and dural calcification with tertiary hyperparathyroidism: A rare entity in chronic renal failure. Eur Radiol. 2002;12(3):11–13. https://doi.org/10.1007/s00330-002-1406-2

[39] Koeppen AH. Merritt's neurology: Edited by LP Rowland, Lippincott Williams & Wilkins, Philadelphia, 2000

[40] Victor M, Ropper AH. Adams and Victor's principles of neurology. McGraw-Hill, Medical Pub. Division, 2001.

How to cite this article: Yalçın ED, Doğan ME. Cone-Beam CT Evaluation of Intracranial Physiological Calcifications by Age and Gender. Clin Exp Health Sci 2024; 14: 1-7. https://doi.org/10.33808/clinexphealthsci.1201994

Clinical and Experimental Health Sciences

MiR-7-5p May Inhibit AML Cell Proliferation Via SKP2, KLF4, OGT Target Genes

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Received: April 14, 2023 **Accepted:** December 16, 2024

ABSTRACT

Objective: Acute Myeloid Leukemia (AML) is distinguished by the differentiation and overgrowth of blast cells. In the current study, we purposed to elucidate the effect of miR-7-5p on AML cellular processes and the expression level of potential target genes.

Methods: miR-7-5p mimic was transfected into AML cells by lipofectamine-mediated method and verified by qRT-PCR. The miR-7-5p's effect on proliferation and apoptosis was investigated by WST-8 and Caspase-3 kit (respectively). miRDB, miRTarBase, Targetscan, miRWalk, https://ongene.bioinfo-minzhao.org/, and http://soft.bioinfo-minzhao.org/lgl/a databases were utilized for in silico identification of possible target genes of miR-7-5p. Relative gene expression of potential target genes was investigated via the qRT-PCR technique.

Results: In the group that is transfected with miR-7-5p, proliferation significantly decreased and apoptosis increased as against the control group. BCL2, SKP2, OGT, KLF4 and EGFR gene expression levels, which were determined as a result of possible target gene analysis by in silico methods and literature search, were investigated in AML cell lines. While the SKP2, KLF4 and OGT expression levels were statistically decreased in the group of transfected with mimic miR-7-5p, no statistically significant change was detected in the expressions of BCL2 and EGFR genes.

Conclusion: miR-7-5p may affect the cancer process in AML by targeting SKP2, KLF4, and OGT genes. It is very important to identify and validate the miR-7-5p target genes, which has the possibility to be a new biomarker in the early diagnosis and therapy of AML. Therefore, our data obtained at mRNA level should be confirmed by further studies.

Keywords: AML cell line, miR-7-5p, SKP2, KLF4, OGT

1. INTRODUCTION

Acute Myeloid Leukemia (AML) is a heterogeneous malignant sickness characterized by unlimited overgrowth and accumulation of abnormal blast cells affected by random genetic differentiation in hematopoietic stem cells (1). AML is the most prevalent leukemia in the adult population, accounting for 80% of all cases (2).

MicroRNAs (miRNAs) have critical roles in cancer pathogenesis including AML. They are a subset of non-protein-coding and endogenous single-stranded RNAs with approximately 20 nucleotides (3, 4). They are regulators of gene expression by binding to the 3' end un-translational regions (UTR) of messenger RNAs (mRNAs) target (5, 6). Each of the miRNAs, which are known as regulator of approximately 60% of all human genes, can regulate expression by targeting more than one gene (7, 8).

miR-7 family is highly conserved and is produced by the miRNA precursor 1-3 (9). miR-7-5p is the most studied member of this miRNA family (10, 11). miR-7-5p has been submitted to be associated with many cancers such as colon (12), pancreas (11), melanoma (13), lung (14), breast (15), gastric (16), glioblastoma (17), lymphoma (18). It has been reported that it is down-regulated in most cancer types and mostly acts as a tumor suppressor. It was indicated that miR-7-5p plays a tumor suppressor role in the development of AML by targeting *OSBPL11* (19). However, the relationship between miR-7-5p and AML has not yet been clearly demonstrated. Therefore, more research studies are needed to determine which possible targets of miR-7-5p can influence the cancer process.

From this point of view, the impact of miR-7-5p on cell viability and apoptosis processes in AML cell lines (HL-60 and NB4 cells) was investigated in this study. Then, possible miR-7-5p target genes were determined via *in silico* methods. The qRT-PCR technique was used to affirm the expressions of possible target genes which were selected as a result of *in silico* analysis in AML cells. For this purpose, gene expressions were compared in AML cells transfected

https://doi.org/*10.33808/clinexphealthsci.1279724*

with miR-7-5p mimic and AML cells inoculated with non-targeting miRNA mimic (nt control).

2. METHODS

2.1. Identification of Possible miR-7-5p Targets by Bioinformatics and In Silico Analysis

In the process of determining the possible miR-7-5p target genes, possible target genes were listed through *in silico* programs such as miRDB, miRTarBase, Targetscan, and miRWalk. Then, starting from the genes with the highest target score (score ≥ 85, genes that have strong evidence "validated via Reporter assay, Western-blot, qRT-PCR") was investigated via https://ongene.bioinfo-minzhao.org/ and http://soft.bioinfo-minzhao.org/lgl/ sites.

2.2. Cell Line Culturing Process

HL-60 and NB4 cell lines are known as cell lines derived from human mature acute myeloblastic leukemia cells and they are frequently used in AML studies. NB4 and HL-60 cells were grown under 37°C, 5% CO_2 conditions, and in RPMI-1640 medium prepared by adding 1% antibiotic and 10% FBS. When the cells multiplied by seeding in a 25 cm^2 cell culture flask reached 70-80% density (confluency), they were transferred to a larger 75 cm^2 cell culture flask and passaged. Other cells that were increased during passage were placed in a freezing medium (RPMI-1640 medium containing 10% DMSO) and placed in cryo tubes to be used in further studies. Then, it was stored in a nitrogen tank at – 196°C.

2.3. Mimic Transfection into NB4 and HL-60 Cells

The miR-7-5p mimic was transfected into cell lines. The non-targeting miRNA oligonucleotide sequence, which is known not to target any gene, was purchased commercially and used in the preparation of control cells (nt control) in the study. Transfection procedures were performed according to the Lipofectamine-2000 protocol.

2.4. Isolation of RNA and cDNA Synthesis for Transfection Validation

AML cells, which were transfected with miR-7-5p and nt mimic 24 hours before, were grown in an oven at the appropriate medium, centrifuged at 1500 rpm (5 minutes), and the pellets were dissolved in Trizol solution (Ambion) and the manufacturer's company RNA was isolated according to the protocol.

In order to control whether the transfection process has taken place and to control the expression level of miR-7-5p in cells transfected into HL-60 and NB4 cell lines, first cDNA synthesis followed by the qRT-PCR was conducted with the use of TaqMan primers and probes. In the transfection validation process, 30 ng RNA was used in total for each of the study and control groups. First of all, cDNA synthesis was performed with the TaqMan MicroRNA Reverse Transcription Kit (ThermoFisher Sci) in accordance with the supplier firm's protocol. Afterward, qRT-PCR processes were performed using TaqMan Universal Master Mix II (ThermoFisher Sci.), miR-7-5p RT probe (ThermoFisher Sci.), and RNU43 RT probe (ThermoFisher Sci.) as to the supplier firm's protocol. The experiments were run in duplicate and the data obtained were evaluated by the $2^{-\Delta\Delta Ct}$ method.

2.5. Investigation of the Influence of miR-7-5p on Cellular Processes as Viability and Apoptosis

Cells, seeded in 96-well plates with 3 replications and $3x10^3$ cells in each well, were incubated at 37°C, 5% CO_2. After 24 hours, miR-7-5p and nt mimic transfection was performed into cells according to the Lipofectamine-2000 protocol. Cell viability was evaluated at the 48th and 72nd hours to determine the proliferation of transfected cells. Using the Multiscan FC microplate (ThermoFisher Sci.) reader device, the proliferation of cells was measured with the Cell Viability Detection 8 Kit (CVDK8) (Eco-tech Biotechnology NutriCulture) according to the supplier firm's protocol and measured at 450 nm absorbance. $2x10^5$ NB4 cells were seeded into a 6-well plate for the purpose of determining the apoptosis situation. miR-7-5p mimic and nt mimic were transfected via Lipofectamine-2000 Reagent according to the supplier firm's protocol. 24 hours later from the transfection, miR-7-5p and nt mimic transfected cells were collected. The Human Caspase 3 Instant ELISA Kit (Invitrogen, ThermoFisher Sci.) protocol was applied and the measurement was conducted at 450 nm absorbance via the Multiscan FC microplate reader (ThermoFisher Sci.).

2.6. cDNA synthesis and the qRT-PCR Procedures to Determine Gene Expression Levels

cDNA synthesis was performed with the "RevertAid First Strand cDNA synthesis" kit (ThermoFisher Sci.) using a total of 1000 ng RNA from RNA samples of HL-60 and NB4 cells transfected with miR-7-5p mimic and nt mimic. Relative expression levels of selected genes, in mimic-transfected HL-60 and NB4 cells, were analyzed by the quantitative RT-PCR method. The qRT-PCR was performed according to the "5X HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne)" protocol. The experiments were conducted in duplicate. The βactin gene was used for normalization.

2.7. Statistical Analysis

SPSS 28 software was used in the statistical evaluation of the data obtained in the study. "Student's t-test" was used to compare the study group and the control group, and the data of the analysis results were demonstrated as mean ± standard deviation. The $2^{-\Delta\Delta Ct}$ method was used in the relative quantitation analysis of the qRT-PCR results. Statistically, the data with $p < .05$ were considered significant. GraphPad Prism 9.3 software, Microsoft Excel, and Paint programs were used for the construction of graphics, figures, and tables. The analysis of overall survival (OS) was performed with the "Kaplan-Meier test" using the GEPIA-2 program.

3. RESULTS

3.1. Possible Interacting Genes of miR-7-5p

About 20 of the genes classified as strong possible interacting genes of miR-7-5p in *in silico* databases were found to be among 803 oncogenes on https://ongene.bioinfo-minzhao.org/ (Figure 1a). Among these 20 genes identified as miR-7-5p targets, 5 of them were found in the "http://soft.bioinfo-minzhao.org/lgl/" database (Figure 1b). The expression levels of these 5 genes [B-Cell CLL/Lymphoma 2 (*BCL2*), S-Phase Kinase Associated Protein 2 (*SKP2*), O-Linked N-Acetylglucosamine (GlcNAc) Transferase (*OGT*), Kruppel Like Factor 4 (*KLF4*) and Epidermal Growth Factor Receptor (*EGFR*) genes] were compared with mimic miR-7-5p and nt transfected AML cells.

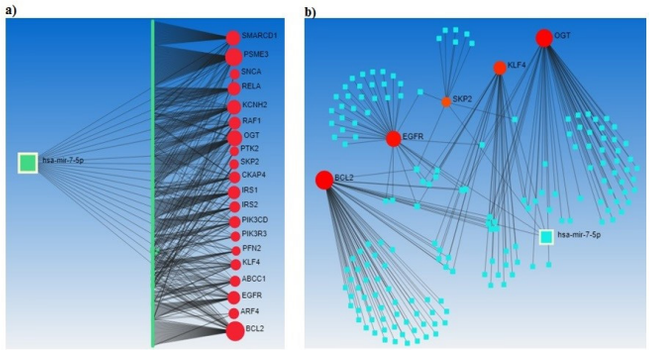

Figure 1. *Bioinformatics analysis results according to searching the miRDB, miRTarBase, Targetscan, miRWalk, https://ongene.bioinfo-minzhao.org/ and http://soft.bioinfo-minzhao.org/lgl/a) databases. a) 20 genes identified to be miR-7-5p targets (517 nodes 622 edges). b) Among these 20 genes, 5 genes were selected for in vitro study (168 miRNAs nodes with 202 edges)*

3.2. Validation of miRNA Mimic Transfection

Verification of the miRNA mimic transfection was showed that the amount of miR-7-5p and nt control mimics transfected into HL-60 and NB4 cells by Lipofectamine-mediated methods increased statistically in the cells and the transfection was successful (Figure 2).

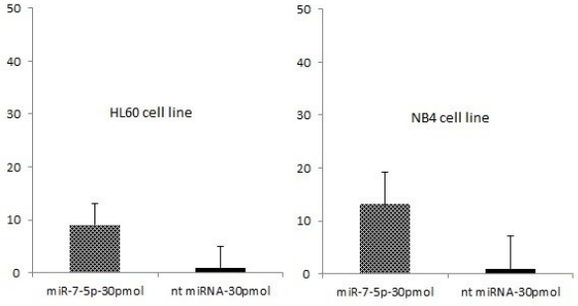

Figure 2. *Transfection validation of miRNA mimic-transfected HL-60 and NB4 cells*

3.3. Impact of miR-7-5p on Cell Proliferation

As a result of the cell proliferation experiment performed to determine the impact of miR-7-5p on the proliferation of cells, the miR-7-5p transfected group in HL-60 and NB4 cells compared to the nt control group at both 48th hour (p=.04; p=.01), It was determined that there was a significant decrease in cell proliferation in both (respectively) and 72 hours (p=.028; p=.002, respectively) (Figure 3).

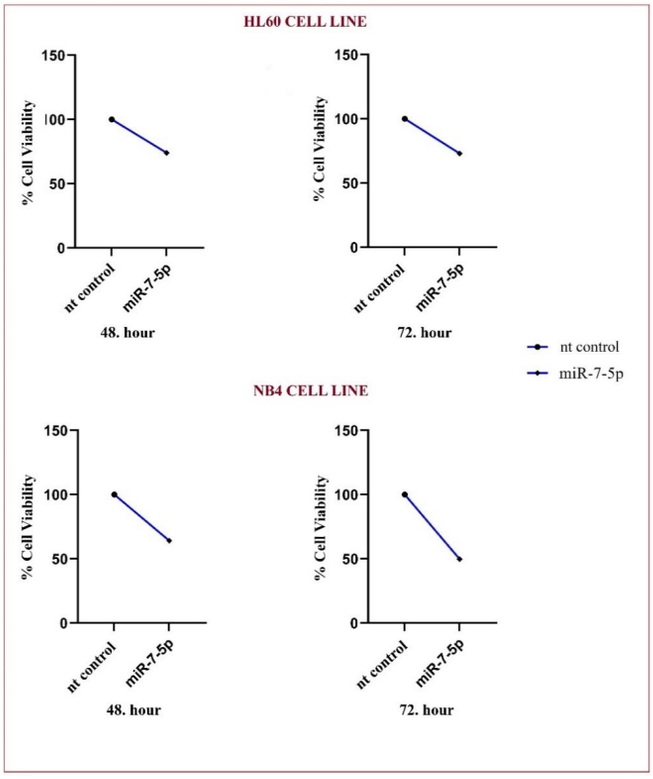

Figure 3. *Proliferation changes in HL-60 and NB4 cells at 48. and 72. Hours*

3.4. Impact of miR-7-5p on Apoptosis

Caspase-3 level was evaluated to determine the impact of miR-7-5p on the apoptosis process and it was found that the cell death in the miR-7-5p transfected group in NB4 cells increased statistically significantly compared to the nt control group (p=.003) (Figure 4).

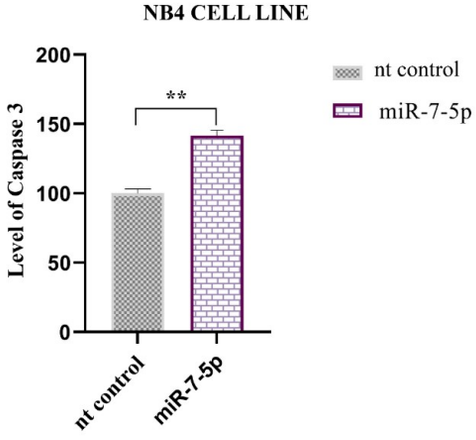

Figure 4. *Effect of miR-7-5p on apoptosis in NB4 cells (** p < .01)*

3.5. miR-7-5p Relation With Expression Levels of OGT, SKP2, BCL2, KLF4 and EGFR

The expression data obtained as a result of qRT-PCR used to look at the expression levels of *OGT*, *SKP2*, *BCL2*, *KLF4,* and *EGFR* genes, which are determined as possible targets of miR-7-5p, were evaluated by the relative quantitation method and analyzed via Student's t-test using βactin gene for normalisation. While the p values obtained in the analyzes were less than 0.05 were considered statistically important, those greater than 0.05 were not considered statistically important. The primer sequences used for qRT-PCR to investigate the expressions of the relevant genes were shown in Table 1. In conclusion, the relative expression levels of *OGT* (p=.012; p=.017), *SKP2* (p=.019; p=.036) and *KLF4* (p=.003; p=.027) genes were reduced in miR-7-5p transfected cells compared to the control group in HL-60 and NB4 cell lines. However, no statistically significant change was detected in the relative expressions of *BCL2* (p=.652; p=.174) and *EGFR* (p=.201; p=.698) genes in miR-7-5p transfected cells compared to the control group in both cell lines (Figure 5). All mean and p values of the gene expression levels were shown in Table 2.

Table 1. *Primer sequences*

Genes	Sequences	Base	Reference
BCL2-F	5′-GATGTGATGCCTCTGCGAAG-3′	20	(43)
BCL2-R	5′-CATGCTGATGTCTCTGGAATCT-3′	22	
SKP2-F	5′-ATGCCCCAATCTTGTCCATCT-3′	21	(44)
SKP2-R	5′-CACCGACTGAGTGATAGGTGT-3′	21	
OGT-F	5′-TGTCACCCTTGACCCAAACTT-3′	21	(45)
OGT-R	5′-GGCACGAAGATAAGCTGCCA-3′	20	
KLF4-F	5′-CCCAATTACCCATCCTTCCTG-3′	21	(46)
KLF4-R	5′-GTCTTCCCCTCTTTGGCTTG-3′	20	
EGFR-F	5′-ATGGTCAAGTGCTGGATG-3′	18	(47)
EGFR-R	5′-GAGGAAGGTGTCGTCTATG-3′	19	
βactin-F	5′-GCCTCGCCTTTGCCGATC-3′	18	(46)
βactin-R	5′-CCCACGATGGAGGGGAAG-3′	18	

Table 2. *Mean and p values of gene expression levels*

Genes	Expression levels in HL-60 cell line		Expression levels in NB4 cell line	
	mean	p-value	mean	p-value
OGT	0,248	.012	0,356	.017
SKP2	0,094	.019	0,534	.036
KLF4	0,386	.003	0,229	.027
BCL2	1,096	.652	1,207	.174
EGFR	0,287	.201	1,325	.698

**significant (p < .05)*

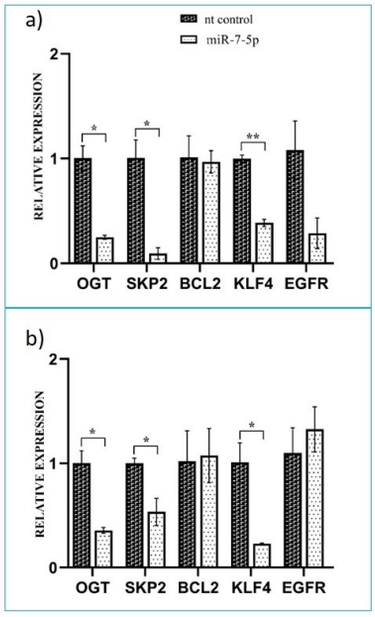

Figure 5. *Comparison of the expression levels of OGT, SKP2, KLF4, BCL2, and EGFR genes in miR-7-5p mimic and non-targeting miRNA mimic transfected AML cells a) HL-60 cell line b) NB4 cell line*

3.6. Overal Survival (OS) Analysis

The overall survival analysis result showed that the *OGT* gene had an indicative role in poor OS in AML patients (Figure 6).

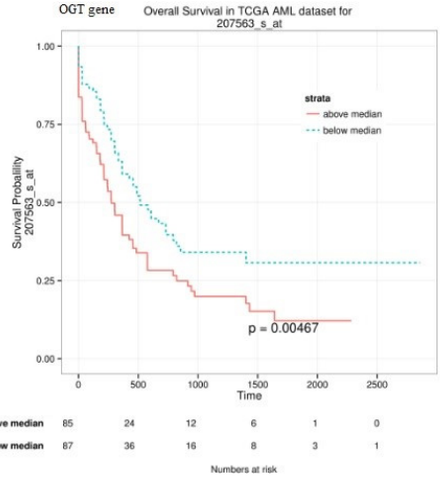

Figure 6. *OGT gene influence on overall AML survival in TCGA. Points to consider when analyzing: Group cutoff: Median, Cutoff – High (%): 50, Cutoff-Low (%): 50, Hazards Ratio (HR): Yes, 95% Confidence Interval: Yes, Axis Units: Months*

4. DISCUSSION

AML is a heterogeneous form of malignant leukemia mostly seen in adults, characterized by excessive accumulation of immature myeloblast cells in the bone marrow/blood (20). One of the factors that play an active role in the formation processes of various cancers, incorporating leukemias, is miRNA deregulation. miRNAs function as both oncogenic and tumor suppressors in the formation processes

of various diseases, including cancers (21, 22). Rearrangement of expression levels in target genes of miRNAs offers potential treatment alternatives for various diseases. It is thought that it will make a great contribution to the development of new treatment strategies in addition to the treatments used today, especially with the rearrangement of the expressions of miRNAs in cancer types (23, 24). In the studies, oncogenic genes are silenced with the rearrangement of miRNA expressions or it is aimed to have a stopping effect in the cancer development process by providing the re-expression of tumor suppressor genes with reduced expression.

miR-7-5p is the most well-known member of the highly conserved miR-7 family (9). It is known that this miRNA behaves as a tumor repressor in many cancers and plays a reduced role in cancer pathogenesis. In the literature, it has been shown in lung cancer (25) and cholangiocarcinoma (26) studies that miR-7-5p plays a role in inhibiting diverse cellular procedures such as cell proliferation, growth, migration, and metastasis, promoting apoptosis and maintaining cell cycle control.

In this project, a substantial decrease in cell proliferation was detected in HL-60 and NB4 cell lines transfected with miR-7-5p mimic in comparison to the control group. Similarly, as a result of the apoptosis experiment, it was determined that miR-7-5p increased the amount of Caspase-3 and drove cells to apoptosis in NB4 cells. Only NB4 cells were used in the apoptosis assay because of the limitation of the project budget., therefore, it could not be compared with the apoptosis process in HL-60 cells. Therefore, apoptosis changes in HL-60 cells should be controlled in further studies so that the effect of miR-7-5p on genes can be better understood by comparing them with the changes in NB4 cells. The expression levels of *BCL2*, *SKP2*, *OGT*, *KLF4*, and *EGFR* genes which were selected as possible targets of miR-7-5p via the bioinformatics approach and *in silico* analysis, were investigated. *SKP2*, *KLF4,* and *OGT* gene expression levels in the miR-7-5p mimic transfected group were found to be statistically significantly decreased compared to the nt control mimic transfected group. But then, it was concluded that there was no substantially significant change in *BCL2* and *EGFR* gene expression levels.

The *SKP2* gene is included in the modulation of the cell cycle and is known to act as a proto-oncogene in tumor pathogenesis in various cancer types known to be overexpressed (27). By directly targeting the expression of the *SKP2* gene, which has a high expression level in breast cancer, miR-7-5p could reduce drug-resistant cancer cell proliferation and epithelial-mesenchymal transition (28). Studies have shown that *SKP2* is a highly oncogenic protein and is strongly associated with AML prognosis (29, 30, 31). On the other hand, there is also a study in the literature reporting that *SKP2* has a weak relationship with AML and that this gene does not play an active role in AML prognosis (32). In our study, we found that *SKP2* was up-regulated in AML cells and its expression was significantly decreased following miR-7-5p transfection. Our findings support the previous studies showing a high association between *SKP2* and AML pathogenesis. Furthermore, it was determined that miR-7-5p and *SKP2* showed a negative correlation in AML cells, as in many other types of cancer, and miR-7-5p played a role in reducing cancer cell proliferation.

The *KLF4* gene is involved in cell cycle control, differentiation, and many developmental processes. In a study on colon cancer in which *KLF4* was reported to play an oncogenic role, the connection between miR-7-5p and *KLF4* was confirmed and it was shown that this miRNA partially reduced the invasion and migration of cancerous cells (33). A study in the literature on AML showed that *KLF4* has high expression levels and promotes monocytic differentiation (34). According to our current study, miR-7-5p and *KLF4* showed a negative correlation in AML cells same as in these literature data.

Disruption in the expression of the *OGT* gene, which is identified to be included in the coordinating of cellular response pathways to stress and cell cycle regulation, has been associated with diabetes, cancer, and cardiac complications (35, 36, 37). Inhibition of O-linked-N-acetylglucosaminylation has been indicated to increase apoptosis tendency in AML cells. Therefore, it is thought that *OGT* may have the potential to be an important target for the formation of a potential therapy for AML patients (38). A study conducted on AML showed that *OGT* expression level was high in AML cells and cell proliferation decreased and apoptosis increased with the inhibition of *OGT* (39). The relevancy between *OGT* and miR-7-5p was also examined on AML cells in our study and it was found a correlation with the literature. Also our current study's overall survival (OS) analysis showed that the *OGT* gene had an indicative role in poor OS in AML patients (Figure 6).

A miRNA can have more than one target gene, and a gene can be targeted by more than one miRNA (40). According to studies in the literature, *EGFR* and *BCL-2* genes are overexpressed in several cancer types including AML and these genes are highly effective for cancer progress. In addition to this, they have potential biomarker properties for cancers (41,42). They can be targeted by miRNAs, mRNAs, or signaling pathways and their expression levels can change with the targeting. Therefore, the roles of these genes in cancer development can be knocked out or knocked down with several procedures. In this case, direct targeting of genes and changing their expression is very important. If there is no direct or indirect relationship between those targeting the gene and the target genes, it is not possible to silence the gene. As in our study, the miR-7-5p directly targeted the *SKP2*, *KLF4,* and *OGT* genes, causing their downregulation. The fact that no statistically significant changes were detected in *BCL2* and *EGFR* genes whose expression levels were examined may suggest that these genes may be under the control of miRNAs other than miR-7-5p in AML cells.

5. CONCLUSION

In conclusion, in our study in which the relevancy of miR-7-5p and AML was examined by cell proliferation, gene expression, and Caspase-3 experiments in HL-60 and NB4 cells, it was determined that the expressions of *SKP2*, *KLF4,* and *OGT* genes were modulated via miR-7-5p at the mRNA level. It is important to confirm these findings with further studies. The limitations of our study include the absence of tissue confirmation. Our results need confirmation of the mRNA and protein levels in patient tissue samples. There are several possible targets of miR-7-5p identified via *in silico* methods. It is very crucial to conclude the definitive interacting genes of miR-7-5p, which may have important potential as a biomarker in processes such as early diagnosis, prognosis follow-up, and discovery of novel treatment approaches for AML.

Acknowledgments: *The authors thank Istanbul University Scientific Research Projects Unit for their project support (Project no: 37653).*

Funding: *The present work was supported by the fund of Istanbul University Scientific Research Projects (Project no: 37653).*
Peer-review: *Externally peer-reviewed.*
Conflict of interest: *The authors declared no conflict of interest.*
Ethics: *Ethical approval was not obtained as a commercially produced cell line was used in the study.*
Author Contribution:
Research idea: IS, MK
Design of the study: IS, MK, SP
Acquisition of data for the study: EM, IS, MK
Analysis of data for the study: EM, IS, MK
Interpretation of data for the study: IS, MK, SO, KC, SP
Drafting the manuscript: EM, IS, MK
Revising it critically for important intellectual content: IS, MK, SO, KC, SP
Final approval of the version to be published: EM, IS, MK, SO, KC, SP

REFERENCES

[1] Short NJ, Ravandi F. How close are we to incorporating measurable residual disease into clinical practice for acute myeloid leukemia? Haematologica. 2019;104(8):1532-41. https://doi.org/10.3324/haematol.2018.208454

[2] Vakiti A, Mewawalla P. Acute Myeloid Leukemia. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2023, StatPearls Publishing LLC.; 2023.

[3] Kaya M, Suer I. The effect of miR-34a-5p on overexpressed AML associated genes. J Ist Faculty Med. 2023;86(1):59-68. https://doi.org/10.26650/IUITFD.1168793

[4] Suer I, Guzel E, Karatas OF, Creighton CJ, Ittmann M, Ozen M. MicroRNAs as prognostic markers in prostate cancer. Prostate. 2019;79(3):265-71. https://doi.org/10.1002/pros.23731.

[5] Suer I, Kaya M. Is the AURKB Gene Involved in Aml Cell Proliferation Since It is Targeted by miR-34a-5p and let-7b-5p? Konuralp Medical Journal. 2023. DOI:10.18521/ktd.1171549.

[6] Kaya M, Karatas OF. The Relationship Between Larynx Cancer and MicroRNAs. Van Med J 2020;27(4):535-41. https://doi.org/10.5505/vtd.2020.80947.

[7] Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009;19(1):92-105. https://doi.org/10.1101/gr.082701.108

[8] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215-33. https://doi.org/10.1016/j.cell.2009.01.002.

[9] Kalinowski FC, Brown RA, Ganda C, Giles KM, Epis MR, Horsham J, Leedman PJ. microRNA-7: a tumor suppressor miRNA with therapeutic potential. Int J Biochem Cell Biol. 2014;54:312-7. https://doi.org/10.1016/j.biocel.2014.05.040.

[10] Gu DN, Jiang MJ, Mei Z, Dai JJ, Dai CY, Fang C, Huang Q, Tian L. microRNA-7 impairs autophagy-derived pools of glucose to suppress pancreatic cancer progression. Cancer Lett. 2017;400:69-78. https://doi.org/10.1016/j.canlet.2017.04.020.

[11] Zhu W, Wang Y, Zhang D, Yu X, Leng X. MiR-7-5p functions as a tumor suppressor by targeting SOX18 in pancreatic ductal adenocarcinoma. Biochem Biophys Res Commun. 2018;497(4):963-70. https://doi.org/10.1016/j.bbrc.2018.02.005.

[12] Zeng CY, Zhan YS, Huang J, Chen YX. MicroRNA-7 suppresses human colon cancer invasion and proliferation by targeting the expression of focal adhesion kinase. Mol Med Rep. 2016;13(2):1297-303. https://doi.org/10.3892/mmr.2015.4643.

[13] Giles KM, Brown RA, Epis MR, Kalinowski FC, Leedman PJ. miRNA-7-5p inhibits melanoma cell migration and invasion. Biochem Biophys Res Commun. 2013;430(2):706-10. https://doi.org/10.1016/j.bbrc.2012.11.086.

[14] Xiao H. MiR-7-5p suppresses tumor metastasis of non-small cell lung cancer by targeting NOVA2. Cell Mol Biol Lett. 2019;24:60. https://doi.org/10.1186/s11658-019-0188-3.

[15] Shi Y, Luo X, Li P, Tan J, Wang X, Xiang T, Ren G. miR-7-5p suppresses cell proliferation and induces apoptosis of breast cancer cells mainly by targeting REGγ. Cancer Lett. 2015;358(1):27-36. https://doi.org/10.1016/j.canlet.2014.12.014.

[16] Yang Z, Shi X, Li C, Wang X, Hou K, Li Z, Zhang X, Fan Y, Qu X, Che X, Liu Y. Long non-coding RNA UCA1 upregulation promotes the migration of hypoxia-resistant gastric cancer cells through the miR-7-5p/EGFR axis. Exp Cell Res. 2018;368(2):194-201. https://doi.org/10.1016/j.yexcr.2018.04.030.

[17] Liu Z, Liu Y, Li L, Xu Z, Bi B, Wang Y, Li JY. MiR-7-5p is frequently downregulated in glioblastoma microvasculature and inhibits vascular endothelial cell proliferation by targeting RAF1. Tumour Biol. 2014;35(10):10177-84. https://doi.org/10.1007/s13277-014-2318-x.

[18] Sorrentino D, Frentzel J, Mitou G, Blasco RB, Torossian A, Hoareau-Aveilla C, Pighi C, Farcé M, Meggetto F, Manenti S, Espinos E, Chiarle R, Giuriato S. High Levels of miR-7-5p Potentiate Crizotinib-Induced Cytokilling and Autophagic Flux by Targeting RAF1 in NPM-ALK Positive Lymphoma Cells. Cancers (Basel). 2020;12(10). https://doi.org/10.3390/cancers12102951.

[19] Jiang D, Wu X, Sun X, Tan W, Dai X, Xie Y, Du A, Zhao Q. Bone mesenchymal stem cell-derived exosomal microRNA-7-5p inhibits progression of acute myeloid leukemia by targeting OSBPL11. J Nanobiotechnology. 2022;20(1):29. https://doi.org/10.1186/s12951-021-01206-7.

[20] Pelcovits A, Niroula R. Acute Myeloid Leukemia: A Review. R I Med J (2013). 2020;103(3):38-40.

[21] Testa U, Pelosi E. MicroRNAs expressed in hematopoietic stem/progenitor cells are deregulated in acute myeloid leukemias. Leuk Lymphoma. 2015;56(5):1466-74. https://doi.org/10.3109/10428194.2014.955019.

[22] Capik O, Sanli F, Kurt A, Ceylan O, Suer I, Kaya M, Ittmann M, Karatas OF. CASC11 promotes aggressiveness of prostate cancer cells through miR-145/IGF1R axis. Prostate Cancer Prostatic Dis. 2021;24(3):891-902. https://doi.org/10.1038/s41391-021-00353-0.

[23] Nair R, Salinas-Illarena A, Baldauf HM. New strategies to treat AML: novel insights into AML survival pathways and combination therapies. Leukemia. 2021;35(2):299-311. https://doi.org/10.1038/s41375-020-01069-1.

[24] Liu Y, Cheng Z, Pang Y, Cui L, Qian T, Quan L, Zhao H, Shi J, Ke X, Fu L. Role of microRNAs, circRNAs and long noncoding RNAs in acute myeloid leukemia. J Hematol Oncol. 2019;12(1):51. https://doi.org/10.1186/s13045-019-0734-5.

[25] Li Q, Wu X, Guo L, Shi J, Li J. MicroRNA-7-5p induces cell growth inhibition, cell cycle arrest and apoptosis by targeting PAK2 in non-small cell lung cancer. FEBS Open Bio. 2019;9(11):1983-93. https://doi.org/10.1002/2211-5463.12738.

[26] Tang Y, Tang Z, Yang J, Liu T, Tang Y. MicroRNA-7-5p Inhibits Migration, Invasion and Metastasis of Intrahepatic Cholangiocarcinoma by Inhibiting MyD88. J Clin Transl

Hepatol. 2021;9(6):809-17. https://doi.org/10.14218/JCTH.2021.00021.

[27] Gstaiger M, Jordan R, Lim M, Catzavelos C, Mestan J, Slingerland J, Krek W. Skp2 is oncogenic and overexpressed in human cancers. Proc Natl Acad Sci U S A. 2001;98(9):5043-8. https://doi.org/10.1073/pnas.081474898.

[28] Wang HF, Dong ZY, Yan L, Yang S, Xu HN, Chen SL, Wang WR, Yang QL, Chen CJ. The N-terminal polypeptide derived from vMIP-II exerts its antitumor activity in human breast cancer through CXCR4/miR-7-5p/Skp2 pathway. J Cell Physiol. 2020;235(12):9474-86. https://doi.org/10.1002/jcp.29755.

[29] Dan W, Zhong L, Zhang Z, Wan P, Lu Y, Wang X, Liu Z, Chu X, Liu B. RIP1-dependent Apoptosis and Differentiation Regulated by Skp2 and Akt/GSK3β in Acute Myeloid Leukemia. Int J Med Sci. 2022 Mar 6;19(3):525-536. https://doi.org/10.7150/ijms.68385. PMID: 35370472; PMCID: PMC8964317.

[30] Thacker G, Mishra M, Sharma A, Singh AK, Sanyal S, Trivedi AK. CDK2-instigates C/EBPα degradation through SKP2 in Acute myeloid leukemia. Med Oncol. 2021 May 17;38(6):69. https://doi.org/10.1007/s12032-021-01523-9. PMID: 34002296.

[31] Thacker G, Mishra M, Sharma A, Singh AK, Sanyal S, Trivedi AK. E3 ligase SCFSKP2 ubiquitinates and degrades tumor suppressor C/EBPα in acute myeloid leukemia, Life Sciences (2020), https://doi.org/10.1016/j.lfs.2020.118041.

[32] Min YH, Cheong JW, Lee MH, Kim JY, Lee ST, Hahn JS, Ko YW. Elevated S-phase kinase-associated protein 2 protein expression in acute myelogenous leukemia: its association with constitutive phosphorylation of phosphatase and tensin homologue protein and poor prognosis. Clin Cancer Res. 2004 Aug 1;10(15):5123-30. https://doi.org/10.1158/1078-0432.CCR-04-0136. PMID: 15297415

[33] Dong M, Xie Y, Xu Y. miR-7-5p regulates the proliferation and migration of colorectal cancer cells by negatively regulating the expression of Krüppel-like factor 4. Oncol Lett. 2019;17(3):3241-6. https://doi.org/10.3892/ol.2019.10001.

[34] Noura M, Morita K, Kiyose H, Matsuo H, Nishinaka-Arai Y, Kurokawa M, Kamikubo Y, Adachi S. Pivotal role of DPYSL2A in KLF4-mediated monocytic differentiation of acute myeloid leukemia cells. Sci Rep. 2020;10(1):20245. https://doi.org/10.1038/s41598-020-76951-0.

[35] Fardini Y, Dehennaut V, Lefebvre T, Issad T. O-GlcNAcylation: A New Cancer Hallmark? Front Endocrinol (Lausanne). 2013;4:99. https://doi.org/10.3389/fendo.2013.00099.

[36] Krause MW, Love DC, Ghosh SK, Wang P, Yun S, Fukushige T, Hanover JA. Nutrient-Driven O-GlcNAcylation at Promoters Impacts Genome-Wide RNA Pol II Distribution. Front Endocrinol (Lausanne). 2018;9:521. https://doi.org/10.3389/fendo.2018.00521.37.

[37] Slawson C, Hart GW. O-GlcNAc signalling: implications for cancer cell biology. Nat Rev Cancer. 2011;11(9):678-84. https://doi.org/10.1038/nrc3114.

[38] Asthana A, Ramakrishnan P, Vicioso Y, Zhang K, Parameswaran R. Hexosamine Biosynthetic Pathway Inhibition Leads to AML Cell Differentiation and Cell Death. Mol Cancer Ther. 2018;17(10):2226-37. https://doi.org/10.1158/1535-7163.MCT-18-0426.

[39] He N, Ma D, Tan Y, Liu M. Upregulation of O-GlcNAc transferase is involved in the pathogenesis of acute myeloid leukemia. Asia Pac J Clin Oncol. 2022;18(5):e318-e28. https://doi.org/10.1111/ajco.13685.

[40] Suer I, Kaya M, Ozgur E. The Effect of miR-34a-5p and miR-145-5p Ectopic Expression on Cell Proliferation and Target Gene Expression in the MDA-MB-231 Cell Line. NKMJ. 2021;9(2):166-73. https://doi.org/10.4274/nkmj.galenos.2021.770512.

[41] Mahmud H, Kornblau SM, Ter Elst A, Scherpen FJ, Qiu YH, Coombes KR, de Bont ES. Epidermal growth factor receptor is expressed and active in a subset of acute myeloid leukemia. J Hematol Oncol. 2016 Aug 3;9(1):64. https://doi.org/10.1186/s13045-016-0294-x. PMID: 27488458; PMCID: PMC4971659

[42] Wei Y, Cao Y, Sun R, Cheng L, Xiong X, Jin X, He X, Lu W, Zhao M. Targeting Bcl-2 Proteins in Acute Myeloid Leukemia. Front Oncol. 2020 Nov 5;10:584974. https://doi.org/10.3389/fonc.2020.584974. PMID: 33251145; PMCID: PMC7674767

[43] Golestani Eimani B, Sanati MH, Houshmand M, Ataei M, Akbarian F, Shakhssalim N. Expression and prognostic significance of bcl-2 and bax in the progression and clinical outcome of transitional bladder cell carcinoma. Cell J. 2014;15(4):356-63.

[44] Zhao H, Pan H, Wang H, Chai P, Ge S, Jia R, Fan X. SKP2 targeted inhibition suppresses human uveal melanoma progression by blocking ubiquitylation of p27. Onco Targets Ther. 2019;12:4297-308. https://doi.org/10.2147/OTT.S203888.

[45] Zhang C, Xie F, Li L, Zhang C, Zhang Y, Ying W, Liu L, Yan X, Yin F, Zhang L. Hepatocyte nuclear factor 1 alpha (HNF1A) regulates transcription of O-GlcNAc transferase in a negative feedback mechanism. FEBS Lett. 2019;593(10):1050-60. https://doi.org/10.1002/1873-3468.13381.

[46] Suer I, Karatas OF, Yuceturk B, Yilmaz M, Guven G, Buge O, Cansiz H, Ozen M. Characterization of stem-like cells directly isolated from freshly resected laryngeal squamous cell carcinoma specimens. Curr Stem Cell Res Ther. 2014;9(4):347-53. https://doi.org/10.2174/1574888x09666140330201632.

[47] Zhang B, Tian Y, Jiang P, Jiang Y, Li C, Liu T, Zhou R, Yang N, Zhou X, Liu Z. MicroRNA-122a Regulates Zonulin by Targeting EGFR in Intestinal Epithelial Dysfunction. Cell Physiol Biochem. 2017;42(2):848-58. https://doi.org/10.1159/000478629.

How to cite this article: Mertoğlu E, Suer İ, Kaya M, Öztürk Ş, Çefle K, Palanduz Ş. MiR-7-5p May Inhibit AML Cell Proliferation Via SKP2, KLF4, OGT Target Genes. Clin Exp Health Sci 2025; 15: 8-14. https://doi.org/10.33808/clinexphealthsci.1279724

Clinical and Experimental Health Sciences

Cost-Effectiveness of Carotid Artery Stenting Compared to Carotid Endarterectomy in Patients with Carotid Stenosis: A Turkish Health System Perspective

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Received: June 15, 2023 **Accepted:** February 28, 2025

ABSTRACT

Objective: This study assesses the cost-effectiveness of carotid artery stenting (CAS) versus carotid endarterectomy (CAE) from the perspective of payers in Türkiye, considering potential complications.

Methods: A decision tree analysis model was employed using data from 61 patients (29 CAS, 32 CAE) treated for carotid stenosis (CS) between 2019-2021. The procedural costs were derived from a university hospital-billing department, while health outcomes such as any stroke and myocardial infarction (MI) and their utility values were based on meta-analyses and established studies. The primary outcome measure was the incremental cost-effectiveness ratio (ICER).

Results: When the model was applied, CAS incurred higher costs ($1.344,41 per patient) compared to CAE ($947,30), resulting in an ICER of $96.345 per QALY. CAE, as a traditional model, demonstrated dominance due to its lower costs and slightly better outcomes. Sensitivity analysis showed that a ±10% change in input parameters, particularly a higher impact was observed in costs and stroke incidence and could alter the ICER about ±$1.225 to $3.500. Budget impact analysis estimated CAS and CAE affecting 4.37% and 3.09% of the healthcare budget, respectively.

Conclusion: CAE demonstrated superior cost-effectiveness over CAS in treating CS within the Turkish healthcare system. Despite CAS's appeal as a less invasive option, its higher costs and marginal effectiveness suggest that CAE should be prioritized unless parameters such as procedural costs and any stroke risks associated with CAS are reduced.

Keywords: Carotid stenosis, carotid artery stenting, carotid endarterectomy, cost-effectiveness analysis, ICER

1. INTRODUCTION

Over the past two decades, carotid artery stenting (CAS) has emerged as an innovative and less invasive alternative to carotid endarterectomy (CAE) for treating carotid artery stenosis (CS) (1,2). It has been reported that approximately 20-25% of all strokes are caused by CS (3,4), and patients with CS are at high risk for developing cardiovascular diseases such as myocardial infarction (MI) (5). Some large randomized controlled trials (RCTs) have shown that while the risk of any stroke is higher with CAS, the incidence of MI is higher after endarterectomy (6-9) in the postoperative period. Additionally, the high stroke risk associated with CAS and the cost of medical equipment such as stents and embolic protection devices raise concerns about its value in the healthcare environment (10).

Several economic analyses have compared CAS and CAE for CS, generally finding that CAE is more cost-effective (1,2,11,13). Moreover, CAE has been proven effective for stroke prevention in selected patients (14). However, the impact of events such as stroke and MI on quality of life and the potential superiority of CAS remains uncertain (15). It is clear that beyond the safety of a treatment technique, economic evaluation is also crucial. While surgical techniques aim to improve patient conditions, procedures that also reduce costs and improve quality of life ensure efficiency (16).

Given the increasing attention to the economic evaluation of healthcare procedures, policymakers and insurers must consider not only the safety but also the cost-effectiveness of new treatments (12). As highlighted, the relative cost-effectiveness of CAS and CAE remains unproven. Therefore, this research performs an economic evaluation under the assumption of complications to assess the cost-effectiveness of CAS versus CAE among patients with both symptomatic and asymptomatic CS in Türkiye's healthcare setting. The perspective of Türkiye's Social Security Institution (SSI) was chosen for this analysis, as it evaluates health technologies based on the national budget's cost burden and serves as the sole reimbursement institution in Türkiye. Most notably, no prior study of such design on CAS and CAE has been conducted in Türkiye.

https://doi.org/*10.33808/clinexphealthsci.1315072*

2. METHODS

We conducted a retrospective cost-effectiveness analysis comparing the costs and outcomes of CAS and CAE for 61 patients (29 treated with CAS and 32 treated with CAE) who underwent surgery for CS between 2019 and 2021. This study was performed from the payer's perspective, focusing on complications occurring within one year after treatment that may impact CAS or CAE outcomes. Major complications considered were MI and any type of stroke, which are common post-treatment complications for CS (8,9). In Türkiye, any disease-related treatments within one year after surgery are billed to the payers. Yet, no complications relating to CAS and CAE after treatment were observed during this period; therefore, data such as utility scores, post-treatment probabilities, and costs related to MI and any stroke were sourced from existing literature.

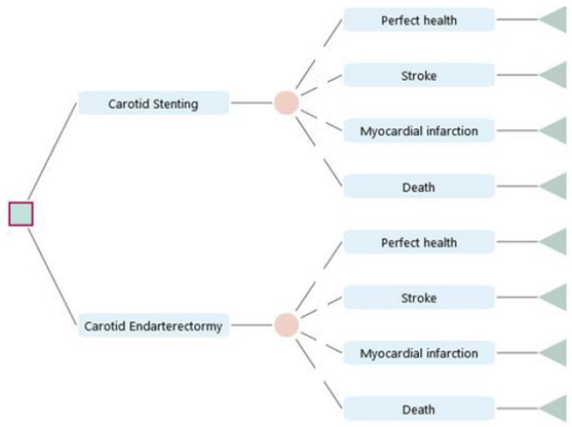

Figure 1. *Simplified decision tree analysis model of health conditions for effectiveness and costs of carotid stenosis. The decision tree model includes all treatment alternatives in relation to the data sources and the possible results. In order to compare the cost-effectiveness of alternatives, we determined data such as the possibilities of outcomes after operation, utilities, and cost parameters separately. While all data related to procedures was inserted into the model on the right side of the tree, we obtained final results on the left side of the tree model for the cost and effectiveness of CAS and CAE separately.*

A decision tree model was employed to estimate the cost-effectiveness of CAS versus CAE. All costs and clinical outcomes were modeled using BYTreePlan (16), based on literature data and expert opinions to analyze expected or weighted costs and outcomes (Figure 1). The model required data on potential complications, providing a weighted average of cost and effectiveness (utilities) data within one year. Accordingly, our study modeled outcomes for any stroke, MI, possible death, and perfect health based on their accessibility in literature and prevalence as complications after CS. No discount rate for cost and health effects was applied since the inputs were limited to one year. In this study, CAS is the intervention, and CAE is the comparator.

To test the effect of input variables on the ICER value, one-way sensitivity analysis was performed (±10%) for the alternative method, CAS (17). The effects of variables were presented with the tornado diagram. Additionally, a budget impact analysis was executed to assess the likely financial effects of the techniques on budget. Due to the lack of reliable data on the incidence and prevalence of CS in Türkiye, the global prevalence of CS from a recently published study (5) was used for this analysis.

2.1. Clinical, cost and baseline utilities for data

The incidences of any stroke, MI, death, or perfect health in the post-procedural (30-day) period for CAS and CAE were pooled from the results of a doctoral thesis, which included 19 randomized controlled trials and was recently defended successfully (18). Regardless of whether patients were symptomatic or asymptomatic, we obtained the transaction cost (index cost) of CAS and CAE after treatment from the billing department of a government university hospital in Ankara, Türkiye. Index costs were categorized into outpatient services, operating costs, pharmaceutical costs, medical equipment, laboratory, radiological, blood costs, anesthesia, hospital bed costs, and other services (e.g., consultation, intravenous drug infusion, and visits in surgical branches). No patients in the CAE and CAS groups experienced MI or any stroke during the one year following treatment. Thus, the costs for any stroke and MI were derived from a published paper in Türkiye, designed as an expert panel (19). In that panel, the annual average cost per patient for any stroke or MI was determined from a healthcare system payer perspective, aligning with our study. Additionally, hospital admission costs and, if needed, imaging and laboratory costs for both CAS and CAE were considered as follow-up costs. Index and follow-up costs are shown in Table 1.

Table 1. *Classification of index costs*

Cost categories	Carotid stenting (CAS)		Carotid endarterectomy (CAE)	
	Total cost (US$)	Average cost per patient (US$)	Total cost (US$)	Average cost per patient (US$)
Index costs	**39.181,58**	**1.351,09**	**30.160,47**	**942,52**
Outpatient cost	291,08	10,04	320,64	10,02
Operation costs	0,00	0,00	20.807,79	650,24
Pharmacy costs	1.199,49	41,36	1.646,82	51,46
Medical equipment cost	29.787,82	1.027,17	2.597,41	81,17
Laboratory costs	358,02	12,35	1.144,38	35,76
Radiology costs	6.856,33	236,43	1.304,51	40,77
Blood center costs	25,08	0,87	506,72	15,83
Anesthesia cost	602,51	20,78	388,62	12,14
Hospital bed costs	264,20	9,11	1.279,13	39,97
Other services costs	88,12	3,04	485,10	15,16
Follow-up costs	**245,73**	**8,47**	**79,47**	**2,48**
Total costs	**39.427,31**	**1.359,56**	**30.239,94**	**945,00**

Quality of life (utility scores) data for any stroke and MI were obtained from a well-established and published study (20). Since no utility weights were available in the literature, we assigned utility values for perfect health and death of 1.0 and

0.0, respectively. The effectiveness of each treatment was measured in quality-adjusted life years (QALY), combining length and quality of life. All inputs, including health state utilities, probabilities, and average cost per patient variables, are presented in Table 2. No cost was assigned for death. As index costs were primarily from 2020, we standardized all costs to 2020 US dollars ($) using the Turkish Consumer Price Index. Another reason for standardizing costs to 2020 was that the prevalence of CS referenced a study published in 2020 (5). All costs were direct medical costs and calculated as averages per patient.

Table 2. *Model variables for utilities and costs associated with CAS and CEA*

Input Variables	CAS	CAE	Sources
Clinical data (probabilities) (%)			(14)
Perfect health (at 30 day)	93.64	94.89	
Any stroke (at 30 day)	4.95	3.34	
MI (at 30 day)	0.53	1.05	
Death (at 30 day)	0.88	0.72	
Cost data (average per patient)			
Index costs and follow-up (US$)	1361,37	946,26	
Any stroke costs (US$)	743,42		(20)
MI costs (US$)	1.120,19		
Total costs (US$)	3.224,98	2.809,87	
Utility data			
Perfect health	1.00		
Any stroke	0.801		(15)
MI	0.804		
Death	0.00		

2.2. Cost-effectiveness analysis

The result is typically summarized as an ICER, defined by the difference in cost between two interventions divided by the difference in their outcomes (QALY) (21). ICER, the primary outcome measure in cost-effectiveness analysis, represents the average incremental cost associated with one additional unit of effect (22). However, ICER alone is insufficient to determine which method is more cost-effective. Therefore, we used Türkiye's gross domestic product (GDP) per capita for 2020 as a threshold value (also known as willingness to pay), consistent with World Health Organization (WHO) recommendations for emerging countries (23). A cost-effectiveness threshold was generated using Microsoft Excel.

2.3. Exclusion Criteria

A key inclusion criterion for this study was that patients underwent CS surgery with CAS or CAE for the first time. Thus, the cost-effectiveness of redo procedures was not considered. Additionally, patients who had restenosis after the operation and those who underwent CS concurrently with coronary bypass surgery were excluded. These patients typically present more complex cases and could significantly affect cost and effectiveness outcomes. Excluding them was intended to provide more precise cost-effectiveness findings for CAS and CAE.

2.4. Ethical Considerations

This study was approved by the Ethics Committee of Ankara University on January 15, 2021 (approval number: 2021/14).

3. RESULTS

3.1. Base case analysis

Out of the 61 participants, 29 (47.5%) underwent CAS, with 20 (68.98%) of them being female, and 32 (52.5%) underwent CAE, with 22 (68.75%) of them being female. Approximately 80.6% of participants were aged 50 or older. The mean post-procedure hospital stay was 2.1 days for CAS and 3.28 days for CAE. The most common risk factors observed in patients were hypertension, cholesterol, diabetes, ischemic heart disorders, and smoking (Table 3). Considering the data presented in Table 2, at 30-day (short-term) outcomes, the rate of perfect health (93.64%) and MI (0.53%) after treatment were lower with CAS, whereas the rates of any stroke (3.34%) and death (0.72%) were lower with CAE. The mean total costs were $415,11 higher per patient for CAS than CAE ($3.224,98 versus $2.809,87), likely due to device costs for the CAS procedure. The utility values for any stroke and MI were similar for both procedures.

Table 3. *Clinical and demographic characteristics of research participants*

Descriptive data		Treatment Procedures			
		Carotid stenting		Carotid endarterectomy	
		n	**(%)**	**n**	**(%)**
Gender	Male	9	31.03	10	31.25
	Female	20	68.98	22	68.75
Total		**29**	**100.00**	**32**	**100.00**
Age	<50	4	13.79	8	25.00
	≥50	25	86.21	24	75.00
Total		**29**	**100.00**	**32**	**100.00**
Average length of hospital stays		2.1		3.28	
Common risk factors		Hypertension, cholesterol, diabetes, ischemic heart disorders and smoking			

3.2. Cost-effectiveness analysis

Applying the decision tree model, the QALY values for CAS and CAE procedures were 0.980 and 0.984, respectively. CAS was associated with a higher increase in cost than CAE (incremental cost of $385,38) and a slight decrease in effectiveness (0.004 QALY). The mean cost per QALY for CAS was $1344,41 versus $947,30 for the CAE group. The estimated ICER for CAS versus CAE treatment was $96.345. Based on study findings, CAS was economically dominated by CAE as it provided fewer QALY gains at increased costs.

Table 4. *Cost-effectiveness of CAS vs. CEA: Turkish healthcare system payer perspective*

Methods	Costs ($)	Incremental Costs ($)	Effectiveness (QALY)	Incremental Effectiveness	Costs / QALY	ICER
CAS	1.317,52	385,38	0.980	-0.004	1.344,41	96.345
CAE	932,14	...	0.984	...	947,30	...

In other words, given the incremental cost of $385,38 and the incremental effectiveness of – 0.004 QALY with CAS compared to CAE, the treatment of CS was $96.345 (Table 4).

To present costs, effects and ICER of alternative intervention strategies, we drew a cost-effectiveness plane. In that plane, ICER was compared to a threshold value (willingness to pay for a unit of health outcome) based on 2020 data, with GDP per capita estimated at $9.592 in Türkiye (24). The threshold was set at one GDP per capita as a high cost-effectiveness point and three times GDP per capita as cost-effective, according to WHO recommendations (23). The cost-effectiveness plane (with costs on the vertical axis and effectiveness units on the horizontal axis) showed that at a $9.592 willingness to pay threshold, ICER was far above both the GDP per capita and the three-times GDP per capita threshold (Figure 2), indicating that CAE was more cost-effective than CAS.

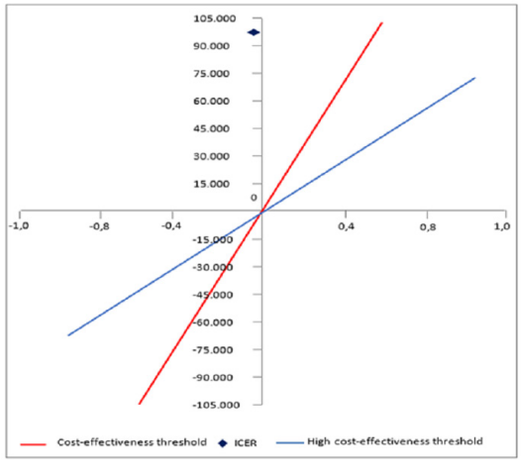

Figure 2. *Cost-effectiveness plane. ICER is a primary outcome measured in cost-effective analysis, representing the average incremental cost related to one additional unit of the measure of effect. However, in economic evaluation, ICER (marked as a diamond in the plane) alone is not enough to explain which method is more cost-effective. Thus, we need a cost-effectiveness plane (threshold) that represents the sort of utilities on the x-axis (horizontal line) and the costs on the y-axis (vertical line). According to WHO, if İCER< threshold (high cost-effectiveness threshold), the new program is very cost-effective; if ICER= (1-3) threshold (cost-effectiveness threshold), the new program is cost-effective; and if ICER>3 threshold, then the new program is not cost-effective.*

Although there was no immediate need for sensitivity analysis due to the high cost and low effectiveness of CAS, we explored how ICER would change if costs were lower and QALY were higher. Since CAS was the intervention tested for its effects, we conducted a one-way sensitivity analysis to estimate the new ICER. Sensitivity analysis was applied by assuming a 10% decrease in costs and a 10% increase in QALY gained with CAS after applying the decision tree model. Even with sensitivity analysis, the ICER value remained within a non-cost-effective threshold, confirming CAE's dominance over CAS. However, if the cost of CAS is significantly reduced, a more cost-effective combination of CAS effectiveness may be possible. The analysis showed that ICER is highly sensitive to changes in QALY, demonstrating robustness (Table 5). In addition, we performed a tornado diagram (Figure 3) for sensitivity analysis, which showed that a ±10% change in input parameters could alter the ICER by about ±$1.225 to $3.500. It was found that parameters, especially such as any stroke and costs, had evident effects on the results, changing the ICER value in favor of CAS as cost per QALY gained.

Table 5. *One-way sensitive analysis for CAS (in terms of Cost and QALY)*

	Changing rate	New ICER (Costs/QALY)
Change interval for costs	-5%	79.876
	-10%	63.420
	-15%	46.938
Change interval for QALY	%5	8.564
	%10	4.100
	%15	2.695

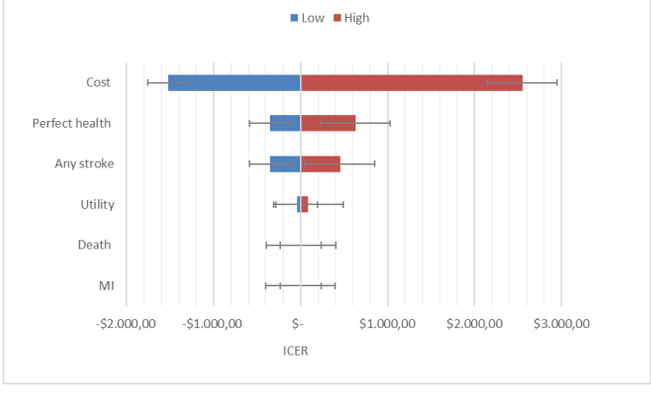

Figure 3. *Sensitivity analysis of tornado diagram for ICER variation. The importance of each input variable on the conclusion is presented from top to bottom. The change (±10%) for each variable is presented in brackets. The tails of each bar indicate the maximum and minimum ICER variation for each variable.*

As a complementary part of the cost-effectiveness analysis, we also conducted a budget impact analysis of both procedures on healthcare expenditures. Reflecting the payer perspective, we considered the premiums obtained by the SSI and its health services spending. Based on the 1.5% CS prevalence (5) in the age range of 30-79, we estimated the target population with CS to be 656.305 individuals using

data from the Turkish Statistical Institute (TURKSTAT) (24). Under the assumption of a 1.5% annual prevalence of CS, the budget impact of the CAS procedure was 4.37%, and for the CAE procedure, it was 3.09% (Table 6).

Table 6. *Budget impact analysis*

Carotid stenosis data (2020)	CAS	CAE
Number of estimated patients	656.305	656.305
Cost ($)	1.317,52	932,14
Carotid stenosis total cost ($)	865.009.990,00	612.332.565,00
Total health expenditures (payer perspective)	19.799.454.789,00	19.799.454.789,00
Proportion in total health expenditures (%)	**4.37**	**3.09**

4. DISCUSSION

Comparing health technologies exclusively in terms of safety and clinical effectiveness is insufficient. We also need to evaluate them in terms of their potential economic and budgetary burdens. Therefore, in this paper, we evaluated the cost-effectiveness of CAS versus CAE in the treatment of CS disease. When evaluating the cost-effectiveness of treatment methods, it is essential to analyze both the cost and benefit values they provide, as well as the cost per incremental effectiveness, or ICER, achieved. This is the first economic evaluation that considers the cost-effectiveness of CAS compared with CAE in the Turkish healthcare system.

We designed our analysis model based on the most prevalent health states after CS surgery associated with either CAS or CAE, as reported in the literature, such as any stroke, MI, and death (6–9). After incorporating survival rates, quality of life benefits, and probabilities associated with any stroke or MI, our cost-effectiveness analysis demonstrated that the ICER for CAS was $96.345. Our results are consistent with other economic evaluation studies. Several previous studies have compared the cost-effectiveness or cost-utility analysis of CAS versus CAE from different perspectives and demonstrated that stenting has a higher cost, mainly due to the cost of devices (stent cost, emboli protection devices, etc.), making CAE more cost-effective over CAS (1,2,11,15,25). On the other hand, some studies indicated that CAS was more cost-effective than CAE despite having higher costs (10,26–28).

After conducting a one-way sensitivity analysis (not only for costs and QALY but also in terms of parameters inserted into the decision tree model), the ICER value still indicated that the cost-effectiveness results for CAE remained favorable. When looking at the impact of costs on the budget, which makes the CAS strategy less effective against CAE, the financial burden of the CAS procedure was higher than that of CAE under the assumption of 1.5% annual prevalence of CS. Consequently, our results show that in the short term, the cost per QALY per patient remains in favor of CAE. However, for the longer term, it should be analyzed how the treatment techniques might show results in terms of ICER using the Markov model if probabilities of transition between health states are detected. Our results were found to be robust and precise when comparing CAS and CAE in terms of financial resources and the benefits provided to patients. However, if sufficient data were available to compare CAS and CAE in terms of health conditions such as redo procedures, restenosis processes, and other possible cases, the ICER for both procedures would likely change further.

There are several limitations to this research. One limitation is the absence of a Markov model, which involves several assumptions and parametric models, such as transition probabilities between health states that we could not find in the literature. Another limitation is that our cost-effectiveness analysis was primarily based on utility, probability, and cost inputs for stroke and MI derived from other studies. Additionally, no administrative or indirect costs were included. Thus, our analysis was performed from the perspective of Turkish health system payers and may not reflect the perspective of other healthcare systems or payers whose costs may differ from our research. Furthermore, the follow-up in our model is limited to one year. Being retrospective and having a relatively small sample size is another limitation of our study. Another limitation is the utility values; since there were no utility values for stroke and MI separately for CAS and CAE in the literature, similar benefit values were assigned during the analysis.

5. CONCLUSION

Our findings support the cost-effectiveness of CAE compared with CAS. However, these results are not generalizable due to the study's limitation to the Turkish healthcare system, different surgical risk types among participants, and the inclusion of both symptomatic and asymptomatic participants. Nevertheless, our results provide important information regarding the implementation of CAE versus CAS in healthcare systems. Although CAS is a relatively novel alternative for the treatment of CS and is more preferred by patients, the standard procedure CAE has proven non-inferiority in terms of cost-effectiveness analysis in our research. If the CAS method is to be prioritized for CS, efforts should focus on reducing the short-term any stroke risk and procedural costs, such as the cost of stenting, to improve CAS's cost-effectiveness. We believe that our study will provide new insights into choosing the best treatment method for patients undergoing CS for healthcare planners, procedure practitioners, payers, and policymakers. It is essential that when health technologies are evaluated, both clinical effectiveness and economic assessment should be taken into consideration.

Acknowledgement: *Thank you for supporting the participants in this study.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by Ethics Committee of Ankara University, (approval date 2021 and number 14.)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: İA, AEE
Design of the study: İA, AEE
Acquisition of data for the study: İA
Analysis of data for the study: İA, AEE
Interpretation of data for the study: İA, AEE
Drafting the manuscript: İA, AEE
Revising it critically for important intellectual content: İA, AEE
Final approval of the version to be published: İA, AEE

REFERENCES

[1] Khan AA, Chaudhry SA, Sivagnanam K, Hassan AE, Suri MFK, Qureshi AI. Cost-effectiveness of carotid artery stent placement versus endarterectomy in patients with carotid artery stenosis. J. Neurosurg. 2012; 117: 89-93. https://doi.org/10.3171/2012.3.JNS111266

[2] Jimenez SV, Carrasco P, Rodriguez G, Doblas M, Orgaz A, Flores A, Maynar M, Gonzalez-Fajardo JA, Fontcuberta J. Cost-effectiveness of carotid surgery. J Vasc Surg. 2018; 57: 177-186. https://doi.org/10.1016/j.avsg.2018.09.013

[3] Erickson KM, Cole DJ. Carotid artery disease: stenting vs endarterectomy. Br J Anaesth. 2010; 105 (S1): i34-i49. https://doi.org/10.1093/bja/aeq319

[4] Hıdıroğlu M, Çetin L, Kunt A, Karakişi O, Küçüker A, Şener E. Early results of carotid endarterectomy for carotid artery diseases. Turkish J Thorac Cardiovasc Surg. 2010; 18(3): 190-195. (Turkish)

[5] Song P, Fang Z, Wang H, Cai Y, Rahimi K, Zhu Y, Fowkes FGR, Fowkes FJI, Rudan I. Global and regional prevalence, burden, and risk factors for carotid atherosclerosis: A systematic review, meta-analysis, and modelling study. Lancet 2020; 8: e721-729. https://doi.org/10.1016/S2214-109X(20)30117-0

[6] Mas JL, Chatellier G, Beyssen B, Branchereau A, Moulin T, Becquemin JP, Larrue V, Lièvre M, Leys D, Bonneville JF, Watelet J, Pruvo JP, Albucher JF, Viguier A, Piquet P, Garnier P, Viader F, Touzé E, Giroud M, Hosseini H, Pillet JC, Favrole P, Neau JP, Ducrocq X. Endarterectomy versus stenting in patients with symptomatic severe carotid stenosis. N Engl J Med. 2006; 355: 1660-1671. https://doi.org/10.1056/NEJMoa061752

[7] Ederle J, Dobson J, Featherstone RL, Bonati LH, Bart van der Worp H, de Borst GJ, Lo TH, Gaines P, Dorman PJ, Macdonald S, Lyrer PA, Hendriks JH, McCollum C, Nederkoorn PJ, Brown MM. Carotid artery stenting compared with endarterectomy in patients with symptomatic carotid stenosis: An interim analysis of a randomized controlled trial. Lancet 2010; 375: 985-997. https://doi.org/10.1016/S0140-6736(10)60239-5

[8] Brott TG, Hobson RW, Howard G, Roubin GS, Clark WM, Brooks W, Mackey A, Hill MD, Leimgruber PP, Sheffet AJ, Howard WJ, Moore WS, Voeks JH, Hopkins LN, Cutlip DE, Cohen DJ, Popma JJ, Ferguson RD, Cohen SN, Blackshear JL, Silver FL, Mohr JP, Lal BK, Meschia JF. Stenting versus endarterectomy for treatment of carotid-artery stenosis. N Engl J Med. 2010; 363: 11-23. https://doi.org/10.1056/NEJMoa0912321

[9] Rosenfield K, Matsumura JS, Chaturvedi S, Riles T, Ansel GM, Metzgeret DC, Wechsler L, Jaff MR, Gray W. Randomized trial of stent versus surgery for asymptomatic carotid stenosis. N Engl J Med. 2016; 374: 1011-1020. https://doi.org/10.1016/j.jvs.2016.06.057

[10] Mahoney EM, Greenberg D, Lavelle TA, Natarajan A, Berezin R, Ishak KJ, Caro JJ, Yadav JS, Gray WA, Wholey MH, Cohen DJ. Costs and cost effectiveness of carotid stenting versus endarterectomy for patients at increased surgical risk: Results from the SAPPHIRE trial. Catheter Cardiovasc Interv. 2011; 77: 463-472. https://doi.org/10.1002/ccd.22869

[11] Kilaru S, Korn P, Kasirajan K, Lee TY, Beavers FP, Lyon RT, Bush HL, Kent KC. Is carotid angioplasty and stenting more cost effective than carotid endarterectomy? J Vasc Surg. 2003; 37: 331-339. https://doi.org/10.1067/mva.2003.124

[12] Vilain KR, Magnuson EA, Li H, Clark WM, Begg RJ, Sem AD, Sternbergh WC, Weaver FA, Gray WA, Voeks JH, Brott TG, Cohen DJ. Costs and cost-effectiveness of carotid stenting versus endarterectomy for patients at standard surgical risk results from the carotid revascularization endarterectomy versus stenting trial (CREST). Stroke 2012; 43: 2408-2416. https://doi.org/10.1161/STROKEAHA.112.661355

[13] Reyhanoğlu H, Aşgün HF, Özcan K, Ertürk M, Durmaz İ. Is contralateral carotis artery occlusion a risk factor for carotid endarterectomy? Turkish J Thorac Cardiovasc Surg. 2016; 24(2): 266-273. https://doi.org/10.5606/tgkdc.dergisi.2016.12356

[14] Balkanay M, Toker ME, Eren E, Erşahin S, Güler M, Yakut C. Cost-effect comparison between coronary artery bypass on beating heart and stent implantation for isolated stenosis of left anterior descending coronary artery. Turkish J Thorac Cardiovasc Surg. 2002; 10: 78-81.

[15] Morris S, Patel NV, Dobson J, Featherstone RL, Richards T, Luengo-Fernandez R, Rothwell PM, Brown MM. Cost-utility analysis of stenting versus endarterectomy in the International Carotid Stenting Study. Int J Stroke. 2016; 11(4): 446-453. https://doi.org/10.1177/1747493016632237

[16] The Excel add-in for tree plan. Available at: https://github.com/ybian/treeplan. Accessed March 8, 2022.

[17] Hagens A, İnkaya AÇ, Yildirak K, Sancar M, van der Schans J, Sancar AA, Ünal S, Postma M, Yeğenoğlu S. COVID-19 vaccination scenarios: A cost-effectiveness analysis for Turkey. Vaccines 2021; 9: 399. https://doi.org/10.3390/vaccines9040399

[18] Aydemir I. Assessment of the cost-effectiveness of carotid endarterectomy and carotid artery stenting methods applied in the treatment of carotid artery stenosis disease, Ph.D., Ankara University, Institute of Health Sciences, Department of Healthcare Management, Ankara, 2022. Available at: https://tez.yok.gov.tr/UlusalTezMerkezi/. Accessed September 22, 2022. (Turkish)

[19] Koçkaya G, Oğuzhan GE, Özin B, Yilmaz KC, Çiftçi O, Çavuş F, Sharaf AM, Buyuktuna N, Buyukısık T, Saylan M. Cost analysis of management of cardiovascular disease comorbidities in Turkey. J Pharm Health Serv Res. 2019; 10: 197-202. https://doi.org/10.1111/jphs.12298 (Turkish)

[20] Jia H, Lubetkin EI, Barile JP, Lohnson WH, Demichele K, Stark DS, Zack MM, Thompson WM. Quality-adjusted life years (QALY) for 15 chronic conditions and combinations of conditions among US adults aged 65 and older. Med Care. 2018; 56: 740-746. https://doi.org/10.1097/MLR.0000000000000943

[21] Drummond MF, Schwartz JS, Jönsson B, Luce BR, Neumann PJ, Siebert U, Sullivan SD. Key principles for the improved conduct of health technology assessments for resource allocation decisions. Int J Technol Assess Health Care 2008; 24(3): 244-258. https://doi.org/10.1017/S0266462308080343

[22] Phillips C. What is cost-effectiveness?, Health Economics, Second edition, 2009. Available at: http://www.bandolier.org.uk/painres/download/whatis/Cost-effect.pdf. Accessed August 28, 2020.

[23] Kazibwe J, Gheorghe A, Wilson D, Ruiz F, Chalkidou K, Chi LY. The use of cost-effectiveness thresholds for evaluating health interventions in low- and middle-income countries from 2015 to 2020: A review. Value Health 25(3): 385-389. https://doi.org/10.1016/j.jval.2021.08.014.

[24] Turkish Statistical Institute/TURKSTAT. Statistical Data Portal 2021. Available at: https://data.tuik.gov.tr/. Accessed February 3, 2022. (Turkish)

[25] Young KC, Holloway RG, Burgin WS, Benesch CG. A cost-effectiveness analysis of carotid artery stenting compared with endarterectomy. J. Stroke Cerebrovasc. Dis 2010; 19(5): 404-409. https://doi.org/10.1016/j.jstrokecerebrovasdis.2009.08.003

[26] Maud A, Vazquez G, Nyman JA, Lakshminarayan K, Anderson DC. Cost-effectiveness analysis of protected carotid artery stent placement versus endarterectomy in high-risk patients. J Endovasc Ther. 2010; 17: 224-229. https://doi.org/10.1583/09-2938.1

[27] Almekhlafi MA, Hill MD, Wiebe S, Goyal M, Yavin D, Wrong JH, Clement FM. When is carotid angioplasty and stenting the cost-effective alternative for revascularization of symptomatic carotid stenosis? A Canadian health system perspective. AJNR Am J Neuroradiol. 2014; 35: 327-332. https://doi.org/10.3174/ajnr.A3682

[28] Featherstone RL, Dobson J, Ederle J, Doig D, Bonati LH, Morris S, Patel NV, Brown MM. Carotid artery stenting compared with endarterectomy in patients with symptomatic carotid stenosis (International Carotid Stenting Study): A randomized controlled trial with cost-effectiveness analysis. Health Technol Assess. 2016; 20(20): 1-94. https://doi.org/10.3310/hta20200

How to cite this article: Aydemir İ, Ezel Esatoğlu A. Cost-Effectiveness of Carotid Artery Stenting Compared to Carotid Endarterectomy in Patients with Carotid Stenosis: A Turkish Health System Perspective. Clin Exp Health Sci 2024; 14: 15-21. https://doi.org/10.33808/clinexphealthsci.1315072

Clinical and Experimental Health Sciences

Evaluation of the Relationship Between $ETCO_2$ and Delta CO_2 Pressure and the Severity of Disease in COVID-19 Pneumonia

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Received: July 13, 2023 **Accepted:** October 23, 2024

ABSTRACT

Objective: In this study, we aimed to determine end-tidal carbon dioxide ($ETCO_2$) and deltaCO_2 levels in patients with Coronavirus Disease 2019 (COVID-19) pneumonia and examine the relationship between these two parameters and the severity of disease.

Methods: Patients with COVID-19 were included in the study. $ETCO_2$ values were recorded and deltaCO_2 values were calculated. They were divided into two groups: mild-moderate and severe patients. An analysis was performed to determine the threshold values for $ETCO_2$ and deltaCO_2 in mild-moderate and severe patient groups.

Results: A total of 83 patients were included in our study. Of the patients, 43 (51.8%) patients had mild/moderate disease and 40 (48.2%) had severe disease. The AUC value was 0.910 for $ETCO_2$ (95% CI; 0.840-0.980, p< .001) and was 0.927 for DeltaCO_2 (95% CI; 0.864-0.990, p< .001). To discriminate patients in the severe group, considering a best cut-off value of 22.5 for $ETCO_2$, the sensitivity and specificity values for this value were 95% and 80%, respectively. Considering a best cut-off value of 11.1 for deltaCO_2, the sensitivity and specificity values for this value were 95% and 77%, respectively. As a result of the DeLong test, the predictive values of deltaCO_2 and $ETCO_2$ for the severe patient group was found to be better than and similar to PCO_2.

Conclusion: We showed that low $ETCO_2$ and high deltaCO_2 values are safe parameters that can be used to predict the severity of disease in patients who apply and are monitored due to COVID-19 pneumonia.

Keywords: COVID-19, $ETCO_2$, deltaCO_2, ARDS, viral pneumonia

1. INTRODUCTION

Pneumonia and respiratory failure are among the most important clinical conditions in patients with COVID-19 (1). Respiratory failure is often hypoxemic, but can also be hypercapnic less frequently (2).

End-tidal carbon dioxide ($ETCO_2$) refers to the pressure, production, and pulmonary excretion of alveolar carbon dioxide (CO_2), cardiac output in general (3). A change in any of these factors impacts the outcome. In recent studies, it has been reported that the sudden increase in $ETCO_2$ during cardiopulmonary resuscitation (CPR) is an effective indicator in predicting the return of spontaneous circulation (ROSC) and that low $ETCO_2$ during CPR is associated with poor outcomes (3-5). Today, $ETCO_2$ monitoring is used in predicting CPR quality and ROSC in cardiac arrest and in the evaluation of the sufficiency of fluid resuscitation in trauma and shock patients (3-6). In cardiopulmonary diseases, the pulmonary blood supply decreases; therefore, the clearance of CO_2 in the alveoli cannot compensate for the excretion of the amount of CO_2 produced in the body (3). For this reason, as the partial pressure of CO_2 in the blood (pCO_2) increases, $ETCO_2$ decreases and the correlation between pCO_2 and $ETCO_2$ deteriorates. This increases the difference in CO_2 pressures. This difference, which should not normally exceed 3-5mmHg, is called "deltaCO_2" (7, 8). DeltaCO_2 has been studied in many subjects such as predicting the severity of disease and mortality in various cardiopulmonary diseases such as pneumonia, pulmonary edema, ARDS, trauma surgery, and pulmonary embolism, and has been determined to be statistically significantly associated with mortality (3-6).

Acute hypoxemic respiratory failure and ARDS are seen in 17-29% of patients followed up due to COVID-19 pneumonia and it has been reported that these patients need intensive care at a rate of 23-32% (7). In some studies, the difference between arterial carbon dioxide ($PaCO_2$), which has been defined as deltaCO_2, and $ETCO_2$ pressure, in patients with ARDS has been evaluated and it has been shown that deltaCO_2 tended to increase as the severity of ARDS increased. It has been suggested that this increase may

https://doi.org/*10.33808/clinexphealthsci.1322620*

alert the clinician in terms of the deterioration of lung functions (8). In the literature, limited studies have been conducted on $ETCO_2$ and $deltaCO_2$ in viral pneumonia. In our study, we aimed to determine $ETCO_2$ and $deltaCO_2$ levels in patients with COVID-19 pneumonia and examine the relationship between these two parameters and the severity of disease.

2. METHODS

This prospective and observational study was carried out between 01/01/2021-06/31/2021 with the approval of the local ethics committee (No. 2012-KAEK-15/228). Patients aged over 18, who applied to the Emergency department (ED) with COVID-19 cardinal findings, who had positive PCR results, and who were diagnosed with pneumonia through thorax imaging were included in the study. Patients' vital signs at admission, comorbidities, laboratory findings at the time of admission, blood gas values, outcomes, and $ETCO_2$ values were recorded. $ETCO_2$ measurements were performed simultaneously while vital signs were recorded. The $ETCO_2$ values of the patients were measured by attaching Capnoxygen® to the patients and using the MasimoRoot® (USA) device using infrared absorption spectroscopy during their examinations and treatments in the ED. The 5th-minute $ETCO_2$ values of the patients were recorded.

After performing measurements for those who were suspected or diagnosed with COVID-19, those who had negative PCR results, who had missing data, who had conditions that could affect $PaCO_2$ and $ETCO_2$ values, those who had a diagnosis of COPD, chronic kidney disease, heart failure, and those who were smokers were excluded from the study.

A radiologist who was unaware of the study results interpreted the radiological findings of the patients. The patients were divided into 3 groups, 0-25%, 25-50%, and 50-100%, according to their rates of pneumonia involvement (9). Considering the COVID-19 Adult Patient Management guideline of the Ministry of Health, those with an involvement of 50% or over and a respiratory rate above 30 or those with a sPO_2 value below 90 were classified as the severe group while the others were classified as the mild-moderate group (2). In-hospital mortality of the patients was recorded.

A 5-unit differences for $deltaCO_2$ levels between the severity groups was considered a clinically significant difference. Accordingly, the sample size was calculated as 34 for each group with a Type 1 error of 5%, a Type 2 error of 20% (80% power), and provided that a two-way analysis was performed. The standard deviation values of $DeltaCO_2$ were obtained from the previous study groups and taken as 12 (8). Considering the possible protocol bias, it was planned to add 10% of patients for each group. Thus, a sample size of 80 patients, 40 in each group, was determined as the minimum number of patients to be included in the study.

2.1. Statistical Analysis

The data were analyzed in the IBM SPSS 20.0 (Chicago, IL, USA) statistical program. The fitness of the distribution of discrete and continuous numerical variables to normal distribution was tested using the Kolmogorov-Smirnov test. Descriptive statistics were presented as median (with an interquartile range of 25-75) for discrete and continuous numerical variables and categorical variables were given as numbers and percentages (%). Categorical variables were evaluated with Chi-square and continuous variables were evaluated using the Mann-Whitney U test. For $ETCO_2$ and $deltaCO_2$, receiver operating characteristic (ROC) analysis was performed. Area under the curve (AUC) values were calculated to discriminate the severity of disease. The difference between ROC curves was examined using the DeLong test.

3. RESULTS

A total of 83 patients were included in our study (Figure 1). Of the patients, 43 (51.8%) patients were in the mild/moderate group and 40 (48.2%) were in the severe group. The $ETCO_2$ value of the patients was measured as 27 (IQR25%-75%; 19-32) and $deltaCO_2$ was 13.2 (9-18.8). According to the comparison between mild-moderate and severe patients, severe patients had higher diastolic blood pressure, pulse, respiratory rate, pCO_2, $deltaCO_2$, temperature, hospitalization rates, and in-hospital mortality rates and lower $ETCO_2$ and saturation (Table 1) ($p< .05$ for all values).

Table 1. *Comparison of demographic characteristics of mild-moderate and severe patient groups*

	Mild-Moderate (n=43)	Severe (n=40)	p-value
Age, median (IQR 25-75)	50 (40-67)	58(49-69)	.072
Sex, n (%)			
Male	19 (44.2)	25(62.5)	.095
Female	24 (55.8)	15(37.5)	
Comorbidities, n (%)			
Hypertension	16 (37.2)	17(42.5)	.623
Coronary Artery	7 (16.3)	10(25)	.325
Disease	5 (11.6)	9(22.5)	.186
Diabetes			
Vital signs, median			
(IQR 25-75)	132 (120-140)	135 (127-145)	.272
Systolic blood pressure	75 (70-80)	80 (75-85)	.032
Diastolic blood pressure	85 (75-95)	96 (81-111)	.007
Pulse	16 (15-20)	22 (16-26)	.009
Respiratory rate	95 (91-96)	85 (83-89)	.018
Saturation	36.9 (36-37.5)	37.6 (36.9-38)	<.001
Temperature	15 (15-15)	15 (15-15)	.018
Glasgow coma score			
$ETCO_2$, median (IQR 25 – 75)	32 (28-34)	19 (17-22)	< .001
Blood gas			
pH	7.41 (7.38-7.43)	7.42 (7.38-7.45)	.136
pCO_2	40.8 (38-44.9)	37.4 (34.5-41.0)	.004
HCO_3 actual	25.2 (22.6-27.4)	24.3 (21-26)	.087
$DeltaCO_2$, median (IQR 25 – 75)	9 (7.8-10.5)	17.3 (15.5-19.7)	<.001
In-hospital mortality n (%)	2 (4.7)	7 (17.5)	<.001

$ETCO_2$: End-tidal Carbon dioxide, HCO3: Serum bicarbonate, pCO2: Partial Carbon dioxide pressure

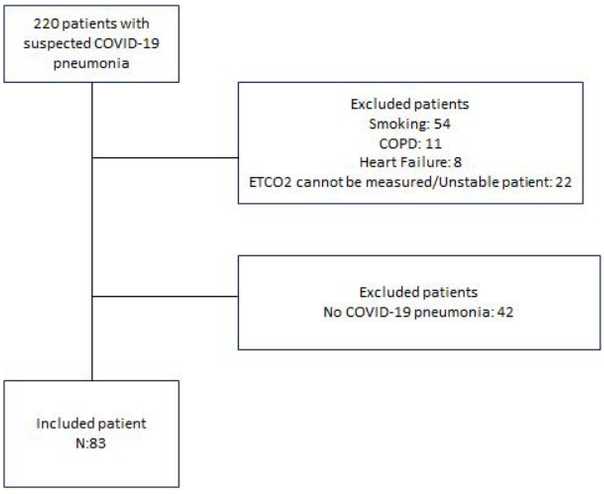

Figure 1. *Flow chart of study*

There was a unidirectional and weak correlation between $ETCO_2$ and HCO_3 and saturation in the severe group (r=0.336 and p= .034, r=0.410 and p= .006, respectively), a reverse and weak correlation between deltaCO_2 and saturation, and a unidirectional and weak correlation between deltaCO_2 and HCO_3 (r=0.350 and p= .027, r=0.395 and p= .009, respectively) whereas there was no correlation in other parameters (p> .05 for all values). No correlation was determined between $ETCO_2$ and deltaCO_2 and parameters in patients in the mild-moderate group (p< .05 for all values) (Table 2).

Table 2. *Correlation of end-tidal CO_2 and deltaCO2 values with some parameters in mild-moderate and severe patient groups*

	Mild-moderate group		Severe group	
$ETCO_2$	Correlation coefficient	p-value	Correlation coefficient	p – value
Systolic blood pressure	-0.008	.961	-0.020	.903
Diastolic blood pressure	0.242	.117	0.070	.666
Pulse	-0.013	.936	-0.028	.863
Respiratory rate	-0.181	.246	0.086	.599
Saturation	0.226	.161	0.410	.006
HCO_3	0.153	.327	0.336	.034
DeltaCO_2				
Systolic blood pressure	0.248	.109	0.145	.371
Diastolic blood pressure	0.104	.507	0.124	.446
Pulse	-0.253	.102	-0.001	.994
Respiratory rate	0.230	.139	0.081	.619
Saturation	-0.192	.218	-0.350	.027
HCO_3	0.062	.703	0.395	.009

$ETCO_2$: End-tidal Carbon dioxide, HCO3: Serum bicarbonate

ROC analysis was performed to determine the threshold values for PCO_2, $ETCO_2$, and deltaCO_2 among the patient groups and AUC was calculated (Figure 2). AUC was 0.670 for PCO_2 (0.565-0.800, p= .04), 0.910 for $ETCO_2$ (95% CI; 0.840-0.980, p< .001), and 0.927 for deltaCO_2 (95% CI; 0.864-0.990, p< .001). When the best cut-off value was taken as 22.5 for $ETCO_2$ to discriminate patients in the severe group, the sensitivity and specificity values for this value were 95% and 80%, respectively. When the best cut-off value was taken as 11.1 for deltaCO_2, the sensitivity and specificity values for this value were 95% and 77%, respectively. As a result of the DeLong test, which was performed to evaluate whether there was a difference between the AUC curves, the predictive values of deltaCO_2 and $ETCO_2$ for the severe patient group were found to be better than and similar to PCO_2 (Table 3) (p< .05 for all values).

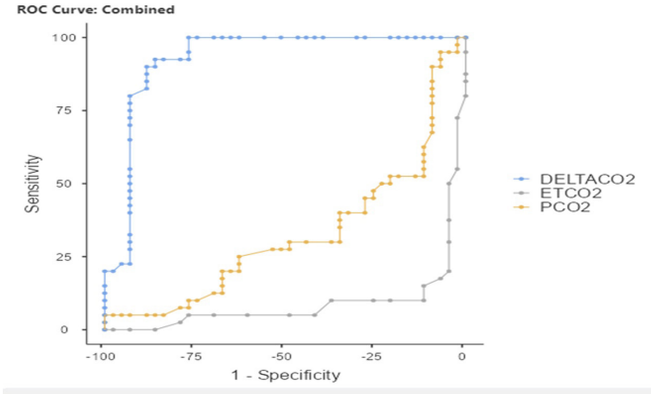

Figure 2. *ROC analysis for PCO2, $ETCO_2$, DeltaCO_2 levels of mild-moderate and severe patient groups*

Table 3. *Comparison of AUC values of PCO2, DeltaCO2, and ETCO2*

	AUC difference	CI (lower)	CI (upper)	p value
PCO_2 vs $ETCO_2$	-0.235	-0.328	-0.142	< .001
PCO_2 vs Delta CO_2	-0.252	-0.399	-0.106	.01
Delta CO_2 vs ETCO2	-0.017	-0.103	0.068	.695

$ETCO_2$: End-tidal Carbon dioxide, pCO2: Partial Carbon dioxide pressure, AUC: Area under curve, CI: confidential interval. The DeLong test was used

4. DISCUSSION

Emergency services have played an important role during the COVID-19 pandemic. The clinical findings of COVID-19 patients monitored in the ED have differed from each other. Thus, determining the severity of the disease and emergency treatment management in these patients is of great importance. In this study, in which we evaluated the relationship between the disease severity and $ETCO_2$ and deltaCO_2 in COVID-19 pneumonia, we found that $ETCO_2$ values measured at admission were low and deltaCO_2 values were high in severe group patients. Furthermore, we showed that deltaCO_2 and $ETCO_2$ levels were associated with saturation and HCO_3 in severe patients. We think that high deltaCO_2 and low $ETCO_2$ levels may be clinically useful in discriminating severe patients and predicting COVID-19 disease severity with high sensitivity and specificity. As a result of the DeLong test, in which we evaluated whether there was a difference

between the AUC curves, we found that $ETCO_2$ alone was as effective as delta $ETCO_2$ in predicting severe patients. $ETCO_2$, which is non-invasively and easily measured at the bedside in the ED, can be measured in a shorter time than analyses such as blood gas which is obtained using invasive methods and can help to identify critical COVID-19 patients quickly and determine the severity of disease, especially in ED where patient admissions are high.

In critical patients with pneumonia, capnography is important for assessing ventilation as well as detecting changes in perfusion and metabolism. Capnography, as a single clinical assessment tool, can provide instant findings on airway integrity, effective breathing, ventilation, perfusion, and metabolism. $ETCO_2$ reflects the pressure, production, and pulmonary excretion of alveolar CO_2 (3). A change in any of these factors affects the outcome (3-5). Today, $ETCO_2$ monitoring is used in the estimation of CPR quality and ROSC in cardiac arrest, as a predictor of mortality in trauma patients, and as a supportive parameter in the evaluation of perfusion in shock patients (3-6). In cardiopulmonary diseases, the pulmonary blood supply decreases; therefore, the clearance of CO_2 in the alveoli cannot compensate for the excretion of the amount of CO_2 produced in the body (3). For this reason, as the partial pressure of CO_2 in the blood (pCO_2) increases, $ETCO_2$ decreases and the correlation between pCO_2 and $ETCO_2$ deteriorates. This increases the difference between the CO_2 pressures, "deltaCO_2". Normally, deltaCO_2 is between 3-5mmHg (7, 8). Several mechanisms are responsible for the change of $ETCO_2$ and indirectly, the change of the deltaCO_2 in COVID-19 pneumonia. One of these mechanisms is the deterioration in the ventilation/perfusion balance. The reason for the decrease in $ETCO_2$ is the ventilation/perfusion changes caused by diseases such as pneumonia and ARDS in which the dead space increases and ventilation is impaired, and diseases such as pulmonary embolism, cardiac arrest, and sepsis in which perfusion is impaired (10). In their study, Kerr et al. compared $PaCO_2$ and $ETCO_2$ values in patients with severe head trauma. They showed that $ETCO_2$ values were close to $PaCO_2$ values in patients without respiratory complications and that $ETCO_2$ values statistically significantly decreased in patients with respiratory system complications (11). In the study conducted by Russell, $PaCO_2$ and $ETCO_2$ values were compared in patients with multi-trauma and it was found that $ETCO_2$ values increased as $PaCO_2$ values increased and that these two values were positively associated with each other (12). In these studies, it was observed that the conditions that did not affect the respiratory system and did not cause metabolic acidemia did not cause a significant change in $ETCO_2$ and deltaCO_2 and that the non-invasively measured $ETCO_2$ value accurately reflected the $PaCO_2$ value.

In their study, Yousuf et al. found a positive correlation between high deltaCO_2 values and the severity of disease in patients who developed ARDS secondary to pneumonia and patients with mild and moderate-severe ARDS and they associated the increase in deltaCO_2 with ARDS and severe tissue damage caused by widespread inflammation in the lung (8). The increased dead space and impaired ventilation caused by this extensive damage can increase deltaCO_2. We obtained statistically similar results in the AUC curves of deltaCO_2 and $ETCO_2$. The measurement of $ETCO_2$ of patients at admission in the ED may be useful in discriminating severe patients without performing invasive tests such as arterial blood gas.

In the literature, the relationship between deltaCO_2 and $ETCO_2$ levels and HCO_3 and $PaCO_2$ values were examined and it was shown that these parameters are associated with each other (13). Uzunosmanoğlu examined the usability of the $ETCO_2$ levels measured at admission in patients with acute gastroenteritis to predict dehydration and the severity of the disease, found a strong positive correlation between the patients' $ETCO_2$ levels and HCO_3, pH, and creatinine values, and attributed low $ETCO_2$ values to metabolic acidosis secondary to dehydration and hyperventilation which develops to compensate for acidosis (14). Similarly, in our study, $ETCO_2$ and deltaCO_2 were found to be positively correlated with HCO_3, albeit weakly, in the severe patient group. We think that this may have arisen due to the compensation mechanisms that occur in the early stage when organ functions are not impaired in patients with severe COVID pneumonia.

Since our study was conducted at a single center, our results cannot be generalized to all centers. Secondly, according to the adult patient guidelines of the Ministry of Health of the Republic of Turkey, hospitalization, examinations, and treatments of patients have shown some differences during the pandemic, and this may have caused differences in our results. While measuring ETCO2, nasal or high-flow oxygen therapy may have caused a slight increase in ETCO2, which may have affected our results. Finally, patients with COPD that may cause hypercapnic respiratory failure or those with neurological diseases were not included in our study. These may have affected our results.

5. CONCLUSION

In our study, we showed that high $ETCO_2$ and low deltaCO_2 values can be used to predict the severity of the disease in patients admitted to and followed up in the ED due to COVID-19 pneumonia. Capnography is an important tool for clinicians in monitoring ventilation since it is non-invasive and enables continuous measurement and instant data collection. Measurement of deltaCO_2, an indicator of increased dead space, may provide more significant findings in patients with hypercapnic respiratory failure. We think that $ETCO_2$, which is measured bedside non-invasively, can be used especially for patients with hypoxic respiratory failure, such as in COVID-19, in crowded ED like ours to discriminate severe patients without any invasive examination.

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by Atatürk Sanatoryum Training and Research Hospital Ethics Committee, Noninvasive Clinic Ethics Committee (Approval date: 12/12/2020 Number: 2012-KAEK-15/228)*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: MEA, EE

Design of the study: EE, ŞKÇ, YÇ

Acquisition of data for the study: MEA, EE

Analysis of data for the study: MEA, EE

Interpretation of data for the study: MEA, EE, ŞKÇ

Drafting the manuscript: MEA, EE, ŞKÇ, YÇ

Revising it critically for important intellectual content: ŞKÇ, YÇ

Final approval of the version to be published: MEA, EE, ŞKÇ, YÇ

REFERENCES

[1] Lai CC, Wang CY, Wang YH, Hsueh SC, Ko WC, Hsueh PR. Global epidemiology of coronavirus disease 2019 (COVID-19): Disease incidence, daily cumulative index, mortality, and their association with country healthcare resources and economic status. Int J Antimicrob Agents. 2020;55(4):105946. DOI: 10.1016/j.ijantimicag.2020.105946.

[2] TC SAĞLIK BAKANLIĞI. "COVID-19 (SARS-CoV2 Enfeksiyonu) Rehberi". Erişim: https://COVID19.saglik.gov.tr/TR-66926/eriskin-hasta-tedavisi.html .(Erişim Tarihi: 30.11.2022) (2022). (Turkish)

[3] Lermuzeaux M, Meric H, Sauneuf B, Girard S, Normand H, Lofaso F, Terzi N. Superiority of transcutaneous CO_2 overend-tidal CO_2 measurement for monitoring respiratory failure in non-intubated patients: A pilot study. J Crit Care. 2016;31(1):150-156. DOI: 10.1016/j.jcrc.2015.09.014

[4] Riaz I, Jacob B. Pulmonary embolism in Bradford, UK: Role of end-tidal CO_2 as a screeningtool. Clin Med (Lond). 2014;14(2):128-133. DOI: 10.7861/clinmedicine.

[5] Kim YW, Hwang SO, Kang HS, Cha KC. The gradient between arterial and end-tidal carbondioxide predicts in-hospital mortality in post-cardiac arrest patient. Am J Emerg Med. 2019;37(1):1-4. DOI: 10.1016/j.ajem.2018.04.025.

[6] Tyburski JG, Collinge JD, Wilson RF, Carlin AM, Albaran RG, Steffes CP. End-tidal CO_2- derived values during emergency trauma surgery correlated without come: A prospective study. J Trauma. 2002;53(4):738-743. DOI: 10.1097/00005373-200210000-00020.

[7] Goh KJ, Choong MCM, Cheong EHT, Kalimuddin S, Duu Wen S, Phua GC, Chan KS, Haja Mohideen S. Rapid progression to acute respiratory distress syndrome: Review of current understanding of critical ıllness from coronavirus disease 2019 (COVID-19) infection. Ann Acad Med Singapore 2020;49:108–118.

[8] Yousuf T, Brinton T, Murtaza G, Wozniczka D, Ahmad K, Iskandar J, Mehta R, Keshmiri H, Hanif T. Establishing a gradient between partial pressure of arterial carbondioxide and end-tidal carbondioxide in patients with acute respiratory distress syndrome. J Investig Med. 2017;65(2):338-341. DOI: 10.1136/jim-2016-000253

[9] Caruso D, Zerunian M, Polici M, Pucciarelli F, Guido G, Polidori T, Rucci C, Bracci B, Tremamunno G, Laghi A. Diagnostic performance of CT lung severity score and quantitative chest CT for stratification of COVID-19 patients. Radiol Med. 2022;127(3):309-317. DOI: 10.1007/s11547-022-01458-9.

[10] Long B, Koyfman A, Vivirito MA. Capnography in the Emergency Department: A Review of Uses, Waveforms, and Limitations. J Emerg Med. 2017;53(6):829-842. DOI: 10.1016/j.jemermed.2017.08.026.

[11] Kerr ME, Zempsky J, Sereika S, Orndoff P, Rudy EB. Relationship between arterial carbon dioxide and end-tidal carbon dioxide in mechanically ventilated adults with severe head trauma. Crit Care Med. 1996;24(5):785-790. DOI: 10.1097/00003246-199605000-00010.

[12] Russell GB, Graybeal JM. Reliability of the arterial to end-tidal carbon dioxide gradient in mechanically ventilated patients with multisystem trauma. J Trauma. 1994;36(3):317-322. DOI: 10.1097/00005373-199403000-00006.

[13] Taghizadieh A, Pouraghaei M, Moharamzadeh P, Ala A, Rahmani F, Basiri Sofiani K. Comparison of end-tidal carbon dioxide and arterial blood bicarbonate levels in patients with metabolic acidosis referred to emergency medicine. J Cardiovasc Thorac Res. 2016;8(3):98-101. DOI: 10.15171/jcvtr.2016.21.

[14] Uzunosmanoğlu H, Emektar E, Dağar S, Çorbacıoğlu ŞK, Çevik Y. Predictive value of capnography for severity of acute gastroenteritis in the emergency department. Am J Emerg Med. 2020;38(6):1159-1162. DOI: 10.1016/j.ajem.2019.158404.

How to cite this article: Altun ME, Emektar E, Çorbacıoğlu ŞK, Çevik Y. Evaluation of the relationship between $ETCO_2$ and delta CO_2 pressure and the severity of disease in COVID-19 pneumonia. Clin Exp Health Sci 2024; 14: 22-26. DOI: 10.33808/clinexphealthsci.1322620

Clinical and Experimental Health Sciences

Retrospective Analysis of Alveolar Osteitis (Dry Socket) Cases Over Two Years

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Received: September 13, 2023 **Accepted:** February 26, 2025

ABSTRACT

Objective: Alveolar osteitis (AO) is a common complication after tooth extraction that negatively affects the quality of life of patients. The aim of this study is to evaluate the etiological risk factors, clinical features and treatment management in alveolar osteitis cases.

Methods: In this study, 123 cases of patients diagnosed with AO in the oral and maxillofacial surgery clinic over a two-year period were retrospectively examined comprehensively. A previously prepared AO patient follow-up form was used to collect patient data.

Results: Among 3278 patients who underwent tooth extraction, 123 cases (47 males, 76 females) diagnosed with AO, aged between 19 and 84 years (mean age: 41.33±12.76 years) were included (AO prevalence: 3.75 %). While 23.6% of the cases had systemic disease, 22.8% were smokers and 8.1% were menstruating or using oral contraceptives. In AO cases, it was observed that the relevant teeth were mostly extracted due to dental caries (53.7%) and most often (56.1%) occurred after mandibular tooth extractions. 59.3% of AO cases occurred after traumatic tooth extraction, and pain (100%), difficulty eating(61.78%), and bad smell/taste (55.28%) were the most common symptoms. While irrigation was performed in 98.37% of the patients, topical alveogel was also applied in 45.5% of the patients.

Conclusion: The results of the study confirm the etiological risk factors stated in the literature in AO cases. In this study, successful results were obtained with the combination of irrigation, topical alveogel application and medical treatment in the treatment of AO cases.

Keywords: Alveolar osteitis, dry socket, retrospective analysis

1. INTRODUCTION

Alveolar osteitis (AO) is one of the common complications after tooth extraction and was named dry socket by Crawford in 1896 (1). AO is defined as "postoperative pain that increases in severity in and around the extraction site, accompanied by a partially or completely ruptured blood clot in the alveolar socket, with or without bad breath, between 1 and 3 days after tooth extraction" (2). While pain, bad odor/taste and difficulty in eating are common in AO cases, symptoms such as swelling, bleeding and fever are observed less frequently (3,4).

The etiology of AO is not known exactly, but some risk factors that play a role in etiology have been described in the literature. Some patient-related risk factors such as age, gender, presence of systemic disease, medication use, oral hygiene, smoking, alcohol use, menstruation, menopause, and oral contraceptive use have been reported (5–7). Clinical and surgical-related risk factors such as the extracted tooth's region, the extraction indication, the extraction difficulty, the extraction socket's condition, and the dentist's experience have also been reported (8–10). It may be caused by a combination of more than one factor, especially mechanical factors that cause the clot to break down or not form, such as diabetes mellitus, smoking, and factors such as traumatic tooth extractions (11–14). However, the presence of risk factors does not always indicate that AO will develop after tooth extraction.

Many combined treatment protocols have been proposed for relief of symptoms and tissue healing in AO cases (15,16). Universal treatment protocols such as irrigation, local agents and use of painkillers are widely used in the treatment of AO cases (17). The irrigation procedure is important in eliminating debris and microorganisms in the socket before placing any agent into the socket, and sterile saline solution and iodopovidone are often used for this purpose. Locally, topical anesthetics, CHX gel, paracetamol gel, zinc oxide eugenol paste, PRF, Alveogyl, SaliCept Patch, topical antibiotics (clindamycin, rifampicin), agents such as Vitamin C are used (18–23). In recent years, new treatment protocols

https://doi.org/*10.33808/clinexphealthsci.1359541*

such as hyaluronic acid, plasma-rich fibrin and low-energy laser therapy have been proposed. While anti-inflammatories and pain relievers are used in the treatment of AO, antibiotics are generally not preferred except for systemic involvement (such as fever and lymphadenopathy). Antibiotics can often be prescribed after tooth extractions (especially surgery extractions) to reduce the risk of AO (24–27).

AO is an annoying condition for patients with symptoms such as severe pain, bad taste in the mouth, and difficulty in eating after tooth extraction. It can negatively affect patients' quality of life and disrupt their daily workflow. When the studies are examined, the etiological risk factors of AO cases are not fully known, and there are different approaches in the literature for prevention and treatment. The aim of this study is to comprehensively examine the demographic variables, clinical findings, and treatment strategies in AO cases seen after tooth extraction in the oral and maxillofacial surgery clinic of a university.

2. METHODS

A retrospective descriptive study was designed and AO cases that developed after tooth extraction at Afyonkarahisar Health Sciences University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery between 30 October 2020 and 30 October 2022 were included in the study. Permission for the study was obtained from the Clinical Research Ethics Committee of Afyonkarahisar Health Sciences University (approval date 04.11.2022 and number 2022/528) and the study was conducted in accordance with the rules of the Declaration of Helsinki.

In this study, 123 AO cases who were diagnosed with AO in the oral and maxillofacial surgery clinic over a two-year period and whose treatment and follow-up were performed in the same clinic were retrospectively examined.

The most significant finding in the diagnosis of alveolar osteitis is an increase in pain severity a few days following tooth extraction. The lack of a blood clot and moderate to severe pain are diagnostic of alveolar osteitis and do not necessitate further laboratory or radiographic tests. Alveolar osteitis can be assumed when a patient presents within the first week after extractions with severe pain (16). Painful conditions occurring a few days after tooth extraction were evaluated as AO in this study.

A previously prepared AO patient follow-up form and patient radiographs were used to collect patient data. This form included information about the patient's socio-demographic data, medical history, etiological risk factors, clinical examination findings, surgical treatment and follow-up processes. Panoramic and periapical radiographs were used to identify retained tooth fragments, bone sequestrations, or other pathological conditions. Cases with missing clinical examination and radiological data were excluded from the study.

Statistical analysis of the data was performed using the SPSS statistical program, version 20 (SPSS Inc, Chicago, IL, USA). Mean and standard deviation values were given in descriptive statistics of continuous data, and number and percentage values were given in nominal data.

3. RESULTS

3.1. Socio-demographic Characteristics

Among a total of 3278 patients who had tooth extraction between 2020-2022, 123 cases (47 men, 76 women) who applied to our clinic with AO symptoms after extraction and were diagnosed with AO were included in the study. In the study, the prevalence of AO was found to be 3.75%.

The sociodemographic characteristics of the cases were given in Table 1. The majority of the cases (61.8%) were females. The age range of the cases is between 19 and 84 years (mean age: 41.33±12.76 years). When the age distribution of the cases was examined, the majority (35.8%) was between the ages of 30-39, followed by those aged 50 and over (26.8%). When the cases were examined according to their educational status, primary school graduates (37.4%) came first, while when the professions of the cases were examined, the first place was housewives (47.2%) (Table 1).

Table 1. *Socio-demographic characteristics of the cases*

		n	%
Age	19-29	23	18.7
	30-39	44	35.8
	40-49	23	18.7
	50 and above	33	26.8
Gender	Male	47	38.2
	Female	76	61.8
Education level	Primary school	46	37.4
	Middle school	15	12.2
	High school	26	21.1
	Undergraduate	36	29.3
Occupation	Housewife	58	47.2
	Student	13	10.6
	Officer	21	17.1
	Employee	25	20.3
	Self employment	6	4.9
	Total	123	100

3.2. Etiological Risk Factors

Etiological risk factors were examined under 2 subheadings: patient-related etiological risk factors, and local anatomic and/or surgery-related etiological risk factors. It was observed that 23.6% of the cases had a systemic disease and 28.5% were using medication regularly. The most common systemic diseases were hypertension and diabetes mellitus. Five of the AO patients were using antiaggregant drugs and one was using anticoagulant drugs. Less than half of the patients (41.6%) reported brushing their teeth twice a day, and 14.6% reported brushing their teeth less than once a day. The rate of smokers was 22.8%, and 15.4% of the cases reported

smoking immediately after tooth extraction. While the rate of those who were menstruating or using oral contraceptives was 13.15% among women, it was 8.1% in total (Table 2).

Table 2. *Etiological risk factors related to the patient*

		n	%
Presence of systemic disease	No diseases	94	76.4
	Hypertension	12	9.75
	Diabetes mellitus	10	8.13
	Thyroid diseases	6	4.87
	Osteoporosis	4	4.87
	Rheumatic diseases	3	2.43
	Asthma	2	1.62
	Other diseases (Heart disease, myasthenia gravis)	2	1.62
Drug use	Yes	35	28.5
	No	88	71.5
Teeth brushing frequency	Less than once a day	18	14.6
	One time per day	55	44.7
	Two times a day	50	41.6
Tobacco use	Yes	28	22.8
	No	95	77.2
Alcohol consumption	Yes	13	10.56
	No	110	89.44
Smoking after tooth extraction	Yes	19	15.4
	No	104	84.6
Menstruation/oral contraceptive use	Yes	10	8.1
	No	113	91.9
	Total	123	100

When the indications for extraction of the relevant tooth in AO cases were examined, it was seen that the teeth were mostly extracted due to tooth decay (53.7%) and pericoronitis (19.5%). In the majority of cases (86.2%), only one tooth was extracted in the same session, while in 4.9%, 3 or more teeth were extracted at the same time.

Traumatic tooth extractions constituted the majority of AO cases (59.3%). Routinely, simple tooth extractions performed using forceps and an elevator were considered atraumatic (nonsurgical) extraction. In contrast, a fracture of the tooth and the need for flap removal were considered a traumatic (surgical) extraction. Impacted dental surgery was performed in 3.3% of the cases. While the extraction sockets were left open in the majority of patients (69.1%) after extraction, the socket was approximated with sutures in 29.3%, and the sockets were closed with sutures in 1.6%. It was observed that local anesthesia containing a vasoconstrictor (68 mg articaine hydrochloride and 0.020 mg epinephrine hydrochloride) was used during tooth extraction in all patients with AO.

It was observed that the majority of the patients (77.2%) were prescribed medication after the relevant tooth extraction. The majority of medications prescribed to patients (28.5%) are solely anti-inflammatory/painkillers. In most patients (65%), no granulation tissue was observed in the extraction sockets during clinical examination (Table 3).

Table 3. *Etiological risk factors related to the extracted tooth and surgical procedure*

		n	%
Tooth extraction indication	Tooth decay	66	53.7
	Periodontitis	8	6.5
	Periapical lesion	11	8.9
	pericoronitis	24	19.5
	tooth root extraction	14	11.4
Number of extracted teeth	One	106	86.2
	Two	11	8.9
	Three and more	6	4.9
Type of tooth extraction	Atraumatic	46	37.4
	Traumatic	73	59.3
	Impacted tooth extraction	4	3.3
Socket closing	Socket open	85	69.1
	Suture approximation	36	29.3
	Full coverage	2	1.6
Post-operative drug use	None	28	22.8
	Antibiotic	6	4.9
	Painkiller/anti-inflammatory	35	28.5
	Mouthwash	6	4.9
	Antibiotic and painkiller	8	6.5
	Antibiotics and mouthwash	2	1.6
	Painkiller and mouthwash	5	4.1
	Antibiotics, painkillers and mouthwash	33	26.8
Post-operative bleeding	Yes	33	26.8
	No	90	73.2
Post-operative granulation tissue	Yes	43	35
	No	80	65
	Total	123	100

3.3. Clinical Signs and Symptoms

Pain was the most important symptom and was observed in all patients. Apart from pain, difficulty in eating (61.78%) and bad odor/taste (55.28%) were observed in most of the cases. The least common symptom was fever (4.06%). In most cases, more than one symptom was observed simultaneously (Figure 1).

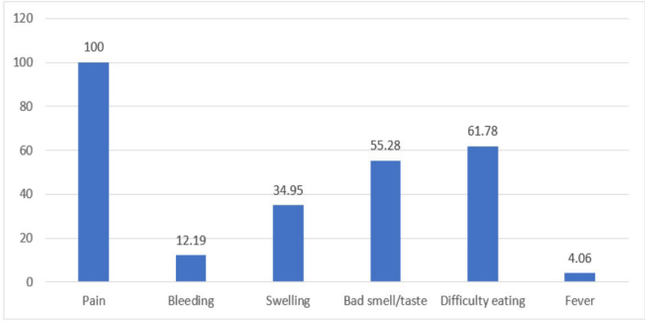

Figure 1. *Distribution of clinical symptoms in alveolar osteitis cases*

While mandibular 3rd molar tooth sockets were the area where AO was most frequently observed (32.5%), this was followed by mandibular 1st molar and mandibular 2nd molar teeth. The place where AO was least common was the mandibular anterior region (1.6%) (Figure 2).

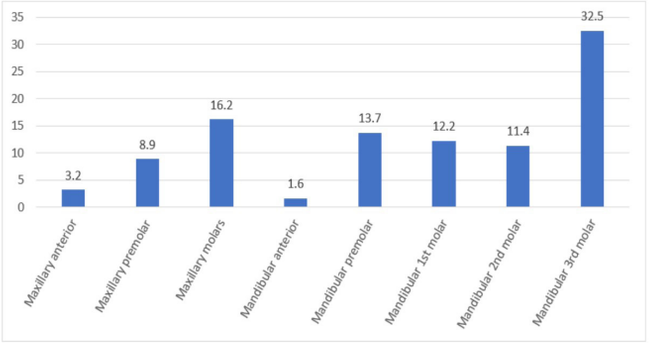

Figure 2. *Distribution of tooth areas where alveolar osteitis occurs*

Clinical symptoms appeared most frequently on the 2nd day after tooth extraction (28.5%), followed by the 3rd day with 23.6% (Figure 3).

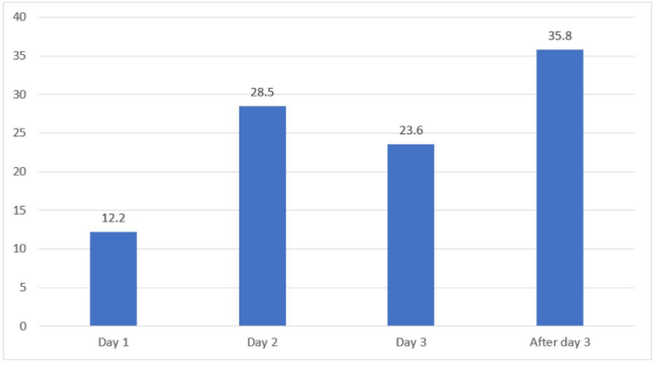

Figure 3. *Distribution of symptoms onset days in alveolar osteitis cases*

3.4. Treatment Management

Much of the current discussion around AO revolves around treatment strategies, although no consensus guidelines have been produced. Therefore, treatment management focuses on symptom relief rather than a specific disease process. Intra-alveolar irrigation is the most widely supported initial therapy technique for alveolar osteitis since it lowers the bacterial load, and eliminates necrotic tissue and clot debris. Except for only 2 patients (98.4%), irrigation was performed to remove debris in the socket and reduce the microorganism load in this study. Two patients did not accept irrigation and were only prescribed medication. The irrigation phase was applied with sterile saline solution, iodopovidone and their combination.

Topical local anesthetic gels can be used to alleviate pain following irrigation. In 45.5% of patients who underwent irrigation and had high pain levels, treatment was supported with alveogel. Curettage of a dry socket is not suggested since it exposes the bone further. In this study, curettage was applied in addition to irrigation to remove foreign bodies such as tooth and bone fragments and debris from the extraction socket in only 3.3% of the cases. Oral analgesics, particularly nonsteroidal anti-inflammatory medications (NSAIDs), can be used in addition to local anesthetics. All patients were treated with analgesic/anti-inflammatory drugs to relieve acute pain. All patients presenting with signs of AO were treated with medication (Figure 4). The most common prescription for patients was a painkiller/mouthwash combination (46.3%), followed by those prescribed only painkillers (26%) (Table 4).

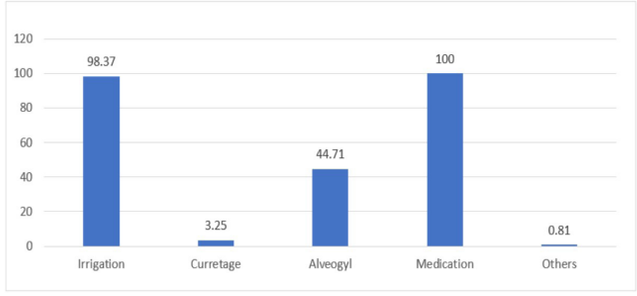

Figure 4. *Distribution of treatment modalities applied in alveolar osteitis cases (%)*

Table 4. *Treatment management of alveoler osteitis cases*

		n	%
Local treatment methods	Irrigation	61	49.6
	Irrigation and curettage	4	3.3
	Irrigation and alveogel	56	45.5
	Other	2	1.6
Medication	Antibiotic	2	1.6
	Painkiller	32	26
	Antibiotics and painkillers	6	4.9
	Painkiller and mouthwash	57	46.3
	Antibiotics, painkillers and mouthwash	26	21.1
	Total	123	100

4. DISCUSSION

AO, which is frequently encountered after tooth extractions, can reduce the quality of life of patients and cause significant workforce losses. On the other hand, when treatment management is done correctly in AO cases, recovery can be achieved without causing serious complications. The cases that developed AO in the oral and maxillofacial surgery clinic were evaluated retrospectively in terms of etiological risk factors, clinical features and treatment approaches. In this study, the prevalence of AO developing after tooth extraction over a two-year period was found to be 3.75%, and this result was found to be consistent with the results of other studies in the literature (between 0.5% and 5%) (16,28).

In previous studies, AO cases were more common in females than in males (6,8,12). In this study, AO was more common in women, similar to the literature. A higher incidence of AO after tooth extraction in non-menopausal females has been associated with estrogen levels, use of oral contraceptives, and hormonal levels changing as a result of the menstrual cycle affecting epithelialization (28–31). The mean age of AO cases in this study was 41.33±12.76 years. Diego et al. (10) in their study, the average age of patients presenting with AO complaints was found to be 39.7 years. AO cases may occur more frequently in older ages due to factors such as decreased healing potential as age progresses, increased systemic disease and drug use, and difficulty in tooth extraction. However, in some studies, no direct correlation was found between AO and the age of the patient (7,8,10).

In the literature, diabetes, oral hygiene and smoking have been reported as risk factors for AO (32,33). In this study, while diabetes was seen in 8.1% of all participants, it constituted 34.5% of those with systemic diseases. In a study conducted in Australia, no significant relationship was found between AO and diabetes (34). Most studies have shown a significant relationship between poor oral hygiene and the occurrence of AO (35,36). In this study, 14.6% of patients stated that they did not brush their teeth even once a day. In most published studies, smoking is one of the most important risk factors for the occurrence of AO (11,14,37). It has been reported that the likelihood of AO increases when the number of cigarettes smoked per day increases or when one smokes immediately after tooth extraction (especially in the first 24 hours) (38–40). In this study, 22.8% of the cases were smokers, and 67.9% of these smokers reported smoking immediately after tooth extraction, which is consistent with Meechan et al. (38) supports study findings reporting the negative effects of smoking on recovery.

In the etiology of AO, the socio-demographic characteristics of the patient as well as the type of tooth extraction and surgical procedures play a role in the emergence of AO. It has been emphasized that the experience of the dentist, especially the traumatic extraction/tooth extraction difficulty, the extraction site and the extraction indication are high-risk factors for AO. In particular, the difficulty of extraction is important in terms of the risk of AO (11,12,41,42). In this study, 59.2% of AO cases were observed after difficult tooth extraction. While this rate was found to be 65% in the study of Halab et al.(10), it was found to be 66.2% in the study of Oginni et al. (12). In the studies conducted, no significant difference was found between the number of teeth extracted and AO, on the contrary, single tooth extractions constitute the majority of AO cases (5,8,11). Taberner-Valverdu et al. (5) reported that 82.35% of AO cases occurred after a single tooth extraction. In this study, it was observed that 86.2% of AO cases occurred after a single tooth extraction, similar to the literature. It should be noted that this result may be related to the fact that single-tooth extraction is more common than multiple-tooth extractions. It has been reported that high doses of local anesthesia and especially its vasoconstrictor properties may be a predisposing factor for dry socket (43). In this study, local anesthesia with a vasoconstrictor (68 mg articaine hydrochloride and 0.020 mg epinephrine hydrochloride) was used in all patients with AO. Local anesthetics especially those with vasoconstrictor, can cause local ischemia and pave the way for the formation of AO.

Previous studies have revealed that AO cases are more common in the mandible than in the maxilla(5,10,12). In the study conducted by Taberner-Valverdu et al. (5), the mandible was 70.59%, maxilla was 29.41%, respectively; In the study conducted by Oginni et al. (4), the mandible was found to be 75.8% and maxilla was found to be 24.2%. In this study, similar to the literature, AO cases occurred in 69.9% of the mandible and 30.1% of the maxilla. AO occurs frequently in the posterior tooth regions of the mandible. In this study, AO cases were most frequently seen in the mandibular 3rd molar tooth region (32.5%), followed by the mandibular 1st molar (12.2%) and 2nd molar (11.4%). 16.2% of the cases were seen in the maxillary molar region. In the study conducted by Oginni et al. (4), AO cases were most frequently seen in the mandibular 1st molar (34.6%) and 2nd molar (27.9%) regions, respectively, followed by the 3rd molar (11.8%) and maxillary 1st molar (10.3%).

A relationship can also be established between AO cases and the indication for extraction of the relevant tooth (2,4,41). In the study conducted by Younis et al. (11), the majority of AO cases were teeth extracted due to caries (41.2%), followed by extractions due to periodontal disease, combined caries/periodontal disease and pericoronitis. Similarly, in this study, it was observed that the majority of teeth extracted before alveolitis (53.7%) were extracted due to tooth decay. Pericoronitis is another common reason for extraction in AO cases, and in this study, the pre-extraction pericoronitis rate in the relevant teeth was found to be 19.5%, which is similar to the pericoronitis rate (16.8%) seen in the study by Leung et al.(8). Studies have shown that the presence of pericoronitis or acute infection before extraction increases the incidence of AO (4,44,45).

In cases of AO, different symptoms may occur that may affect the daily life of the patient. AO patients often have severe pain; difficulty in eating, bad odor/taste, swelling and bleeding are other common symptoms, and these symptoms often occur together (4,9,40). In this study, clinical symptoms such as pain (100%), difficulty in eating (61.78%), bad smell/taste (55.28%) and swelling (34.95%) were observed in AO cases. Although rare, systemic symptoms such as lymphadenopathy and fever may be observed in addition to local findings in some cases. Severe pain, which is the first symptom in AO cases, usually begins between days 1 and 4 (2). In the study conducted by Oginni et al (4), pain occurred between days 1 and 3 in 85.7% of the patients. In this study, consistent with the literature, alveolitis symptoms appeared in 64.3% of the patients between days 1 and 3.

The etiology and pathophysiology of AO are not fully known and the effectiveness of treatment methods is still debated. However, a number of measures can reduce the occurrence

of AO. Blum explained these common precautions as follows: Careful planning, minimum trauma-maximum care, ensuring the presence of a blood clot after the extraction, reducing the preoperative plaque level and maximum oral hygiene, encouraging the patient to quit smoking, Performing tooth extraction between days 23 and 28 of the menstrual cycle in patients using oral contraceptives, giving the patient comprehensive verbal instructions before and after surgery, and communicating these instructions in writing for maximum compliance (2).

Some treatment procedures can be followed in the treatment management of AO cases. As a result of a comprehensive literature review conducted in Argentina, 39 different routine treatment protocols for AO were identified (15). In AO cases, procedures that aim to create new granulation tissue in the socket for 7-10 days, accelerate healing, and relieve symptoms during this healing process come to the fore (16,17,46). These procedures can be listed as irrigation, dressing with topical agents and drug therapy. Blum summarized these procedures as follows: no stimulus should remain at the extraction site, local anesthetic for pain, irrigation with warm sterile saline, no curettage, prescription of analgesics, and use of CHX mouthwash (2).

Irrigation procedures are of great importance for AO cases. In studies, different procedures and agents are recommended by different authorities (15,46,47). As a result of the comprehensive screening of 17 studies conducted by Garola et al. (15), sterile saline solution was used in commonly in all studies. In addition, there are also studies using a combination with povidoneniod, clindamycin, rifampicin, and hydrogen peroxide irrigations (21,48). When we look at AO treatment protocols, irrigation agents alone are ineffective in relieving symptoms. Topical agents are applied to the socket to heal the socket and relieve symptoms (17,20,22). It is recommended that these agents should not be left on for a long time and should be renewed in order to avoid causing a foreign body reaction (16). Similar to the literature, an irrigation procedure with a combination of sterile saline and povidone-iodine was applied to all but 2 of the AO cases. Alveogel has long been the first choice agent in the treatment of AO due to its antimicrobial, anesthetic and analgesic properties. In this study, in addition to sterile saline povidone-iodine combination irrigation in AO treatment management, alveogyl was routinely applied to the socket every other day in 56 patients (45.5%).

Although there is insufficient evidence to support the use of antibiotics in the treatment management of AO, antibiotics continue to be widely prescribed for prophylaxis and postoperative purposes (2). If the patient's immune system is not suppressed or there are no systemic symptoms such as fever, weakness, and lymphadenopathy, systemic antibiotics are considered unnecessary in AO cases (49). Prescribing long-acting local anesthesia and systemic analgesics are the most effective methods for controlling pain and relieving other symptoms (16). In this study, while analgesics were prescribed to all AO cases, antibiotics were prescribed only to 27.64%. The majority of patients (67.47%) were also prescribed a 0.12% CHX mouthwash.

5. CONCLUSION

AO is a condition frequently encountered after tooth extraction in oral and maxillofacial surgery practice, which negatively affects the daily life of the patient with severe pain and bad odor/taste. The lack of a definitively effective treatment method in the treatment of AO shows the importance of risk factors and protective/preventive activities involved in the etiology. Atraumatic tooth extraction, primary closure of the extraction socket, patient compliance with post-operative recommendations, and good oral hygiene are the basic principles to be followed in reducing AO cases. In addition, continuous education of dentists about the risk factors and management of AO cases and raising patients' awareness about AO after tooth extraction may reduce the number of patients presenting to the hospital with AO after tooth extraction.

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by the Clinical Research Ethics Committee of Afyonkarahisar Health Sciences University (approval date 04.11.2022 and number 2022/528). This study was carried out in fullcompliance with the principles of the Declaration of Helsinki.*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: ÖE
Design of the study: ÖE
Acquisition of data for the study: ÖE, İÇ
Analysis of data for the study: ÖE
Interpretation of data for the study: ÖE
Drafting the manuscript: ÖE, İÇ
Revising it critically for important intellectual content: ÖE
Final approval of the version to be published: ÖE, İÇ

REFERENCES

[1] Crawford JY: Dry sockets after extraction. Dent Cosmos 38:929, 1896

[2] Blum IR. Contemporary views on dry socket (alveolar osteitis): A clinical appraisal of standardization, aetiopathogenesis and management: A critical review. Int J Oral Maxillofac Surg. 2002;31(3):309-317. https://doi.org/10.1054/ijom.2002.0263

[3] Araújo MG, Silva CO, Misawa M, Sukekava F. Alveolar socket healing: what can we learn? Periodontol 2000. 2015;68(1):122-134. https://doi.org/10.1111/prd.12082

[4] Oginni FO, Fatusi OA, Alagbe AO. A clinical evaluation of dry socket in a Nigerian teaching hospital. Journal of Oral and Maxillofacial Surgery. 2003;61(8):871-876. https://doi.org/10.1016/S0278-2391(03)00248-9

[5] Taberner-Vallverdú M, Camps-Font O, Gay-Escoda C, Sánchez-Garcés MA. Previous dry socket as a risk factor for alveolar osteitis: A nested case-control study in primary healthcare services. J Clin Exp Dent. 2022;14(6):479-485. https://doi.org/10.4317/jced.59586

[6] Rakhshan V. Common risk factors of dry socket (alveolitis osteitis) following dental extraction: A brief narrative review. J Stomatol Oral Maxillofac Surg. 2018;119(5):407-411. https://doi.org/10.1016/j.jormas.2018.04.011

[7] Ghosh A, Aggarwal VR, Moore R. Aetiology, Prevention and Management of Alveolar Osteitis—A Scoping Review. J Oral Rehabil. 2022;49(1):103-113. https://doi.org/10.1111/joor.13268

[8] Lee CTY, Zhang S, Leung YY, Li SKY, Tsang CC, Chu CH. Patients' satisfaction and prevalence of complications on surgical extraction of third molar. Patient Prefer Adherence. 2015;9:257-263. https://doi.org/10.2147/PPA.S76236

[9] Christensen J, Hauge Matzen L, Wenzel A. Should removal of lower third molars be included in the pre-graduate curriculum for dental students? An evaluation of post-operative complications after student operations. Acta Odontol Scand. 2012;70(1):42-48. https://doi.org/10.3109/00016357.2011.575082

[10] Halab D, Escobar J, Muoz C, Uribe S. Logistic regression analysis of risk factors for the development of alveolar osteitis. J Oral Maxillofac Surg. 2012;70(5):1040-1044. https://doi.org/10.1016/j.joms.2011.11.024

[11] Abu Younis MH, Abu Hantash RO. Dry socket: frequency, clinical picture, and risk factors in a palestinian dental teaching center. *Open Dent J*. 2011;5:7-12. Published 2011 Feb 7. https://doi.org/10.2174/1874210601105010007

[12] Oginni FO. Dry Socket: A prospective study of prevalent risk factors in a Nigerian Population. J Oral Maxillofac Surg. 2008;66(11):2290-2295. https://doi.org/10.1016/j.joms.2008.01.063

[13] Parthasarathi K, Smith A, Chandu A. Factors affecting incidence of dry socket: A prospective community-based study. J Oral Maxillofac Surg. 2011;69(7):1880-1884. https://doi.org/10.1016/j.joms.2010.11.006

[14] Noroozi AR, Philbert RF. Modern concepts in understanding and management of the "dry socket" syndrome: comprehensive review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(1):30-35. https://doi.org/10.1016/j.tripleo.2008.05.043

[15] Garola F, Gilligan G, Panico R, Leonardi N, Piemonte E. Clinical management of alveolar osteitis. A systematic review. Med Oral Patol Oral Cir Bucal. 2021;26(6):e691-e702. https://doi.org/10.4317/medoral.24256

[16] Chow O, Wang R, Ku D, Huang W. Alveolar osteitis: A review of current concepts. J Oral Maxillofac Surg. 2020;78(8):1288-1296. https://doi.org/10.1016/j.joms.2020.03.026

[17] Daly BJ, Sharif MO, Jones K, Worthington HV, Beattie A. Local interventions for the management of alveolar osteitis (dry socket). Cochrane Database Syst Rev. 2022;9(9):CD006968. Published 2022 Sep 26. https://doi.org/10.1002/14651858.CD006968.pub3

[18] Halabi D, Escobar J, Alvarado C, Martinez N, Muñoz C. Chlorhexidine for prevention of alveolar osteitis: A randomised clinical trial. J Appl Oral Sci. 2018;26:e20170245. https://doi.org/10.1590/1678-7757-2017-0245

[19] Reeshma S, Dain CP. Comparison of platelet-rich fibrin with zinc oxide eugenol in the relief of pain in alveolar osteitis. Health Sci Rep. 2021;4(3). https://doi.org/10.1002/hsr2.354

[20] Burgoyne CC, Giglio JA, Reese SE, Sima AP, Laskin DM. The efficacy of a topical anesthetic gel in the relief of pain associated with localized alveolar osteitis. J Oral Maxillofac Surg. 2010;68(1):144-148. https://doi.org/10.1016/j.joms.2009.06.033

[21] Çebi AT. Evaluation of the effects of intra-alveolar irrigation with clindamycin, rifampicin and sterile saline in alveolar osteitis treatment. J Stomatol Oral Maxillofac Surg. 2020;121(6):680-683. https://doi.org/10.1016/j.jormas.2020.01.004

[22] Taberner-Vallverdú M, Nazir M, Sánchez-Garcés MÁ, Gay-Escoda C. Efficacy of different methods used for dry socket management: A systematic review. Med Oral Patol Oral Cir Bucal. 2015;20(5):e633-e639. https://doi.org/10.4317/medoral.20589

[23] Supe NB, Choudhary SH, Yamyar SM, Patil KS, Choudhary AK, Kadam VD. Efficacy of alvogyl (Combination of Iodoform + Butylparaminobenzoate) and zinc oxide eugenol for dry socket. Ann Maxillofac Surg. 2018;8(2):193-199. https://doi.org/10.4103/ams.ams_167_18

[24] Marcussen KB, Laulund AS, Jørgensen HL, Pinholt EM. A systematic review on effect of single-dose preoperative antibiotics at surgical osteotomy extraction of lower third molars. J Oral Maxillofac Surg. 2016;74(4):693-703. https://doi.org/10.1016/j.joms.2015.11.017

[25] Gbotolorun OM, Dipo-Fagbemi IM, Olojede AO, Ebigwei S, Adetoye JO. Are systemic antibiotics necessary in the prevention of wound healing complications after intra-alveolar dental extraction? Int J Oral Maxillofac Surg. 2016;45(12):1658-1664. https://doi.org/10.1016/j.ijom.2016.08.023

[26] Ndukwe KC, Braimah RO, Owotade JF, Aregbesola SB. Comparative efficacy of amoxicillin/clavulanic acid and levofloxacin in the reduction of postsurgical sequelae after third molar surgery: A randomized, double-blind, clinical trial in a nigerian university teaching hospital. Niger J Surg. 2016;22(2):70-76. https://doi.org/10.4103/1117-6806.179830

[27] Gazal G, Al-Samadani KH, Alsaidalani HM, Karbouji GA, Alharbi AM. A comparison of pre-emptive co-amoxiclav, postoperative amoxicillin, and metronidazole for prevention of postoperative complications in dentoalveolar surgery: A randomized controlled trial. Int J Environ Res Public Health. 2022;19(7):4178. https://doi.org/10.3390/ijerph19074178

[28] Almeida LE, Pierce S, Klar K, Sherman K. Effects of oral contraceptives on the prevalence of alveolar osteitis after mandibular third molar surgery: A retrospective study. Int J Oral Maxillofac Surg. 2016;45(10):1299-1302. https://doi.org/10.1016/j.ijom.2016.05.022

[29] Catellani JE, Harvey S, Erickson SH, Cherkin D. Effect of oral contraceptive cycle on dry socket (localized alveolar osteitis). J Am Dent Assoc. 1980;101(5):777-780. https://doi.org/10.14219/jada.archive.1980.0420

[30] Leimola-Virtanen R, Pennanen R, Syrjänen K, Syrjänen S. Estrogen response in buccal mucosa. A cytological and immunohistological assay. Maturitas. 1997;27(1):41-45. https://doi.org/10.1016/S0378-5122(97)01113-4

[31] Cohen ME, Simecek JW. Effects of gender-related factors on the incidence of localized alveolar osteitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;79(4):416-422. https://doi.org/10.1016/S1079-2104(05)80120-9

[32] Saravanan K. Assessment of post extraction complications in Indians. Bioinformation. 2021;17(12):1120-1125. https://doi.org/10.6026/973206300171120

[33] 33. Gadicherla S, Smriti K, Roy S, Pentapati KC, Rajan J, Walia A. Comparison of extraction socket healing in non-diabetic,

prediabetic, and type 2 diabetic patients. Clin Cosmet Investig Dent. 2020;12:291-296. https://doi.org/10.2147/CCIDE.S264196

[34] Power DJ, Sambrook PJ, Goss AN. The healing of dental extraction sockets in insulin-dependent diabetic patients: a prospective controlled observational study. Aust Dent J. 2019;64(1):111-116. https://doi.org/10.1111/adj.12669

[35] Peñarrocha-Diago M, Sanchis JM, Sáez U, Gay C, Bagán J V. Oral hygiene and postoperative pain after mandibular third molar surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92(3):260-264. https://doi.org/10.1067/moe.2001.115722

[36] Tjernberg A. Influence of oral hygiene measures on the development of alveolitis sicca dolorosa after surgical removal of mandibular third molars. Int J Oral Surg. 1979;8(6):430-434. https://doi.org/10.1016/S0300-9785(79)80081-2

[37] Kuśnierek W, Brzezińska K, Nijakowski K, Surdacka A. Smoking as a risk factor for dry socket: A systematic review. Dent J (Basel). 2022;10(7):121. https://doi.org/10.3390/dj10070121

[38] Meechan JG, Macgregor ID, Rogers SN, Hobson RS, Bate JP, Dennison M. The effect of smoking on immediate post-extraction socket filling with blood and on the incidence of painful socket. Br J Oral Maxillofac Surg. 1988;26(5):402-409. https://doi.org/10.1016/0266-4356(88)90093-9

[39] Heng CK, Badner VM, Clemens DL, Mercer LT, Mercer DW. The relationship of cigarette smoking to postoperative complications from dental extractions among female inmates. Oral Surgery, Oral Medicine, Oral Pathology. 2007;104(6):757-762. https://doi.org/10.1016/j.tripleo.2007.04.020

[40] Sweet JB, Butler DP. The relationship of smoking to localized osteitis. J Oral Surg. 1979;37(10):732-735.

[41] Heasman PA, Jacobs DJ. A clinical investigation into the incidence of dry socket. Br J Oral Maxillofac Surg. 1984;22(2):115-122. https://doi.org/10.1016/02664356(84)90023-8.

[42] Larsen PE. Alveolar osteitis after surgical removal of impacted mandibular third molars. Oral Surgery, Oral Medicine, Oral Pathology 1992;73(4):393-397. https://doi.org/10.1016/0030-4220(92)90312-E

[43] Meechan JG, Venchard GR, Rogers SN. Local anaesthesia and dry socket. A clinical investigation of single extractions in male patients. Int J Oral Maxillofac Surg. 1987;16(3):279-284. https://doi.org/10.1016/S0901-5027(87)80148-0

[44] Isik BK, Gürses G, Menziletoglu D. Acutely infected teeth: to extract or not to extract?. Braz Oral Res. 2018;32:e124. https://doi.org/10.1590/1807-3107bor-2018.vol32.0124

[45] Martis C, Karabouta I, Lazaridis N. Extractions of impacted mandibular wisdom teeth in the presence of acute infection. Int J Oral Surg. 1978;7(6):541-548. https://doi.org/10.1016/S0300-9785(78)80071-4

[46] Alexander RE. Dental extraction wound management: a case against medicating postextraction sockets. J Oral Maxillofac Surg. 2000;58(5):538-551. https://doi.org/10.1016/s0278-2391(00)90017-x

[47] Cardoso CL, Rodrigues MTV, Ferreira O, Garlet GP, De Carvalho PSP. Clinical concepts of dry socket. J Oral Maxillofac Surg. 2010;68(8):1922-1932. https://doi.org/10.1016/j.joms.2009.09.085

[48] Suchánek J, Ivančaková RK, Mottl R, Browne KZ, Pilneyová KC, Pilbauerová N, Schmidt J, Suchánková Kleplová T. Hyaluronic acid-based medical device for treatment of alveolar osteitis-clinical study. Int J Environ Res Public Health 2019;16(19):3698. https://doi.org/10.3390/ijerph16193698

[49] Fazakerley M, Field EA. Dry socket: A painful post-extraction complication (a review). Dent Update. 1991;18(1):31-34.

How to cite this article: Ekici Ö, Çalışkan İ. Retrospective Analysis of Alveolar Osteitis (Dry Socket) Cases Over Two Years. Clin Exp Health Sci 2025; 15: 27-34. https://doi.org/10.33808/clinexphealthsci.1359541

Clinical and Experimental Health Sciences

The Effects of Prenatal Optimism and Spiritual Intelligence on Childbirth Attitudes

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Received: September 19, 2023 **Accepted:** March 05, 2025

ABSTRACT

Objective: This study was conducted to determine the effects of prenatal optimism and spiritual intelligence on childbirth attitudes in pregnant women.

Methods: This cross-sectional study was completed with the participation of 288 pregnant women. The data were collected using a Personal Information Form, the Childbirth Attitudes Questionnaire, the Life Orientation Test, and the Scale for Spiritual Intelligence.

Results: The mean total score of the Scale for Spiritual Intelligence, Life Orientation Test, and Childbirth Attitudes Questionnaire of the participants were determined to be 47.95±7.81, 18.43±4.85, and 39.26±10.89, respectively. It was determined that while spiritual intelligence levels did not have a significant effect on the childbirth attitudes variable, prenatal optimism levels had a significant effect on childbirth attitudes, where the former explained approximately 5% of the variance in the latter (R^2=0.047, p=.001).

Conclusion: According to the results of this study, an increase in the prenatal optimism of pregnant women helps their fear of childbirth decrease.

Keywords: Prenatal, pregnancy, optimism, spiritual intelligence, childbirth attitude

1. INTRODUCTION

The prenatal period is a period of getting physically and psychologically prepared for the changes to be experienced along with the transition to motherhood. Pregnancy is accepted as a stressful life event that necessitates coping strategies and adaptation in women (1). The health condition of the fetus, the fear of childbirth, physical changes, and increased healthcare needs make pregnant women more prone to depression, anxiety, and stress (2). Stressful life events such as the perinatal period can erode the individual's coping resources and cause increased use of ineffective coping strategies under continuous stress. As a personality trait, optimism plays a significant role in the self-management of behaviors and serves an important function in terms of adaptation to various stressors in life (3). As individuals with high levels of optimism will make more efforts to manage a stress factor, it is believed that optimism can play a protective role against negative health outcomes (3,4). In the relevant literature, it is seen that high levels of optimism have been associated with positive childbirth outcomes and lower levels of stress, anxiety, and peripartum depression (4,5).

A coping mechanism against depression, anxiety, and stress in pregnancy is to turn toward faith and values that involve one's spirituality and ethical, cultural, and world views (6). Spiritual intelligence is a form of adaptation and problem-solving behaviors that help the individual adapt to internal and external integrity. An increase in spiritual intelligence is aimed at increasing the spiritual well-being and adaptation levels of pregnant women (7). Recent research has shown that spiritual intelligence is positively related to increasing spiritual well-being and resilience against stress (8), and if spirituality is considered, the fears and psychological anxieties of pregnant women can be alleviated (6,7,9).

Childbirth-related attitudes refer to tendencies, emotions, and behaviors developed about labor. These attitudes, which are particularly developed towards the unknown in one's first pregnancy, usually take the form of fear. Statistics show that 10-15% of pregnant women experience the fear of childbirth (7). The fear of childbirth is an emotional stressor that affects the mental health and well-being of the mother-to-be throughout the pregnancy period (10). Excessive fear of childbirth puts the pregnant woman at risk of emotional imbalance, and this negatively affects the relationship between the mother and her baby (7). This situation also brings along interventional labor and an

https://doi.org/10.33808/clinexphealthsci.1362979

increased risk of C-section requirements (10). Therefore, the care provided to the pregnant woman experiencing fear of childbirth is important in terms of increasing her motivation and power to manage problems related to her pregnancy (7). As optimism and spirituality may be an alternative mechanism of coping with stress and fear in this period, this study was conducted to determine the effects of prenatal optimism and spiritual intelligence on childbirth-related attitudes in pregnant women.

2.METHODS

2.1. Design

This study was designed as a cross-sectional study and was conducted between February 2022 and July 2022.

2.2. Setting and Participants

The population of the study consisted of pregnant women who presented to the pregnancy outpatient clinic of a Medical Faculty Hospital between the dates when the study was conducted. The sample size of the study was calculated to be 278 individuals using the G*Power 3.1.9.2 software with a two-point deviation from a known mean childbirth attitudes score, which was 39.90±11.36 (11), 0.05 margin of error, 0.17 effect size, and 90% power. The study was completed with 288 pregnant women. Pregnant women who were 18 years old or older, literate, could communicate in Turkish, agreed to participate in the study voluntarily, did not experience any complications related to the prenatal period, and had a healthy fetus were included in the study.

2.3. Data Collection Instruments

The data were collected using a "Personal Information Form", the "Childbirth Attitudes Questionnaire", the "Life Orientation Test", and the "Scale for Spiritual Intelligence."

2.3.1.Personal Information Form: The 16-item form was prepared by the researchers by reviewing the literature to determine the sociodemographic characteristics of the participants (e.g., age, education level, employment status) and factors that could potentially affect their optimism, spiritual intelligence, and childbirth attitudes in the prenatal period (12-14).

2.3.2.Childbirth Attitudes Questionnaire (CAQ): The scale was developed by Lowe (2000) to measure pregnant women's fears of childbirth (15). The Turkish validity and reliability study of the scale was conducted by Dönmez et al. (2014). It is a 16-item 4-point Likert-type scale. There are no inversely scored items on the scale. The total score of the scale is calculated by taking the average of the scores of 16 items. The minimum and maximum scores to be obtained from the scale are 16 and 64. A high score indicates high levels of anxiety and fear. The Cronbach's alpha coefficient of the scale was reported as .82, while this value was found to be .91 in our study (12).

2.3.3.Life Orientation Test (LOT): The scale was developed by Scheier and Carver (1987) to evaluate the optimism levels of individuals (16). The Turkish validity and reliability study of the scale was conducted by Aydın and Tezer (1991). It is a 12-item 4-point Likert-type scale. Items 3, 8, 9, and 12 on the scale are coded in reverse, and items 2, 6, 7, and 10 are filler questions that are not included in the scoring of the scale. The minimum and maximum scores to be obtained from the scale are 0 and 32. A high score suggests a high level of optimism. Cronbach's alpha coefficient for the scale was reported as .77, while this value was found to be .77 in our study (13).

2.3.4.Scale for Spiritual Intelligence (SSI): The scale developed by Kumar and Mehta (2011) aims to determine the spiritual intelligence levels of individuals (17). The Turkish validity and reliability study of the scale was conducted by Tekin and Ekşi (14). The 5-point Likert-type scale consists of 20 items. There are 9 inversely coded items on the scale (items 1, 4, 7, 9, 13, 14, 15, 16, and 17). The scale has four subscales (understanding self, human values, compassion, and conscience). The total score is obtained by adding the scores of all subscales. The minimum and maximum scores are to be obtained on a scale of 20 and 100. A high score shows a high level of spiritual intelligence. Cronbach's alpha coefficient for the scale was reported as .85, while this value was found to be .71 in our study (14).

2.4. Ethics

An explanation about the study was made to potential participants, they were informed about the informed consent text, and the consent of those who agreed to participate was obtained. Additionally, the participants were informed that they were free to quit the study at any step. Ethical approval for the study was obtained from the Non-Invasive Clinical Research Ethics Committee of Selcuk University, Faculty of Health Sciences. (Decision No.: 2022/58).

2.5. Research Questions

1. Do the socio-demographic characteristics of pregnant women affect their prenatal optimism scores?
2. Do the socio-demographic characteristics of pregnant women affect their spiritual intelligence scores?
3. Do the socio-demographic characteristics of pregnant women affect their childbirth attitudes?
4. Do the prenatal optimism and spiritual intelligence scores of pregnant women during pregnancy affect their childbirth attitudes?

2.6. Data Analysis

The data collected in the study were analyzed using the SPSS 25.0 statistics software. Skewness and kurtosis values were calculated for the normality test. The skewness values were observed to vary between – 0.094 and 0.014, while the kurtosis values ranged from – 0.739 to 0.186. When skewness and kurtosis values are between – 1.5 and +1.5, it is considered that the data are normally distributed (18). In the analyses of the data in this study, frequencies, percentage distributions, mean, and standard deviation values were used, and independent-sample t-test, ANOVA, linear regression analysis, and Tukey's test were employed as parametric methods. The level of statistical significance was set at p< .05.

3. RESULTS

The mean total SSI, LOT, and CAQ scores of the participants were determined to be 47.95±7.81, 18.43±4.85, and 39.26±10.89, respectively (Table 1).

This section presents some characteristics of the participants and a comparison of the mean scores on the total scale and the subscales according to these characteristics. Statistically significant differences were found in the mean SSI 'understanding self' subscale scores of the participants based on their age group, family type, employment and educational status (p< .05) This result showed that the levels of understanding self among the participants in the age group of 36-41 were higher compared to the levels among those in other age groups. Similarly, the levels of understanding self among the participants residing in districts were determined to be significantly higher compared to those residing in provinces. The mean total SSI score and the mean understanding self and conscience subscale scores of the participants with extended families were determined to be significantly higher compared to the mean scores of those with nuclear families. The understanding self and conscience subscale scores of the participants varied significantly based on the education levels of their spouses (p< .05). The mean understanding self and conscience subscale scores of the participants whose spouses had university or higher degrees were significantly lower in comparison to the scores of those in the other groups. The mean compassion subscale score of the participants with low levels of income was found to be significantly lower than the mean score of those in the other income groups. Statistically significant differences were also observed the mean total LOT scores of the participants based on their age groups, where the life orientation scores in the 18-23 age group were observed to be significantly lower. The mean total LOT scores of the participants were found to vary to a significant extent based on the education levels of their spouses (p< .05). Accordingly, as the education levels of their spouses increased, the optimism levels of the participants also increased. Regarding the family types of the participants, there was a statistically significant difference in their mean total CAQ scores (p< .05). It was seen that the CAQ scores of the participants with nuclear families were significantly higher (Table 2).

Statistically significant differences were found in the mean SSI 'understanding self' subscale scores of the participants based on their attendance at prenatal follow-ups (p<.05). The mean human values subscale scores of the participants were determined to significantly differ based on their parity and whether they were having a planned pregnancy (p< .05) (Table 3).

Table 4 presents the results of the multiple linear regression analysis of the factors affecting the mean total SSI, LOT, and CAQ scores of the participants. Accordingly, the spiritual intelligence scores of the participants did not have a significant effect on their childbirth attitudes (p> .05). The life orientation scores of the participants had a negative and significant effect on their childbirth attitudes (p<.001). As the life orientation (optimism) scores of the participants decreased, their anxiety and fear levels increased. The variable of life orientation explained 5% of the total variance in childbirth attitudes (R^2=.047) (Model 1). It was seen that having an extended family positively and significantly affected the childbirth attitudes of the participants (p< .01, R^2=.035) (Model 2). In Model 3, it was determined that the educational levels of the spouses of the participants had a positive and significant effect on the life orientation scores of the participants (p< .01, R^2=.031). In Model 4, the family type variable was determined to be a significant factor affecting the spiritual intelligence statuses of the participants (p< .01, R^2=.050) (Table 4).

***Table 1**: SSI, LOT, and CAQ scores and cronbach's alpha values*

Scales	Mean±SD	Minimum-Maximum	Cronbach's alpha
Scale for Spiritual Intelligence (SSI)	47.95±7.81	21-67	.714
Life Orientation Test (LOT)	18.43±4.85	7-32	.778
Childbirth Attitudes Questionnaire (CAQ)	39.26±10.89	16-64	.915

SSI: Scale for Spiritual Intelligence, LOT: Life Orientation Test, and CAQ: Childbirth Attitudes Questionnaire

Table 2. *Some sociodemographic characteristics of the participants and comparisons of their SSI, LOT, and CAQ scores based on these characteristics*

Characteristics	n	%	Understanding self subscale Mean±SD	Human values subscale Mean±SD	Compassion subscale Mean±SD	Conscience subscale Mean±SD	SSI Mean ±SD	LOT Mean±SD	CAQ Mean±SD
Age group			17.98 ±2.99	11.50±2.58	12.15±3.13	6.70±2.90	48.35±7.32	17.57±4.10	37.95±11.48
18-23 (1)	77	26.7	17.58±3.01	11.30±2.91	12.60±3.29	6.36±2.11	47.85±7.28	18.31±4.41	40.38±3.63
24-29 (2)	129	43.8	16.41±3.62	10.96±2.49	12.78±3.68	6.38±2.79	46.55±9.14	19.88±5.90	38.25±11.50
30-35 (3)	60	20.8	18.60±3.14	12.12±3.16	12.76±3.47	7.12±2.90	50.60±8.14	18.16±5.75	40.20±13.28
36-41 (4)	25	8.7	F=3.993/**p= .008**	F=1.121/p= .341	F=0.492/p= .688	F=0.796/p= .497	F=1.684/p= .171	F=2.694/**p= .046**	F=1.059/p=
p*			**Difference 1,2,4>3**					**Difference 3>1,2,4**	.367
Family type			17.32± 3.13	11.30±2.78	12.44±3.24	6.33±2.42	47.41±7.62	18.59±4.92	40.06±10.70
Nuclear family	249	86.5	18.89±3.34	11.69±2.68	13.07±3.92	7.71±3.04	51.38±8.28	17.35±4.27	34.15±10.84
Extended family	39	13.5	**p= .004**	p= .418	p= .277	**p= .002**	**p= .003**	p= .138	**p= .002**
p**									
Employment status			16.64±2.88	11.71±2.49	13.03±3.53	6.50±2.36	47.89±7.10	19.05±5.76	37.89±12.02
Employed	56	19.4	17.75±3.24	11.27±2.83	12.41±3.29	6.53±2.60	47.96±7.99	18.28±4,60	39.59±10.60
Unemployed	232	80.6	**p= .020**	p= .284	p= .212	p= .937	p= .947	p= .353	p= .293
p**									
Educational status			17.68±3.28	10.96±3.06	11.61±3.72	6.81±2.48	47.08±8.05	17.73±4.66	40.15±12.23
Primary School (1)	60	20.8	17.95±3.35	11.51±2.70	12.79±3.42	6.69±2.76	48.95±8.23	18.46±4.86	39.14±10.21
Secondary/High School (2)	146	50.7	16.68±2.70	11.36±2.66	12.74±2.77	6.01±2.15	40.80±6.66	18.85±4.96	38.84±11.12
University and above (3)	82	28.5	F=4.332/**p= .014**	F=0.828/p= .438	F=2.898/p= .057	F=2.368/p= .096	F=2.489/p= .085	F=0.939/p= .392	F=0.268/p=
p*			**Difference 2>3,1**						.765
Spousal educational status						6.81±2.83			
Primary School (1)			17.75± 3.07	11.08±2.71	11.90±3.41	6.74±2.59	47.55±7.73	17.63±4.18	41.28±10.48
Secondary/High School (2)	60	20.8	17.91±3.18	11.47±2.83	12.70±3.43	5.78±2.09	48.82±7.80	18.16±4.93	39.00±11.16
University and above (3)	157	54.5	16.53±3.19	11.33±2.68	12.70±3.04	F=3.990/**p= .020**	46.36±7.75	19.69±5.01	38.15±10.52
p*	71	24.7	F=4.786/**p= .009**	F=0.426/p= .653	F=1.368/p= .256	**Difference**	F=2.552/p= .080	F=3.497/**p= .032**	F=1.450/p=
			Difference 2>3,1			**1,2>3**		**Difference 3>2,1**	.236
Income level			17.40±4.32	11.02±3.12	11.48±3.36	6.84±3.20	46.74±8.80	17.62±5.22	39.48±11.97
Less than expenses (1)	50	17.4	17.62±2.86	11.44±2.73	12.54±2.99	6.38±2.37	48.00±7.52	18.44±4.46	39.47±10.50
Equal to expenses (2)	206	71.5	17.18±3.31	11.31±2.41	14.12±4.68	6.90±2.54	49.53±8.02	19.62±6.35	37.62±11.77
Higher than expenses (3)	32	11.1	F=0.314/p= .731	F=0.480/p= .619	F=6.328/**p= .002**	F=1.028/p= .359	F=1.260/p= .285	F= 1.676/p= .189	F=0.408/p=
p**					**Difference 3>2,1**				.666

**One-Way ANOVA, **Independent-Samples t-Test, p<0.05. Note: p-values indicating significant differences are shown in bold.*

Table 3. *Some obstetric characteristics of participants and comparison of SSI, LOT and CAQ scores based on these characteristics*

Characteristics	n	%	Understanding self subscale Mean±SD	Human values subscale Mean±SD	Compassion subscale Mean±SD	Conscience subscale Mean±SD	SSI Mean ±SD	LOT Mean±SD	CAQ Mean±SD
Parity									
Primiparous	123	42.7	17.43±3.01	11.85±2.56	12.75±3.09	6.36±2.48	48.40±7.38	18.36±4.44	40.03±11.26
Multiparous	165	57.3	17.61±3.35	10.98±2.86	12.36±3.52	6.64±2.61	47.61±8.13	118.47±5.14	38.69±10.60
p**			p= .625	**p= .008**	p= .333	p= .365	p= .398	p= .845	p= .304
Current pregnancy									
Planned	251	81.2	17.51±3.10	11.17±2.70	12.49±3.22	6.42±2.51	47.60 ±7.41	18.62±4.73	38.91±10.89
Unplanned	37	12.8	17.70±3.87	12.62±2.89	12.81±4.08	7.21±7.76	50.35±9.92	17.08±5.46	41.67±10.71
p**			p= .778	**p= .003**	p= .654	p= .078	p= .113	p= .070	p= .151
Attends regular prenatal follow-ups	244	84.7	17.36±3.07	11.33±2.75	12.43±3.40	6.43±2.53	47.56±7.52	18.42±4.80	39.06±10.99
Yes	44	15.3	18.47±3.74	11.50±2.90	13.06±2.97	7.04±2.64	50.09±9.09	18.47±5.18	40.38±10.33
No			**p= .035**	p= .712	p= .211	p= .143	p= .088	p= .948	p= .460
p**									
Preference of mode of delivery									
C-section	73	25.3	17.68±3.39	11.24±3.02	12.32±3.84	6.38±2.39	47.64±8.60	18.38±4.92	40.86±10.85
Vaginal delivery	215	74.7	17.48±3.14	11.39±2.68	12.60±3.16	6.57±2.61	48.06±7.55	18.44±4.83	38.72±10.87
p**			p= .652	p= .693	p= .582	p= .587	p= .695	p= .924	p= .148

**One-Way ANOVA, **Independent-Samples t-Test, p<0.05. Note: p-values indicating significant differences are shown in bold.*
SSI: Scale for Spiritual Intelligence, LOT: Life Orientation Test, and CAQ: Childbirth Attitudes Questionnaire

Table 4. *Evaluation of factors affecting SSI, LOT, and CAQ scale scores by multiple linear regression-analysis*

	B	t	p	95% CI	
				Lower	Upper
Model 1: The effects of spiritual intelligence and life orientation on CAQ scores					
SSI Total Score	0.049	0.820	.413	-0.095	0.230
LOT Total Score	-0.202	-3.416	**.001**	-0.716	-0.192
R=.218 R^2=.047 Durbin-Watson=1.809 (p= .001)					
Model 2: The effect of family type on CAQ scores					
Family Type	-.186	-3.204	**.002**	-9.548	-2.281
R=.186 R^2=.035 Durbin-Watson=1.913 (p= .002)					
Model 3: The effects of age and spousal education status on LOT scores					
Age	0.091	1.716	.087	-0.013	0.196
Spousal Education Status	1.123	2.665	**.008**	0.293	1.952
R=.177 R^2=.031 Durbin-Watson=1.666 (p= .011)					
Model 4: Some sociodemographic and obstetric variables on SSI scores					
Age	0.77	0.554	.580	-0.196	0.349
Family Type	-7.879	-3.958	**.000**	-11.798	-3.960
Educational Status	-0.856	-0.717	.474	-3.207	1.495
Spousal Education Status	-1.017	-0.855	.393	-3.359	1.325
Employment Status	1.777	0.946	.345	-1.921	5.474
Social Security Status	2.774	1.596	.112	-0.648	6.196
Income Level	0.063	0.051	.960	-2.391	2.518
Parity	-2.759	-1.857	.064	-5.685	0.167
Pregnancy Planning Status	2.961	1.488	.138	-0.957	6.880
Attending Regular Prenatal Follow-Ups	2.012	1.077	.282	-1.666	5.960
R=.299 R^2=.050 Durbin-Watson=1.896 (p= .010)					

B: regression coefficient, t: test statistic, p: statistical significance,
Note: p-values indicating significant differences are shown in bold.
SSI: Scale for Spiritual Intelligence, LOT: Life Orientation Test, and CAQ: Childbirth Attitudes Questionnaire

4. DISCUSSION

The results obtained in this study provide information about the effects of prenatal optimism and spiritual intelligence on childbirth attitudes in pregnant women.

Eliminating inequality in health-related issues for pregnant women, understanding their beliefs and attitudes regarding the childbirth process, and increasing their access to quality childbirth services have been the focus of international maternal health policies. Besides, to optimize a woman's childbirth experience and outcomes, it is considered important to evaluate her psychological and psychosocial aspects in addition to the physiology of pregnancy and childbirth (19). In this study, it was determined that as the optimism levels of the participants increased, their anxieties and fears regarding childbirth decreased. In the relevant literature, some studies have shown that optimism and fear of childbirth are negatively and significantly correlated (20,21). Optimism helps individuals believe in their skills and make positive inferences from society and their environment, and thus, it enables them to encounter more positive outcomes (22). This situation shows that optimists have a higher chance of adopting active coping strategies and reevaluating a situation positively when a significant target for them is obstructed. Moyer reported that in comparison to pessimistic pregnant women, pregnant women who had higher levels of optimism during labor could be relieved more easily, and as a result, the probability of non-progressing labor could be reduced (4). In another study, it was stated that optimism in pregnancy was related to a better quality of life (23). The results of this study were consistent with those in the literature. These results show that an emotional state such as optimism can improve psychological and physical well-being and strengthen the individual's capacity for overcoming unexpected situations.

Spiritual intelligence refers to a series of skills, capacities, and spiritual resources that can enhance individual adaptation in daily life (7). Individuals with higher spiritual intelligence levels have a holistic attitude towards the difficulties of life, and they are more flexible and self-conscious individuals (24). However, the findings obtained in this study showed that spiritual intelligence did not affect the childbirth attitudes of the participants significantly. In the study conducted by Hatami et al., which revealed similar results to those in this study, the relationship between spiritual intelligence and childbirth attitudes was not found to be statistically significant (7). Likewise, in the study carried out by Mokhtari et al., no significant relationship was found between fertility factors and spiritual intelligence and resilience (25). On the other hand, Mohammadi Rizi et al. found a negative and significant relationship between spiritual intelligence and childbirth attitudes in pregnant women (26). Similarly, in another study, a statistically significant relationship was reported between spiritual intelligence and fear of childbirth in pregnant

women, and mothers with no fear of childbirth had higher levels of spiritual intelligence (24). The results of this study differed from those obtained in the relevant literature. It is thought that this difference may have stemmed from the fact that all participants in this study were predominantly Muslims, and therefore, they may have similar endurance, tolerance, and mental skills.

4.1. Limitations

This study was conducted at a single center, so the results cannot be generalized to all pregnant women. The results of this study should only be used to inform the practices in this province. However, it is thought that this study, which is considered noteworthy in terms of the effects of affirmations in the prenatal period on childbirth-related attitudes, will contribute to the literature.

5. CONCLUSION

The findings of this study showed that an increase in the optimism levels of pregnant women is an effective factor in reducing their fears of childbirth. However, it was also determined that the spiritual intelligence levels of the participants did not affect their fears of childbirth.

Childbirth is a stressful event that requires mental adjustment. Pregnant women who experience anxiety and fear of childbirth need relatively more support than other pregnant women. It is important that when healthcare professionals encounter women who experience the fear of childbirth during pregnancy, they approach them from an individualized and biopsychosocial perspective. Optimism is one of the psychological determinants necessary for the formation of positive childbirth attitudes. For this reason, interventions aimed at providing accurate information about childbirth, increasing the optimism levels of individuals, reducing their stress, and eliminating their anxiety will contribute to the process of developing childbirth-related coping strategies and increasing positive childbirth outcomes among pregnant women.

Optimism is a potentially promising variable that could help protect women from the development of fear of childbirth in an easily accessible, acceptable, and effective way. In this context, it is believed that increasing optimism levels in pregnant women will contribute to their experience of a positive labor process.

Acknowledgements: *The authors thank all women who volunteered to participate in the study and sincerely shared their views.*
Funding: *This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.*
Conflicts of interest: *The authors declare that there is no conflict of interests.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Non-Interventional Clinical Research Ethics Committee of Selçuk University, Faculty of Health Sciences (Approval date: 27.01.2022; Number: 2022/58)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: HBO
Design of the study: HBO, ANA
Acquisition of data for the study: HBO, ANA
Analysis of data for the study: HBO, ANA
Interpretation of data for the study: ANA
Drafting the manuscript: HBO, ANA
Revising it critically for important intellectual content: HBO
Final approval of the version to be published: HBO, ANA

REFERENCES

[1] Mohamadirizi S, Mohamadirizi S, Khani B. Prenatal optimism and its relationship with fetal and maternal characteristics in primiparous women. Int J Pediatr. 2015; 3(5):897– 901.

[2] Khodakarami B, Golalizadeh BF, Soltani F, Soltanian A, Mohagheghi H. Prognostic role of spiritual intelligence components in pregnant women's depression, anxiety, and stress. Health Spiritual Med Ethics. 2016;3(2):16-23.

[3] Rauch SA, Defever E, Oetting S, Graham-Bermann SA, Seng JS. Optimism, coping, and posttraumatic stress severity in women in the childbearing year. Psychol Trauma. 2013;5(1):77-83. https://doi.org/10.1037/a0022214

[4] Moyer CA, Elsayed Y, Zhu Y, Wei Y, Engmann CM, Yang H. Is generalized maternal optimism or pessimism during pregnancy associated with unplanned cesarean section deliveries in China? J. Pregnancy. 2010(1):1-10. https://doi.org/10.1155/2010/754938

[5] Lobel M, DeVincent CJ, Kaminer A, Meyer BA. The impact of prenatal maternal stress and optimistic disposition on birth outcomes in medically high-risk women. Health Psychology 2000;19(6):544-553. https://doi.org/10.1037/0278-6133.19.6.544

[6] Khodakarami B, Golalizadeh BF, Soltani F, Soltanian A, Mohagheghi H. Impact of a counseling program on depression, anxiety, stress, and spiritual intelligence in pregnant women. Journal of Midwifery and Reproductive Health 2017;5(2):858-866. https://doi.org/10.22038/jmrh.2016.7755

[7] Hatami A, Badrani MR, Kamboo MS, Jahangirimehr A, Hemmatipour A. An investigation of the relationship of spiritual intelligence and resilience with attitude to fear of childbirth in pregnant women. J Evolution Med Dent Sci. 2019;8(1):24-28. https://doi.org/10.14260/jemds/2019/6

[8] Zolfaghary F, Osko S, Bakouei F, Pasha H, Adib-Rad H. Spiritual ıntelligence as a coping strategy to manage job stress for midwives in northern Iran: A cross-sectional study. J Relig Health. 2023;62(5):3301–3312. https://doi.org/10.1007/s10943-023-01863-y

[9] Lysne CJ, Wachholtz AB. Pain, spirituality, and meaning making: What can we learn from the literature? Religions 2011;2(1):1-16. https://doi.org/10.3390/rel2010001

[10] Aksoy AN. Fear of childbirth: review of the literature. ODU Journal of Medicine. 2015; 2(3):161-165. (Turkish)

[11] Dönmez S, Kısa S, Özberk H. Investigation of fear of childbirth, attitude and state anxiety among primigravid women. Ethno Med. 2016;10(4):488-497. https://doi.org/10.1080/09735070.2016.11905522

[12] Dönmez S. Hande D, Çelik N, Yeniel ÖA, Kavlak O. Turkish version of the childbirth attitudes questionnaire. Turkiye Klinikleri J Gynecol Obst. 2014;24(4):212-218. (Turkish)

[13] Aydın G, Tezer E. The relationship of optimism to health complaints and academic performance. Journal of Psychology 1991; 26 (7):2-9.

[14] Tekin ÖE, Ekşi H. Adapting the scale for spiritual intelligence to Turkish. Spiritual Psychology and Counseling 2019;4(2):123-141. https://doi.org/10.12738/spc.2019.4.2.0062

[15] Lowe NK. Self-efficacy for labor and childbirth fears in nulliparous pregnant women. J Psychosom Obstet Gynaecol. 2000;21(4):219-224. https://doi.org/10.3109/01674820009085591

[16] Scheier ME, Carver CS. Dispositional optimism and physical well-being: The influence of generalized outcome expectancies on health. J Pers. 1987;55(2):169-210. https://doi.org/10.1111/j.1467-6494.1987.tb00434.x

[17] Kumar V, Mehta M. Gaining adaptive orientation through spiritual and emotional intelligence. Chauhan In AK, Nathawat SS editors. New Facets of Positivism. Delhi, India: Macmillan Publishers. 2011:281-301.

[18] Tabachnick BG, Fidell LS. Using Multivariate Statistics. 5th ed. Boston: Pearson; 2013.

[19] Haines HM, Rubertsson C, Pallant JF, Hildingsson I. The influence of women's fear, attitudes and beliefs of childbirth on mode and experience of birth. BMC Pregnancy Childbirth. 2012;12(1):55-69. https://doi.org/10.1186/1471-2393-12-55

[20] Gourounti K, Kouklaki E, Lykeridou K. Validation of the Childbirth attitudes questionnaire in greek and psychosocial characteristics of pregnant women with fear of childbirth. Women Birth. 2015;28(3):44-51. https://doi.org/10.1016/j.wombi.2015.02.004

[21] Goutaudier N, Bertoli C, Séjourné N, Chabrol H. Childbirth as a forthcoming traumatic event: Pretraumatic stress disorder during pregnancy and its psychological correlates. J Reprod Infant Psychol. 2019;37(1):44-55. https://doi.org/10.1080/02646838.2018.1504284

[22] Abdos M, Aghili M, Sanagoo A, Kavosi A. Investigating effectiveness of happiness training on psychological well-being and optimism of pregnant women with a history of spousal abuse. JCBR. 2018;2(4):33-40. https://doi.org/10.29252/jcbr.2.4.33

[23] Lagadec N, Steinecker M, Kapassi A, Magnier AM, Chastang J, Robert S, Nadia G, Ibanez G. Factors influencing the quality of life of pregnant women: A systematic review. BMC Pregnancy Childbirth. 2018;18(1):1-14. https://doi.org/10.1186/s12884-018-2087-4

[24] Abdollahpour S, Khosravi A. Relationship between spiritual intelligence with happiness and fear of childbirth in Iranian pregnant women. Iranian J Nursing Midwifery Res. 2018;23(1):45-50. https://doi.org/10.4103/ijnmr.IJNMR_39_16

[25] Mokhtari F, Torabi F, Pirhadi M. Relationship between fertility characteristics with spiritual intelligence and resilience in infertile couples. J Educ Health Promot. 2022;11(44):1-7. https://doi.org/10.4103/jehp.jehp_97_21

[26] Mohammadi RS, Mohebi DZ, Torabi F, Mohamadirizi S. The relationship between spiritual intelligence and fear of childbirth in low–risk pregnant women. J Babol Univ Med Sci. 2017;19(9):26-31. https://doi.org/10.22088/jbums.19.9.26

How to cite this article: Özçalık BH, Ataş NA. The Effects of Prenatal Optimism and Spiritual Intelligence on Childbirth Attitudes. Clin Exp Health Sci 2025; 15: 35-41. https://doi.org/10.33808/clinexphealthsci.1362979

Clinical and Experimental Health Sciences

Comparison of the cVEMP and oVEMP Responses with Different Stimuli in Healthy Individuals

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Received: October 24, 2023 **Accepted:** March 5,2025

ABSTRACT

Objective: Vestibular evoked myogenic potentials (VEMP) are electromyographic responses induced by auditory, tactile, or electrical stimulation. Electrode placement, intensity, and the type of stimulus applied to influence the amplitude and latency of VEMP responses. The study aims to investigate the effect of differences in stimulus intensities and stimulus types on VEMP results.

Methods: Twenty participants (40 ears) between the ages of 18 and 30 (22.7±1.8) took part in the study. Results from the cervical VEMP (cVEMP) and ocular VEMP (oVEMP) tests were examined using six different characterized stimuli (click, LS CE Chirp, 500 Hz – 1000 Hz Tone Burst, and 500 Hz – 1000 Hz LS CE Chirp) at intensities of 100 dB nHL, 90 dB nHL, and 80 dB nHL.

Results: In cVEMP and oVEMP testing, there was no significant difference between the amplitudes of 500 Hz tone burst (TB) and 500 Hz LS CE chirp stimuli; however, the p1 and n1 latencies of chirp stimuli were found to be significantly shorter. There was no significant difference between p1-n1 latency and the asymmetry ratio of frequency-specific stimuli. No difference was seen between click and chirp stimuli in any of the assessments.

Conclusion: The chirp stimulus is an effective alternative for TB. It is encouraged that each clinic develops its normative data because of the differences in recording parameters.

Keyword: S CE Chirp, narrow band, vestibular evoked myogenic potentials, frequency-specific, otolith organs

1. INTRODUCTION

Vestibular-evoked myogenic potentials (VEMP) are electromyographic responses in which otolith organs are triggered by auditory, vibrotactile, or electrical stimulation[1]. Ocular vestibular evoked potential (oVEMP) is a short-latency response that reflects vestibuloocular reflex projection to the inferior oblique muscle. Cervical vestibular evoked myogenic potential (cVEMP) reveals the inhibition and excitability of the sternocleidomastoid (SCM) muscle because of the vestibulocollic reflex. The cVEMP test assesses the saccule and inferior vestibular nerve integrity, while the oVEMP test assesses the utricle and superior vestibular nerve and their central projections[2]. The VEMP test is important for the diagnosis of disorders such as Meniere's disease, endolymphatic hydrops, vestibular schwannoma, superior semicircular canal dehiscence, and vestibular neuronitis [3-6]. The VEMP tests have also gained popularity in neurology and neurosurgery patients because they provide important information about the location of pathology[7-11].

To measure VEMP responses, high-intensity audio stimuli (90–110 dB nHL) at rates between 3-6 Hz are often presented as either monoaural or binaural through headphones.

Different types of acoustic stimuli are used in VEMP response recording. Studies indicate that click and tone burst (TB) stimuli can produce VEMP responses [12] . The TB stimulus is frequently chosen in clinics because otolith neurons are particularly active at low-frequency region [13]. The chirp stimulus, in addition to the click and TB stimuli, has attracted interest since it is relatively new [14, 15]. The Level Specific Claus Elberling Chirp (LS CE Chirp) was produced after the chirp stimulus was developed considering the tonotopic feature of the cochlea. However, since low-frequency energy is sent first in the time domain, it is thought that it may also

https://doi.org/10.33808/clinexphealthsci.1367956

be effective in stimulating otolith organs. Studies in which the effects of stimuli are evaluated at a single intensity support this hypothesis [15].

The amplitude and latency of VEMP responses are influenced by factors that depend on the individual and recording parameters, such as electrode location, the intensity and type of stimulus used, and the individual's age [12]. For this reason, each clinic should establish its guidelines and determine its normative data. Disclosing the differences in findings based on the stimuli applied can assist clinicians in the diagnosis by helping them in choosing the appropriate parameters for the test and evaluating the results. Our study aims to investigate the amplitude and latency difference between click, TB, and LS CE Chirp stimuli at various frequencies and intensities in cVEMP and oVEMP tests.

2. METHODS

The study was conducted in the Audiology Skills and Practice Laboratory of the Bezm-i Alem Vakıf University and was approved by the decision numbered 71306642-050.01.04 of the Bezm-i Alem Vakıf University Non-Invasive Ethics Committee. In the study, 20 participants between the ages of 18 and 30 were involved. Each participant underwent cVEMP and oVEMP testing who has bilateral Type A tympanograms, hearing thresholds of 20 dB HL or better between 0.25 and 8 kHz in a conventional pure-tone audiometry test, no neurological or metabolic diseases, and no complaints or history of dizziness or vertigo. Additionally, to rule out subclinical peripheral or central vestibular pathology, spontaneous nystagmus and gaze-evoked nystagmus were evaluated using videonystagmography (VNG module; Interacoustics A/S, Denmark). The VNG, Fukuda, and Romberg tests were within normal limits.

The Interacoustics Eclipse EP25 (Interacoustics A/S, Denmark) was used to record VEMPs after the patients' skin had been cleaned with Nuprep gel. For the cVEMP test, a ground electrode was placed on the forehead, active electrodes were placed on the middle of the SCM muscle, and the reference electrode was positioned at the sternal notch. The participants were positioned in a sitting position, and when the stimulus was given, the ipsilateral SCM muscle was contracted by turning their heads to the contralateral direction of the stimulus. In the oVEMP test, the active electrodes were placed on the inferior oblique muscles, the reference electrodes were placed under the active electrodes, and the ground electrode was placed on the forehead. Participants were asked to look 30° upwards in a sitting position, and stimuli were given monaurally to the right and left ears and recorded from the contralateral electrodes. Table 1 displays the variables used in cVEMP and oVEMP recordings. The response's repeatability was examined using a double-trace recording. By integrating two traces into one wave component, the analyses of the p1-n1 amplitude, p1 latency, n1 latency, p1-n1 latency, and asymmetry ratio were carried out by experienced audiologists.

Click, LS CE Chirp, NB CE Chirps (500 Hz and 1000 Hz), and TB (500 Hz and 1000 Hz) were used to elicit cVEMP with different intensities (100 dBnHL, 90 dBnHL, and 80 dBnHL) and oVEMPs with 100dBnHL intensity. In the case of stimulus and left-right ear tests, the tests were interrupted for 15 minutes to prevent muscle fatigue. Test parameters for oVEMP and cVEMP had been shown in Table 1.

Table 1. *cVEMP and oVEMP test recording parameters. µV: Microvolt, CE: Claus Elberling, dB: Decibel, EMG: Electromyography, Hz: Hertz, LS: Level specific, ms: Millisecond, nHL: normal hearing level, TB: Tone burst*

	cVEMP				oVEMP			
	Click	LS CE Chirp	TB 500-1000 Hz	LS CE Chirp 500-1000 Hz	Click	LS Chirp	TB 500-1000 Hz	LS CE Chirp 500-1000 Hz
Rate	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
EMG Activity	49.9-150.6 µV	49.9-150.6 µV	49.9-150.6 µV	49.9-150.6 µV	25.6-70.0 µV	25.6-70.0 µV	25.6-70.0 µV	25.6-70.0 µV
Polarity	Rarefaction	Rarefaction	Rarefaction	Rarefaction	Rarefaction	Rarefaction	Rarefaction	Rarefaction
Intensity	100-90-80 dB nHL	100-90-80 dB nHL	100-90-80 dB nHL	100-90-80 dB nHL	100 dB nHL	100 dB nHL	100 dB nHL	100 dB nHL
Time Window	80 ms	80 ms	80 ms	80 ms	80 ms	80 ms	80 ms	80 ms
Sweep	150-200	150-200	150-200	150-200	150-200	150-200	150-200	150-200

The data were analyzed using the IBM SPSS 25.0 software (IBM, Ehningen, Germany) and reported as mean ± standard deviation (SD). The Kolmogorov-Smirnov test and the Q-Q plots were used to assess normality. The Wilcoxon signed-rank test was used to compare right and left ears. The nonparametric Kruskal-Wallis test and post hoc analysis with Bonferroni correction were used to compare the variables. All analyses were carried out using a 95% confidence interval, and a result of $p<0.05$ was accepted as statistically significant.

3. RESULTS

Twenty participants (40 ears), 10 males and 10 females, between the ages of 18 and 30 (22.7 ± 1.8), took part in the study. The results of cVEMPs were studied using stimulus intensities of 100 dB nHL, 90 dB nHL, and 80 dB nHL through 6 different stimuli, and oVEMPs were studied at 100 dBnHL intensity with 6 different stimuli. Data from 40 ears were pooled because there was no significant difference between ears.

3.1. cVEMP

The p1-n1 amplitude, p1 latency, n1 latency, p1-n1 latency, and asymmetry rate values with different stimuli at 100 dB nHL were listed in Table 2. and Figure 1 shows statistically different values of the p1-n1 amplitude, p1 latency, n1 latency for different stimuli. The P1-N1 amplitude was not statistically different between click and chirp stimuli (p>.05). These stimuli were compared to frequency-specific stimuli, amplitudes were significantly higher in both TB and NB LS CE Chirp stimuli only at 500 Hz (p<.001).

The p1 latency of the 500 Hz TB stimulus at 100 dB nHL intensity was the longest and only 1000 Hz TB stimuli latencies did not show a significant difference. When the n1 latencies were examined, the longest TB latency was obtained at 500 Hz The absolute p1 and n1 wave latencies of the click and LS CE Chirp stimuli did not differ significantly (p>.05).

The response rates of different stimuli, p1-n1 amplitude values, p1 latency, n1 latency, p1-n1 latency, and asymmetry rates of the cVEMP test at 90 dB nHL are shown in Table 3. The largest amplitude value was obtained at 500 Hz LS CE Chirp when the p1-n1 amplitude of the cVEMP responses at 90 dB nHL was compared, although the difference was significant only at 1000 Hz LS CE Chirp (p=.001), 1000 Hz TB (p<.001), click (p=.012), and LS CE Chirp (p<.001). No difference was observed between click and LS CE Chirp stimuli (p>.05). There were significant differences in p1 latencies between 500 Hz LS CE Chirp and 500 Hz TB (p<0.001), 500 Hz LS CE Chirp and 1000 Hz TB (p=.003), 1000 Hz LS CE Chirp and 1000 Hz TB (p=0.021), 1000 Hz LS CE Chirp and 500 Hz TB (p<.001), click and 500 Hz TB (p=.006), LS CE Chirp and 500 Hz TB (p=.009). N1 latencies significantly differ between 500 Hz LS CE Chirp and 500 Hz TB (p<.001), 500 Hz LS CE Chirp and 1000 Hz TB (p=.003), 1000 Hz LS CE Chirp and 1000 Hz TB (p=.007), 1000 Hz LS CE Chirp and 500 Hz TB (p<.001), click and 500 Hz TB (p=.004), LS CE Chirp and 500 Hz TB (p<.001).

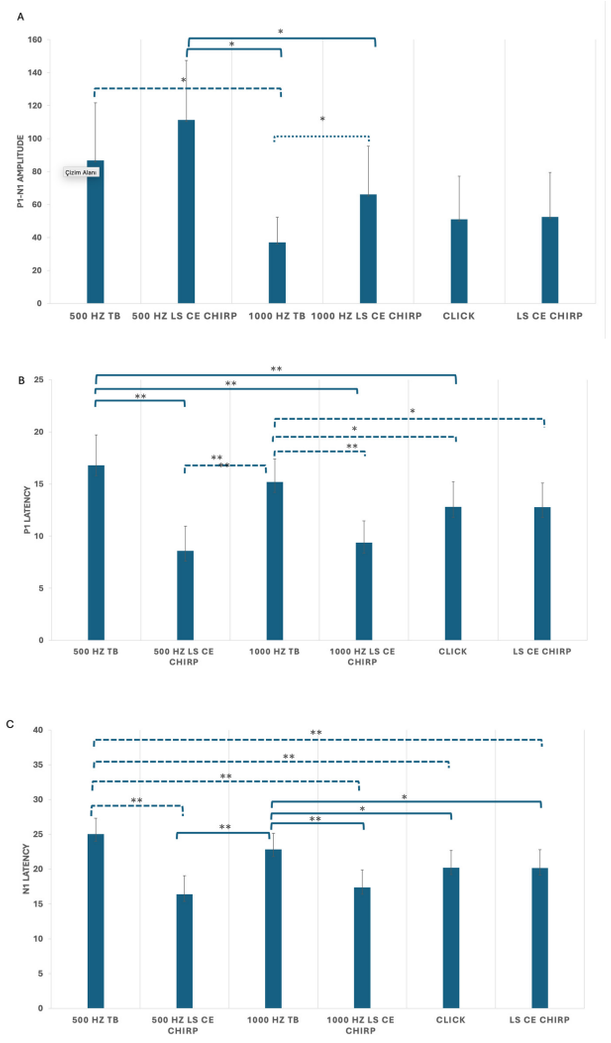

Figure 1. *cVEMP 100 dB nHL responses comparisons for different types of stimuli. A. P1-N1 Amplitudes. B P1 latencies. C. N1 Latencies. **: p<0.001, *: p<0.001µV: Mikrovolt, CE: Claus Elberling, Hz: Hertz, LS: Level specific, ms: Millisecond, nHL: normal hearing level, TB: Tone burst.*

Table 2 *100 dB nHL responses for the cVEMP. µV: Mikrovolt, CE: Claus Elberling, Hz: Hertz, LS: Level specific, ms: Millisecond, nHL: normal hearing level, TB: Tone burst*

	Vemp Responses(%)	P1-N1 Amplitude (µV)	P1 Latency (ms)	N1 Latency (ms)	P1-N1 Latency (ms)	Asymmetry Ratio (%)
500 Hz TB	40(100%)	86,77 ± 34,91	16,80±2,09	25,03±2,29	8,30±1,35	18±09
500 Hz LS CE Chirp	40(100%)	111,26 ± 36,08	8,60±2,36	16,38±2,70	8,17±1,76	15±10
1000 Hz TB	40(100%)	37,08 ± 15,26	15,20±2,21	22,84±2,32	7,65±1,81	16±11
1000 Hz LS CE Chirp	40(100%)	66,17 ±29,26	9,37±2,10	17,39±2,52	7,95±1,70	17±12
Click	40(100%)	50,96 ±26,27	12,80±2,42	20,21±2,47	7,42±1,88	17±11
LS CE Chirp	40(100%)	52,47 ± 26,91	12,78±2,31	20,16±2,64	7,36±2,09	19±13

Table 3. *90 dB nHL responses for the cVEMP. µV: Microvolt, CE: Claus Elberling, Hz: Hertz, LS: Level specific, ms: Millisecond, nHL: normal hearing level, TB: Tone burst*

	Vemp Response (%)	P1-N1 Amplitude (µV)	P1 Latency (ms)	N1 Latency (ms)	P1-N1 Latency (ms)	Asymmetry Ratio (%)
500 Hz TB	39(97,5%)	39,82 ± 22,79	18,02±2,52	25,53±2,48	7,82±2,58	19±11
500 Hz LS CE Chirp	40(100%)	49,55 ±30,80	13,61±2,43	20,88±3,63	7,59±2,21	19±10
1000 Hz TB	34(85%)	14,28 ± 6,69	16,15±4,06	23,61±5,02	7,46±2,94	16±15
1000 Hz LS CE Chirp	33(82,5%)	24,24 ± 12,65	13,84±2,77	21,02±3,08	7,17±2,21	19±17
Click	26(65%)	25,89 ±12,56	15,25±3,09	22,76±4,02	7,51±2,50	18±12
LS CE Chirp	28(70%)	19,57 ± 9,11	15,02±4,49	21,27±5,32	6,33±2,82	13±13

When p1-n1 latency values were compared, no significant difference was found for stimuli at 90 dB nHL and 100 dB nHL intensity levels (p>.05). There was no significant difference between the asymmetry ratios (p>.05).

Due to statistically insufficient data, responses to all stimuli with the intensity of 80 dB nHL in the cVEMP test could not be analyzed. However, the stimulus with the highest response at 80 dB nHL intensity was a 500 Hz narrow band (NB) LS CE Chirp observed in 16 ears (40%).

3.2. oVEMP

The response values of the oVEMP test at different stimuli at 100 dB nHL are given in Table 4 and Figure 2 shows the statiscally significant dişfferences of P1-N1 amplitudes, p1 an n1 latencies. The highest p1 amplitude was recorded at 500 Hz LS CE Chirp. The longest p1 and n1 latencies were recorded at 500 Hz TB; There was no significant difference between the click and LS CE Chirp stimuli (p>.05).

No significant difference was observed between p1-n1 latency values and the asymmetry ratio between stimuli results (p>.05).

Table 4. *100 dB nHL responses for the oVEMP. µV: Microvolt, CE: Claus Elberling, Hz: Hertz, LS: Level specific, ms: Millisecond, nHL: normal hearing level, TB: Tone burst*

	Vemp Responses (%)	P1-N1 Amplitude (µV)	P1 Latency (ms)	N1 Latency (ms)	N1-P1 Latency (ms)	Asymmetry Ratio (%)
500 Hz TB	40(100%)	6,76 ± 5,72	17,28 ±1,53	11,99 ± 1,34	5,59 ± 1,10	13 ± 11
500 Hz LS CE Chirp	40(100%)	10,77 ±8,23	9,26 ± 2,43	4,10 ± 2,32	5,40 ± 1,20	14 ± 10
1000 Hz TB	40(100%)	5,89 ± 3,62	16,02 ± 0,84	10,71 ± 0,88	5,30 ± 1,00	18 ± 12
1000 Hz LS CE Chirp	40(100%)	6,17 ± 4,88	10,51 ± 0,80	5,25 ± 1,25	4,59 ± 2,57	20 ± 14
Click	40(100%)	4,15 ± 3,58	13,64 ± 1,43	8,00 ± 1,26	5,58 ± 1,09	21 ± 16
LS CE Chirp	40(100%)	3,75 ± 2,91	13,54 ±1,40	8,37 ± 1,31	5,19 ± 1,32	21 ± 19

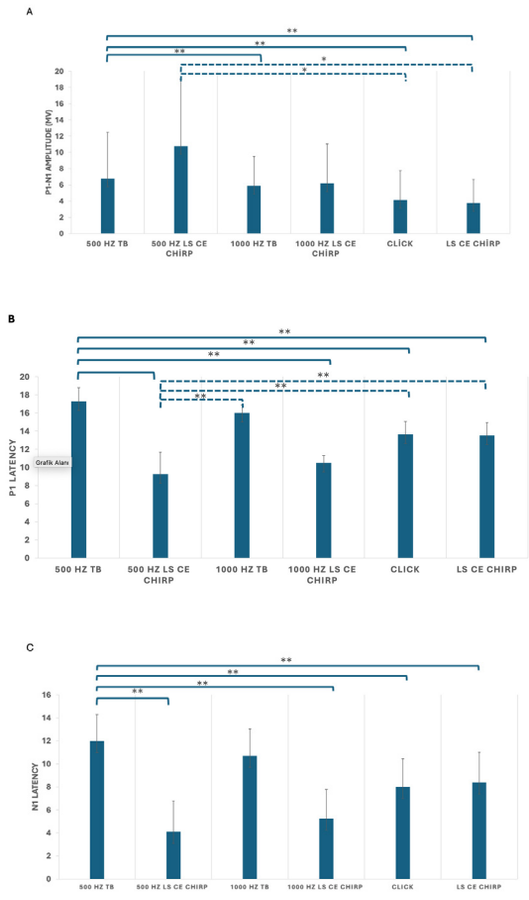

Figure 2. *oVEMP 100 dB nHL responses comparisons for different types of stimuli. A. P1-N1 Amplitudes. B P1 latencies. C. N1 Latencies. **: p<0.001, *: p<0.001µV: Mikrovolt, CE: Claus Elberling, Hz: Hertz, LS: Level specific, ms: Millisecond, nHL: normal hearing level, TB: Tone burst.*

4. DISCUSSION

Results of VEMPs are influenced by recording parameters such as electrode location, stimulus type, intensity level, polarity, and frequency, as well as patient-related features including age and gender. Selection of the stimulus is an essential component of the VEMP test since it affects the response's latency and amplitude values [14]. Although studies have been conducted to investigate the differences between the stimuli utilized in the literature, there is no consensus on the chirp stimulus type and normative values [15].

The 500 Hz TB stimulus is often used in clinics since it activates the otoliths [13] and produces the biggest amplitude VEMP amplitudes [16]. However, the shortest latencies and the largest amplitudes were obtained in the 500 Hz LS CE Chirp stimulus compared with others. Similar results were obtained in different studies using NB Chirp stimuli [14, 15, 17]. It has been hypothesized that the use of chirp stimuli results in more efficient activation of the macula with enhanced synchronization, which accounts for the short delay [15]. Another hypothesis is that since the developers of the NB CE Chirp stimulus set its onset time earlier than in TB, shorter wave latencies are obtained in chirp [18]. In our study, the reason that the p1 and n1 wave latencies in the cVEMP and oVEMP tests were shorter than all other stimuli in the 500 Hz and 1000 Hz LS CE Chirp stimuli is assumed to be linked to the difference in the onset time of the stimuli.

The NB LS CE Chirp stimulus had a larger p1-n1 amplitude at 500 Hz than the TB stimulus in the cVEMP and oVEMP tests, but this difference was not statistically significant (p>.05). Although there is research in the literature that claims there is no difference [19], contrary to our finding, there are studies that claim the TB stimulus amplitude [20]

or NB CE Chirp stimulus amplitude is higher [14, 15, 17]. The chirp stimulus was developed by modeling the tonotopic structure of the cochlea to provide a more synchronized firing by simultaneously delivering the auditory stimulus to low and high frequencies [21]. However, unlike the cochlea, otolith organs do not have a tonotopic organization, thus stimulation influences differently. Although the stimulus used in the VEMP test is auditory, the irregular afferent neurons of the utricle and/or saccule are stimulated [13]. These factors would be the reason for the absence of a statistically significant difference between the NB LS CE Chirp and TB stimuli. It was determined that there was no difference between LS CE Chirp and Click stimuli for the same reason. The findings support the idea that the NB LS CE Chirp stimulus could have a similar impact on eliciting a response as the TB stimulus.

The asymmetry ratio did not differ between stimuli, similar to other studies in the literature [15, 19]. The interaural asymmetry ratio is an important parameter in the presence of unilateral peripheral vestibular pathology [22]. The asymmetry ratio did not differ because individuals without dizziness complaints were included in our study. As a result, the sensitivity and specificity of the various stimuli used to evaluate the asymmetry ratio could not be investigated. In future studies, studying the stimulus differences within the pathologic group will help physicians decide which stimulus to use in the assessment.

In our study, the cVEMP and oVEMP tests both generated 100% response rates for all stimuli at 100 dB nHL intensity. The 500 Hz LS CE Chirp had the highest response rate (100%) at 90 dB nHL intensity. Adult VEMP response rates range from 80% to 100% [23]) and they decline with aging[14, 24]. Although our results are in line with previous research, the difference in 90 dB nHL could be due to some of the energy from the 500 Hz LS CE Chirp stimulus being scattered towards 1000 Hz, increasing the rate of excitation [14]. On the other hand, it should be noted that in our study, the difference between the number of responses observed in recordings with 500 Hz TB and 500 Hz LS CE Chirp stimulus was only one ear. In our study, the lowest response was obtained in the click stimulus (65%), in accordance with the literature [23, 24]. This was attributed to the fact that the VEMP response is most effectively produced at 500 Hz[13], and wideband stimulus is insufficient to stimulate the otolith organs.

The data obtained in our study is expected to be helpful for physicians in terms of stimulus selection and normalization values. Our findings provide clinicians with valuable insights for stimulus selection and establishing normative values . The small sample size is considered one of the limitations of the study. In addition, it is believed that it would be beneficial to investigate the difference between stimuli in the population with unilateral vestibular hypofunction and/or central disease in future studies.

5. CONCLUSIONS

We compared the wave amplitudes, P1 and N1 latencies, and P1-N1 latencies of the cVEMP and oVEMP at different frequency and intensity levels. The results showed that while P1 and N1 latencies were significantly shorter with LS CE Chirp stimuli, which had the highest response rate, there was no significant difference in response amplitude between the commonly used 500 Hz TB stimulus and the novel 500 Hz LS CE Chirp stimulus.

These findings suggest that the NB LS CE Chirp stimulus may be a suitable alternative to the TB stimulus; however, further research is needed, particularly in patients with vestibular disorders. Additionally, since VEMP application procedures may vary across clinics, each clinic should establish its own normative data.

Acknowledgement: *We thank the study participants and control subjects for their contributions to this study. Niyazi O. Arslan and Ziya Aydin are Scholarship Holders of the Ministry of National Education of the Republic of Türkiye.*

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by Ethics Committee of Bezmialem Vakıf University (approval date 27.01.2017. and number 71306642-050.01.04)*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: NB

Design of the study: NB

Acquisition of data for the study: ZA, BDC, NÖA

Analysis of data for the study: NB, HH, ZA, BDC, NÖA, ÖGT

Interpretation of data for the study: HH,

Drafting the manuscript: HH

Revising it critically for important intellectual content: NB, ÖGT

Final approval of the version to be published: NB

REFERENCES

[1] Murofushi T. Clinical application of vestibular evoked myogenic potential (VEMP). Auris Nasus Larynx, 2016; 43(4): 367-376. https://doi.org/10.1016/j.anl.2015.12.006

[2] Rodriguez AI, Marler E, Fitzpatrick D, Creutz T, Cannon SA, Thomas MLA, Janky KL. Optimization of cervical and ocular vestibular evoked myogenic potentialtesting using an impulse hammer in adults, adolescents, and children. Otology & Neurotology, 2020; 41(6):817-827. https://doi.org/10.1097/MAO.0000000000002632

[3] Seo YJ, Kim J, Choi JY, Lee WS. Visualization of endolymphatic hydrops and correlation with audio-vestibular functional testing in patients with definite Meniere's disease. Auris Nasus Larynx, 2013;40(2):167-172. https://doi.org/10.1016/j.anl.2012.07.009

[4] Chiarovano E, Cynthia Darlington C, Vidal PP, Lamas G, Waele C. The role of cervical and ocular vestibular evoked myogenic potentials in the assessment of patients with vestibular

schwannomas. PLoS One, 2014; 9(8):e105026 https://doi.org/10.1371/journal.pone.0105026

[5] Noij KS, SD. Rauch, Vestibular evoked myogenic potential (VEMP) testing for diagnosis of superior semicircular canal dehiscence. Frontiers in Neurology, 2020;11:695; 1-8. https://doi.org/10.3389/fneur.2020.00695

[6] Magliulo A, Iannella G, Gagliardi S, Re M. 1-year follow-up study with C-VEMPs, O-VEMPs and video head impulse testing in vestibular neuritis. European Archives of Oto-Rhino-Laryngology, 2015; 272: 3277-3281. https://doi.org/10.1007/s00405-014-3404-9

[7] Guo X, Liu X, Ye S, Liu X, Yang X, Fan D. Eye movement abnormalities in amyotrophic lateral sclerosis. Brain Sciences, 2022; 12(4): 489. https://doi.org/10.3390/brainsci12040489

[8] Eleftheriadou A, Deftereos SN, Zarikas V, Panagopoulos G, Sfetsos S, Karageorgioub CL, Ferekidou E, Kandilorosd D, Korres S. The diagnostic value of earlier and later components of vestibular evoked myogenic potentials (VEMP) in multiple sclerosis. Journal of Vestibular Research, 2009;19(1-2): 59-66. https://doi.org/10.3233/VES-2009-0342

[9] Bal N, ŞengülY, Behmen MB, Powel A, Louis ED. Vestibular reflexes in essential tremor: abnormalities of ocular and cervical vestibular-evoked myogenic potentials are associated with the cerebellum and brainstem involvement. Journal of Neural Transmission, 2023;1-7. https://doi.org/10.1007/s00702-023-02652-3

[10] Matsuzaki M, Murofushi T, Mizuno M. Vestibular evoked myogenic potentials in acoustic tumor patients with normal auditory brainstem responses. European archives of oto-rhino-laryngology, 1999; 256: 1-4. https://doi.org/10.1007/s004050050112

[11] Tsutsumi T, Tsunoda A, Noguchi Y, Komatsuzaki A. Prediction of the nerves of origin of vestibular schwannomas with vestibular evoked myogenic potentials. Otology & Neurotology, 2000;21(5):712-715. https://doi.org/10.1016/b878-0-323-01830-2.50022-5

[12] Eleftheriadou A, E Koudounarakis. Vestibular-evoked myogenic potentials eliciting: An overview. European archives of oto-rhino-laryngology, 2011;268(3): 331-339. https://doi.org/10.3233/VES-2009-0342

[13] Curthoys IS. A critical review of the neurophysiological evidence underlying clinical vestibular testing using sound, vibration and galvanic stimuli. Clinical Neurophysiology, 2010;121(2):132-144. https://doi.org/10.1016/j.clinph.2009.09.027

[14] Mat Q, Duterme JP, Tainmont S, Lelubre C, Manto M. Optimizing ocular vestibular evoked myogenic potentials with narrow band CE-chirps. Ear and Hearing, 2021;42(5):1373-1380. https://doi.org/10.1097/AUD.0000000000001031

[15] Reddy TM, Heinze B, Biagio-de Jager L, Maes L. Cervical and ocular vestibular evoked myogenic potential: A comparison of narrowband chirp, broadband chirp, tone burst and click stimulation. International Journal of Audiology, 2023; 62(6):579-586. https://doi.org/10.1080/14992027.2022.2064924

[16] Akin FW, Murnane OD, Proffitt TM. The effects of click and tone-burst stimulus parameters on the vestibular evoked myogenic potential (VEMP). Journal of the American Academy of Audiology, 2003;14(09); 500-509. https://doi.org/10.3766/jaaa.14.9.5

[17] Karaçaylı C, Karaçaylı C, Öçal FCA, Çoban VK, Satar B. Normative data of ocular vestibular evoked myogenic potentials in response to chirp stimulus. The Journal Of International Advanced Otology, 2020;16(3): 378. https://doi.org/10.5152/iao.2020.6354

[18] Speidel DP, BeckL. Demystifying the CE-Chirp. Hearing Review, 2016;23(2):28. https://hearingreview.com/hearing-products/accessories/components/demystifying-ce-chirp

[19] Ocal FCA, Karacayli C, Coban VK, Satar B. Can narrow band chirp stimulus shake the throne of 500 Hz tone burst stimulus for cervical vestibular myogenic potentials? Journal of Audiology & Otology, 2021;25(2): 98. https://doi.org/10.7874/jao.2020.00486

[20] Özgür A, Çelebi Erdivanlı Ö, Özergin Coşkun Z, Terzi S, Yiğit E, Demirci M, Dursun E. Comparison of tone burst, click and chirp stimulation in vestibular evoked myogenic potential testing in healthy people. International Advanced Otology, 2015;11(1): 33-35 https://doi.org/10.5152/iao.2015.927

[21] Cebulla M, Stürzebecher E, Don M, Müller-Mazzotta J. Auditory brainstem response recording to multiple interleaved broadband chirps. Ear and Hearing, 2012;33(4): 466-479. https://doi.org/10.1097/AUD.0b013e318241e85a

[22] Bas B, Keseroglu K, Er S, Ozdek A, Korkmaz MH. Is chirp more effective than click and tone-burst during oVEMP test? Annals of Medical Research, 2021; 27(3): 0819-0824. https://doi.org/10.5455/annalsmedres.2019.11.693

[23] Isaradisaikul S, Navacharoen N, Hanprasertpong C, Kangsanarak J. Cervical Vestibular-Evoked Myogenic Potentials: Norms and Protocols. International Journal of Otolaryngology, 2012; 2012(1): 913515. https://doi.org/10.1155/2012/913515

[24] Janky KL, Shepard N. Vestibular evoked myogenic potential (VEMP) testing: normative threshold response curves and effects of age. Journal of the American Academy of Audiology, 2009;20(08): 514-522. https://doi.org/10.3766/jaaa.20.8.6

How to cite this article: Bal N, Husam H, Aydın Z, Demirci Cebeci B, Arslan NÖ, Gedik Toker Ö. Comparison of the cVEMP and oVEMP Responses with Different Stimuli in Healthy Individuals. Clin Exp Health Sci 2025; 15: 42-47. DOI: 10.33808/clinexphealthsci.1367956

Clinical and Experimental Health Sciences

Determining the Association Between Pesticide Safety Behaviors and Health Literacy of Farmers Registered in WhatsApp Groups of Antalya Provincial Agriculture and Forest Directorate: A Descriptive-Correlational Study

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Received: October 31, 2023 **Accepted:** February 16, 2025

ABSTRACT

Objective: Pesticide use safety practices are essential in preventing pesticide exposure risk. Health literacy should be at a sufficient level to acquire these safety practices. This study aimed to determine the association between pesticide safety practices and health literacy of farmers registered in the WhatsApp groups of the Antalya Provincial Agriculture and Forest Directorate.

Methods: The sample of this descriptive-correlational study consisted of 221 farmers registered in Whatsapp groups belonging to five districts of Antalya. Research data were collected between March and August 2022 using an online Pesticide Safety Behaviors and Health Literacy Google form. Descriptive statistics, Multiple Linear Regression Analysis, and Pearson Correlation analysis were used to evaluate the data.

Results: There was a high positive correlation between pesticide safety behaviors and health literacy mean scores of farmers ($r=.844$, $p<.01$). According to the multiple linear regression results, the most significant variables affecting pesticide safety behavior were, respectively, training on pesticide use ($\beta=0.426$), higher levels of education ($\beta=0.347$), female gender ($\beta=0.195$), and older age ($\beta=0.110$). Health literacy was affected by higher levels of education ($\beta=0.591$), female gender ($\beta=0.340$), and employment status ($\beta=0.088$).

Conclusion: The study group showed adequate pesticide safety behaviors and health literacy levels. The most crucial factor in exhibiting safe behaviors was receiving training on pesticides. Higher levels of education level and female gender positively affected both variables. To minimize the risk of pesticide exposure for farmers., it was recommended to plan community-based participatory training interventions and raise awareness by making cross-sectoral cooperation.

Keywords: Farmworkers, pesticide exposure, health literacy, public health nursing

1. INTRODUCTION

In agriculture, pesticides are commonly used to control plant growth and prevent or eliminate harmful pests. According to the latest data from the Food and Agriculture Organization (FAO) for 2021, it is known that more than four million tons of pesticides are used in the world (1). According to the Turkish Ministry of Agriculture and Forestry Plant Protection Products Statistics for 2017, 60,020 thousand tons of pesticides are used for agricultural purposes in Türkiye. Antalya province mostly uses pesticides (2).

Pesticide use behaviors of farmers are affected by many factors, such as age (3), education level (4), risk perception (5), legal regulations, socioeconomic level (6,7), climate (8) and cultural planting practices (4), comfort (6, 7), pesticide knowledge level (5, 9). It is known that farmers are generally at a low level of education (10, 11), and a low education level is associated with low health literacy (12-14). "Health literacy" refers to a person's or community's capacity to obtain, comprehend, assess, and utilize information related to their well-being for decision-making purposes. In studies on pesticide knowledge and the behavior of farmers, it is reported that individuals are aware of the health risks of pesticides but are not adequately protected (7, 15, 16). This situation makes us think there may be a problem in individuals' behavior change and positive attitude development during evaluating and applying the acquired knowledge. Understanding farmers' pesticide use behavior is essential to protect their health and the environment. A study conducted in İzmir determined that 86.9% of the farmers used pesticides, but 59.3% did not take any protective measures (10). In a study conducted in Türkiye, farmers' knowledge and attitudes about pesticide use were

https://doi.org/10.33808/clinexphealthsci.1383819

low (17). In a systematic review that included 121 studies to reveal the personal protective equipment (PPE) and pesticide safety behaviors of farmers, the protection behaviors of the employees were insufficient (18).

Approximately 78% of the world's poorest people live in rural areas and work largely dependent on agricultural land (19, 20). Existing studies have shown that HL levels among agricultural workers and rural residents tend to be lower than in other population groups (21, 22). Farmers are vulnerable to the adverse health effects of exposure to pesticides. Studies have shown that individuals with low education/literacy have higher risks of pesticide exposure (23). The low literacy level of agricultural workers can cause difficulties in understanding and applying the risk and use signs on pesticide labels (24). It can also lead to a lack of knowledge about alternative pest management and new technologies in agriculture (25). In addition, data on agricultural and protective behavior factors related to HL among farmers in Türkiye are limited. It was aimed to investigate whether individuals' abilities to acquire, understand, apply, and evaluate health-related information would affect their likelihood of exhibiting pesticide protective behaviors. It is expected that the obtained results will guide the interventions of public health nurses. In this direction, initiatives can be planned to increase both the health literacy and pesticide safety behaviors of agricultural workers.

1.1. Research Questions

1. What was the level of safe pesticide use behaviors of farmers?
2. What variables affected farmers' pesticide safety behaviors?
3. What was the level of Health Literacy of farmers?
4. What were the variables that affected the Health Literacy levels of farmers?
5. Was there a relationship between farmers' safe pesticide use behaviors and their Health Literacy levels?

2. METHODS

2.1. Type and time of Research

This descriptive-correlational type study was conducted following the Strengthening Reporting of Observational Studies in Epidemiology (STROBE) checklist (26). Research data were collected between March and August 2022 using an online Google form.

2.2. Population and Sample

Antalya province is a region where pesticides are used intensively in many agricultural fields, especially greenhouse agriculture. In Antalya, which has eighteen districts, the highest pesticide consumption is in Kumluca, Finike, Aksu, Demre, and Serik districts. "WhatsApp" groups were established by the Antalya Provincial Directorate of Agriculture and Forestry to conduct farmer training during the Covid-19 pandemic. The groups were formed from individuals registered in the "Farmer Registration System" through District Directorates. The planned trainings cover plant production, good agricultural practices, organic farming, and pest control. The population of this study consists of 2500 farmers registered to WhatsApp groups in five districts with the highest pesticide consumption.

We used the sample determination formula to determine the number of samples needed when the universe is known. The sample size was calculated by taking the frequency of safe pesticide use behavior p=0.37, d=0.05 from the Masruri et al (27) study. It was determined that the minimum sample size to be included in the study was 290 farmers. No sampling method was used in the study, and farmers over 18 who agreed to participate were included. There are no exclusion criteria from the study. The data was sent to all individuals in the WhatsApp group list. Data collection forms were created through Google Forms, and individuals were expected to respond. A total of 211 agricultural workers participated in the study. When the targeted sample could not be reached online, the power of the study with the collected data was calculated with the G*Power program. In the post hoc correlation analysis of the relationship between pesticide safety behaviors and Health Literacy level for the statistical power of the study, the power of the study was found to be 0.97 in the 95% confidence interval (r=.844).

2.3. Data Collection

The research data were collected online with a data collection form consisting of three parts. In the first part of the questionnaire, there were 10 questions about the descriptive characteristics of individuals and how many years they had worked in agriculture, how often they applied pesticides, and whether they had received training before. The second part of the data collection form had questions about pesticide safety behaviors. The Health Literacy Scale was used in the third section of the data collection form.

2.3.1. Pesticide Safety Behaviors Questionnaire

This section was generated by researchers by the "Guidelines for Personal Protection when Handling and Applying Pesticide –Inter Code of Conduct on Pesticide Management" prepared in cooperation with the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (28). In this section, there were 20 questions to determine the behavior of farmers before, during, and after pesticide application. Four questions were about reading labels ***before pesticide application***, following directions, choosing less risky ones, and mixing by hand; 11 questions were about ***pesticide application***, whether individuals eat or drink something, use personal protective equipment, and pay attention to weather conditions; five questions were about bathing, changing

clothes and washing ***after the application.*** The questions were designed in a five-point Likert type (Never=1 point), (Rarely=2 points), (Sometimes=3 points), (Often=4 points), (Always=5 points). The questions were designed in a five-point Likert type ("Never" (1 point), "Rarely" (2 points), "Sometimes" (3 points), "Often" (4 points), and "Always" (5 points). To measure the attention of farmers, some questions (14.,22.,23.,25.,28.) were formed in a negative form. These questions were reverse-coded. A maximum of 100 and a minimum of 20 points can be obtained from this part of the questionnaire. The increase in the mean score was interpreted as a good level of pesticide safety behaviors. In evaluating the measurement reliability of the data collection form, the Cronbach-alpha coefficient was calculated as 0.77. The questionnaire showed sufficient measurements.

2.3.2. Health Literacy Scale

The original Health Literacy Scale scale was 25-item, which was simplified by Toçi et al. 2013 (29) from the 47-item Health LiteracyS-E.U (Health Literacy Survey in Europe) form developed by Sorensen et al. 2013 (30). The standard deviation of the original scale was 0.95, and the internal consistency coefficients (Cronbach's alpha) determined for the subscales vary between 0.90 and 0.94 (30). The Turkish validity and reliability of the Health Literacy scale used in this study were conducted by Bayik Temel and Aras (31). There were 25 questions on the scale. The scale consisted of access to information (5 questions), understanding information (7 questions), appraisal/evaluation (8 questions), and application/use (5 questions). The Cronbach Alpha value was 0.92. The scale was in a five-point Likert type (I have no difficulty: 5 points, I have little difficulty: 4 points, I have some difficulty: 3 points, I have many difficulties: 2 points, I am unable to do it: 1 point). The minimum score on the scale was 25, and the maximum score was 125. All items were positive.

2.4. Statistical Analysis

Researchers analyzed the research data with the IBM SPSS 20.0 program (Akdeniz University licensed). Data were shown as numbers, percentages, mean, and standard deviation. The normality curve of the data was checked before the analyses were made. The kurtosis skewness values of the total scores of both scales were found between – 1.5 and +1.5. Data showed a normal distribution.

Dependent variables, pesticide safety behaviors, and variables affecting Health Literacy were evaluated with multiple linear regression analysis. Educational level, gender, marital status, income, employment, total years of work, and previous training in pesticide safety were independent variables. Categorical independent variables were transformed into dummy variables. Gender (Female:1, Male:0), Education Level (High school and above:1, Secondary school and below:0), Income (Income Equal to expense or high:1, low:0), Employment (Working in own field: 1, wage earner, tenant/shareholder: 0), training on pesticide use (Yes:1, No:0), Marital Status (Married:1, Single:0). Pearson Correlation analysis performed to determine the correlation between Health Literacy and pesticide safety behaviors—data analyzed at a 95% confidence interval. Sample adequacy was evaluated for the analysis used in the study. For each independent variable, 10-20 data is required for multiple linear regression. The study's sample size was sufficient for analysis (32).

2.5. Research Ethics

Ethical consent was obtained from the Akdeniz University Clinical Research Ethics Committee (Number:70904504/829, Date:21.12.2021), and informed consent was obtained from participants. The Antalya Provincial Agriculture and Forest Directorate granted institutional permission.

3. RESULTS

The mean age of the farmers was 43.99±10.91 (min.:24, max.:69), and 44% were women. Among participants, 42.5% were in secondary school or below, and 57.5% were at high school or higher education level. The income of 95% of the participants is less than and equal to their expenses. The percentage of those working in their fields is 81%, and 84.2% are married. Among participants, 59.3% had received pesticide safety training before, and the mean working year was 19±10.91. The mean score of pesticide safety behaviors was 77.45±7.61 (min:20, max:100), and the mean Health Literacy score was 90.87±12.43 (min: 25, max: 125). (Table 1)

Table 1. *Scales and sub-dimensions (n=221)*

	Min.	Max.	Mean
Pesticide Safety Behavior Score	**54**	**93**	**77.45±7.61**
Before Pesticide Practice	7	20	16.00±2.58
During Pesticide Practice	25	55	42.96±5.37
After Pesticide Practice	10	25	18.48±3.23
Health Literacy Score	**66**	**116**	**90.87±12.43**
Accessing Information	10	25	18.52±3.66
Understanding Information	15	34	25.19±4.36
Appraisal/Evaluation	18	40	30.07±4.41
Practice/Use	8	25	17.09±3.30

Multiple linear regression analysis was applied to determine the variables affecting pesticide safety behaviors. The correlation between all independent variables included in the model and pesticide safety behaviors was R=0.840. Independent variables explained 69% of the change in the mean pesticide safety behavior score (R^2 =0.692). There was a slight difference between the Adjusted R^2 =0.692 value and the R^2=0.705 value corrected for sampling error. This means that there were enough samples taken, and the margin of error in the measurements was minimal. At least one of the independent variables had a significant effect that could

explain the dependent variable (F=56,031, p=.000). Significant variables affecting pesticide safety behaviors were older age (β=0.110, p=.034), female gender (β=0.195, p=.000), higher levels of education (β=0.347, p=.000), and training on pesticide use (β=0.426, p=.000). Training on pesticides was the variable that most affected the pesticide safety behavior score. Among the significant variables, a one-unit increase in the standard deviation of training on pesticides caused an increase of 0.426 units in the pesticide safety behavior score. Although not statistically significant, the increase in total working years and being single had an adverse effect on the pesticide safety behavior score (Table 2).

Table 2. *Variables affecting pesticide safety behaviors*

Variables	Beta (β)	Standard Error	*p-value*
Age	**0.110**	0.036	**.034***
Gender	**0.195**	0.641	**.000***
Education Level	**0.347**	0.936	**.000***
Income	0.014	1.359	.723
Employment	0.024	0.820	.572
Training on Pesticides	**0.426**	0.939	**.000***
Total Working Years	-0.044	0.031	.318
Marital status	-0.097	1.033	.051
R	**0.840**		
R^2	**0.705**		
Adjusted R^2	**0.692**		
F and *P* value	**56.031**		**.000***

**p<.05*

The independent variables affecting the level of Health Literacy were evaluated with multiple regression analysis. The correlation between the independent variables included in the model and Health Literacy was R=0.827. Independent variables explain 67% of the change in the mean Health Literacy score (R2 = 0.675). At least one of the independent variables had a significant effect that could explain the dependent variable (F=77,295, p=.000). Significant variables affecting the level of Health Literacy were determined as female gender (β=0.340, p=.000), higher levels of education (β=0.591, p=.000), and working in own field (β=0.088, p=.000) (Table 3).

Table 3. *Variables affecting Health Literacy*

Variables	Beta (β)	Standard Error	*p-value*
Age	0.057	0.056	.245
Gender	0.340	1.066	**.000***
Education Level	0.591	1.099	**.000***
Income	-0.041	2.258	.299
Employment	0.088	1.311	**.035***
Marital Status	-0.072	1.675	.147
R	**0.827**		
R2	**0.684**		
Adjusted R2	**0.675**		
F and *P* value	**77.295**		**.000***

*p<.001

There was a high positive correlation between pesticide safety behaviors and the Health Literacy mean scores of farmers (r=.844, p<.01). In general, there were significant relationships between the sub-dimensions of both scales. The highest correlation was between pesticide safety behaviors and the appraisal/evaluation sub-dimension (r=.780). There was a high level of relationship between pesticide safety behaviors and the sub-dimensions of appraisal/evaluation (r=.780) and understanding information (r=.702) and a moderate relationship between the sub-dimensions of practice/use (r=.630) and access to information (r=.626) (Table 4).

Table 4. *Determining the correlation between pesticide safety behaviors and sub-dimensions of the Health Literacy scale*

Scales and Sub-Dimensions		1	2	3	4	5	6	7	8	9
Pesticide Safety Behaviors (1)	r	1	.553*	.810*	.584*	**.845***	.626*	.702*	.780*	.630*
	p		**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
Before Pesticide Practice (2)	r		1	.150*	.207*	.447*	.339*	.343*	.449*	.316*
	p			**.025**	**.002**	**.000**	**.000**	**.000**	**.000**	**.000**
During Pesticide Practice (3)	r			1	.126	.684*	.523*	.558*	.621*	.524*
	p				.062	**.000**	**.000**	**.000**	**.000**	**.000**
After Pesticide Practice (4)	r				1	.495*	.335*	.451*	.445*	.360*
	p					**.000**	**.000**	**.000**	**.000**	**.000**
Health Literacy (5)	r					1	.768*	.857*	.874*	.751*
	p						**.000**	**.000**	**.000**	**.000**
Accessing Information (6)	r						1	.637*	.524*	.420*
	p							**.000**	**.000**	**.000**
Understanding Information (7)	r							1	.640*	.459*
	p								**.000**	**.000**
Appraisal/ Evaluation (8)	r								1	.621*
	p									**.000**
Practice/Use (9)	r									1
	p									

** p< .01*

4. DISCUSSION

It was aimed to determine the relationship between pesticide safety behaviors and health literacy of the Whatsapp group of farmers, where training and information were provided on agricultural issues such as crop cultivation, pest control, and irrigation management. The assumption is that the ability of farmers to acquire, understand, evaluate, and apply information about their health will bring safe use behaviors to protect them from the harmful effects of pesticides. The results of the study were examined under three main headings.

4.1. Farmers' Pesticide Safety Behavior Levels and Affecting Variables

The mean score was 77.45±7.61 out of 100 points for farmers' pesticide safety behaviors. This study group's pesticide safety behaviors were sufficient, unlike some studies in the literature (5, 7). We can explain this because the farmers in the WhatsApp group were in contact with the staff of the District Agriculture Directorate. Contacting agricultural professionals within the framework of programs that maintain environmental and human health, such as Integrated Pest Control and Good Agricultural Practices, led to safe behaviors. This result was in line with the increase in pesticide safety behavior in farmers trained with IPM programs in the literature (33, 34, 35, 36).

As expected, individuals who had received training in pesticide-safe behaviors also scored higher. The meta-analyses determined that education interventions significantly affected behavior change (37). As in many studies, this study's high education level brought safe pesticide applications (6,7, 36, 38). As literacy rates continue to increase and information becomes more readily available, individuals are expected to become more knowledgeable about pesticides. This study shows that their pesticide safety scores reflect the need for more farmers with low education levels to access correct information and adapt to the recommended safety guidelines.

Research results show that, similar with literacy, employees exhibited more safety behaviors as age increasesThis situation can be explained by the fact that with advancing age, individuals are more likely to be exposed to/encounter training and information activities regarding safe behaviors, as in Wang et al.'s (2017) study. (39). Another important variable that significantly affects safe pesticide use behaviors is female gender. Contrary to some, this study found that women exhibit better safety behaviors (40, 41). The reason for this may be that, unlike other agricultural countries, women in Antalya, Türkiye are actively involved in agricultural work and have responsibilities. Women in agriculture in Türkiye are in a decision-making position in many stages, such as preparing pesticides, spraying, and applying the recommended harvest time. In general, it is known that women's risk perceptions cause positive attitudes toward safety (42). Based on these results, the fact that women who actively work with pesticides are aware of the health risks and have a high-risk perception has brought about safe pesticide behaviors.

4.2. Farmers' Health Literacy Levels and Affecting Variables

The mean Health Literacy score of farmers was 90.87±12.43. The Health Literacy levels of farmers were evaluated with different measurement tools in different cultures. This situation made it difficult to compare with different studies. As in this study, the health literacy level of agricultural workers in Thailand was found to be relatively high (43). In a different study, it was found that the health literacy levels of agricultural workers in Türkiye were limited and problematic (44). It was seen that the health literacy level of agricultural workers was similar to different groups in Türkiye (31, 45, 46, 47).

As in other agricultural studies, higher education levels were expected to affect Health Literacy positively (43, 48). With advanced literacy skills, individuals' access to information, transportation, evaluation, and transportation processes becomes more accessible. In this study, unlike the Turkish Health Literacy Study, the Health Literacy level of women was higher than that of men (49). It is thought that this situation may be since men in rural areas do not care about their health-related decisions.

4.3.Farmers' Correlation of Pesticide Safety Behaviors and Health Literacy

There was a strong positive correlation between farmers' safe pesticide use behaviors and their Health Literacy levels**.** The results of the Pobhirun et al. (50) study on sweet corn farmers also supported this finding. This result was significant regarding individuals obtaining information on the harms of pesticides, their correct use, reducing exposure, understanding this information, evaluating it through functional processes, and exhibiting appropriate behavior. Especially the ability of farmers working with pesticides to read the labels on the boxes, comply with the instructions, and not enter the field within the recommended time is indirectly related to Health Literacy. Interventions to improve farmers' Health Literacy levels will also increase individuals' awareness.

5. CONCLUSION

The study results showed a strong correlation between the pesticide safety behaviors and Health Literacy levels of the farmers. In this context, the lack of sufficient information and training for agricultural workers on the use of personal protective equipment and safe practices in pesticide use should be completed. Based on these results, it is important that public health nurses regularly provide training to agricultural workers regarding pesticide safety, covering all employees. Another striking result is that social environments such as WhatsApp groups where information exchange takes

place are effective in individuals exhibiting safe behaviors. In this context, public health nurses will play important roles in reaching agricultural workers, planning initiatives in cooperation with stakeholders such as Provincial/District Agriculture Directorates, and protecting and improving health. Similarly, according to the Occupational Health and Safety regulations of the Ministry of Environment and Urbanization, annual blood cholinesterase/pseudocholinesterase measurement is recommended for pesticide applicators in preventing pesticide exposure. Public health nurses will play an active role in guiding individuals to health screenings with their counseling and case management roles.

5.1. Limitations of the Research

One of the limitations of the study was that 57.5% of the individuals were at high school or higher education level, which was likely to affect the study results. Another limitation is the lack of access to those who are older and have lower levels of education. Also predicted sample size could not be reached. One disadvantage was that online research responses might be answered hastily and without much consideration.

Acknowledgement: *We would like to thank all participants involved in the study and Antalya Provincial Directorate of Agriculture and Forestry*
Funding: *The authors received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by the Akdeniz University Clinical Research Ethics Committee (approval date 21.12.2021and number 70904504/829)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: DA, SÖ
Design of the study: DA, SÖ
Acquisition of data for the study: DA, SÖ
Analysis of data for the study: DA, SÖ
Interpretation of data for the study: DA, SÖ
Drafting the manuscript: DA
Revising it critically for important intellectual content: SÖ
Final approval of the version to be published: DA, SÖ

REFERENCES

[1] FAO.FAOSTAT Pesticides Use Dataset 2020. Access Date:20.02.2023. [Available from: http://www.fao.org/faostat/en/#data/RP].

[2] Turkish Ministry of Agriculture and Forestry GDoFaC. The Usage Situations of 2017 Plant Protection Products on the Basis of Provinces as Grouped (2017). [Available from: https://www.tarimorman.gov.tr/Konular/Bitki-Sagligi-Hizmetleri/Bitki-Koruma-Urunleri-Ve-Makinalari/Istatistik-Bilgileri. Access Date: 21.02.2023.

[3] Berni I, Menouni A, Ghazi El I, Duca RC, Kestemont MP, Godderis L, Jaafari SE. Understanding farmers' safety behavior regarding pesticide use in Morocco. Sustainable Production and Consumption. 2021;25:471-483. https://doi.org/10.1016/j.spc.2020.11.019

[4] Bagheri A, Emami N, Allahyari MS, Damalas CA. Pesticide handling practices, health risks, and determinants of safety behavior among Iranian apple farmers. Human and Ecological Risk Assessment: An International Journal. 2018;24(8):2209-2223. https://doi.org/10.1080/10807039.2018.1443265

[5] Jallow MFA, Awadh DG, Albaho MS, Devi VY, Thomas BM. Pesticide risk behaviors and factors influencing pesticide use among farmers in Kuwait. Science of The Total Environment. 2017;574:490-498. https://doi.org/10.1016/j.scitotenv.2016.09.085

[6] Bhandari G, Atreya K, Yang X, Fan L, Geissen V. Factors affecting pesticide safety behaviour: The perceptions of Nepalese farmers and retailers. Science of The Total Environment. 2018;631-632:1560-1571. https://doi.org/10.1016/j.scitotenv.2018.03.144

[7] Sharifzadeh MS, Abdollahzadeh G, Damalas CA, Rezaei R, Ahmadyousefi M. Determinants of pesticide safety behavior among Iranian rice farmers. Science of The Total Environment. 2019; 651: 2953-2960. https://doi.org/10.1016/j.scitotenv.2018.10.179

[8] Shammi M, Sultana A, Hasan N, Mostafizur Rahman M, Saiful Islam M, Bodrud-Doza M, Uddin K. Pesticide exposures towards health and environmental hazard in Bangladesh: A case study on farmers' perception. Journal of the Saudi Society of Agricultural Sciences. 2020;19(2):161-173. https://doi.org/10.1016/j.jssas.2018.08.005

[9] Bagheri A, Emami N, Damalas CA, Allahyari MS. Farmers' knowledge, attitudes, and perceptions of pesticide use in apple farms of northern Iran: impact on safety behavior. Environmental Science and Pollution Research International. 2019;26(9):9343-9351 https://doi.org/10.1007/s11356-019-04330-y

[10] Ergonen AT, Salacin S, Ozdemir MH. Pesticide use among greenhouse workers in Turkey. Journal of Clinical Forensic Medicine.2005;12(4):205-208. https://doi.org/10.1016/j.jcfm.2004.10.017

[11] Gün S. Pesticide Use in Turkish Greenhouses: Health and Environmental Consciousness. Polish Journal of Environmental Studies. 2009;18(4):607-615.

[12] Van der Heide I, Wang J, Droomers M, Spreeuwenberg P, Rademakers J, Uiters E. The relationship between health, education, and health literacy: Results from the Dutch Adult Literacy and Life Skills Survey. Journal of Health Communication. 2013;18(1):172-184. https://doi.org/10.1080/10810730.2013.825668

[13] Joveini H, Rohban A, Askarian P, Maheri M, Hashemian M. Health literacy and its associated demographic factors in 18-65-year-old, literate adults in Bardaskan, Iran J Educ Health Promot. 2019;8:244. https://doi.org/10.4103/jehp.jehp_26_19

[14] Sudhakar S, Aebi ME, Burant CJ, Wilson B, Wenk J, Briggs FBS, Pyatka N, Blixen C, Sajatovic M. Health literacy and education level correlates of participation and outcome in a remotely delivered epilepsy self-management program. Epilepsy & Behavior. 2020;107:107026. https://doi.org/10.1016/j.yebeh.2020.107026

[15] Gesesew HA, Woldemichael K, Massa D, Mwanri L. Farmers knowledge, attitudes, practices and health problems associated with pesticide use in Rural Irrigation Villages, Southwest Ethiopia. PloS one. 2016;11(9):0162527.

https://doi.org/10.1371/journal.pone.0162527

[16] Taghdisi MH, Amiri BB, Dehdari T, Khalili F. Knowledge and practices of safe use of pesticides among a group of farmers in Northern Iran. Int J Occup Environ Med. 2019;10(2):66-72. https://doi.org/10.15171/ijoem.2019.1479

[17] Cevik C, Ozdemir R, Ari S. Relationship between farmers' knowledge and attitudes towards pesticide use and their sociodemographic characteristics: A cross-sectional study from north-western Turkey. Roczniki Panstwowego Zakladu Higieny. 2020;71(3):341-348.
https://doi.org/10.32394/rpzh.2020.0123

[18] Sapbamrer R, Thammachai A. Factors affecting use of personal protective equipment and pesticide safety practices: A systematic review. Environ Res. 2020;185:109444.
https://doi.org/10.1016/j.envres.2020.109444

[19] Food and Agriculture Organization of the United Nations (FAO) (2024). Reduce Rural Poverty. Available at: https://www.fao.org/reduce-rural-poverty/our-work/family-farming/en/ (Accessed 15 June 2024).

[20] The World Bank (2014). For Up to 800 Million Rural Poor, a Strong World Bank Commitment to Agriculture. Available at: https://www.worldbank.org/en/news/feature/2014/11/12/for-up-to-800-million-rural-poor-a-strong-world-bank-commitment-to-agriculture. (Accessed 15 June 2024).

[21] Aljassim N, Ostini R. Health literacy in rural and urban populations: a systematic review. Patient Educ Couns. 2020;103:2142–2154
https://doi.org/10.1016/j.pec.2020.06.007

[22] Bassem R, Fahim AE, Mosaad SE, Waheed A. Occupational health literacy among agricultural workers. Egypt J Occup Med. 2023;47:33–45.
https://doi.org/10.21608/EJOM.2022.157730.1288

[23] Mueller W, Jones K, Fuhrimann S, Ahmad ZNBS, Sams C, Harding AH et al. Factors influencing occupational exposure to pyrethroids and glyphosate: An analysis of urinary biomarkers in Malaysia, Uganda and the United Kingdom. Environmental Research. 2024; 242:117651
https://doi.org/10.1016/j.envres.2023.117651

[24] Rother HA. Pesticide labels: Protecting liability or health? – Unpacking "misuse" of pesticides. Current Opinion in Environmental Science & Health. 2018;4: 10-15.
https://doi.org/10.1016/j.coesh.2018.02.004

[25] Ahmad A., Shahid M., Khalid S. Zaffar H, Naqvi T, Pervez A, Naqvi T, Bilal M, Ali MA, Abbas G, Nasim W. Residues of endosulfan in cotton growing area of Vehari, Pakistan: an assessment of knowledge and awareness of pesticide use and health risks. Environ Sci Pollut Res. 2019;26:20079–20091. https://doi.org/10.1007/s11356-018-3169-6

[26] von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370(9596):1453-1457.
https://doi.org/10.1016/S0140-6736(07)61602-X

[27] Masruri B, Dehdari T, Yekzamani P, Moghadasi N, Ashtarinezhad A. Assessment of knowledge and practice of pistachio farmers in terms of pistachio pesticide safety. Human and Ecological Risk Assessment: An International Journal. 2021;27:595-605. https://doi.org/10.1080/10807039.2020.1744111

[28] Food and Agriculture Organization of the United Nations & World Health Organization. Guidelines for personal protection when handling and applying pesticide: international code of conduct on pesticide management. Food and Agriculture Organization of the United Nations. Published [1 February 2020]. https://apps.who.int/iris/handle/10665/330917

[29] Toçi E, Burazeri G, Sorensen K, Jerliu N, Ramadani N, Roshi E, Brand R. Health literacy and socioeconomic characteristics among older people in Transitional Kosovo. Journal of Advances in Medicine and Medical Research. 2013;3(4):1646-1658.

[30] Sørensen K, Van den Broucke S, Pelikan JM, Fullam J, Doyle G, Slonska Z, Kondilis B, Stoffels V, Osborne RH, Brand H. Measuring health literacy in populations: Illuminating the design and development process of the European Health Literacy Survey Questionnaire (HLS-EU-Q). BMC Public Health. 2013;13:948.

[31] Bayık Temel A, Aras Z. Evaluation of validity and reliability of the Turkish Version of Health Literacy Scale. Florence Nightingale Journal of Nursing. 2017;25(2):85-94.
https://doi.org/10.17672/fnhd.94626

[32] Çokluk O. Logistic regression analysis: Concept and application. Educational Sciences: Theory and Practice.2010;10(3):1357-1407.

[33] Jørs E, Konradsen F, Huici O, Morant RC, Volk J, Lander F. Impact of training bolivian farmers on integrated pest management and diffusion of knowledge to neighboring farmers. J Agromedicine. 2016;21(2):200-208.
https://doi.org/10.1080/1059924x.2016.1143428

[34] Clausen AS, Jors E, Atuhaire A, Thomsen JF. Effect of integrated pest management training on ugandan small-scale farmers. Environmental Health Insights. 2017;11:1178630217703391. https://doi.org/10.1177/1178630217703391

[35] Maddah D, Ghach W, Abi Farraj N, Yehya M, Khatib JA, Alami NH. The first community-based intervention to promote safe pesticide use by developing knowledge, attitudes, and practices among Lebanese farmers. Human & Ecological Risk Assessment. 2020;26(10):2824-2835.
https://doi.org/10.1080/10807039.2019.1688639

[36] Galli A, Winkler MS, Doanthu T, Fuhrimann S, Huynh T, Rahn E, Stamm C, Staudacher P, Huynh TV, Loss G. Assessment of pesticide safety knowledge and practices in Vietnam: A cross-sectional study of smallholder farmers in the Mekong Delta. Journal of Occupational and Environmental Hygiene. 2022;19(9):509-523.
https://doi.org/10.1080/15459624.2022.2100403

[37] Ayaz D, Öncel S, Karadağ E. The effectiveness of educational interventions aimed at agricultural workers' knowledge, behaviour, and risk perception for reducing the risk of pesticide exposure: A systematic review and meta-analysis. Int Arch Occup Environ Health. 2022;95:1167–1178.
https://doi.org/10.1007/s00420-022-01838-8

[38] Diomedi BZ, Nauges C. Pesticide-handling practices: The case of coffee growers in Papua New Guinea. Australian Journal of Agricultural and Resource Economics. 2016;60:112–129. https://doi.org/10.1111/1467-8489.12106

[39] Wang J, Deng Y, Ma Y. Relationships between safe pesticide practice and perceived benefits and subjective norm, and the moderation role of information acquisition: Evidence from 971 Farmers in China. Int J Environ Res Public Health. 2017;14(9):962. https://doi.org/10.3390/ijerph14090962

[40] Christie ME., Van Houweling E. & Zseleczky L. Mapping gendered pest management knowledge, practices, and pesticide exposure pathways in Ghana and Mali. Agric Hum Values. 2015;32:761–775.
https://doi.org/10.1007/s10460-015-9590-2

[41] Kasner EJ, Keralis JM, Mehler L, Beckman J, Bonnar-Prado, J, Lee SJ, Diebolt-Brown B, Mulay P, Lackovic M, Waltz J, Scwartz A, Mitchell Y, Morage-McHaley S, Roisman R, Gergely R, Calvert G. Geder differences in acute pesticide-related illnesses and injuries among farmworkers in the United States, 1998–2007. American Journal of Industrial Medicine. 2012;55(7):571–583. https://doi.org/10.1002/ajim.22052

[42] Znajmiecka-Sikora M, Sałagacka M. Analysis of the relationship between psychological gender and risk perception style and attitudes towards safety in a group of women and men. International Journal of Occupational Safety and Ergonomics. 2022;28(1):364-375
https://doi.org/10.1080/10803548.2020.1760527

[43] Montgomery H., Morgan S, Srithanaviboonchai K, Ayood P, Siviroj P, Wood M. Correlates of health literacy among farmers in Northern Thailand. International Journal of Environmental Research and Public Health. 2020;17(19):7071
https://doi.org/10.3390/ijerph17197071

[44] Koç N, Adana F. Perception of Health, Health Literacy Levels of Farm Labourers and Related Factors. Journal of Public Health Nursing. 2021;3(3):171-183.
https://doi.org/10.54061/jphn.995982

[45] Bayık Temel A, Çimen Z. Kronik hastalığı olan yaşlı bireylerde sağlık okuryazarlığı, sağlık algısı ve ilişkili faktörler. Ege Üniversitesi Hemşirelik Fakültesi Dergisi. 2017;33(3):105-125. (Turkish)

[46] Kuloğlu Y, Uslu K. Geleceğin sağlık çalışanlarında sağlık okuryazarlık düzeyinin sağlık algısı üzerindeki etkisi. Doğuş Üniversitesi Dergisi. 2022;23(1):255-277.
https://doi.org/10.31671/doujournal.955317 (Turkish)

[47] Yıldızeli F, Alabaz D, Gözüyeşil E. Ebeveynlerin çocukluk çağı aşılarının kabulünün sağlık okuryazarlığı ile ilişkisi. J Pediatr Inf. 2021;15(2):91-99.
https://doi.org/10.31125/hunhemsire.966461 (Turkish)

[48] Tusun RB, Emiroğlu ON. Vegetable growers' views on pesticide use in greenhouse agriculture and evaluation of health literacy status. Journal of Samsun Health Sciences. 2022; 7(3):897-914. https://doi.org/10.47115/jshs.1161593 (Turkish)

[49] Republic of Turkey Ministry of Health General Directorate of Health Promotion. Turkish Health Literacy and Related Factors Research. 2018. [Access Date:29.02.2022].
https://sggm.saglik.gov.tr/TR,57003/turkiyenin-saglik-okuryazarligi-duzeyi-olculdu.html#. (Turkish)

[50] Pobhirun T, Pinitsoontorn S. The association between health literacy and pesticide use behaviors among sweet corn farmers in the Pak Chong district of Thailand: A cross-sectional study. F1000Research. 2019;11(8):448.
https://10.12688/f1000research.18398.2

How to cite this article: Ayaz D, Öncel S. Determining the Association Between Pesticide Safety Behaviors and Health Literacy of Farmers Registered in WhatsApp Groups of Antalya Provincial Agriculture and Forest Directorate: A Descriptive-Correlational Study. Clin Exp Health Sci 2025; 15: 48-55. DOI: 10.33808/clinexphealthsci.1383819

Clinical and Experimental Health Sciences

Antimicrobial Effectiveness of Hypochlorous Acid in Infected Dentin Tubules: A Pilot Study

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Received: January 3, 2024 **Accepted:** January 6, 2025

ABSTRACT

Objective: To assess the disinfection capacity of HOCl, NaOCl, and CHX on dentinal tubules.

Methods: Enterococcus faecalis suspension was supplemented to the dentin blocks. The groups were created according to the irrigation solution, 2.5% NaOCl was used in Group 1, 2% CHX in Group 3, and 200 ppm HOCl in Group 3. All the irrigants (50 µL) were dropped on dentin for 30 seconds, 1 min, and 3 min. Four samples were selected for each solution group to form its control group. To observe the bacterial growth 10 ml of a sample taken from the tubes was cultivated on Mueller-Hinton agar. After the incubation, the total number of colonies was determined.

Results: In Group 1, the number of colonies in the samples taken for all three-time intervals was 0, and the solution efficiency was found to be 100%. In Group 2, the success rate was 97.4%, 99.2%, and 99,9% for 30 sec., 1 min, and 3 min, respectively. In Group 3, the success rate was 63%, 86.3%, and 93,4% for 30 sec., 1 min, and 3 min, respectively.

Conclusion: HOCl has a success rate of antimicrobial effect of more than 90% at the end of the 3 min duration on dentinal tubules.

Keywords: Antimicrobial effect, dentin tubules, hypochlorous acid, ırrigation solution.

1. INTRODUCTION

Biofilm formation was observed in the root canal system due to pulp necrosis, leading to dentin infection. (1). Mechanical methods are usually used to remove the biofilm during root canal treatment, however, the high complexity of the root canal anatomy such as lateral canals and isthmuses decreases the efficiency of endodontic instruments (1). To obtain a disinfected root canal system without bacterial accumulation, various endodontic irrigation solutions especially chlorine-containing solutions such as sodium hypochlorite (NaOCl) are used during root canal treatment (2).

Hypochlorous acid (HOCl) dissociated into H^+ and OCl^- ions in an aqueous solution is accepted as a powerful oxidizing compound. HOCl destroys viruses by denaturation and aggregating proteins with nitrogen-centered radicals (3). Inactivation or destruction of microbes exists with the disinfection mechanism including the breaking down of the cell wall of microbes or viruses (4,5). Currently, various forms of hypochlorous acid solutions are suggested as a disinfectant because of their ideal disinfectant properties such as being non-toxic to surface contact, non-corrosive and inexpensive for coronaviruses in oral and maxillofacial surgery offices (6). Therefore, hypochlorous acid-containing solutions as recommended by the United States Environmental Protection Agency (USEPA) against COVID-19 (7). HOCl solutions are considered one of the ideal disinfectants due to being inexpensive, easy to use, quickly effective in large areas, and having a wide range of bactericidal and viricidal effects based on concentrations above 50 ppm at least 3 min of contact time (6).

The in vitro replication of bacterial invasion in dentinal tubules usually evaluated with cultural methods has been investigated for more than a century (8). In these methods, the root canals of extracted teeth or prepared dentin blocks were used as media for the growth of bacteria. Bacterial penetration for up to 500 mm can be observed in dentinal tubules using the dentin block model for nearly 2 decades

Clin Exp Health Sci 2025; 15: 56-62
ISSN:2459-1459

https://doi.org/10.33808/clinexphealthsci.1414218

in antimicrobial efficacy studies (9). Recently, confocal laser scanning microscopy (CLSM) helped many researchers to visualize both live and dead bacteria within infected dentin, and it has been accepted as a suitable method (10-14). However, there are still some roadblocks to collecting specimens from dentin tissue containing heavy and equal bacterial load, as simulating clinical infection using traditional in vitro methods is known to be difficult (15).

To knowledge there is no previous study evaluating the antimicrobial activity of HOCl solution as an irrigation solution in endodontic treatment. The purpose of this study was to assess the disinfection capacity of HOCl, sodium hypochlorite (NaOCl), and chlorhexidine (CHX) on dentinal tubules.

2. METHODS

2.1. Preparation of Dentin Block

Thirty extracted human mandibular premolar teeth were used in this study according to the protocol approved by the Research Ethics Committee of the Faculty of Medicine Akdeniz University (KAEK-904). The teeth were stored in 0.01% NaOCl solution before use to prevent dehydration. The coronal part of the teeth was separated from the root by a diamond disk and root canal preparation was finished with Protaper Next X3 rotary file. Each cylindrical dentin block was sectioned vertically and the cement layer was removed to obtain standard dentin blocks with the size of 4x4x2 mm described as in the previous study (16). A total of 60 dentin blocks were obtained and four of them were excluded from the study due the distruption of the dentin blocks. Each sample was irrigated with 17% EDTA solution for 1 min for smear layer removal. After irrigation with sterile water for 10 min, the outer layer of dentin blocks was covered by resin composite. The samples were sterilized by autoclaving for 20 min at 121 ° C. The sterility of the dentin blocks was checked by incubating each sample for 24 hours at 37 ° C in 5 ml of brain heart infusion agar.

2.2. Dentin Infection with Enterococcus faecalis

Enterococcus faecalis (E. faecalis) ATCC 29212 was cultured (100 µL of seeding was done) on brain heart infusion agar (Becton-Dickinson, Sparks, MD) at 35°C ± 2°C for 18 to 20 h. After incubation, the bacteria were suspended in brain heart infusion broth (BHI) (Becton-Dickinson, Sparks, MD) and the spectrophotometric standardization method was used to provide $3X10^6$ colony-forming units (CFU)/mL (OD405=0.05).

E. faecalis ATCC 29212 suspension of 500 µl was supplemented to the dentin specimen in each eppendorf tube and centrifugation was repeated twice at 5600 g for 5 min. A fresh bacterial solution was added in tubes between each centrifugation. And after centrifugation, the solution that penetrated the dentin blocks was discarded to facilitate bacterial recovery. All dentin blocks were then incubated in BHI broth at 37 ° C for 18-24 hours. The same procedure was repeated 2 days later. All samples were incubated at 37 ° C for a total of 5 days. Randomly chosen 5 samples were separated for SEM examination to verify the presence of bacteria in dentin tubules. For the randomization, the random number generator was used.

2.3. SEM Examination

Before disinfection protocol, one dentin block from each group was selected for SEM examination to verify the presence of *E. faecalis* ATCC 29212 in dentin tubules. Glutaraldehyde (2.5%) and osmium tetroxide (1%) were used in the fixation procedure for 30 min and 1 hour, respectively. The samples were dehydrated using ethanol and sputter-coated with gold-palladium in a vacuum evaporator (Polaron SC7620, Quorum Technologies Ltd., UK). The penetration of *E. faecalis* in dentin tubules was observed by SEM (LEO-1430 VP SEM system, Carl Zeiss AG, Jena, Germany)) at a magnification of 5000-7000x operating at 15.00 kV**.** (Figure 1).

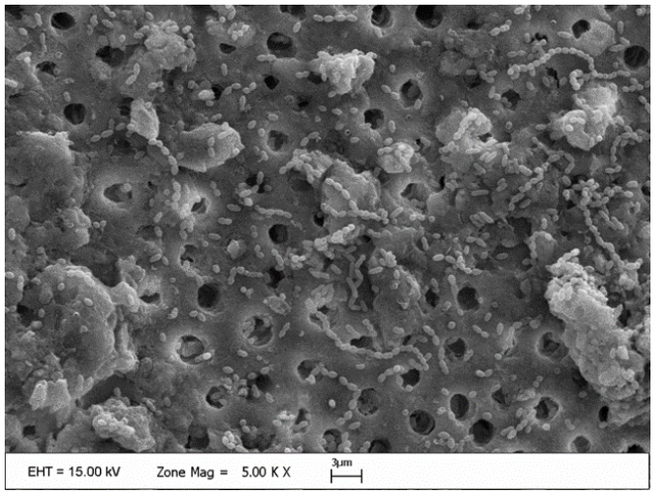

Figure 1. *SEM image of infected dentin surface and occluded by E. faecalis after centrifugation.*

2.4. Dentin Disinfection

At the end of the incubation period, each tooth was removed from Eppendorf tubes and washed with sterile distilled water for one min, and left to dry in sterile petri dishes. A total of 60 samples were divided into three groups according to the irrigation solution used for the disinfection procedure.

2.5. Application of Irrigation Solution

Four samples were selected for each group to form its control group. Only sterile saline solution was applied to these samples and the mean value was calculated. Sodium hypochlorite solution (2.5% NaOCl) was used as an irrigation solution in Group 1, and 0.2% Chlorhexidine (CHX) (Klorhex, Drogsan Pharmaceutical Ind. And Trade Inc), and 200 ppm HOCI were the other irrigation solutions for Group 2 and Group 3. HOCl solution was prepared by using Toucan

Eco Active (ECA Australasia Pty Ltd., Australia) according to manufacturer's recomendation. All the irrigants were dropped on the inner side of the dentin wall as a droplet of 50 µL for 30 sec, 1 min, and 3 min. 2.5% NaOCl solution was obtained from 5% NaOCl (Wizard, Wizard, RehberKimya, Istanbul, Turkey) by diluting 1:1 with distilled water.

The samples were then irrigated with sterile saline solution for 1 min and one sample was chosen from each group (sample with 3 min process) for confocal laser scanning microscope (CLSM) examination. The remaining samples are placed in tubes containing 1 ml of sterile saline to agitate the samples using a Vortex mixer (Onilab LCC, California, USA) for 1 min Then, 100 µl of bacterial suspension prepared in 1/100 dilution was added to tubes containing 4 ml Muller Hinton Agar (MHA), and kept in a 60 °C water bath, and were mixed and spread on MHA plates. The plates were incubated at 35°C ± 2°C for 18 to 20 h. At the end of the incubation period, the colonies that proliferated in each petri dish were counted. The results were multiplied by 10^3 to get the number in 1 ml. After the experiment, the numbers of colonies formed in the petri dishes for each disinfectant and the application period are indicated in the tables. The samples chosen for CLSM examination for visualization of damaged and survived bacteria cells were stained with the SYTO 9/propidium iodide (LIVE/DEAD, BacLigth; Invitrogen, Eugene, OR) as previously reported (1). The samples were observed using a confocal laser scanning microscope (ZEISS LSM 800, Jena, Germany) with 20X magnification at a resolution of 1024x1024 **(**Figure 2).

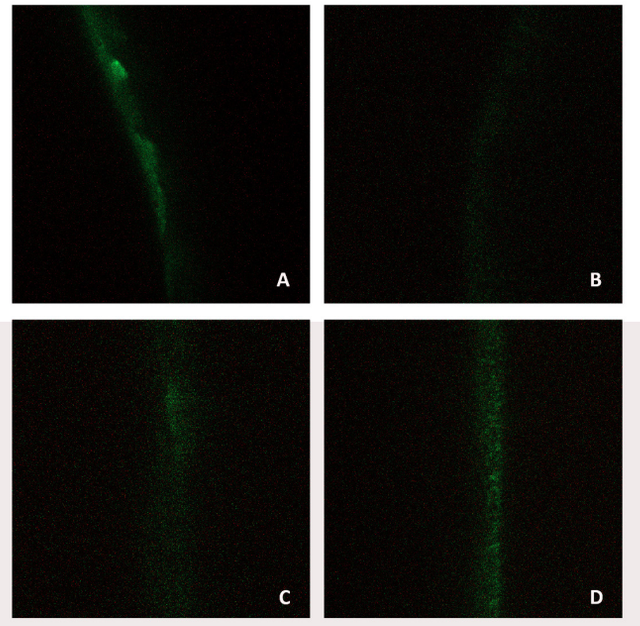

Figure 2. *Representative CLSM images obtained by staining with cell viability stain after the application of different solutions for 3 min.* ***(A)*** *Control (NaCl),* ***(B)*** *2.5% NaOCl,* ***(C)*** *2% CHX,* ***(D)*** *200 ppm HOCl.*

3. RESULTS

For each solution used in the study, 100 µL of seeding was done and the average colony values found at 30 seconds and first min, and the third min in 1 mL were shown in Table 1 in detail.

Table 1. *The mean and standart deviation values of colonies found at 30 second, first minute and third minute when 100 µL of seeding was done in 1 mL.*

	Group 1 (2.5% NaOCl)				Group 2 (2% CHX)				Group 3 (200 ppm HOCl)			
	Control	*30 sec.*	*1 min*	*3 min*	*Control*	*30 sec.*	*1 min*	*3 min*	*Control*	*30 sec.*	*1 min*	*3min*
Colony numbers x 10$^{3)}$	10.10^4	0	0	0	35.10^4	910^3 16710^2	2510^2 2710^2	$2,510^2$ 510^2	40.10^4	14810^3 11810^3	5510^3 39.210^3	26.310^3 13.310^3
Inactivated Factor (IF) Ncontrol / Nsample	-	100000	100000	100000	-	38,88	140	1400	-	2,7	7,27	15,2
inactivated	-	5	5	5	-	1,58	2,14	3,14	-	0,43	0,86	1,81
Efficency *100/Ncontrol	-	100%	100%	100%	-	97.4%	99.2%	99.9%	-	63%	86.3%	93.4%

**NaOCl: sodium hypochlorite, CHX: Chlorhexidine, HOCl: Hypochlorous acid, Sec.: Second, Min.: Minute.*

Number of colonies were determined at specific time intervals (30 seconds and first min, and the third min) following the application of the irrigation solution. The success rates of the solutions were calculated by comparing the number of colonies obtained in the experimental group with those in the control group. In Group 1 (%2.5 NaOCl), the number of colonies in the samples taken for all three-time intervals was 0, and the solution efficiency was found to be 100%. In Group 2 (2%CHX) the success rate was 97.4%, 99.2%, and 99,9% for 30 sec., 1 min, and 3min, respectively. In Group 3 (200 ppm HOCl) the success rate was 63%, 86.3%, and 93,4% for 30 sec., 1 min, and 3min, respectively. Because this study was a pilot study with an insufficient sample size for statistical analysis, no analyses were performed.

4. DISCUSSION

In this study the disinfection capacity of HOCl, NaOCl, and CHX on dentinal tubules was evaluated by using culture method. Furthermore, a singular biofilm model was preffered

in the current research to minimize any changes resulting from bacterial interactions and to guarantee consistency and uniformity (13). According to the findings, HOCl demonstrates an antibacterial efficacy of 90% after a 3-minute exposure on dentinal tubules.

HOCl solutions became popular because of their easy access and use, quick effect in many areas, and wide range of bactericidal and viricidal effects based on concentrations above 50 ppm at least 3 min of contact time (6). There are various studies evaluating the effectiveness of HOCl both in inert surfaces and in vivo conditions. It has been shown that HOCl can inactivate several types of viruses including coronaviruses in less than one min (19). To decontaminate inert surfaces containing enteric viruses, HOCl at a concentration of 200 ppm is successful in a one min contact time (6). In an animal study, the systemic and gastrointestinal effects of ingesting HOCl, from the perspective of its use in mouthwash, were evaluated and there are no abnormalities in histopathological and enamel roughness tests and systemic effects (18). Also the effectiveness of HOCl in cleaning biofilm-contaminated implant surfaces by reducing the lipopolysaccharide concentration of *P. gingivalis* (20). In this study, the disinfection capacity of HOCl, NaOCl, and CHX solutions on dentinal tubules were evaluated.

Wang et. al (21) showed that over 60% of E.faecalis cells in a 1-day-old biofilm inside dentin were eliminated by 6% NaOCl in 3 minutes. In contrast, less bacteria were killed when 3-week-old biofilms in dentin were exposed to the same solution (21). Furthermore, a prior study indicated that to replicate the clinical environment challenge, the bacteria were cultured for 3 weeks to establish mature biofilm formation (13). Ma et al.(16) suggested that a 24-hour incubation time following centrifugation allowed bacteria to potentially recover from any damage they had incurred. Furthermore, histologic sections stained using the Brown and Brenn method and scanning electron microscope (SEM) examinations have revealed that only a small number of dentinal tubules are infiltrated by bacteria even after an extended period of incubation (23). In the present study, all samples were incubated for a total of 6 days and the presence of bacteria in dentin tubules was verified by SEM examination.

Various methods have been used to evaluate the effectiveness of endodontic irrigation solutions. The dentin block model was one of the first and most widely used methods for an in-vitro examination to evaluate the antimicrobial efficacy of irrigation solutions (16, 22, 24). Ma et. al (16) evaluated the effectiveness of several disinfecting solutions by using a noninvasive CLSM method and found that NaOCl and Qmix had higher antimicrobial effects deep into dentin tubules than the other solutions. In a previous study, the antimicrobial activity of a 2.5% NaOCl/9% etidronic acid (HEBP) solution on *E. faecalis* was assessed and it was shown that HEBP did not affect the activity of NaOCl to eliminate *E. Faecalis* located in dentinal tubules (22). In the present study, the dentin blocks were contaminated with *E. faecalis* and the effectiveness of the solutions was evaluated by using culturing method.

The successful and long prognosis of the teeth with endodontic treatment is widely dependent on the disinfection of pathogen microorganisms and the elimination of bacterial biofilm from the root canal system. *E. faecalis* which is a facultative anaerobe is one the most commonly isolated bacteria from the root canal system of unsuccessful endodontic cases. *E. faecalis* in the form of biofilm The extracellular polysaccharide matrix produced by biofilm, makes the bacteria more resistant to antimicrobial agents (25). NaOCl is the most effective irrigating solution with its strong disinfection property and dissolution capacity of necrotic debris and other organic materials (26). However, its cytotoxic effect and causing metal corrosion, bad taste and smell noticed by the patient during root canal treatment, risk of injury to the oral mucosa, skin, and eye area, obstruction, and sensitivity of the airway are the main risks and disadvantages of NaOCl (26, 27).

Chlorhexidine is also a popular irrigation solution in endodontics with its property of broadspectrum antimicrobial effect. The previous study (28). showed that CHX exhibited a well antibacterial effect on *E. faecalis* followed by NaOCl. However, there was no agreement on the antimicrobial efficacy of CHX against NaOCl in recent studies because of the contradictory results (29). Therefore, although CHX has considerably lower toxicity than NaOCl (30), in the previous studies it was shown that CHX is a cytotoxic agent irrelevant to the cell type by disrupting the cell membranes (31, 32). The cytotoxic effects on stem cells from human exfoliated deciduous teeth (SHED) cells were also demonstrated by using CHX as an irrigation solution in different concentrations (32). It was known that, at an effective antimicrobial concentration range, HOCl which is produced by immune cells has a lower cytotoxic effect on mammalian cells when compared with NaOCl and H_2O_2 (33).

The efficacy of HOCl on *Staphylococcus aureus* growing as a biofilm on glass (34), *S. epidermidis and P. aeruginosa* biofilms (35), biofilms in chronic wounds (36) and bacteria in biofilms formed by tested ocular pathogens (37) were indicated in the previous studies. However, there is not any study that evaluates the disinfection capacity of HOCl on dentinal tubules. In the present study, at the end of the waiting period of 3 min, it was observed that HOCl showed similar antimicrobial activity to CHX. In the previous studies, the inactivation capacity of 200 ppm HOCl against coronavirus, noroviruses, and other enteric viruses in less than 1 min was shown (18, 38). This can be explained by the fact that biofilms reduce the effectiveness of antimicrobial agents.

In the previous review article, it was shown that although the neutral chlorine-containing solutions have an antimicrobial effect, this antimicrobial capability is lower than NaOCl concentrations used in daily practice in endodontic treatment (39). Furthermore, the modification of pH values below 7.5 decrease the dissolution capability of organic tissue (39). The other usage of HOCl in dentistry is as a chemotherapeutic

agent for the treatment of periodontitis (40). HOCl plays an important role that activating tyrosine kinase signaling cascades, generating an increase in the production of inflammatory mediators, and growth factors (40). Chen et. al (20) evaluated the antibacterial activity of HOCL, NaOCl, and CHX on the titanium alloy surfaces of biofilm-contaminated implants. It was indicated that all these irrigants showed an antibacterial effect and killed the majority of bacteria. However, HOCl significantly lowered the LPS concentration of P. gingivalis when compared with the other irrigants. Besides all the advantages of HOCL, the difficulty of storage, short expiration date, and having a proper generator to possess HOCl are the disadvantages of this strong oxidizing agent (41).

Various approaches, such as molecular techniques, colorimetric techniques, bioluminescence, , and microscopic techniques (SEM, and CLSM) are employed to assess the antibacterial efficacy of different disinfection protocols in endodontics (42). Nevertheless, these approaches have some limitations. Morphological and structural features of microbial biofilms can be identified by capturing high-resolution images of the root canal surface using SEM analysis, however it is not possible to ascertain the viability of the bacterial cells (38). Furthermore, SEM analysis does not allow for the visualization of the multi-layered biofilm in three dimensions. Fluorescent dyes in CLSM analysis enable visualization of the three-dimensional structure and geographical distribution of biofilm (14). They also facilitate the assessment of quantitative parameters including the ratio of live to dead bacteria and biofilm bio-volume (14). However, previous suggestions indicate that the contents of root canal samples may contain autofluorescent and detrital components that could be mistaken for bacteria. Additionally, background fluorescence was sometimes seen in the canal lumen (16). In addition, using culture methods to evaluating the antimicrobial effect of an irrigation solutions on dentin blocks have some limitations such as that the exact part of the root canal space in which the recovered bacteria comes from can not be determined; the survived bacteria remained from residual biofilms attached to dentinal tubules on root canal walls.

5. CONCLUSION

Within the limitations of this in vitro model, HOCl has a success rate of antimicrobial effect of more than 90% at the end of the 3 min duration on dentinal tubules that biofilm-contaminated dentin discs. Further studies evaluating the disinfection capacity and antimicrobial effect of HOCl for endodontic treatment are needed. Therefore, the use of HOCl in regenerative endodontic treatments, which are frequently applied in today's endodontics, should be investigated by searching the cytotoxic effect on stem cells.

Funding: *The authors received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Akdeniz University Faculty of Medicine (KAEK-904)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: SK, DK, AK
Design of the study: SK, DK, AK, NB, KY
Acquisition of data for the study: SK, NB
Analysis of data for the study: SK, AK, AYÇ
Interpretation of data for the study:SK, AK
Drafting the manuscript: SK, AK
Revising it critically for important intellectual content: AK, AYÇ
Final approval of the version to be published: SK, DK, AK, NB, KY, AYÇ

REFERENCES

[1] Nair P, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol. 2005;99(2):231-52. https://doi.org/10.1016/j.tripleo.2004.10.005

[2] Shuping GB, Ørstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod. 2000;26(12):751-5. https://doi.org/10.1097/00004.770.200012000-00022

[3] Winter J, Ilbert M, Graf P, Özcelik D, Jakob U. Bleach activates a redox-regulated chaperone by oxidative protein unfolding. Cell. 2008;135(4):691-701. https://doi.org/10.1016/j.cell.2008.09.024

[4] Suman R, Javaid M, Haleem A, Vaishya R, Bahl S, Nandan D. Sustainability of Coronavirus on different surfaces. J Clin Exp Hepatol. 2020;10(4):386-90. https://doi.org/10.1016/j.jceh.2020.04.020

[5] Chen C, Zhang X-J, Wang Y, Zhu L-X, Liu J. Waste water disinfection during SARS epidemic for microbiological and toxicological control. Biomed Environ Sci. 2006;19(3):173-8.

[6] Block MS, Rowan BG. Hypochlorous acid-a review. Oral Maxillofac Surg. 2020;78(9):1461-6. https://doi.org/10.1016/j.joms.2020.06.029

[7] United States Environmental Protection Agency. About List N: Disinfectants for Coronavirus (COVID-19) https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2., LAST UPDATED ON MAY 24, 2022.

[8] Miller WD. The micro-organisms of the human mouth: the local and general diseases which are caused by them. SS White Dental Mfg. Company; 1890.

[9] Haapasalo M, Ørstavik D. In vitro infection and of dentinal tubules. J Dent Res. 1987;66(8):1375-9. https://doi.org/10.1177/002.203.4587066.008.1801

[10] Zapata RO, Bramante CM, de Moraes IG, Bernardineli N, Gasparoto TH, Graeff MS, et al. Confocal laser scanning microscopy is appropriate to detect viability of Enterococcus faecalis in infected dentin. J Endod. 2008;34(10):1198-201. https://doi.org/10.1016/j.joen.2008.07.001

[11] Parmar D, Hauman C, Leichter J, McNaughton A, Tompkins G. Bacterial localization and viability assessment in human ex vivo dentinal tubules by fluorescence confocal laser scanning microscopy. Int Endod J. 2011;44(7):644-51. https://doi.org/10.1111/j.1365-2591.2011.01867.x

[12] Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading

dentinal tubules. J Endod. 2004;30(11):778-81. https://doi.org/10.1097/00004.770.200411000-00007

[13] Akdere, S. K., Aydin, Z. U., Erdönmez, D. Antimicrobial effectiveness of different irrigation activation techniques on teeth with artificial internal root resorption and contaminated with Enterococcus faecalis: a confocal laser scanning, icroscopy analysis. Lasers Med Sci. 2023;*38*(1), 89. https://doi.org/10.1007/s10103.023.03748-8

[14] Swimberghe R, Coenye T, De Moor R, Meire M. Biofilm model systems for root canal disinfection: a literature review. Int Endod J. 2019; 52(5):604–628. https:// doi. org/10. 1111/iej. 13050

[15] Shen Y, Stojicic S, Haapasalo M. Bacterial viability in starved and revitalized biofilms: comparison of viability staining and direct culture. J Endod. 2010;36(11):1820-3. https://doi.org/10.1016/j.joen.2010.08.029

[16] Ma J, Wang Z, Shen Y, Haapasalo M. A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. J Endod. 2011;37(10):1380-5. https://doi.org/10.1016/j.joen.2011.06.018

[17] Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M (2014) Efect of long-term exposure to endodontic disinfecting solutions on young and old Enterococcus faecalis bioflms in dentin canals. J Endod 40(4):509–514. https://doi.org/10.1016/j.joen.2013.11.026

[18] Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect. 2020;104(3):246-51. https://doi.org/10.1016/j.jhin.2020.01.022

[19] Morita C, Nishida T, Ito K. Biological toxicity of acid electrolyzed functional water: Effect of oral administration on mouse digestive tract and changes in body weight. Arch Oral Biol. 2011;56(4):359-66. https://doi.org/10.1016/j.archoralbio.2010.10.016

[20] Chen C-J, Chen C-C, Ding S-J. Effectiveness of hypochlorous acid to reduce the biofilms on titanium alloy surfaces in vitro. Int J Mol Sci. 2016;17(7):1161. https://doi.org/10.3390/ijms17071161

[21] WangZ, ShenY,HaapasaloM. Effectiveness of endodontic disinfecting solutions against young and old Enterococcus faecalis biofilms in dentin canals. J Endod. 2012;38(10):1376–9. https://doi.org/10.1016/j.joen.2012.06.035

[22] Arias-Moliz MT, Ordinola-Zapata R, Baca P, Ruiz-Linares M, Ferrer-Luque CM. Antimicrobial activity of a sodium hypochlorite/etidronic acid irrigant solution. J Endod. 2014;40(12):1999-2002. https://doi.org/10.1016/j.joen.2014.07.031

[23] Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol. 1990;6(4):142–9. https://doi.org/10.1111/j.1600-9657.1990.tb00409.x

[24] Wang D, Shen Y, Hancock RE, Ma J, Haapasalo M. Antimicrobial effect of peptide DJK-5 used alone or mixed with EDTA on mono-and multispecies biofilms in dentin canals. J Endod. 2018;44(11):1709-13. https://doi.org/10.1016/j.joen.2018.07.018

[25] Wang D, Shen Y, Ma J, Hancock RE, Haapasalo M. Antibiofilm effect of D-enantiomeric peptide alone and combined with EDTA in vitro. J Endod. 2017;43(11):1862-7. https://doi.org/10.1016/j.joen.2017.06.037

[26] Golob BS, Olivi G, Vrabec M, El Feghali R, Parker S, Benedicenti S. Efficacy of photon-induced photoacoustic streaming in the reduction of Enterococcus faecalis within the root canal: different settings and different sodium hypochlorite concentrations. J Endod. 2017;43(10):1730-5. https://doi.org/10.1016/j.joen.2017.05.019

[27] Afhkami F, Ahmadi P, Chiniforush N, Sooratgar A. Effect of different activations of silver nanoparticle irrigants on the elimination of Enterococcus faecalis. Clin Oral Invest. 2021;25:6893-9. https://doi.org/10.1007/s00784.021.03979-5

[28] Sinha DJ, Nandha KD, Jaiswal N, Vasudeva A, Tyagi SP, Singh UP. Antibacterial effect of Azadirachta indica (neem) or Curcuma longa (turmeric) against Enterococcus faecalis compared with that of 5% sodium hypochlorite or 2% chlorhexidine in vitro. Bull Tokyo Dent Coll. 2017;58(2):103-9. https://doi.org/10.2209/tdcpublication.2015-0029

[29] Ruksakiet K, Hanák L, Farkas N, Hegyi P, Sadaeng W, Czumbel LM, et al. Antimicrobial efficacy of chlorhexidine and sodium hypochlorite in root canal disinfection: a systematic review and meta-analysis of randomized controlled trials. J Endod. 2020;46(8):1032-41 https://doi.org/10.1016/j.joen.2020.05.002

[30] Tatnall F, Leigh I, Gibson J. Comparative study of antiseptic toxicity on basal keratinocytes, transformed human keratinocytes and fibroblasts. Skin Pharmacol Physiol. 1990;3(3):157-63. https://doi.org/10.1159/000210865

[31] Lee TH, Hu CC, Lee SS, Chou MY, Chang YC. Cytotoxicity of chlorhexidine on human osteoblastic cells is related to intracellular glutathione levels. Int Endod J. 2010;43(5):430-5. https://doi.org/10.1111/j.1365-2591.2010.01700.x

[32] Tu Y-Y, Yang C-Y, Chen R-S, Chen M-H. Effects of chlorhexidine on stem cells from exfoliated deciduous teeth. J Formos Med Assoc. 2015;114(1):17-22. https://doi.org/10.1016/j.jfma.2012.12.008

[33] Wang L, Bassiri M, Najafi R, Najafi K, Yang J, Khosrovi B, et al. Hypochlorous acid as a potential wound care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. J Burns Wounds. 2007;6.

[34] Luppens SB, Reij MW, van der Heijden RW, Rombouts FM, Abee T. Development of a standard test to assess the resistance of Staphylococcus aureus biofilm cells to disinfectants. Appl. Environ. Microbiol. 2002;68(9):4194-200. https://doi.org/10.1128/AEM.68.9.4194-4200.2002

[35] Sandvik EL, McLeod BR, Parker AE, Stewart PS. Direct electric current treatment under physiologic saline conditions kills Staphylococcus epidermidis biofilms via electrolytic generation of hypochlorous acid. PloS one. 2013;8:e55118. https://doi.org/10.1371/journal.pone.0055118

[36] Claudio Milanese M. A new acid-oxidizing solution: assessment of its role on methicillin-resistant Staphylococcus aureus (MRSA) biofilm morphological changes. Wounds. 2015;27(10):265-73.

[37] Romanowski EG, Stella NA, Yates KA, Brothers KM, Kowalski RP, Shanks RM. In vitro evaluation of a hypochlorous acid hygiene solution on established biofilms. Eye Contact lens. 2018;44(2):S187. https://doi.org/10.1097/ICL.000.000.0000000456

[38] Park GW, Boston DM, Kase JA, Sampson MN, Sobsey MD. Evaluation of liquid-and fog-based application of Sterilox hypochlorous acid solution for surface inactivation of human

norovirus. Appl Environ Microbiol. 2007;73(14):4463-8. https://doi.org/10.1128/AEM.02839-06

[39] Rossi-Fedele G, Guastalli AR, Doğramacı E, Steier L, De Figueiredo J. Influence of pH changes on chlorine-containing endodontic irrigating solutions. Int Endod J. 2011;44(9):792-9. https://doi.org/10.1111/j.1365-2591.2011.01911.x

[40] Mainnemare A, Megarbane B, Soueidan A, Daniel A, Chapple I. Hypochlorous acid and taurine-N-monochloramine in periodontal diseases. J Dent Res. 2004;83(11):823-31. https://doi.org/10.1177/154.405.910408301101

[41] Kameda T, Oka S, Igawa J-I, Sakamoto M, Terada K. Can hypochlorous acid be a powerful sanitizer to replace alcohol for disinfection?-Its bactericidal, degradation of the solutions under various storage condition, and steel rust effects. Dent Mat J. 2022;41(1):167-83. https://doi.org/10.4012/dmj.2021-146

[42] Basrani B (2015) Endodontic irrigation: chemical disinfection of the root canal system. Springer International Publishing, Switzerland.

How to cite this article: Koç S, Kırıcı D, Kuştarcı A, Bulut N, Yılmaz K, Çoban AY. Antimicrobial Effectiveness of Hypochlorous Acid in Infected Dentin Tubules: A Pilot Study. Clin Exp Health Sci 2025; 15: 56-62. DOI: 10.33808/clinexphealthsci.1414218

Clinical and Experimental Health Sciences

A Questionnaire-Based Study on Use of Plants in Diabetic Patients

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Received: 05.02.2024 **Accepted:** 05.03.2025

ABSTRACT

Objective: Among patients diagnosed with diabetes mellitus, we aimed to investigate the rates of use of medicinal plants, detailed information on use and purchase, socio-demographic characteristics associated with the tendency to use plants, and the status of reporting to health care professionals.

Methods: The study was conducted in family health centers where patients diagnosed with diabetes mellitus visited. A questionnaire form of objective questions was filled for patients. The questionnaire included socio-demographic characteristics (age, gender, education) of the patients, disease condition, medicines used, and whether or not they used plants. If so, more information was obtained on the plants that are the name, used part, preparation method, frequency of administration, the place from which the plants was obtained, the person who advised the product, the knowledge of the physician and his/her attitude about herbals.

Results: 100 people (66% women, 34% men) participated in the study between the ages of 24-80, 13% of them have Type 1 and 87% have Type 2 diabetes. Only 11% of patients use plants. Eight plants have been identified. The most commonly used plant (36.36%) is cinnamon. Among the usage of the plants, decoction is placed on the top, with 78%. 62.5% of the patients obtain the plants from herbal shops. Friends or relatives are the primary sources of information regarding medicinal plant use (73%).

Conclusion: By increasing the knowledge of physicians about plants, it has been seen that patients can share their usage of plants more easily with physicians.

Keywords: Diabetic patients, medicinal plant, traditional medicine, Türkiye

1. INTRODUCTION

Diabetes is a severe, chronic disease that develops when the pancreas produces insufficient insulin or when the body is unable to use the insulin that it does produce adequately. Diabetes is a major public health issue, with both the number of cases and the prevalence of diabetes continuously rising over the last several decades. Diabetes can lead to complications in many parts of the body, including heart attack, stroke, kidney failure, leg amputation, eyesight loss, and nerve damage (1). The most common type of diabetes is type 2 diabetes which accounts for more than 90% of all diabetes globally. In 2021, diabetes was estimated to affect 537 million adults. Diabetes was the cause of more than 6.7 million deaths (2).

The burden of disease is gradually increasing today as the elderly population rises, and alternative treatment approaches for diseases are gaining importance. Over the last decade, there has been an increasing interest in the use of traditional medicine around the world (3). According to World Health Organization data, nearly 4 billion people worldwide attempt to solve their health problems with herbal drugs in the first place (4). Today, complementary and alternative medicine (CAM) is favored as a supportive treatment to decrease the side effects of medications used to treat chronic diseases and interactions that may occur when multiple drugs are used. In Europe, between 20 to 65% of the general population uses complementary and alternative medicine and herbal medicines are one of the most popular CAM methods among patients with chronic diseases (5, 6).

Medicinal plants have been used since ancient times in the treatment of diabetes. In the Egyptian Papyruses, the works of Hippocrates, and Chinese medicine and Ayurveda texts, plants were mentioned for diabetic treatment. Metformin, which has been used successfully in the treatment of diabetes for the last fifty years, is obtained from *Galega officinalis* L. (7). More than 1200 organisms (such as marine algae, fungi, plants) have been utilized ethnopharmacologically

https://doi.org/10.33808/clinexphealthsci.1431781

or experimentally to treat symptoms of diabetes. Fabaceae, Asteraceae, and Lamiaceae are the most frequently cited plant families for diabetes. In antidiabetic activity studies conducted on plants traditionally used against diabetes, the effect was found in 81% of the studied plants, while this rate dropped to 47% in activity studies on randomly selected plants (8). The possible mechanisms of action of plants in diabetes include inhibition of α-glucosidase and α-amylase, the effects on glucose uptake and glucose transporters, the enhancement of insulin secretion and of pancreatic β-cell proliferation, the inhibition of protein tyrosine phosphatase 1B activity and antioxidant activity (9). According to the studies, *Momordica charantia*, *Lagerstroemia speciosa*, *Trigonella foenum-graecum*, *Gymnema sylvestre* are an example of a hypoglycemic effective plant; *Panax quinquefolius*, *Lagerstroemia speciosa*, *Cinnamomum cassia* for those with increased insulin sensitivity; and *Plantago ovata*, *Amorphophallus konjac*, *Trigonella foenum-graecum* for those that inhibit carbohydrate absorption (10).

The objective of this study is to determine the use of medicinal plants in diabetic patients, including detailed usage, plant supply, source of usage information, socio-demographic features, and notifying the physician about herbal uses. In addition, it will help to raise health professionals' awareness by making determinations about the usage of plants in diabetic patients.

2. METHODS

2.1. Ethical Considerations

Patients diagnosed with diabetes mellitus (DM) who visited the family health center (in İstanbul) were informed about the study. Only individuals who agreed to participate and signed a consent form were included. Informed consent was obtained from all patients. All principles of the Helsinki declaration were followed throughout the study. The study was considered ethically appropriate by the Istanbul University Istanbul Faculty of Medicine, Clinical Research Ethics Committee (09.12.2016/21).

2.2. Study Design and Sampling

This survey-based study was conducted with 100 patients diagnosed with DM between February and May 2017. A questionnaire form with objective questions was filled for patients who visited family health centers in Fatih district, İstanbul. Patients over 18 years of age with diabetes were included in this study.

2.3. Questionnaire

The questionnaire included the socio-demographical characteristics (age, gender, education) of the patients, disease condition, medicines used, whether or not they use plants. If so, more information was obtained on the plants that are the name, used part, preparation method, frequency of administration, the place from which the plant was obtained, the person who advised the product, the knowledge of the physician and his/her attitude about herbals. The studies in the literature were investigated during survey design, and questions about diabetes were constructed for this study (11, 12).

2.4. Data Analysis

Data were analyzed using PAST software. Both chi-square analysis and Fisher's exact test was used to determine the statistical significance of differences between groups. Chi-square test was used for comparison of nonnumerical data. Fisher's exact test was used due to smaller sample sizes. A p value less than .05 was taken to indicate statistical significance.

Referred plants by the patients were bought from the local herbal market in the study district and the scientific name was identified as far as possible. The plants were identified by Assoc. Prof. Dr. Bahar Gürdal. A literature search on antidiabetic activities of identified plants was made.

3. RESULTS

100 patients aged between 24 and 80 years (average age 61.08 ± 9.85) participated in the study. Male/female ratio was 34/66. Forty-six patients (46%) had completed primary school, 8 patients (8%) intermediary school, 14 patients (14%) high school, 14 patients (14%) university and 18 patients (18%) had no education (Table 1).

Table 1. *Socio-demographic characteristics of the participants*

Variable	Frequency (%)	Plants User	
		Yes (%)	No (%)
Gender (p>0.05)			
female	66 (66%)	9 (13.6%)	57 (86.4%)
male	34 (34%)	3 (8.8%)	31 (91.2%)
Age (p>0.05)			
<40	2 (2%)	–	2 (100%)
40-50	9 (9%)	2 (22.2%)	7 (77.8%)
51-60	40 (40%)	2 (5%)	38 (95%)
>60	49 (49%)	7 (14%)	42 (86%)
Education (p>0.05)			
no education	18 (18%)	1 (5.5%)	17 (94.5%)
primary school	46 (46%)	7 (15%)	39 (85%)
intermediary school	8 (8%)	–	8 (100%)
high school	14 (14%)	2 (14%)	12 (86%)
university	14 (14%)	1 (7%)	13 (93%)

11% of female patients have Type 1 DM and 55% of them have Type 2 DM. This percentage for males is 2% Type 1 and 32% Type 2 diabetics. 44% of the patients have DM history in their family and 56% of them don't have. Duration of the diagnosis of DM and the duration of treatment were 0-3

years in 31%, 4-10 years in 46%, 11-20 years in 19% and more than 20 years in 4% patients. Only diabetes is diagnosed in 20% of patients. Besides DM, 23% of the patients have hypertension, 8% have hypertension and cardiac disease, 6% have hypertension and hyperlipidemia. Other 43% suffer from more than 2 diseases (osteoporosis, asthma, rheumatism, chronic obstructive pulmonary disease, gout, goiter, prostatitis, cancer, glaucoma) except DM.

Of the 100 patients surveyed, 11% said that they used plants and 89% did not use plants. Male/female ratio of the plant users was 3/9. As a result, the use of eight plants are documented, these are used in single or mixed by diabetic patients (Table 2). Plants are cinnamon (4 patients), olive leaves (one patient), pomegranate flower/lemon (one patient), cinnamon / olive leaves (3 patients), mint/ oregano (one patient) and cinnamon bark/ mahaleb/ black cumin (one patient).

Table 2. *List of the plants and their used part*

Plant name	Latin name of plant	Used part
Black cumin	*Nigella sativa* L.	Seed
Cinnamon	*Cinnamomum cassia* (L.) J.Presl	Bark
Lemon	*Citrus limon* (L.) Osbeck	Fruit juice
Mahaleb	*Prunus mahaleb* L.	Seed
Mint	*Mentha × piperita* L.	Leaves
Olive	*Olea europaea* L.	Leaves
Oregano	*Origanum onites* L.	Leaves
Pomegranate	*Punica granatum* L.	Flower

The patients haven't provided plant samples which are used by them. The scientific (botanical) names of the cinnamon, oregano, and mint specified by the patients could not be exactly determined. Referred plants were purchased from herbal shops in the study district and identified as *Cinnamomum cassia* (L.) J.Presl, *Origanum onites* L., *Mentha × piperita* L. It is thought that patients also mentioned these plants. As the species used in other plants (olive, pomegranate, lemon, mahaleb, black cumin) are already known, no samples were obtained from them. The answer to the question 'Where do you obtain plants?' was from herbal shops (6 patients), friends/relatives (3 patients), nature/ own garden (1 patient), and pharmacy (1 patient) (Figure 1). Preparation methods are decoction (7 patients), infusion (one patient) and mixing with yogurt (one patient) (Figure 2). The answer to the question 'How long have you been using these plants for your DM?' was within 1-5 years (6 patients), more than 5 years (2 patients). Three patients could not state a clear period.

Friend or relative were the primary sources of information regarding medicinal plant use (73%). This is followed by the internet (18%) and television programs (9%) (Figure 3). Six patients didn't inform their physician about using plants beside those five patients informed. When asking 'How was your physician's approach to you about your plant usage?' 46% of patients reported that physicians didn't have knowledge about herbal medicine; 18% of patients declared that physicians avoided commenting; 27% of the patients stated that physicians informed and motivated the utilization; 9% of the patients reported that physicians opposed herbal medicines (Table 3).

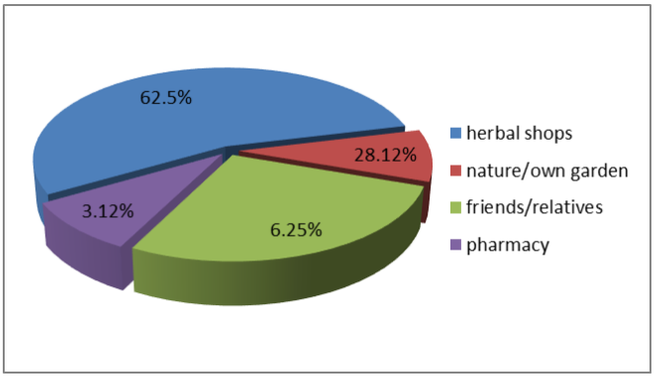

Figure 1. *The way of obtaining plants*

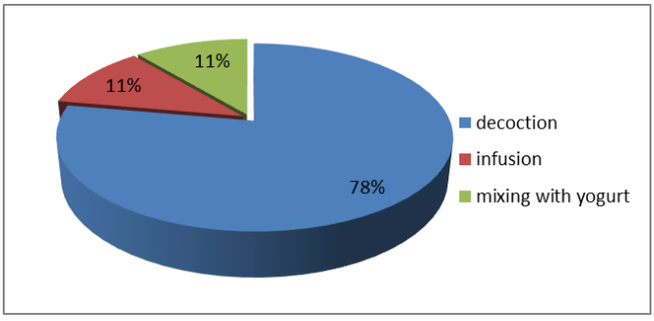

Figure 2. *Preparation methods of plants used*

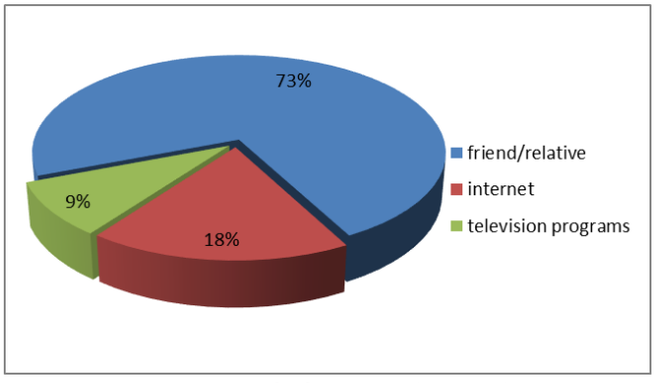

Figure 3. *The main source of information about medicinal plant use*

Table 3. *Physician's approach to plant usage*

Variable		n (%)
Informing to physician	Yes	5 (45%)
	No	6 (55%)
Physician's approach about plant usage	Opposed	1 (9%)
	Avoided commenting	2 (18%)
	Informed and motivated the utilization	3 (27%)
	Not have knowledge	5 (46%)

4. DISCUSSION

Herbal medicines are sold and accessible at herbal stores and supermarkets as well as pharmacies. In most instances, these herbal medicines are made available to patients without a prescription, and possible adverse reactions, contraindications, and interactions with prescription medications and foods are rarely identified (13). Studies on the usage of herbal products are crucial for revealing the situation and increasing awareness among health professionals and patients. There are only a few studies on the use of plants by diabetic patients in Turkey, and this study will make a valuable contribution to this field.

In our study, the number of patients who used plants for diabetes was 11%. There was no relationship between medicinal plants use with gender, age, and education. In the related surveys which were done in Turkey, different use ratios were reported. Ceylan et al. (14) determined that 41% of diabetic patients used at least one of CAM practice in Ankara. Sex, age, marital status, occupation, and monthly income level were not found to be significant factors ($p > 0.05$). Duration of diabetes in the patients, educational status, birthplace, and family type were found to be significant factors in using CAM ($p < 0.05$). The most preferred practice was the use of herbal medicine (88.1%), followed by acupuncture and meditation practices (5.3%). Öztürk et al. (12) investigated the use of herbal products in type 2 DM patients in Istanbul and reported 52.1% of patients used herbal products. There was no correlation between herbal products usage, its frequency, the type of product, and the advisor and gender, marital status, or educational status. In another study about herbal medicine use among DM patients in Northern Cyprus, they reported 32% of patients used herbal medicine (11). All of the patients declared that the plants they used had a positive effect on them. Soner et al. (15) investigated the uses of herbal medicine in a population of Turkish hospital patients. 48.8% of the participants used herbal medicines and the majority of patients used them to stay healthy. Although there was no linear relationship between age groups and HM usage, there was a clear linear relationship between educational level, monthly family income, and HM use.

There are several studies on herbal medicine usage among diabetic patients in different countries. According to interviews with type 2 diabetes patients, 58% of them reported the use of herbal medicine in North Sudan (16). Gender, education, diabetes duration, and family income were all significant characteristics associated with herbal medicine use. Black seed, cinnamon, olive, and fenugreek were the most commonly used plants. The first three plants were also recorded in our study. As in our study, family and friends were the primary sources of information about medicinal plant use. The majority of patients (63.8%) did not notify their physicians about utilizing herbs. This rate is 55% in our study. Ali-Shtayeh et al. (17) evaluated the CAM uses of Palestinian diabetic patients. 51.9% of the patients used herbs. No statistically significant association was found between plant uses and gender, age, marital status, educational level, presence of other chronic diseases, presence of other diabetic family members, diabetes type or duration of diabetes. All plants except mahaleb and mint, which were mentioned in our study, were also reported in that study. The primary sources of plant recommendations were the same to those found in our study. Due to the widespread use of bush medicine in Trinidad and Tobago, Mahabir and Gulliford (18) investigated the use of bush medicine by diabetic patients there. In the Caribbean, herbal remedies derived from medicinal plants are referred to as “bush medicines.” of patients 42% stated using bush medicines and 58% of bush medicine users reported using it for diabetes. In the survey, 103 plants were identified. There were no significant differences between bush medicine use with age and gender. Al-Asadi and Salih (19) assessed the prevalence of herbal remedy use among diabetes patients in Nassyria (Iraq). Only 17.3% of respondents utilized herbal remedy. As in our study, the rate of medicinal plant use was found to be low. Cinnamon and black cumin were the two most commonly used plants. These two plants were found in our results as well. Other plants used were identified as aloe, fenugreek, wormwood, bitter apple, Syrian rue, and garlic. Patients with a higher degree of education were shown to be more frequent plant users. Only 5.9% of plant users disclosed their usage to their physician. This ratio was 45% in our study. Plant uses were mainly recommended to patients by their friends. In Oriental Morocco, uses of herbal medicines among diabetic patients were evaluated (20). Plant usage was reported to be 54.8% in diabetic patients. *Salvia officinalis* L., *Trigonella foenum-graecum* L., *Olea europaea* L., *Artemisia herba-alba* Asso, and *Origanum vulgare* L. were the most commonly used plants. 60% of plant usage has been recorded as a mixture of plants (more than one plants combined). Diabetic patients used single or mixed plants in our study as well. The source on plant use coincides with our study as friends/family and media. In Jordan, the usage of herbal medicine by diabetes patients evaluated and 16.6% of the participants stated that they used plants (21). Plants used by diabetic patients were determined to be green tea, aniseed, ginger, chamomile, sage, fenugreek, blackseed, white lupin, germander, garlic, cinnamon, and olive leaves. Three plants overlap with our study. In Kanpur division (India), Dixit and Tiwari (22) interviewed with 44 healers to determine anti-diabetic plants. Thirty-five species used by healers for the treatment of diabetes have been identified. The most often used plants were *Aloe vera* L., *Syzygium cumini* L., *Momordica charantia* L., *Phyllanthus emblica* L., *Ocimum sanctum* L., *Trigonella foenum-graceum* L., *Catharanthus roseus* (L.) G.Don, *Carica papaya* L., *Allium sativum* L., *Cassia fistula* L., *Allium cepa* L., and *Tecoma stans* (L.) Juss. ex Kunth.

In our study, eight plants used by diabetic patients were determined. *In vitro* and *in vivo* studies have shown that some of them have anti-diabetic properties. *Nigella sativa* seeds contain essential and fixed oils, alkaloids, proteins, and saponin. The main fatty acids are linoleic acid, palmitic

acid, and oleic acid. The major compounds of its essential oil are thymoquinone, trans-anethole, p-cymene, and α-pinene (23, 24). Different mechanisms of antidiabetic effect of *Nigella sativa* have been shown such as it reduces appetite, glucose absorption in intestine, blood glucose level, hepatic gluconeogenesis, triglycerides, cholesterol, body weight and simulates glucose induced secretion of insulin from beta-cells in pancreas. Its *in vivo* antihyperglycemic effects have been attributed to insulinotropic and insulin-like properties (25, 26). Major compounds of *Cinnamomum cassia* are determined as cinnamaldehyde, coumarin and essential oils. Because o-hydro-xyphenylacetaldehyde (o-HPA), a coumarin metabolite, is hepatotoxic, long-term intake of *C. cassia* may pose a health risk (27, 28). Kumar et al. (29) investigated antidiabetic activity of *C. cassia* on streptozotocin-induced diabetic rats. They prepared de-coumarinated water-soluble polyphenol-rich extracts of *C. cassia* and compared with a standard aqueous cinnamon extract. When compared to an aqueous cinnamon extract, polyphenol-rich *C. cassia* extracts significantly improved blood sugar, serum insulin, lipid profile, and liver antioxidant enzymes. Another study evaluated blood glucose and plasma insulin levels in rats given extracts from *Cinnamomum cassia* and *C. zeylanicum* (30). The *C. cassia* extract was found to be superior to the *C. zeylanicum* extract. Plasma insulin levels were raised. Extracts showed that insulin stimulatory effect. Al-Baidhani et al. (31) investigated the activity of herbal prescriptions which are used for diabetes. Therefore α-amylase and α-glucosidase inhibitory activities were studied. Prescriptions included mix plants. Prescription, which contains extracts of *Prunus mahaleb* L. and *Prunus dulcis* (Mill.) D.A.Webb, was shown to be the most effective α-amylase and α-glucosidase inhibitor. Ethanol extract of olive leaves was investigated in streptozotocin-induced diabetic rats. The antidiabetic activity of olive leaves was found to be more effective than that of glibenclamide (32). In another study, the antidiabetic effect of olive leaves was shown *in vivo* (33). As a result, improvement in glucose levels and in levels of inflammatory and metabolic markers was demonstrated. Huang et al. (34) evaluated *in vivo* antidiabetic activity of *Punica granatum*. Its flower extract showed that it inhibited the glucose loading-induced increase of plasma glucose levels, enhanced cardiac PPAR-g mRNA expression, and restored the down-regulated cardiac glucose transporter (GLUT)-4 mRNA. Additionally, gallic acid is found to be mostly responsible for this activity. Bagri et al. (35) studied the antidiabetic effect of *Punica granatum* flowers on streptozotocin-induced diabetic rats and aqueous extract was used. It showed benefits in the control of diabetes, abnormalities in lipid profiles, and oxidative stress through the activation of pancreatic antioxidant enzymes.

Plants used by the public have the potential to become drugs if they are supported by *in vitro*/*in vivo* activity studies and their effects are proven by clinical studies. Medicinal plants can help prevent diabetes and its complications, as well as improve treatment and quality of life. The important point is that when using plants in chronic diseases, the side effects, and interactions should be evaluated by the physician, and the importance of patient-physician cooperation.

5. CONCLUSION

Both in our study and in other studies, friends and relatives were indicated as the first source of recommending herbal products. There may be caused potential interactions and side effects. Prescription drugs are used in diabetes and also other chronic diseases. Therefore, accurate assessment of drug-herb interactions is critical. This requires the physician to be aware of the usage of herbal products. These studies provide important contributions to the determination of the use of herbal products by the patients. It contributes to raising awareness in both patients and physicians.

Acknowledgement: *We thank all the patients who participated in this survey and shared valuable information.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Clinical Research Ethics Committee of Istanbul University Pharmacy Faculty, (approval date 15.12.2016 and number 2016/1420)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: BG
Design of the study: BG
Acquisition of data for the study: BG, BT
Analysis of data for the study: BG, BT
Interpretation of data for the study: BG, BT
Drafting the manuscript: BG, BT
Revising it critically for important intellectual content: BG, BT
Final approval of the version to be published: BG, BT

REFERENCES

[1] WHO. Global report on diabetes. World Health Organization, 2016.
[2] IDF. IDF Diabetes Atlas 10th edition. International Diabetes Federation. Brussels, Belgium, 2021. https://www.diabetesatlas.org
[3] WHO. Traditional medicine. Fifty-Sixth World Health Assembly, A56/18, 14.10, 31 March 2003.
[4] WHO. Noncommunicable Diseases. World Health Organization 2018. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases
[5] Çakmak S, Nural N. Complementary and alternative medicine applications in chronic diseases. J Intern Med Nurs-Special Topics 2017;3(2):57-64.
[6] Ernst E, Fugh-Berman A. Complementary and alternative medicine: What is it all about? Occup Environ Med. 2002;59(2):140-144.
[7] Parildar H, Serter R, Yesilada E. Diabetes mellitus and phytotherapy in Turkey. J Pak Med Assoc. 2011;61(11): 1116.
[8] Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine 1995;2(2):137-189. https://doi.org/10.1016/S09447113(11)80059-0
[9] Governa P, Baini G, Borgonetti V, Cettolin G, Giachetti D, Magnano AR, Miraldi E, Biagi M. Phytotherapy in the

management of diabetes: A review. Molecules 2018;23(1):105. https://doi.org/10.3390/molecules23010105

[10] Aslan M, Orhan N. Diyabet tedavisinde kullanılan bitkisel ürünler ve gıda destekleri. MİSED 2010;23-24:27-38.(Turkish)

[11] Özkum D, Akı Ö, Toklu HZ. Herbal medicine use among diabetes mellitus patients in Northern Cyprus. J Med Plant Res. 2013;7(22):1652-1664. https://doi.org/10.5897/JMPR12.1207

[12] Öztürk S, Gündoğdu YP, Gürsu M, Yamak M, Özkan O, Şar F, Yenigün M, Kazancıoğlu R. Use of herbal products in type 2 diabetic patients. Medical Bulletin of Haseki 2015;53(3):214-219.

[13] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. https://doi.org/10.3389/fphar.2013.00177

[14] Ceylan S, Azal O, Taşlipinar A, Türker T, Açikel CH, Gulec M. Complementary and alternative medicine use among Turkish diabetes patients. Complement Ther Med. 2009;17:78-83. https://doi.org/10.1016/j.ctim.2008.07.003

[15] Soner BC, Sahin AS, Sahin TK. A survey of Turkish hospital patients' use of herbal medicine. Eur J Integr Med. 2013;5(6):547-552. https://doi.org/10.1016/j.eujim.2013.08.004

[16] Ali BAM, Mahfouz MS. Herbal medicine use among patients with type 2 diabetes in North Sudan. Annu Res Rev Biol 2014;4(11):1827-1838.

[17] Ali-Shtayeh MS, Jamous RM, Jamous RM. Complementary and alternative medicine use amongst Palestinian diabetic patients. Complement Ther Clin Pract. 2012;18(1):16-21. https://doi.org/10.1016/j.ctcp.2011.09.001

[18] Mahabir D, Gulliford MC. Use of medicinal plants for diabetes in Trinidad and Tobago. Rev Panam Salud Publ. 1997;1(3):174-179.

[19] Al-Asadi JN, Salih N. Herbal remedies use among diabetic patients in Nassyria, Iraq. Middle East J Fam Med. 2012;10(10):40-46.

[20] Alami Z, Aynaou H, Alami B, Hdidou Y, Latrech H. Herbal medicines use among diabetic patients in Oriental Morocco. J Pharmacogn Phytotherapy 2015;7(2):9. https://doi.org/10.5897/JPP2014.0338

[21] Wazaify M, Afifi FU, El-Khateeb M, Ajlouni K. Complementary and alternative medicine use among Jordanian patients with diabetes. Complement Ther Clinl Pract. 2011;17(2): 71-75. https://doi.org/10.1016/j.ctcp.2011.02.002

[22] Dixit S, Tiwari S. Investigation of anti-diabetic plants used among the ethnic communities of Kanpur division, India. J Ethnopharmacol. 2020;253:112639. https://doi.org/10.1016/j.jep.2020.112639

[23] Ghosheh OA, Houdi AA, Crooks PA. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa* L.). J Pharm Biomed Anal. 1999;19(5):757-762. https://doi.org/10.1016/S0731-7085(98)00300-8

[24] Nickavar B, Mojab F, Javidnia K, Amoli MAR. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Z Naturforsch C. 2003;58(9-10):629-631. https://doi.org/10.1515/znc-2003-9-1004

[25] Benhaddou-Andaloussi A, Martineau LC, Spoor D, Vuong T, Leduc C, Joly E, Burt A, Meddah B, Settaf A, Arnason JT, Prentki M, Haddad PS. Antidiabetic activity of *Nigella sativa* seed extract in cultured pancreatic β-cells, skeletal muscle cells, and adipocytes. Pharm Biol. 2008;46(1-2):96-104. https://doi.org/10.1080/138.802.00701734810

[26] Mathura ML, Gaura J, Sharmaa R, Haldiya KR. Antidiabetic properties of a spice plant *Nigella sativa*. J Endocrinol Metab. 2011;1(1):1-8. https://doi.org/10.4021/jem12e

[27] Shinjyo N, Waddell G, Green J. A tale of two cinnamons: A comparative review of the clinical evidence of *Cinnamomum verum* and *C. cassia* as diabetes interventions. J Herb Med. 2020;21:100342. https://doi.org/10.1016/j.hermed.2020.100342

[28] Zaidi SF, Aziz M, Muhammad JS, Kadowaki M. Diverse pharmacological properties of *Cinnamomum cassia*: A review. Pak J Pharm Sci. 2015;28(4):1433-1438.

[29] Kumar K, Issac A, Ninan E, Kuttan R, Maliakel B. Enhanced anti-diabetic activity of polyphenol-rich de-coumarinated extracts of *Cinnamomum cassia*. J Funct Foods. 2014;10:54-64. https://doi.org/10.1016/j.jff.2014.05.008

[30] Verspohl EJ, Bauer K, Neddermann E. Antidiabetic effect of *Cinnamomum cassia* and *Cinnamomum zeylanicum in vivo* and *in vitro*. Phytother Res. 2005;19(3):203-206. https://doi.org/10.1002/ptr.1643

[31] Al-Baidhani R, Rezadoost H, Hamidi A, Motevali SM, Mirzajani F. The α-amylase and α-glucosidase inhibitory effects of some traditional antidiabetic prescriptions based on bioautography using LC-ESI/MSMS. J Medicinal Plants. 2020;21(81): 33-50. https://doi.org/10.52547/jmp.21.81.33

[32] Eidi A, Eidi M, Darzi R. Antidiabetic effect of *Olea europaea* L. in normal and diabetic rats. Phytother Res. 2009;23(3): 347-350. https://doi.org/10.1002/ptr.2629

[33] Guex CG, Reginato FZ, de Jesus PR, Brondani JC, Lopes GHH, de Freitas Bauermann L. Antidiabetic effects of *Olea europaea* L. leaves in diabetic rats induced by high-fat diet and low-dose streptozotocin. J Ethnopharmacol. 2019;235:1-7. https://doi.org/10.1016/j.jep.2019.02.001

[34] Huang TH, Peng G, Kota BP, Li GQ, Yamahara J, Roufogalis BD, Li Y. Anti-diabetic action of *Punica granatum* flower extract: activation of PPAR-γ and identification of an active component. Toxicol Appl Pharmacol. 2005;207(2):160-169. https://doi.org/10.1016/j.taap.2004.12.009

[35] Bagri P, Ali M, Aeri V, Bhowmik M, Sultana S. Antidiabetic effect of *Punica granatum* flowers: Effect on hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. Food Chem Toxicol. 2009;47(1):50-54. https://doi.org/10.1016/j.fct.2008.09.058

How to cite this article: Gürdal B, Toprak B. A Questionnaire-Based Study on Use of Plants in Diabetic Patients. Clin Exp Health Sci 2024; 14: 63-68. DOI: 10.33808/clinexphealthsci.1431781

Clinical and Experimental Health Sciences

Effect of Ultraviolet Protective Agents and Plasma Applications on the Color Stability of Maxillofacial Silicones

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Received: February 12, 2024 **Accepted:** March 4, 2025

ABSTRACT

Objective: The purpose of the present study was to evaluate the color stability of maxillofacial silicones after applying plasma and ultraviolet protectives.

Methods: Six different color specimen groups (clear, white, yellow, red, blue, and mixed) from additional polymerized maxillofacial silicone elastomer were prepared. The surface of the polymerized silicone was modified with argon and oxygen plasma. Then, five UV protective agents (benzophenone-3, 2-ethylhexyl salicylate, titanium dioxide, Ethylhexyl methoxycinnamate, and TiO2-ZnO) were applied to the modified silicones. Specimens of each color and UV group were aged with an accelerated aging and thermocycling device. The color difference (ΔE) of maxillofacial silicones was statistically analyzed by 4-way ANOVA (α=0.05).

Results: The silicone specimens coated with 2-ethylhexyl salicylate (UV-ES) showed the lowest ΔE values in all color groups and aging regimes. The red color generally showed the highest ΔE values, and the white color showed the lowest ΔE values. When the silicone surfaces were modified with oxygen and argon plasma, oxygen plasma exhibited significantly higher ΔE values than the argon plasma in red color groups, whereas in yellow color groups, argon plasma exhibited higher ΔE values than the oxygen plasma.

Conclusion: Coating the silicone surface with UV-ES followed by oxygen or argon plasma revealed a positive impact on the color stability of silicone elastomer. Plasma treatment and UV-ES coating may be used to enhance the clinical lifetime of silicone facial prostheses.

Keywords: Color; maxillofacial silicone; UV Protective, plasma, thermocycling

1. INTRODUCTION

All over the world, there are a considerable number of patients with maxillofacial defects resulting from cancer, trauma, or congenital diseases. These patients anticipate high-quality prosthetic reconstructions depending on advanced technology (1,2). A maxillofacial prosthesis with a natural appearance and comfortable facial tissues improves quality of life (3). The esthetic result of a maxillofacial prosthesis is relevant to many patient concerns, while color was the most important determinant of the esthetics of these prostheses (4,5).

Silicone elastomers have been considered favorable materials for maxillofacial prostheses for over 50 years because of their biocompatibility, adequate strength, flexibility, and suitability for intrinsic coloration. Initially, intrinsically colored silicone prostheses can successfully reproduce individual skin color and translucency when manipulated by an experienced and skilled prosthodontist. However, silicone maxillofacial prostheses cannot preserve initial physical properties during clinical use. Deterioration in mechanical and physical properties, discoloration, and retentive substrate delamination frequently occur (6-9). Color change of the prosthesis during usage is the most prominent problem among these complications. After the color change gets through to a recognizable level, the replacement of a maxillofacial prosthesis is required. Clinical observations revealed that the mean lifetime of silicone facial prostheses is up to 2 years (3,10,11).

Color degradation of maxillofacial silicones has been caused by environmental factors, including ultraviolet (UV) light, air pollution, humidity, body secretions, and patients' daily habits, such as cleaning and disinfection processes or smoking. Previous research showed that UV

https://doi.org/10.33808/clinexphealthsci.1398224

light is the most important factor for the color change of maxillofacial prostheses (2-4). To prolonge the longevity of the maxillofacial prostheses by improving color stability, several studies have been performed. These studies include the addition of UV protective chemicals, namely nano-oxides, UV absorbers, UV filters, and hindered amine light stabilizers in the polymer structure (4,5,12-16). Considering previous research, incorporating UV protectives in bulk silicone elastomer during prosthesis fabrication may result in unfavorable material properties. As the material surface is primarily exposed to environmental factors, modifying the surface layer of polymerized material with UV protectives might be a reasonable attempt. Bishal et al. (12) developed and investigated the effectiveness of a technique including coating the surface of a maxillofacial silicone with TiO_2 thin film to enhance the color stability of the material. It was reported that TiO_2 nano-coating reduced discoloration of the maxillofacial silicon compared with non-coated specimens after artificial aging. Furthermore, TiO_2-coated specimens showed clinically acceptable color change.

The purpose of the present study was to investigate the effect of 5 different UV protective agents on the color change of surface-modified maxillofacial silicones after artificial aging procedures. The null hypothesis of the study was that; coating the silicone surfaces with 5 UV protectives following both oxygen and argon plasma treatments would similarly decrease the color change after aging.

2. METHODS

A high-temperature curing platinum-catalyzed maxillofacial silicone (M511; Technovent Ltd, Newport, UK) was used in the present study. For specimen fabrication, the base and the catalyzer of the silicone elastomer were mixed at a ratio of 10:1 according to the manufacturer's instructions. To fabricate 6 color groups, pigments were mixed into silicone 0.2% by weight, and unpigmented, white, red, yellow, blue, and mixture (red, yellow, and blue) groups were obtained. Each group was mixed on a glass plate until the color was evenly distributed. Air bubbles in the silicone mixture were removed using a vacuum chamber and placed into disk-shaped stone molds (15 mm in diameter and 2 mm in thickness). The molds were kept in an oven for 1 hour at 100 ° C for polymerization. Polymerized specimens were evaluated under magnification (Dental Loupe opt-on; Orange Dental GmbH & Co KG, Osnabrück, Germany) for defect and porosity. Excess material at the edges of the specimens was trimmed, and specimens were cleaned in an ultrasonic cleaner (Eurosonic Energy; Euronda SpA, Vincenza, Italy) for 10 minutes to remove the stone mold residue. Before modifying the surfaces of specimens with plasma and UV protectives, parameters that provide the optimum roughness on the silicone surface were investigated for oxygen and argon plasma. For this purpose, 7 disc-shaped silicone specimens (1 was for control without plasma treatment, and 6 were for plasma treatments) were fabricated as described for specimen fabrication. The surfaces of the specimens were cleaned with acetone and kept for 15 minutes for drying. Specimens were treated with oxygen or argon gas in the plasma device as described by Güngör et al. (17). Each plasma treatment was applied for 5, 10, or 15 minutes (18-21). Immediately after plasma treatment, the surface topography of each specimen was analyzed by atomic force microscopy (AFM) (Park Systems). Also, the surface topography of one specimen without plasma treatment was analyzed to investigate the effect of plasma on the silicone surface. Table 1 shows the surface roughness of the specimens after the plasma treatments. According to the AFM images and the mean surface roughness values of each specimen, 10 minutes of plasma treatment provided an optimum surface for both oxygen and argon gases (Fig. 1).

Table 1. *Surface roughness of specimens after plasma treatments*

Specimen	Plasma type	Time (minutes)	Surface roughness (µm)
1	No plasma	-	1.068
2	Argon	5	1.676 µm
3	Argon	10	3.320 µm
4	Argon	15	1.637 µm
5	Oxygen	5	0.368 µm
6	Oxygen	10	3.073 µm
7	Oxygen	15	1.393 µm

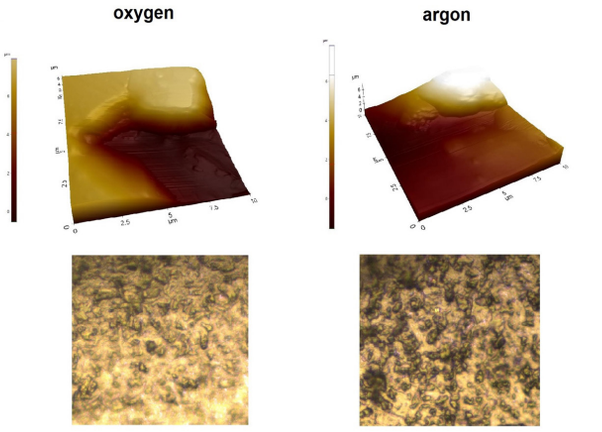

Figure 1. *AFM images of oxygen and argon plasma coated (10 minutes) silicone surfaces.*

Five UV protective agents; benzophenone-3 (UV-BP), ethylhexyl methoxycinnamate (UV-EM), titanium dioxide (UV-TD), 2-ethylhexyl salicylate (UV-ES), and sunscreen including titanium dioxide and zinc oxide (UV-SS) were applied onto the silicone specimens of 6 color (unpigmented, white, red, yellow, blue, and a mixture of red, yellow, and blue) groups. Control groups without UV protective coating were also prepared to evaluate the color change of the silicon material, and control groups were subjected to similar aging procedures with UV protective added groups. Table 2 shows the materials used in the study.

Table 2. *Materials used in study*

Material	Composition	Brand and Manufacturer	Lot no
Maxillofacial silicone, Part A	Platinum catalyzed, adding curing heat-temperature-vulcanized silicone elastomer	M511; Technovent Ltd	B16AJ
Maxillofacial silicone, Part B			
Yellow pigment	Dry pigment	Yellow P206, Technovent Ltd	12A
White pigment	Dry pigment	White 205, Technovent Ltd	12B
Blue pigment	Dry pigment	Blue P216, Technovent Ltd	12A
Red pigment	Dry pigment	Brillant Red, Technovent Ltd	12A
UV-BP	Benzophenone-3	Tokyo Chemical Industry Co	GVUTM-AD
UV-EM	Ethylhexyl methoxycinnamate	Sigma-Aldrich	BCBN1923V
UV-TD	Titanium dioxide	Sigma-Aldrich	SZBD03300V
UV-ES	2-ethylhexyl salicylate	Sigma-Aldrich	MKBX8153V
UV-SS	Sunscreen including titanium dioxide and zinc oxide	Kiehl's	

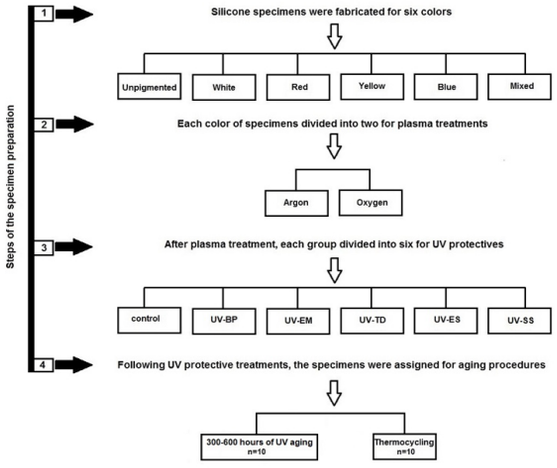

Figure 2. *Study design and number of specimens in each group.*

The powdery UV protectives UV-BP and UV-TD that were dissolved in the liquid silicone, UV protectives in liquid form (UV-ES and UV-EM) and sunscreen were applied onto the prepared surfaces as a film layer immediately after plasma treatment. The surfaces of control specimens were cleaned with acetone before UV protective application. Then, specimen surfaces were coated with a liquid silicone layer approximately 0.01 mm in thickness. The specimens were stored in a light protective box for 24 hours. They were subjected to accelerated aging procedures namely weathering (UV light-heat-humidity) and thermocycling. A different specimen was fabricated for the thermocycling procedure of each group. In total, 144 groups were prepared including 6 UV groups (5 UV protectives and control), 6 colors, 2 plasma treatments, and 2 aging (n=10). The study design is stated in Figure 2. Color measurements were performed initially and after 300 hours and 600 hours of UV aging on the same specimen. The UV aging procedure was performed as described in the previous studies.[21,22] After 300 aging, the specimens were dried and kept in a dark room for 24 hours. Within 48 hours, color measurements were made, and the aging procedure proceeded. After 600 hours color measurements were repeated. The color parameters including L∗, a∗, and b∗ were measured using a spectrophotometer (CM-2300d; Konica Minolta, Inc).

The color change (Delta-E: ΔE) of a specimen was calculated using the equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

(ΔL*: difference in lightness, Δa*: difference in a value, and Δb*: difference in b value).

The ΔE results were analyzed in terms of the normality by the Kolmogorov-Smirnov test (P>.05). The data was tested regarding homogeneity of the variances using the Levene test. The mean and standard deviation of the ΔE data were calculated as descriptive statistics. Statistical analysis of the ΔE data was performed by 4-way analysis of variance (ANOVA) with UV protective, surface treatment, aging, and color as independent variables to evaluate the effects of all factors. Mean values were compared by Tukey HSD test at the 0.05 significance level using the statistical software SPSS v25 (IBM Inc.).

3. RESULTS

A statistically significant interaction was found among UV protectives, surface treatment (plasma gas type), aging, and color (P<0.001) according to the results of 4-way ANOVA (Table 3). The mean values, standard deviations, and comparisons for ΔE data are shown in Table 4.

Table 3. *4-way ANOVA results for ΔE*

Source	df	Adj SS	Adj MS	F-value	*P*-value
UV protective	5	93965	18793.0	1797.91	<.000
Surface treatment	1	2285	2284.6	218.57	<.000
Aging	2	2150	1075.0	102.85	<.000
Color	5	107104	21420.7	2049.30	<.000
UV protective×Surface treatment	5	5654	1130.7	108.18	<.000
UV protective×Aging	10	18588	1858.8	177.83	<.000
UV protective×Color	25	64858	2594.3	248.19	<.000
Surface treatment×Aging	2	2961	1480.3	141.61	<.000
Surface treatment×Color	5	5878	1175.7	112.48	<.000
Aging×Color	10	6256	625.6	59.85	<.000
UV protective×Surface treatment×Aging	10	2372	237.2	22.70	<.000
UV protective× Surface treatment ×Color	25	14605	584.2	55.89	<.000
UV protective×Aging× Color	50	8615	172.3	16.48	<.000
Surface treatment×Aging×Color	10	6261	626.1	59.89	<.000
UV protective×Surface treatment×Aging×Color	50	6930	138.6	13.26	<.000
Error	1944	20320	10.5		
Total	2159	368799			

Table 4. *Mean values, standard deviations (±), and comparisons for ΔE data*

UV protective	Surface treatment	Aging	Unpigmented	White	Yellow	Red	Blue	Mixed
Control	Argon	300 hours of UV aging	6.13 (±0.7) $Ca1^{A}$	4.08 (±0.3) $Cab1^{AB}$	15.31 (±0.6) $Aa1^{B}$	9.78 (±1) $Bb2^{D}$	14.14 (±0.5) $Aa1^{AB}$	14.45 (±0.8) $Aa1^{BC}$
		600 hours of UV aging	7.25 (±0.8) $Ca1^{AB}$	4.52 (±0.3) $Da1^{C}$	13.39 (±0.6) $ABa1^{C}$	5.39 (±0.4) $CDc2^{C}$	12.37 (±0.6) $Bab1^{BC}$	15.32 (±0.6) $Aa1^{BC}$
		Thermocycling	5.79 (±1.4) $Da1^{B}$	2.08 (±0.4) $Eb1^{B}$	10.17 (±4.4) $BCb1^{D}$	14.12 (±7.9) $Aa2^{E}$	11.24 (±2.1) $Bb1^{D}$	8.4 (±2.3) $Cb1^{D}$
	Oxygen	300 hours of UV aging	7.13 (±1.7) $Da1^{BC}$	3.06 (±0.3) $Ea1^{BC}$	14.45 (±1.6) $Ba1^{B}$	18.9 (±2.6) $Ab1^{C}$	9.77 (±0.9) $Ca2^{D}$	14.59 (±0.6) $Ba1^{CD}$
		600 hours of UV aging	6.66 (±1.1) $Da1^{C}$	3.82 (±0.5) $Ea1^{BC}$	12.2 (±1.7) $Cb1^{CD}$	25.09 (±1.9) $Aa1^{C}$	11.78 (±1.6) $Ca1^{D}$	14.89 (±1.5) $Ba1^{C}$
		Thermocycling	7.1 (±1.8) $CDa1^{B}$	2.34 (±0.5) $Ea1^{B}$	9.28 (±11.9) $BCc1^{C}$	19.43(±10.9) $Ab1^{C}$	10.41 (±2.1) $Ba1^{CD}$	5.54 (±2.7) $Db2^{DE}$
UV-BP	Argon	300 hours of UV aging	8.1 (±0.7) $CDa2^{A}$	5.96 (±0.6) $Da1^{A}$	13.84 (±1) $Aa1^{B}$	11.59 (±1.5) $ABb2^{D}$	10.07 (±0.7) $BCb2^{C}$	11.33 (±1.1) $Ba2^{D}$
		600 hours of UV aging	7.99 (±0.8) $Ba1^{A}$	7.55 (±0.9) $Ba1^{A}$	13.56 (±0.8) $Aa1^{C}$	6.05 (±1.2) $Bc2^{C}$	11.63 (±2.2) $Aab2^{C}$	12.87 (±1.1) $Aa2^{C}$
		Thermocycling	4.56 (±0.5) $Cb1^{B}$	2.03 (±0.5) $Db1^{B}$	13.34 (±5.4) $Ba1^{C}$	27.02 (±5.9) $Aa1^{C}$	13.01 (±1.5) $Ba1^{D}$	11.08 (±3.3) $Ba1^{C}$
	Oxygen	300 hours of UV aging	10.28 (±1.9) $Ca1^{A}$	4.88 (±2) $Dab1^{B}$	8.97 (±3.1) $Cab2^{C}$	19.32 (±1.7) $Ab1^{C}$	15.13 (±1.9) $Ba1^{C}$	16.07 (±1) $Ba1^{C}$
		600 hours of UV aging	9.41 (±2.1) $Ca1^{AB}$	4.99 (±1.2) $Da2^{B}$	9.75 (±3) $Ca2^{D}$	25.27 (±1.7) $Aa1^{C}$	14.62 (±1.5) $Ba1^{C}$	15.11 (±0.4) $Ba1^{C}$
		Thermocycling	5.18 (±0.7) $CDb1^{B}$	2.95 (±0.8) $Db1^{B}$	7.03 (±2.2) $BCb2^{C}$	8.74 (±4.9) $ABc2^{D}$	10.53 (±2) $Ab2^{CD}$	7.28 (±1) $BCb2^{CD}$
UV-EM	Argon	300 hours of UV aging	6.15 (±0.4) $Cab1^{A}$	4.39 (±0.5) $Ca1^{AB}$	15.44 (±2.4) $Aa1^{B}$	4.74 (±1) $Cb2^{E}$	14.34 (±0.8) $ABa1^{A}$	12 (±0.7) $Ba1^{CD}$
		600 hours of UV aging	6.73 (±0.3) $Ca1^{AB}$	4.91 (±0.4) $Ca1^{BC}$	16.95 (±10.8) $Aa1^{B}$	5.57 (±0.7) $Cb2^{C}$	13.72 (±0.7) $Ba1^{BC}$	13.71 (±0.7) $Ba1^{BC}$
		Thermocycling	4.11 (±0.9) $Cb1^{B}$	1.34 (±0.4) $Db1^{B}$	10.18 (±2.1) $Bb1^{D}$	21.32 (±9.9) $Aa1^{D}$	10.83 (±9.5) $Bb1^{D}$	3.28 (±1.1) $CDb1^{E}$
	Oxygen	300 hours of UV aging	6.73 (±0.9) $Da1^{C}$	3.60 (±0.7) $Ea1^{BC}$	13.74 (±2.4) $Ba1^{B}$	16.92 (±2.1) $Ab1^{C}$	10.65 (±1.2) $Cb2^{D}$	12.35 (±1.1) $BCb1^{D}$
		600 hours of UV aging	6.91 (±1.2) $Da1^{C}$	3.53 (±0.7) $Ea1^{BC}$	15.02 (±0.8) $BCa1^{B}$	25.57 (±0.8) $Aa1^{C}$	12.69 (±1.3) $Ca1^{CD}$	15.50 (±1.1) $Ba1^{C}$
		Thermocycling	5.04 (±0.4) $Ba1^{BC}$	2.59 (±0.3) $Ba1^{B}$	3.78 (±2.2) $Bb2^{D}$	4.10 (±1.9) $Bc2^{E}$	8.22 (±5.1) $Ac2^{D}$	3.75 (±1.7) $Bc1^{E}$
UV-TD	Argon	300 hours of UV aging	6.04 (±0.8) $Da1^{A}$	2.89 (±0.4) $Eab1^{B}$	28.49 (±1.3) $Bc1^{A}$	37.43 (±1.7) $Ab2^{A}$	12.86 (±1) $Cb2^{AB}$	15.23 (±0.9) $Cb2^{B}$
		600 hours of UV aging	5.41 (±0.4) $Da1^{B}$	3.29 (±0.4) $Da1^{C}$	30.58 (±1.7) $Bb1^{A}$	38.45 (±1.6) $Ab2^{A}$	14.30 (±0.6) $Cb2^{B}$	16.11 (±0.9) $Cb2^{B}$
		Thermocycling	4.69 (±0.8) $Ea1^{B}$	0.93 (±0.3) $Fb1^{B}$	40.73 (±2.5) $Ca1^{A}$	62.53 (±3.4)$Aa2^{A}$	49.33 (±7.1) $Ba1^{A}$	37.99 (±3.8) $Da1^{A}$
	Oxygen	300 hours of UV aging	6.05 (±0.4) $Ea1^{C}$	2.31 (±0.3) $Fa1^{C}$	22.78 (±5.3) $Db2^{A}$	61.6 (±2.6) $Ab1^{A}$	45.27 (±1.8) $Bb1^{A}$	35.6 (±4.0) $Cab1^{A}$
		600 hours of UV aging	6.26 (±0.9) $Ea1^{C}$	2.39 (±0.5) $Fa1^{C}$	22.34 (±5.9) $Db2^{A}$	60.7 (±4.5) $Ab1^{A}$	48.64 (±2.3) $Ba1^{A}$	34.83 (±4.6) $Cb1^{A}$
		Thermocycling	2.6 (±0.8) $Db2^{C}$	2.11 (±0.4) $Da1^{B}$	37.43 (±1.8) $Ca2^{A}$	68.88 (±2.2) $Aa1^{A}$	44.06 (±5.3) $Bb2^{B}$	37.05 (±4.1) $Ca1^{A}$
UV-ES	Argon	300 hours of UV aging	6.2 (±0.8) $CDa1^{A}$	3.85 (±0.6) $Da1^{AB}$	7.41 (±0.9) $Cb2^{C}$	20.91 (±1.3) $Ab1^{B}$	11.72 (±0.8) $Bb1^{BC}$	10.27 (±0.3) $Bb1^{D}$
		600 hours of UV aging	6.6 (±0.3) $Ca1^{AB}$	4.77 (±0.8) $Ca1^{C}$	9.66 (±0.4) $Ba2^{D}$	14.68 (±1.2) $Ac1^{B}$	12.8 (±0.3) $Ab1^{BC}$	15.26 (±0.4) $Aa1^{BC}$
		Thermocycling	6.44 (±0.8) $CDa1^{B}$	1.63 (±0.7) $Eb1^{B}$	5 (±1.8) $Dc2^{E}$	25.12 (±3.7) $Aa1^{C}$	18.21 (±3) $Ba1^{C}$	8.04 (±1.2) $Cc1^{D}$
	Oxygen	300 hours of UV aging	7.51 (±0.5) $BCa1^{BC}$	2.88 (±0.6) $Dab1^{BC}$	13.86 (±1) $Aa1^{B}$	5.1 (±0.8) $CDb2^{D}$	6.77 (±1.2) $Cb2^{E}$	9.26 (±0.7) $Bb1^{E}$
		600 hours of UV aging	7.85 (±1) $Ca1^{BC}$	3.59 (±0.3) $Da1^{BC}$	12.86 (±1.6) $Ba1^{BC}$	16.71 (±1.2) $Aa1^{D}$	10.52 (±1.2) $Ba2^{D}$	15.92 (±0.2) $Aa1^{C}$
		Thermocycling	5.88 (±0.9) $Ca1^{B}$	1.10 (±0.3) $Db1^{B}$	8.93 (±2) $ABb1^{C}$	6.53 (±1.7) $BCb2^{DE}$	11.16 (±2.6) $Aa2^{C}$	8.13 (±2.3) $BCb1^{C}$
UV-SS	Argon	300 hours of UV aging	6.43 (±0.6) $Db2^{A}$	6.16 (±0.5) $Da1^{A}$	16.12 (±1.9) $Bb1^{B}$	15.47 (±1.4) $Bb2^{C}$	11.93 (±1.8) $Cc2^{ABC}$	21.44 (±0.5) $Ac2^{A}$
		600 hours of UV aging	7.61 (±0.5) $Cab2^{AB}$	7.27 (±0.8) $Ca1^{AB}$	17.48 (±1.7) $Bb1^{B}$	15.03 (±0.8) $Bb2^{B}$	22.61 (±0.8) $Ab2^{A}$	23.73 (±0.5) $Ab2^{A}$
		Thermocycling	9.09 (±0.7) $Da2^{A}$	6.93 (±0.3) $Da1^{A}$	34.73 (±11.5) $Ba1^{B}$	41.1 (±3.6) $Aa2^{B}$	30.04 (±16) $Ca2^{B}$	32.62 (±5) $Ba1^{B}$
	Oxygen	300 hours of UV aging	9.26 (±2.1) $Cb1^{AB}$	8.03 (±0.9) $Ca1^{A}$	8.28 (±2.8) $Cc2^{C}$	38.17 (±3.7) $Ab1^{B}$	23.48 (±4.6) $Bc1^{B}$	23.65 (±3.7) $Bc1^{B}$
		600 hours of UV aging	10.36 (±1.4) $CDb1^{A}$	7.89 (±0.2) $Da1^{A}$	11.23 (±0.6) $Cb2^{CD}$	38.64 (±5.4) $Ab1^{B}$	26.4 (±3.8) $Bb1^{B}$	26.75 (±2.2) $Bb1^{B}$
		Thermocycling	14.14 (±0.3) $Ea1^{A}$	6.58 (±1.5) $Fa1^{A}$	33.36 (±15.2) $Ca1^{B}$	56.3 (±8) $Aa1^{B}$	48.8 (±8.6) $Ba1^{A}$	29.57 (±4.8) $Da2^{B}$

UV-BP, benzophenone-3; UV-EM, ethylhexyl methoxycinnamate; UV-TD, titanium dioxide; UV-ES, 2-ethylhexyl salicylate; UV-SS, sunscreen.Same uppercase letters indicate that ΔE values of silicone color groups were not significantly different in same protective agent, surface treatment, and aging groups (P>0.05). Same lowercase letters indicate that the ΔE values of aging groups were not significantly different in same protective agent, surface treatment, and silicone color groups (P>0.05). Same numbers indicate that ΔE values of surface treatment groups were not significantly different in same protective agent, aging, and silicone color groups (P>0.05). Same superscripts indicate that ΔE values of protective agent groups were not significantly different in same surface treatment, aging, and silicone color groups (P>0.05).

After UV aging for 300 hours, white, yellow, and mixed color groups of argon-treated and UV-ES-coated specimens resulted in lower ΔE compared with control groups with no UV protection and the differences were significant in yellow and mixed groups (P<0.05). Also, blue and mixed groups (argon treated and UV-BP covered) showed significantly lower ΔE values than control groups. Comparing UV protective coated groups after oxygen treatment, yellow color groups of UV-BP and UV-SS revealed lower ΔE than control groups. Also, red, blue, and mixed colors of UV-ES coated groups showed significantly lower ΔE values than control groups. Following UV aging for 600 hours, the highest color changes were noted for all groups regardless of surface treatment and UV protective coating. In all aging procedures, the highest color change (ΔE) was generally observed in the red groups than other color groups within each surface treated and coated group. The lowest ΔE values were found in white groups. In control groups (with no coating), the lowest ΔE values were detected in unpigmented, white, and yellow groups after 300 hours, and the red, blue, and mixed colors showed the lowest ΔE values after thermocycling. UV protective coated groups showed significantly highest and lowest ΔE values after 600 hours and thermocycling, respectively (P<0.05).

4. DISCUSSION

In the present study, the surfaces of a maxillofacial silicone elastomer were modified with plasma application. Then they were coated with 5 different UV protectives to decrease the color change of silicone elastomers. The null hypothesis of coating the silicone-colored surfaces with 5 UV protectives following both oxygen and argon plasma treatments would similarly decrease the color change of the material after aging was rejected because of the significant differences among the experimental groups.

Particularly UV light and environmental effects cause discoloration on the silicone facial prostheses (2,4) UV light causes continuing polymerization of the silicone chains. During this prolonged polymerization process, subproducts are released. Thereby decomposition of color pigments and disruption of the chains of polymer occur (4,7,10). This process resulted in the discoloration of the material. Previous literature includes experimental studies on protecting the maxillofacial silicones against the damaging effects of UV light. In these studies; opacifiers, UV protectives, and thermochromic pigments were incorporated into the silicone (2,4,5,9-11,15,16). The addition of opacifier powders as a white pigment in silicones enhanced optical and mechanical properties (2,4,5,15,16). However, the long-term effects of these protectives on the mechanical properties, optical properties, and biocompatibility of silicone are unknown when incorporated into the bulk material. Therefore, in the present study, coating the surface of polymerized silicone elastomer with UV protectives was planned. Previously only one study by Bishal et al (12) used a similar approach. They coated the surface of intrinsically pigmented silicone with a nanolayer of TiO_2. TiO_2 was found to be effective in reducing the color change of the silicone elastomer after artificial aging. In this study, reduced color changes in some UV-protective coated groups after artificial aging have been observed. These findings may address further research on the surface modification of polymerized maxillofacial silicone elastomers instead of intrinsically modifying the material before polymerization to provide color stability.

In the present study, oxygen or argon plasma treatments were applied to the specimen surfaces with the aims of cleaning, enhancing surface energy and wettability, and providing a chemical bonding between the coating material and the specimen. This study revealed that coating the silicone elastomer with UV-ES followed by oxygen or argon plasma treatments revealed significantly lower color difference values for each color group after aging than the control group (P<0.05). No characteristic difference was noted between the values of groups those oxygen and argon surfaces treated before UV-ES coating except for the red group. In the red-colored specimen group, ΔE value of argon-treated and UV-ES coated specimens was higher than un-coated control specimens and color change of oxygen-treated and UV-ES coated specimens. This finding may have resulted from chemical reactions of 3 components, namely argon-treated silicone surface, red pigment, and UV-ES. Plasma is an ionized form of a gas. Ions are negatively and positively charged particles of atoms. When the ions have adequate energy, they break covalent bonds on the surface layer of the material (18-20). Thus, hydrophilicity and wettability properties of the polymer surface can be improved by plasma application (18,20). The AFM analyses of the plasma-treated silicone surfaces revealed that argon plasma created more surface roughness than oxygen plasma. Differences in surface topographies of oxygen and argon plasma-treated specimens can result from different reactive properties of oxygen and argon atoms. Beyond surface topography, the chemical reactive potential of the silicone surface with pigments and UV protectives may differ by ionized oxygen and argon.

As previously reported, the type of pigment plays an important role in silicone discoloration (4,9). Red-pigmented silicone showed the highest discoloration under different aging conditions (9,11). UV protectives that reduce discoloration with maxillofacial silicone elastomers would be valuable, especially for reddish prostheses. Coating the colored and cured silicone surface with UV-ES would also be functional because it is a clear liquid and does not change the color and translucency of the final prosthesis, unlike opacifiers.

In the present study, one type of maxillofacial silicone (M511) was evaluated for the effectiveness of coating the silicone surface with UV protectives. Different maxillofacial silicone elastomers should be evaluated in terms of color stability after surface coating with UV protective agents, especially UV-ES. In future studies, the color change of different types of maxillofacial silicones can be investigated. Furthermore, silicone surface coating methods might be improved.

Artificial aging is a fast method of evaluating the long-term properties of clinical materials. Two aging procedures were applied in the present study. One is accelerated weathering which includes temperature, UV light, and humidity to test materials (22,23). UV light has been reported as responsible for the degradation of polymers and colorants (8,11). Therefore, a similar aging procedure is widely used to test silicone elastomers (2,4,6,11). However, differences in climate around the world cause the maxillofacial prosthetic material to be exposed to various factors. (1) Although accelerated aging with weathering devices is a fast and effective method for prosthetic materials, other factors affecting prostheses during daily use such as air pollution, hygiene procedures, and patient habits cause color degradation (15). In this study, thermocycling aging was additionally applied to a specimen group for simulating water cleaning of the prostheses using warm or cold water. Depending on the findings of the current study thermocycling also affected the color difference values. This result may reveal that intrinsically colored maxillofacial prostheses can show a color change when they are exposed to a wet environment at varying temperatures.

The study includes some limitations. First, the thickness of the UV protective and liquid silicone layer was not precisely measured and was not objectively standardized. In this study, the hypothesis was that only the surface of the coating of a colored and cured silicone with UV protectives would protect the silicone from discoloration. Thereby, the material would be enhanced without changing its optimally developed polymer formulation. Further studies are needed for more precious surface coating, especially for UV-ES. Another limitation of the present study is the M511 silicone elastomer coated with UV protectives. In clinical practice, various silicone elastomers and pigments were used. Other commercially available maxillofacial silicones should be tested in terms of color stability when coated with UV-ES. In the present study, the color differences after aging procedures were calculated with the CIELab formula to compare the results with the previous studies that used mostly the CIELab values. However further studies evaluating the color stability of UV-protective coated maxillofacial silicone elastomers by using the CIEDE2000 formula is required.

5. CONCLUSIONS

Within the limitations of this study, it can be concluded that; UV aging of 600 hours was the aging procedure that caused the highest ΔE values and thermocycling caused the lowest ΔE values. After aging procedures, the highest color change (ΔE) was generally observed in the red color groups than other groups while the lowest ΔE values were noted in white colors. Coating the silicone surface with UV-ES followed by oxygen or argon plasma revealed significantly lower ΔE for each color group after aging compared to the control groups. UV-ES coating might be a promising approach to prolong the clinical lifetime of silicone maxillofacial prostheses.

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors do not have any financial interest in the companies whose materials are included in this article.*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: BTB

Design of the study: SKN

Acquisition of data for the study: SKN, BTN, MBG, ND, MET, YKA

Analysis of data for the study: YKA

Interpretation of data for the study: SKN, BTN, MBG, ND, MET, YKA

Drafting the manuscript: MBG

Revising it critically for important intellectual content: SKN, BTB, MBG

Final approval of the version to be published: SKN, BTN, MBG, ND, MET, YKA

REFERENCES

[1] Chang T-L, Garrett N, Roumanas E, Beumer III J. Treatment satisfaction with facial prostheses. J Prosthet Dent. 2005;94(3):275–280. https://doi.org/10.1016/j.prosdent.2005.06.002.

[2] Han Y, Zhao Y, Xie C, Powers JM, Kiat-amnuay S. Color stability of pigmented maxillofacial silicone elastomer: effects of nano-oxides as opacifiers. J Dent. 2010;38(2):e100–105. https://doi.org/10.1016/j.jdent.2010.05.009.

[3] Karakoca S, Aydin C, Yilmaz H, Bal BT. Retrospective study of treatment outcomes with implant-retained extraoral prostheses: survival rates and prosthetic complications. J Prosthet Dent. 2010;103(2):118–126. https://doi.org/10.1016/S0022-3913(10)60015-7.

[4] Han Y, Powers JM, Kiat-Amnuay S. Effect of opacifiers and UV absorbers on pigmented maxillofacial silicone elastomer, part 1: Color stability after artificial aging. J Prosthet Dent. 2013;109(6):397–401. https://doi.org/10.1016/S0022-3913(13)60327-3.

[5] Akash R, Guttal SS. Effect of incorporation of nano-oxides on color stability of maxillofacial silicone elastomer subjected to outdoor weathering. J Prosthodont. 2015;24(7):569–575. https://doi.org/10.1111/jopr.12252.

[6] Lemon JC, Chambers MS, Jacobsen ML, Powers JM. Color stability of facial prostheses. J Prosthet Dent. 1995;74(6):613–618. https://doi.org/10.1016/s0022-3913(05)80314-2.

[7] Hatamleh MM, Watts DC. Effect of extraoral aging conditions on color stability of maxillofacial silicone elastomer. J Prosthodont. 2010;19(7):536–543. https://doi.org/10.1111/j.1532-849X.2010.00627.x.

[8] Beatty MW, Mahanna GK, Jia W. Ultraviolet radiation-induced color shifts occurring in oil-pigmented maxillofacial elastomers. J Prosthet Dent. 1999;82(4):441–446. https://doi.org/10.1016/s0022-3913(99)70031-4.

[9] dos Santos DM, Goiato MC, Moreno A, Pesqueira AA, Haddad MF. Influence of pigments and opacifiers on color stability of an artificially aged facial silicone. J Prosthodont. 2011;20(3):205–208. https://doi.org/10.1111/j.1532-849X.2010.00657.x

[10] Kheur M, Sethi T, Coward T, Kakade D, Rajkumar M. Evaluation of the effect of ultraviolet stabilizers on the change in color of pigmented silicone elastomer: An in vitro study. J Indian Prosthodont Soc. 2016;16(3):276. https://doi.org/10.4103/0972-4052.176535.

[11] Kiat-amnuay S, Beerbower M, Powers JM, Paravina RD. Influence of pigments and opacifiers on color stability of silicone maxillofacial elastomer. J Dent. 2009;37(1):e45–e50. https://doi.org/10.1016/j.jdent.2009.05.004

[12] Bishal AK, Wee AG, Barão VA, Yuan JC-C, Landers R, Skotja C, Takoudis CG. Color stability of maxillofacial prosthetic silicone functionalized with oxide nanocoating. J Prosthet Dent. 2019;121(3):538–543. https://doi.org/10.1016/j.prosdent.2018.06.007

[13] Griniari P, Polyzois G, Papadopoulos T. Color and structural changes of a maxillofacial elastomer: the effects of accelerated photoaging, disinfection and type of pigments. J Appl Biomater Funct Mat. 2015;13(2):87–91. https://doi.org/10.5301/jabfm.5000229.

[14] 14. Kheur MG, Kakade D, Trevor CJ, Lakha TA, Sethi T. Effect of newly developed pigments and ultraviolet absorbers on the color change of pigmented silicone elastomer. J Indian Prosthodont Soc. 2017;17(4):395-400. https://doi.org/10.4103/jips.jips_148_17.

[15] Tran NH, Scarbecz M, Gary JJ. In vitro evaluation of color change in maxillofacial elastomer through the use of an ultraviolet light absorber and a hindered amine light stabilizer. J Prosthet Dent. 2004;91(5):483–490. https://doi.org/10.1016/S002.239.130400112X.

[16] Bangera BS, Guttal SS. Evaluation of varying concentrations of nano-oxides as ultraviolet protective agents when incorporated in maxillofacial silicones: An in vitro study. J Prosthet Dent. 2014;112(6):1567–1572. https://doi.org/10.1016/j.prosdent.2014.07.001

[17] Güngör MB, Nemli SK, Inal CB, Bağkur M, Dilsiz N. Effect of plasma treatment on the peel bond strength between maxillofacial silicones and resins. Dent Mater J. 2020 Mar. 31;39(2):242-250. https://doi.org/10.4012/dmj.2018-259.

[18] Masood SH, Mohamed SA. The effect of plasma treatment on the bonding of soft denture liners to heat-cured acrylic resin denture base material and some surface properties of acrylic resin polymer. J Bagh Coll Dentistry. 2012;24(3):29–35.

[19] Gray J, Norton P, Griffiths K. Surface modification of a biomedical poly (ether) urethane by a remote air plasma. Appl Surf Sci. 2003;217(1-4):210–22. doi:10.1016/S0169-4332(03)00552-X

[20] Hauser J, Zietlow J, Köller M, Esenwein SA, Halfmann H, Awakowichz P, Steinau HU. Enhanced cell adhesion to silicone implant material through plasma surface modification. J Mater Sci Mater Med. 2009;20(12):2541–2548. https://doi.org/10.1007/s10856.009.3826-x

[21] Nishigawa G, Maruo Y, Oka M, Oki K, Minagi S, Okamoto M. Plasma treatment increased shear bond strength between heat-cured acrylic resin and self-curing acrylic resin. J Oral Rehabil. 2003;30(11):1081–1084. https://doi.org/10.1046/j.1365-2842.2003.01198.x.

[22] Bankoğlu Güngör M, Karakoca Nemli S, Turhan Bal B, Kaşko Arıcı Y. Effect of ultraviolet protective agents on maxillofacial silicone elastomer, part 2: Mechanical properties after artificial aging. J Prosthet Dent. 2023;129(4):658-668. https://doi.org/10.1016/j.prosdent.2021.06.032.

[23] Turhan Bal B, Bankoğlu Güngör M, Karakoca Nemli S, Aydın C, Kaşko Arıcı Y. Effect of ultraviolet protective agents on maxillofacial silicone elastomer, part 1: Color stability after artificial aging. J Prosthet Dent. 2023;129(3):513-519. https://doi.org/10.1016/j.prosdent.2021.06.033.

How to cite this article: Karakoça Nemli S, Turhan Bal B, Bankoğlu Güngör M, Dilsiz N, Eskitoros Togay M, Kasko Arıcı Y. Effect of Ultraviolet Protective Agents and Plasma Applications on the Color Stability of Maxillofacial Silicones. Clin Exp Health Sci 2024; 14: 69-75. DOI: 10.33808/clinexphealthsci.1398224

Clinical and Experimental Health Sciences

Analysis of Sleep Quality and Related Factors in Patients with Type 2 Diabetes Mellitus

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Received: February 27, 2024 **Accepted:** February 26, 2025

ABSTRACT

Objective: Diabetes Mellitus is one of the most common diseases which affects life quality. The aim of the study was to examine sleep quality and sleeping related factors in patients with Type 2 Diabetes Mellitus (T2DM).

Methods: An observational, cross-sectional study was conducted between 01.09.2018-01.12.2018 in Sisli Hamidiye Etfal Research and Training Hospital. Sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI) questionnaire. Moreover, we used Patient Health Questionaire-9 (PHQ-9) scale to diagnose depression degree.

Results: The study included 227 individuals with T2DM, with a mean age of 60.26 ± 9.13 years, 59.5% of whom were female. The mean disease duration was 10.46 ± 7.65 years, and 70% had accompanying hypertension. Poor sleep quality (PSQI ≥ 6) was identified in 54.2% of participants, with significantly poorer sleep quality observed in females, obese individuals, unemployed participants, and those with diabetes-related complications such as neuropathy and retinopathy ($p < .05$). Although no significant correlation was found between PSQI and glycemic control markers (HbA1c or fasting blood glucose), participants with higher levels of these markers tended to report poorer sleep. Depression severity (PHQ-9) was strongly associated with poor sleep quality ($p < .001$), with higher scores observed in females, low-income and unemployed participants, obese individuals, and those with diabetes-related complications.

Conclusion: This study underscores the high prevalence of sleep disturbances among individuals with T2DM, with factors such as female gender, obesity, unemployment, hypertension, and diabetes-related complications like neuropathy and retinopathy significantly impairing sleep quality. The bidirectional relationship between poor sleep and depression highlights the need for integrated mental health support in diabetes care. Although no significant correlations were found between sleep quality and glycemic markers, trends suggest a potential link, warranting further research.

Keywords: Diabetes Mellitus, Sleep Disorders, Quality of Life, Diabetic Neuropathy, Pittsburgh Sleep Quality Index

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by defects in insulin secretion and action, requiring continuous care (1). The prevalence of diabetes is increasing globally. 10.5% of the American population (roughly 35 million individuals) were living with DM in 2018, and the incidence in Türkiye was calculated at 13.5% in the same year (2).

Among the various forms of DM, Type 2 Diabetes Mellitus (T2DM) is linked with comorbidities like cardiovascular disease, diabetic neuropathy, retinopathy, and nephropathy. These complications adversely affect quality of life (3-4). Research indicates that sleep disturbances are prevalent with T2DM compared to the general population (5-6).

Sleep is a physiological need for maintaining health, quality of life, and daily performance (7). Sleep disturbances, including insufficient or irregular sleep, contribute to cognitive dysfunction, psychological stress in general health (8). In T2DM, these disruptions are further compounded by complications such as peripheral neuropathy and psychological stress. Painless diabetic neuropathy has been shown to be associated with poor sleep quality (9).

The relationship between T2DM and sleep quality is bidirectional. Poor sleep exacerbates glycemic dysregulation by increasing insulin resistance, reducing glucose tolerance, and elevating stress hormone levels (10). Conversely,

https://doi.org/10.33808/clinexphealthsci.1442009

T2DM-related complications, including sleep apnea, restless leg syndrome, and other sleep disorders, worsen metabolic outcomes (11). Chronic sleep deprivation is a risk factor for the development of T2DM, mediated through mechanisms like obesity and metabolic syndrome (12).

This article examines the interplay between T2DM and sleep quality, focusing on the prevalence of sleep disturbances, and underlying mechanisms for improving outcomes of individuals with T2DM.

2. METHODS

Individuals who had been diagnosed with T2DM, who applied to the Family Practice Outpatient Clinic of Health Scince University (HSU) Sisli Hamidiye Etfal Training and Research Hospital for routine check-ups between 01.09.2018-01.12.2018, were included in the study. Patients who had T1DM diagnosis, who used medicine that could affect sleep quality, who had a psychiatric disorder, who received treatment for a diagnosed sleep problem, and who did not agree to participate were excluded from the study.

In this study, a power analysis was conducted to ensure the adequacy of the sample size by using open source R software (Version 4.3.3.). The sample size required for this study was calculated based on an effect size (d) of 0.415, a significance level (α) of 0.05, and a desired power of 0.80. Using these parameters, the sample size was determined to be 227 individuals with 95% reliability, calculated based on the number of patients with Type 2 diabetes who visited the outpatient clinic during the same period in the previous year, which is consistent with typical sample size estimations for non-parametric tests like the Wilcoxon-Mann-Whitney test (13).

2.1. Data Collection Tools

Sociodemographic Information Form, Pittsburgh Sleep Quality Index (PSQI), and Patient Health Questionnaire-9 (PHQ-9) were used in order to collect data for the study, and the questionnaire was administered face-to-face. The Sociodemographic Information Form was developed by the researchers in line with similar studies, and it consisted of descriptive questions such as the individual's age, gender, marital status, educational status, and disease (how long the patient has been diagnosed with the disease).

Following the administration of the questionnaire, the patients' HbA1c and fasting blood glucose (FBG) values obtained in the routine check-ups were examined over the system. Those who had a HbA1c level of 7 and below were evaluated as under control. Those who had a FBG level of 130 and below were evaluated as normal FBG (14).

2.2. Pittsburgh Sleep Quality Index

Pittsburgh Sleep Quality Index (PSQI) is a scale used to determine the sleep quality of an individual within the last month, whether the individual experienced a sleep disorder, and the severity of this disorder. The scale has a total of 24 questions. With the scale, sleep is evaluated under 7 subscales, which are subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The total PSQI score is calculated by adding up the scores obtained from the seven subscales. The score of each subscale varies between 0 and 3. The total PSQI score, on the other hand, ranges from 0 to 21. Those who score 5 and below in total are evaluated to have "good" sleep quality. The Turkish reliability and validity study of the scale was conducted by Agargun et al. (1996), which they reported a Cronbach's alpha coefficient of 0.77 for the overall scale (15). Consistent with Agargun et al., we found Cronbach's alpha coefficient of 0.755, indicating good internal consistency (The mean values (M) and standard deviations (SD) for the subscales of PSQI were as follows: subjective sleep quality (M = 0.634, SD = 0.965), sleep latency (M = 1.59, SD = 1.06), sleep duration (M = 1.04, SD = 1.07), habitual sleep efficiency (M = 1.39, SD = 0.67), sleep disturbances (M = 0.25, SD = 0.78), and use of sleeping medication (M = 0.41, SD = 0.76).

2.3. Patient Health Questionaire-9

PHQ-9 is a scale developed to diagnose depression and to determine its severity by questioning the 9 diagnostic criteria included in the Diagnostic and Statistical Manual of Mental Disorders (DSM-4). Kroenke, Spitzer, and Williams introduced the Patient Health Questionnaire-9 (PHQ-9) as a brief measure for assessing depression severity (16). The authors reported a high internal consistency for the PHQ-9, with a Cronbach's alpha of 0.89, indicating that the items on the scale are strongly correlated and effectively measure the underlying construct of depression severity. The Turkish validity and reliability of the scale was conducted by Sari et al. in 2016. In their study, they reported the **Cronbach's alpha** for the PHQ-9 to be **0.84,** which reflects a high level of internal consistency in this population as well (17).

In this study, "not at all" is scored as 0, and "nearly every day" is scored as 3. The minimum score to be obtained from the scale is 0, while the maximum score is 27. According to the original questionnaire scoring evaluation, 1-4 points indicate minimal depression, 5-9 points mild depression, 10-14 points moderate depression, 15-19 points moderately severe depression, and 20-27 points severe depression. As the score obtained increases, the severity of depression increases as well (17). In our study, we found a high internal consistency, with a Cronbach's alpha of 0.874. M and SD for the nine criteria of PHQ-9 were as follows, respectively: M = 0.894 (SD = 1.07), M = 1.00 (SD = 1.06), M = 1.12 (SD = 1.28), M = 1.34 (SD = 1.08), M = 0.67 (SD = 1.03), M = 0.55 (SD =

0.95), M = 0.37 (SD = 0.77), M = 0.33 (SD = 0.74), and M = 0.22 (SD = 0.60).

2.4. Statistical Method

SPSS 16.0 (Chicago, U.S.A.) for Windows was used for statistical analyses. Frequencies and percentages were calculated for categorical variables. Standard deviation, minimum, maximum, and median were employed for numerical variables. Prior to conducting the statistical analyses, the normality of the data for each variable was assessed using the Shapiro-Wilk test. The results of this test revealed that the normality assumption was not satisfied for the variables ($p<.05$). Consequently, non-parametric methods were employed to analyze the data. Mann-Whitney U test was performed to compare two independent groups, while the Kruskal-Wallis test was used for comparisons involving more than two independent groups. Due to the violation of the normality assumption, Spearman's rank correlation was utilized to investigate the relationship between the variables. Significance level was set at $p<.05$.

3. RESULTS

227 individuals with T2DM were included in the study. 135 (59.5%) of the participants were female, and 92 (40.5%) were male. The mean age of the participants was found as 60.26±9.13 (min=40, max=87). When the participants were asked about the duration of their diabetes disease, the mean duration was determined to be 10.46±7.65 (min=1, max=35) years. Those who were treated for 11 years and above was 87 (38.3%) individuals. The number of the patients diagnosed with accompanying hypertension was 159 (70%). The number of patients who had one or a few of the complications related to diabetes (retinopathy, neuropathy, nephropathy, or diabetic foot) was determined to be 37 (38.3%). And, the number of the patients who were using insulin injection in addition to oral medication was found as 80 (35.2%).

The mean score of the participants obtained from PSQI was determined to be 6.79±4.06 (min=0, max=19). The rate of those with poor sleep quality was 54.2% (n=123). As shown in Table 1, it was determined that there was no significant relationship of sleep quality with age, education level, income level, and smoking status ($p\geq.05$). It was found that the sleep quality of female patients was significantly poorer than that of male patients, as was the sleep quality of obese patients than that of non-obese patients (p=.000). The sleep quality of unemployed patients was significantly poorer compared to employed patients (p=.001). The sleep quality of the patients who had the disease for 11 years and above was determined to be poorer than those who had the disease for 10 years and below (p=.024). The PSQI scores of the diabetic patients with accompanying hypertension were higher compared to those with no accompanying hypertension (p=.001). It was also found that the sleep quality of the patients who had diabetic complications of retinopathy (p=.047); and neuropathy (p=.049) was significantly poorer compared to those who did not have these complications.

According to the laboratory test results of the patients, it was found that BFG mean value was 149.26±53.78 (min=56, max=384), and HbA1c mean value was 7.39±1.52 (min=5.10, max=13.0). When the relationship between PSQI, HbA1c, and BFG was examined through correlation analysis (Table 3), no significant association was found between PSQI and HbA1c or between PSQI and BFG. Similarly, no significant difference in PSQI scores was observed between the group with T2DM under control and the group with uncontrolled T2DM in terms of HbA1c and BFG values (p=.343 and p=.107, respectively). However, as shown in Table 1, participants with elevated HbA1c and BFG levels demonstrated poorer sleep quality.

The mean score of the patients obtained from PHQ-9 was 6.52±5.42 (min=0, max=23) as presented in Table 2. Women had significantly higher PHQ-9 scores (7.93 ± 5.67) compared to men (4.46 ± 4.30), indicating a significant difference in PHQ-9 between genders (p=.000). Participants in the low-income group (≤1700 TL) had the highest mean PHQ-9 scores (7.91 ± 5.77), followed by the medium-income group (6.00 ± 5.26) and the high-income group (5.18 ± 4.58). The differences across income levels were statistically significant (p=.017). Unemployed participants exhibited significantly higher PHQ-9 scores (7.97 ± 5.86) compared to employed individuals (4.81 ± 4.29) (p=.000). Participants with BMI ≥ 30 had significantly higher PHQ-9 (7.86 ± 5.55) compared to those with a BMI of 25–29.9 (4.90 ± 4.81), with a p-value of .000. There was also no statistically significant difference in PHQ-9 scores between participants with a disease duration of <11 years (6.21 ± 4.79) and those with ≥11 years (7.02 ± 6.31) (p =.302). Participants with HbA1c ≥ 7 had higher PHQ-9 scores (6.94 ± 5.86) compared to those with HbA1c < 7 (6.13 ± 4.98), but the difference was not statistically significant (p=.260). Participants on insulin and oral treatment had significantly higher PHQ-9 scores (7.69 ± 6.42) compared to those on oral treatment only (5.88 ± 4.70) (p=0.016). Those with diabetes-related complications reported significantly higher PHQ-9 scores (7.53 ± 6.24) than participants without complications (5.89 ± 4.76) (p=.038). Participants with a PSQI score ≥6 exhibited significantly higher PHQ-9 scores (8.83 ± 5.66) compared to those with scores <6 (3.79 ± 3.56) (p=.000), suggesting a strong association between poor sleep quality and higher depression levels. As stated in Table 3, there was no significant correlation between PHQ-9 and HbA1c (r = 0.019, p=.770) or between PHQ-9 and BFG (r = 0.098, p=.793).

Table 1. *Analyses performed on the factors affecting the sleep quality of the diabetic patients for PSQI*

PSQI Scores					
(N;%)	**Mean±Std.**	**p**		**Mean±Std.**	**p**
Gender			**HbA1c**		
Women (n=135; 59.5%)	7.95±4.11	**.000**	<7(n=118; 52.0%)	6.57±3.91	.343
Men (n=92; 40.5%)	5.10±3.34		≥ 7(n=109; 48.0%)	7.04±4.22	
Income level			**Blood Fasting Glucose**		
Low(≤1700 TL)(n=76; 33.5%)	7.50±4.20	.130	<131(n=105; 46.3%)	**6.32**±3.72	.107
Medium(1701-3499 TL)(n=118; 52.0%)	6.58±3.93		≥131(n=122;53.7%)	7.19±4.29	
High(≥3500 TL)(n=33; 14.5%)	5.94±4.07		**Treatment Style**		
Education Status			Only oral treatment (n=147; 64.8%)	6.50±3.77	.248
Below high school(n=182; 80.2%)	6.92±3.99	.247	Insulin and oral treatment(n=80; 35.2%)	7.33±4.52	
High School and over(n=45; 19.8%)	6.29±4.34		**Hypertension disease**		
Employment			Yes(n=159; 70.0%)	7.32±4.11	**.001**
Unemployed(n=123; 54.2%)	7.62±4.21	**.001**	No (n=68; 30.0%)	5.56±3.68	
Employed(n=104; 45.8%)	5.82±3.67		**Complications Related to Diabetes**		
Smoking Status			Yes(n=87;38.3%)	7.32±4.44	.170
Yes(n=50; 22.0%)	6.76±4.04	.944	No(n=140;61.7%)	6.46±3.78	
No(n=177; 78.0%)	6.80±4.08		**Diabetic retinopathy**		
Body Mass Index distribution			Yes(n=31; 13.7%)	8.58±5.22	**.047**
25-29.9(n=103; 45.4%)	5.65±3.50	.000	No(n=196; 86.3%)	6.51±3.78	
≥ 30(n=124; 54.6%)	7.74±4.26		**Diabetic nephropathy**		
Duration of disease			Yes(n=35; 15.4%)	7.57±4.76	.286
<11 years (n=140; 61.7%)	6.24±3.69	.024	No(n=192; 84.6%)	6.65±3.92	
≥11 years (n=87; 38.3)	7.62±4.46		**Diabetic neuropathy**		
Diabetic foot			Yes(n=62; 27.3%)	7.74±4.60	**.049**
Yes(n=9; 4.0%)	6.78±6.30	.698	No(n=165; 72.7%)	6.45±3.79	
No(n=218; 96.0%)	6.79±3.96				

Note: Mann-Whitney U and Kruskal-Wallis tests were used.

Table 2. *The relationship of PHQ-9 results with sociodemographic information*

PHQ-9 Scores		
(N;%)	**Mean±Std.**	**p**
Gender		
Women(n=135; 59.5%)	7.93±5.67	**.000**
Men(n=92; 40.5%)	4.46±4.30	
Income level		
Low(≤1700 TL)(n=76; 33.5%)	7.91±5.77	**.017**
Medium(1701-3499 TL)(n=118; 52.0%)	6.00±5.26	
High(≥3500 TL)(n=33; 14.5%)	5.18±4.58	
Education Level		
Below high school(n=182; 80.2%)	6.74±5.51	.216
High School and over(n=45; 19.8%)	5.62±5.01	
Employment		
Unemployed(n=123; 54.2%)	7.97±5.86	**.000**
Employed(n=104; 45.8%)	4.81±4.29	
Smoking Status		
Yes(n=50; 22.0%)	5.48±4.24	.073
No(n=177; 78.0%)	6.81±5.69	
Body Mass Index		
25-29.9(n=103; 45.4%)	4.90±4.81	**.000**
≥ 30(n=124; 54.6%)	7.86±5.55	
Duration of Disease		
<11 years (n=140; 61.7%)	6.21±4.79	.302
≥11 years (n=87; 38.3%)	7.02±6.31	
HbA1c		
<7(n=118; 52.0%)	6.13±4.98	.260
≥ 7(n=109; 48.0%)	6.94±5.86	
Blood Fasting Glucose		
<131(n=105; 46.3%)	6.26±5.21	.497
≥131(n=122; 53.7%)	6.75±5.61	
Treatment Style		
Only oral treatment(n=147; 64.8%)	5.88±4.70	**.016**
Insulin and oral treatment(n=80; 35.2%)	7.69±6.42	
Complications Related to Diabetes		
Yes(n=87;38.3%)	7.53±6.24	**.038**
No(n=140;61.7%)	5.89±4.76	
PSQI Scores Avarage		
<6 points(n=104; 45.8%)	3.79±3.56	**.000**
≥6 points(n=123; 54.2%)	8.83±5.66	

Note: Mann-Whitney U and Kruskal-Wallis tests were used.

Table 3. *Correlation of HbA1c, BFC, PHQ-9, and PSQI*

		HbA1c	BFC	PHQ-9	PSQI
HbA1c	r	-	-	—	-
	p	.	-	-	-
BFC	r	.629*	-	-	-
	p	<.001	.	-	-
PHQ-9	r	.019	.018	-	-
	p	.770	.793	.	-
PSQI	r	.081	.096	.614*	-
	p	.222	.149	<.001	.

**Spearman's correlation coefficient is significant at the .05 level (2-tailed).*

4. DISCUSSION

Sleep is a fundamental human need, and disruptions in sleep quality can have profound effects on both physical and mental health. This study is the first to reveal that factors such as unemployment, diabetes-related neuropathy and retinopathy, and hypertension significantly impair sleep quality in patients with T2DM.

In this study, 54.2% of participants had poor sleep quality, which aligns closely with the prevalence reported by Solinska et al. (53%) (18). Similarly, Keskin et al. identified poor sleep quality in 60.9% of their participants (mean PSQI = 6.18 ± 3.42) (19), and Tsai et al. reported a comparable prevalence (20). However, studies conducted in Iran and Egypt showed divergent results, with one reporting a lower prevalence of 38% (21) and the other a much higher rate of 81% (PSQI ≥ 8) (22). These variations likely stem from differences in cultural, environmental, and living conditions across populations.

Gender differences were evident, with women exhibiting significantly poorer sleep quality than men (OR = 1.24). This finding is consistent with previous studies (19, 20, 22, 23) and may be attributed to women's heightened emotional sensitivity and greater exposure to stressors (24). Employment also played a role, with employed participants showing better sleep quality. This may be due to more structured daily routines and better regulation of sleep-wake cycles, as suggested in prior research (22, 25).

Obesity emerged as a critical factor influencing sleep quality. Consistent with studies by Hung et al. (26) and Keskin et al. (19), higher BMI was positively associated with poorer sleep quality. Excess weight can contribute to physical conditions such as hypoventilation and hypoxemia, which disrupt sleep (27). Additionally, the relationship between sleep disturbances and neuroendocrine changes, including increased appetite and altered glucose metabolism, highlights a complex interplay that could exacerbate both obesity and T2DM (28-29).

Although our study did not find significant correlations between PSQI scores and HbA1c or fasting blood glucose (BFG) levels, participants with higher HbA1c and BFG levels tended to report poorer sleep quality. This finding aligns with Şahin et al. (31) but contrasts with studies by Keskin et al. (19) Tsai et al. (20), and Barakat et al. (22), which showed poorer sleep quality in patients with uncontrolled HbA1c. The lack of statistical significance in our study may be attributed to the relatively small sample size.

The relationship between treatment style and sleep quality remains unclear, with conflicting findings reported in the literature (31). Our study found no significant association. However, diabetes-related complications, particularly neuropathy and retinopathy, were strongly linked to poorer sleep quality. These findings contrast with Solinska et al., who did not observe such associations (18). Neuropathy symptoms such as pain, tingling, and electric shock-like sensations, which worsen at night, are likely contributors

to disrupted sleep (32). Further research is needed to substantiate these results.

Depression was another critical determinant of poor sleep quality. In this study, PSQI scores increased with the severity of depression, with each 1-point increase in PSQI corresponding to a 1.25-fold increase in PHQ-9 scores. This finding is consistent with Shamshigaran et al., who also reported a strong association between psychological distress and sleep quality (21). Factors such as female gender, unemployment, obesity, combined therapy, and diabetes-related complications were linked to higher PHQ-9 scores. For instance, Kara et al. highlighted that patients undergoing combined insulin and oral therapy reported greater depressive symptoms, likely due to the psychological burden associated with complex treatment regimens (33).

Overall, this study identifies key factors contributing to poor sleep quality in individuals with T2DM, including female gender, obesity, unemployment, hypertension, and depression. Moreover, diabetes-related complications such as neuropathy and retinopathy were found to have a detrimental impact on sleep, a less frequently reported observation. The bidirectional relationship between sleep disturbances and depression underscores the importance of addressing both conditions comprehensively (34).

In conclusion, it is essential to routinely evaluate and manage sleep disorders and depression in patients with T2DM, particularly in those who are female, obese, unemployed, or suffering from diabetes-related complications. Although this study found no significant correlations between fasting blood glucose, HbA1c, and sleep quality, future research with larger sample sizes is recommended to better understand these associations.

LIMITATIONS OF THE STUDY

In this current study, the fact that groups differing in terms of diabetes duration and complications were evaluated together may have been a limitation in showing the strength of the effect. Conducting studies with similar groups and observing the situation after treatment may reveal the latent relationships clearly. The study being carried out in a single center and inadequate number of the patient population are the limitations of the study.

5. CONCLUSION

In the study, we determined the factors affecting sleep quality in diabetic patients as being female, obesity,being unemployed, having diabetes-related neuropathy and retinopathy, having hypertension, and having a high level of depression.

Therefore, all patients, especially female, obese, unemployed, diabetic patients with complications, should be evaluated and treated for sleep disorders and depression. Accordingly, in order to improve sleep quality in patients with Type 2 DM, it can be recommended to get them to lose weight, to prevent development of complications by getting them to have primary care check-ups, and to evaluate them psychologically in a regular manner. Although there was no significant correlation between fasting blood glucose and HbA1c and sleep quality in our study, we think that further studies should be conducted on this subject.

Funding:*Theauthor(s) receivednofinancialsupportfortheresearch.*
Conflicts of interest: *The authors declare that they have n oconflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Board of Sisli Hamidiye Etfal Health Application and Research Center (HARC) (Approval date: 07.08.2018; Number: 2064)*
Author Contributions:
Research idea: ESE, GZÖ
Design of the study: ESE, GZÖ
Acquisition of data for the study: ESE
Analysis of data for the study: ESE
Interpretation of data for the study: ESE
Drafting the manuscript: GZÖ
Revising it critically for important intellectual content: GZÖ
Final approval of the version to be published: ESE, GZÖ

REFERENCES

[1] American Diabetes Association. 6. Glycemic Targets: *Standards of Medical Care in Diabetes—2020. Diabetes Care* 2020; 43 (Supp. 1): S66–S76. https://doi.org/10.2337/dc20-S006

[2] Yılmaz MB, Kılıçkap M, Abacı A, Barçın C, Bayram F, Karaaslan D, Göksülük H, Kayıkçıoğlu M, Özer N, Süleymanlar G, Şahin M, Tokgözoğlu L, Satman İ. Temporal changes in the epidemiology of diabetes mellitus in Turkey: A systematic review and meta-analysis. Archives of the Turkish Society of Cardiology 2018; 46(7):546-555. https://doi.org/10.5543/tkda.2018.88225

[3] Garg P, Duggal N. Type 2 diabetes mellitus, its impact on quality of life and how the disease can be managed-a review. Obesity Medicine. 2022; 35: 100459 https://doi.org/10.1016/j.obmed.2022.100459

[4] Gebremariam GT, Biratu S, Alemayehu M, Welie AG, Beyene K, Sander B, Gebretekle GB. Health related quality of life of patients with type 2 diabetes mellitus at a tertiary care hospital in Ethiopia. PLoS ONE 2022; 17(2): e0264199. https://doi.org/10.1371/journal.pone.0264199

[5] Plantinga L, Rao MN, Schillinger D. Prevalence of self-reported sleep problems among people with diabetes in the United States, 2005-2008. Preventing Chronic Disease 2012;9: E76. https://doi.org/10.5888/pcd9.110244

[6] Khandelwal D, Dutta D, Chittawar S, Kalra S. Sleep disorders in type 2 diabetes. Indian Journal of Endocrinology and Metabolism 2017;21(5): 758-761. https://doi.org/10.4103/ijem.IJEM_156_17

[7] World Health Organization. Regional Office for Europe.(2004). WHO technical meeting on sleep and health: Bonn Germany, 22–24 January 2004. World Health Organization. Regional Office for Europe. Available from: https://apps.who.int/iris/handle/10665/349782

[8] Crivello A, Barsocchi P, Girolami M, Palumbo F. The meaning of sleep quality: A survey of available technologies. IEEE Access. 2019;(7):167374–167390. https://doi.org/10.1109/ACCESS.2019.2953835

[9] Choi D, Kim BY, Jung CH, Kim CH, Mok JO. Association between sleep quality and painless diabetic peripheral neuropathy assessed by current perception threshold in type 2 diabetes mellitus. Diabetes Metab J. 2021 May;45(3):358-367. https://doi.org/10.4093/dmj.2019.0219

[10] Reutrakul S, Van Cauter E. Sleep influences on obesity, insulin resistance, and risk of type 2 diabetes. *Metabolism.* 2018 Jul;84:56-66. https://doi.org/10.1016/j.metabol.2018.02.010

[11] Anothaisintawee T, Reutrakul S, Van Cauter E, Thakkinstian A. Sleep disturbances compared to traditional risk factors for diabetes development: Systematic review and meta-analysis. Sleep Medicine Reviews. 2016;30:11-24. https://doi.org/10.1016/j.smrv.2015.10.002

[12] Knutson KL, Ryden AM, Mander BA, Van Cauter E. Role of sleep duration and quality in the risk and severity of type 2 diabetes mellitus. Archives of Internal Medicine. 2006;166(16):1768-74. https://doi.org/10.1001/archinte.166.16.1768

[13] Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2 nd ed. Lawrence Erlbaum Associates.1988.

[14] American Diabetes Association. 6. Glycemic Targets: *Standards of Medical Care in Diabetes—2020. Diabetes Care* 2020; 43 (Supp. 1): S66-S76. https://doi.org/10.2337/dc20-S006

[15] Agargün MY, Kara H, Anlar Ö. Pittsburgh Uyku Kalitesi İndeksi'nin geçerliği ve güvenirliği. Türk Psikiyatri Dergisi 1996; 7(2): 107–115.

[16] Kroenke K, Spitzer RL, Williams JB. The PHQ-9: Validity of a brief depression severity measure. Journal of General Internal Medicine. 2001;16(9):606-13. https://doi.org/10.1046/j.1525-1497.2001.016009606.x.

[17] Sari YE, Kokoglu B, Balcioglu H, Bilge U, Colak E, Unluoglu I. Turkish reliability of the Patient Health Questionnaire-9. Biomedical Research. 2016 Special Issue 460-462.

[18] Solińska M, Śliwińska A, Kosmalski M, Drzewoski J. The phenotype of elderly patients with type 2 diabetes mellitus and poor sleep quality. *International Journal of Environmental Research and Public Health,* 2020;*17*(16): 5992. https://doi.org/10.3390/ijerph17165992

[19] Keskin A, Ünalacak M, Bilge U, Yildiz P, Güler S, Burak SE, Muzaffer B. Effects of sleep disorders on hemoglobin a1c levels in type 2 diabetic patients. Chinese Medical Journal, 2015; 128(24): 3292–3297. https://doi.org/10.4103/0366-6999.171415

[20] Tsai Y-W, Kann N-H, Tung T-H, Chao Y-J Lin C-J, Chang KC, Chang SS, Chen JY. Impact of subjective sleep quality on glycemic control in type 2 diabetes mellitus. Family Practice, 2012; 29(1):30–35. https://doi.org/10.1093/fampra/cmr041

[21] Shamshirgaran SM, Ataei J, Malek A, Iranparvar-Alamdari M, Aminisani N. Quality of sleep and its determinants among people with type 2 diabetes mellitus in Northwest of Iran. World Journal of Diabetes, 2017;8(7):358-364. https://doi.org/10.4239/wjd.v8.i7.358

[22] Barakat S, Abujbara M, Banimustafa R, Batieha A, Ajlouni K. Sleep quality in patients with type 2 diabetes mellitus. Journal of Clinical Medicine Research 2019;11(4):261–266. https://doi.org/10.14740/jocmr2947w

[23] Cho E-H, Lee H, Ryu OH, Choi MG, Kim S-W. Sleep disturbances and glucoregulation in patients with type 2 diabetes. Jornal of Korean Medical Science 2014; 29(2):243-247. https://doi.org/10.3346/jkms.2014.29.2.243

[24] Arslan I. Bilinçli farkındalık, depresyon düzeyleri ve algılanan stres arasındaki ilişki. Birey ve Toplum 2018; 8(2):73–86. https://doi.org/10.20493/birtop.477445

[25] Aktaş H, Şaşmaz CT, Kılınçer A, Mert E, Gülbol S, Külekçioğlu D, Kılar S, Yüce R, İbik Y, Uğuz E, Demirtaş A. Yetişkinlerde fiziksel aktivite düzeyi ve uyku kalitesi ile ilişkili faktörlerin araştırılması. Mersin Univ Saglık Bilim Derg. Mart 2016;8(2):60-70.

[26] Hung H-C, Yang Y-C, Ou H-Y, Wu J-S, Lu F-H, Chang CJ. The association between self-reported sleep quality and overweight in a Chinese population. Obesity, 2013;21(3):486–492. https://doi.org/10.1002/oby.20259

[27] Güzel A. Sleep related hypoventilation and hypoxemia disorders. Güncel Göğüs Hastalıkları Serisi, 2014;2(2):213–224. https://doi.org/10.5152/gghs.2014.0011

[28] Spiegel K, Knutson K, Leproult R, Tasali E, Cauter E. Sleep loss: A novel risk factor for insulin resistance and Type 2 diabetes. Journal of Applied Physiology 2005;99(5):2008–2019. https://doi.org/10.1152/japplphysiol.00660.2005

[29] Van Cauter E, Spiegel K, Tasali E, Leproult R. Metabolic consequences of sleep and sleep loss. Sleep Medicine, 2008;9(1):23–28. https://doi.org/10.1016/S1389-9457(08)70013-3

[30] Şahin S, Haliloglu Ö, Korkmaz ÖP, Durcan E, Şahin HR, Yumuk VD, Damcı T, ilkova HM, Siva ZO. Does treatment with sodium-glucose co-transporter-2 inhibitors have an effect on sleep quality, quality of life, and anxiety levels in people with Type 2 diabetes mellitus? Turkish Journal of Medical Sciences, 2012;51(2):735-742. https://doi.org/10.3906/sag-2008-37

[31] Rajendran A, Parthsarathy S, Tamilselvan B, Seshadri KG, Shuaib M. Letter: prevalence and correlates of disordered sleep in southeast asian indians with type 2 diabetes. Diabetes & Metabolism Journal 2012;36(4):314-315. https://doi.org/10.4093/dmj.2012.36.1.70

[32] Galer BS, Gianas A, Jensen MP. Painful diabetic polyneuropathy: Epidemiology, pain description, and quality of life. Diabetes Research and Clinical Practice 2000;47(2):123–128. https://doi.org/10.1016/S0168-8227(99)00112-6

[33] Kara AM, Kara T. Relationship of treatment method with medication adherence, quality of life and depression in patients diagnosed with t2dm. The Medical Bulletin of Haseki, 2019; 57(4):377-385. https://doi.org/10.4274/haseki.galenos.2019.4874

[34] Ma L, Zhang C. The function and structure of precuneus is associated with subjective sleep quality in major depression. Frontiers in Psychiatry 2021; 12:831524. https://doi.org/10.3389/fpsyt.2021.831524

How to cite this article: Esen ES, Öztürk GZ. Analysis of sleep quality and related factors in patients with Type 2 Diabetes Mellitus. Clin Exp Health Sci 2025; 15: 76-82. DOI: 10.33808/clinexphealthsci.1442009

Clinical and Experimental Health Sciences

Computerized Tomography-Based Scoring Systems (Marshall and Rotterdam Score) versus Physiological Scoring Systems (GCS and APACHE II Score) in Predicting Mortality in Traumatic Brain Injury

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Received: March 23, 2024 **Accepted:** March 6,2025

ABSTRACT

Objective: Since traumatic brain injury (TBI) has high mortality rates, it is essential to identify patients with poor prognosis. In this study, the mortality prediction performances of the Glasgow Coma Scale (GCS), Acute Physiology and Chronic Health Assessment-II (APACHE-II), Marshall, and Rotterdam scores were compared in patients with TBI in the intensive care unit (ICU) of a tertiary center.

Methods: Patients followed up in the ICU due to moderate to severe TBI between January 2020 and January 2022 were retrospectively reviewed. Patients were classified as survivor and nonsurvivor groups. The patient's clinical characteristics and the scoring systems' performance in predicting 28-day mortality were investigated.

Results: A total of 150 patients were included in the study, and 82.4% (n=98) were male. GCS scores were significantly lower in the nonsurvivor group, while APACHE-II, Marshall, and Rotterdam scores were significantly higher ($p < .001$ for all). GCS, APACHE-II, and Rotterdam scores were independent predictors of mortality ($p = .002$, $p = .012$, and $p = .003$, respectively). Receiver operating characteristics curve analysis revealed that GCS cut-off value was ≥6.5, area under the curve (AUC)=0.851, APACHE-II cut-off value was ≥ 21.5, AUC=0.866, Marshall cut-off value was ≥ 3.5, AUC=0.827 and Rotterdam cut-off value was ≥ 3.5, AUC=0.864.

Conclusion: GCS, APACHE-II, Marshall, and Rotterdam scores are valid in predicting mortality in patients with TBI. Their performance in predicting mortality is ranked from highest to lowest as APACHE-II, Rotterdam, GCS, and Marshall.

Keywords: Acute Physiology and Chronic Health Evaluation-II (APACHE-II); Glasgow Coma Scale; in-hospital mortality; scoring system; traumatic brain injury.

1. INTRODUCTION

Traumatic brain injury (TBI) is defined as a disruption in the normal function of the brain due to a blow to the head or a penetrating head injury. It is also called the ''silent epidemic'' due to the increase in its incidence worldwide. Its annual incidence has been reported to be between 27 and 69 million (1). TBI has become a critical health problem that causes economic loss for both the affected individual and society, as well as being a significant cause of morbidity and mortality (2). Early diagnosis and classification in the acute phase of severe TBI cases are essential for better management and predicting outcomes.

TBI is classified as mild (GCS=13-15), moderate (GCS=9-12), and severe (GCS=3-8) using the Glasgow Coma Scale (GCS), a physiological scoring system frequently used in the intensive care unit (ICU) (3). It has been reported that the reliability and accuracy of GCS may be low because the assessment of verbal and motor responses may not be optimal in patients with severe TBI who are orotracheal intubated or under the influence of sedative drugs (4). Another physiological scoring system, Acute Physiology and Chronic Health Assessment-II (APACHE-II) is also used in the ICU for mortality prediction in trauma patients and non-trauma patients (5-8). Another option for early and objective assessment of TBI severity is a morphological classification based on computed tomography (CT) scans, which is the preferred method due to rapid image acquisition. Marshall and Rotterdam scores, which are CT-based scoring systems, have also been reported to help predict mortality in patients with TBI (9-10).

https://doi.org/10.33808/clinexphealthsci.1457777

Although the mortality prediction performances of physiological scoring systems and CT-based scoring systems in patients with TBI are compared in the literature, there is no consensus on their superiority. This study aims to compare the prognostic values of GCS, APACHE-II, Marshall, and Rotterdam scores in predicting mortality in patients with TBI who are followed in the ICU of a tertiary center.

2. METHODS

This retrospective observational study was started after the approval of the Istanbul Kanuni Sultan Süleyman Training and Research Hospital Clinical Trials Review Board and Ethics Committee (date: 11.11.2023, KAEK/2023.10.142). The study was conducted following the principles of the Declaration of Helsinki. The data of TBI patients who were followed up and treated in the two years between January 2020 and January 2022 at the ICU of the University of Health Sciences Türkiye, Istanbul Kanuni Sultan Süleyman Training and Research Hospital, were scanned from hospital records and patient files and included in the study.

Inclusion criteria are as follows: (1) age ≥18 years; (2) isolated head trauma; (3) admitted to ICU within 24 hours after trauma; (4) abbreviated injury score (AIS) ≥ 3; (5) patients with moderate to severe TBI (GCS<13). Exclusion criteria included: (1) performing cardiopulmonary resuscitation within the first 24 hours after trauma; (2) staying in the ICU for less than 24 hours or death of the patient within the first 24 hours; (3) absence of brain tomography imaging; (4) COVID-19 suspected or positive; (5) missing data.

Demographic data, primary diagnoses, trauma type and etiology, operation status, ICU, mechanical ventilator (Mv) and hospital stay, discharge location, GCS and APACHE-II, Marshall and Rotterdam scores at ICU admission, and 28-day mortality of the patients were obtained from the hospital information system and patient files. The diagnoses of all patients with moderate to severe TBI were based on their histories and cranial tomography findings performed in the emergency department. The same radiologist calculated Marshall and Rotterdam scores by examining the cranial tomography.

2.1 Physiological Scoring Systems in TBI

GCS was defined in 1974 to determine the severity of trauma. It is frequently used as a significant predictor of prognosis in patients with head trauma. It is a popular, simple, reliable, and repeatable method for assessing trauma patients' consciousness levels. GCS scores range between 3 and 15 points, depending on patients' eye opening, verbal responses, and motor movements. Low scores indicate poor prognosis (11). The APACHE-II score evaluates 12 physiological parameters, as well as the patient's age and previous health status. The highest score is 71; high scores indicate poor prognosis (12). APACHE-II is not a trauma-specific scoring system like GCS. Only GCS is evaluated for trauma patients. However, it has been stated that APACHE-II is superior to GCS in trauma patients due to the evaluation of increasing age and chronic health problems.

2.2 Radiological Scoring Systems in TBI

Cranial CT scanning, the preferred method in the acute evaluation after severe TBI, objectively assesses structural damage. Its objectivity provides diagnostic information for operative intervention decisions and objective prognosis data. The Marshall and Rotterdam classifications, the two most common radiographic TBI classifications, are strongly associated with outcome. The Marshal and Rotterdam scoring systems, which score between 1 and 6 points based on cranial tomography findings, further underscore the objectivity of this diagnostic tool. The three main findings evaluated in the Marshall score are the status and amount of midline shift, the status of the basal cisternae, and high or mixed-density lesions, which depend on the lesion volume. In the Rotterdam score, in addition to the condition of the basal cisterns, the presence and amount of midline shift, as in the Marshal score, epidural mass lesion, intraventricular hemorrhage, or traumatic subarachnoid hemorrhage are evaluated (13-15).

The study's primary outcome was 28-day mortality in patients with moderate to severe TBI. It compared the mortality prediction performance of physiologic scoring systems (GCS and APACHE II score) and CT-based scoring systems (Marshal and Rotterdam score) at ICU admission. In this context, G* Power 3.1 program was used to determine the sample size. For t-tests, when $p < .05$, effect size = 0.5, and the power of the study was determined as 80%, it was calculated that 140 patients should be included in the study.

2.3. Statistical analysis

Statistical analysis was performed using the SPSS 25.0 (SPSS Inc., Chicago, USA) program. Data were expressed as number of patients, percentage, median (range), mean, and standard deviation. The normality of the data was evaluated with the Shapiro-Wilks test and histogram. Mann-Whitney U test was used to evaluate quantitative data that did not show normal distribution. Qualitative data were analyzed using Chi-square and Fisher exact tests. Multivariate regression analysis was used to determine whether GCS, APACHE-II, Marshal and Rotterdam score differed significantly between the groups and were independent predictors of mortality. Receiver operating characteristics (ROC) curve analysis was performed to determine the prognostic value of GCS, APACHE-II, Marshall and Rotterdam scores. Youden index (sensitivity+specificity-1) was used to determine the cut-off values in ROC analysis. The significance level was accepted as $p < .05$.

3. RESULTS

The study included 150 TBI patients who were followed up and treated in the ICU (Figure 1). The majority of the patients

were male (81.6% n=124). No significant difference was observed between the groups in terms of gender (p = .640). The mean age was significantly higher in the nonsurvivor group (55.1±23.7 vs. 44.5±20.2 years, p = .024). Blunt trauma was detected in 97.4% of the patients (n=148), and 55.3% (n=84) underwent surgery. The surgical status did not affect mortality (p = .274). The mean GCS score was significantly lower in the nonsurvivor group (4.2±1.9 vs. 8.7±3.3, p < .001), while APACHE-II (29.7±6.9 vs.18.4±7.9, p < .001), Marshall (4.7±1.0 vs. 2.8±1.3, p < .001) and Rotterdam (4.4±1.1 vs. 2.5±1.2, p < .001) scores were significantly higher. The revised trauma score was significantly lower in the nonsurvivor group (1.2±0.4 vs. 2.5±0.7, p < .001). Percutaneous tracheostomy was performed in the ICU for 10.5% of the total population (n=15). The tracheostomy procedure did not differ significantly between the groups (p = .096). In the nonsurvivor group, ICU and mechanical ventilation length of stay were significantly lower (p < .001 for both). However, hospital length of stay did not differ significantly between the groups (18.8±18.9 vs. 17.8±8.8 days, p =.187). After their treatment, patients were discharged to the ward (69.1%), palliative care (3.9%), external center (3.3%), and home (2%) in order of frequency. Brain death was diagnosed in 1.3% (n=2) of the entire population. The 28-day mortality rate was 20.7% (n=31) (Table 1).

When Marshall and Rotterdam CT scores were examined, Marshall class 2 (42.1%) and Rotterdam class 2 (34.9%) were detected most frequently in the entire population. Marshall class 5 (64.5%) and Rotterdam class 4 (29%) were observed most frequently in the nonsurvivor group (Table 2).

When trauma etiologies were evaluated, falls from the same level or heights (44.7%, n=68) and traffic accidents (38.2%, n=58) were the most common causes of TBI (Table 3).

The most common primary diagnoses in patients with TBI were acute subdural hematoma (30.9%, n=47) and subarachnoid hemorrhage (24.3%, n=37). Primary diagnoses did not differ significantly between the groups (p = .276) (Table 4).

Table 1. *Clinical characteristics of patients*

Variable	Overall (n=150)	Survivor (n=119)	Nonsurvivor (n=31)	p-value
Age (years)	46.8±21.4 (18-90)	44.5±20.2 (18-90)	55.1±23.7 (18-89)	**.024**
Gender, n (%)				.640
Female	28 (18.4)	21 (17.6)	7 (21.2)	
Male	124 (81.6)	98 (82.4)	26 (78.8)	
Trauma severity, n (%)				**< .001**
Moderate	68 (44.7)	66 (55.5)	2 (6.1)	
Severe	84 (55.3)	53 (44.5)	31 (93.9)	
Trauma type, n (%)				.206
Blunt	148 (97.4)	117 (98.3)	31 (93.9)	
Penetrating	4 (2.6)	2 (1.7)	2 (6.1)	
RTS score	2.2±0.8 (1-4)	2.5±0.7 (1-4)	1.2 ±0.4 (1-2)	**< .001**
GCS score	7.7 ±3.5 (3-12)	8.7 ±3.3 (3-12)	4.2 ±1.9 (3-10)	**< .001**
APACHE-II: score	20.8 ±9.0 (6-52)	18.4 ±7.9 (6-45)	29.7 ±6.9 (15-52)	**< .001**
Marshall score	3.2 ±1.5 (1-6)	2.8 ±1.3 (1-6)	4.7 ±1.0 (2-6)	**< .001**
Rotterdam score	2.9 ±1.4 (1-6)	2.5 ±1.2 (1-6)	4.4 ±1.1 (2-6)	**< .001**
Neurosurgery, n (%)	84 (55.3)	63 (52.9)	21 (63.6)	.274
Tracheostomy, n (%)	15 (10.5)	9 (7.6)	6 (18.2)	.096
Duration of ICU (days)	11.3±13 (2-95)	9.3±9.9 (2-60)	18.8±18.9 (2-95)	**< .001**
Duration of Mv (days)	6.5±11.2 (0-75)	3.6±8.1 (0-50)	16.7±14.6 (2-75)	**< .001**
Duration of hospital (days)	18±11.7 (2-95)	17.8±8.8 (4-60)	18.8±18.9 (2-95)	.187
Discharge				-
Ward	105 (69.1)			
Palliative care	6 (3.9)			
Transfer to outer center	5 (3.3)			
Home	3 (2)			
Brain death, n (%)	2 (1.3)			-
Mortality (28-day)	31 (20.7)			-

Values are expressed as number of patients, percentage, mean ± standard deviation (min-max). RTS: Revised trauma score, GCS: Glasgow Coma Scale, APACHE-II: Acute Physiology and Chronic Health Assessment-II, ICU: Intensive care unit, Mv: Mechanical ventilation

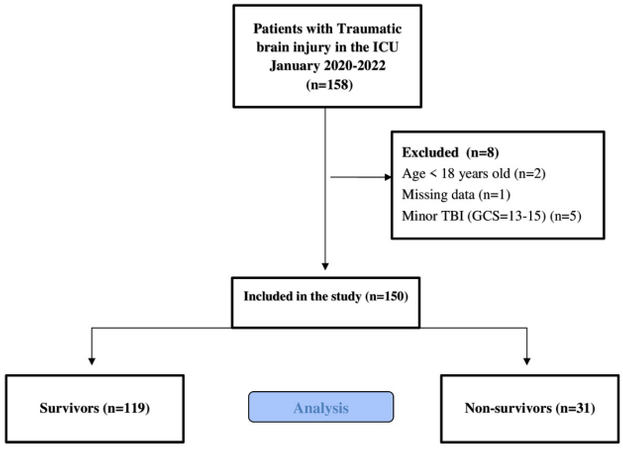

Figure 1. *Flow chart of the study*

Table 2. *Marshall and Rotterdam scores by groups*

Variable	Overall (n=150)	Survivor (n=119)	Nonsurvivor (n=31)
Marshall CT score			
I	10 (6.6)	10 (8.4)	0
II	64 (42.1)	62 (52.1)	2 (6.4)
III	14 (9.2)	11 (9.2)	3 (9.6)
IV	10 (6.6)	7 (5.8)	3 (9.6)
V	48 (31.6)	28 (23.5)	20 (64.5)
V	6 (3.9)	1 (0.8)	5 (16.1)
Rotterdam CT score			
I	21 (13.8)	21 (17.6)	0
II	53 (34.9)	51 (42.8)	2 (6.4)
III	31 (20.4)	27 (22.6)	4 (12.9)
IV	15 (9.9)	6 (5)	9 (29)
V	24 (15.8)	12 (10)	12 (3.9)
V	8 (5.3)	2 (1.6)	6 (19.3)

Values are expressed as the number of patients and percentage.

Table 3. *Trauma etiologies of patients with traumatic brain injury*

	Overall (n=150)	Survivor (n=119)	Nonsurvivor (n=31)
Falls	68 (44.7)	44 (37)	24 (72.7)
Traffic accidents	58 (38.2)	51 (42.9)	7 (21.2)
Fight/Battering	16 (10.5)	16 (13.4)	0
Work accidents, crush	6 (3.9)	6 (3.9)	0
Gunshot wounds/explosions	4 (2.6)	2 (1.7)	2 (6.1)

Values are expressed as the number of patients and percentage.

In multivariate regression analysis, GCS, APACHE-II, and Rotterdam scores were independent predictors of mortality (p =.002, p =.012, and p =.003, respectively) (Table 5).

In the ROC analysis of the prognostic values of the scoring systems in mortality prediction, the cut-off value for the GCS score was 6.5, and the area under the curve (AUC)=0.851 (95%CI, 0.788-0.914). The cut-off value for the APACHE-II score was 21.5, and the AUC=0.866 (0.806-0.926). The cut-off value for the Marshall score was 3.5, and the AUC=0.827 (0.755-0.899). The cut-off value for the Rotterdam score was 3.5, and the AUC=0.864 (0.799-0.929) (Table 6).

Table 4. *Primary diagnoses in traumatic brain injuries*

	Overall (n=150)	Survivor (n=119)	Nonsurvivor (n=31)	p-value
Primary diagnosis				.276
Acute subdural hematoma	47 (30.9)	34 (28.6)	13 (27.7)	
Subarachnoid hemorrhage	37 (24.3)	27 (22.7)	10 (30.3)	
Epidural hematoma	30 (19.7)	26 (21.8)	4 (12.1)	
İntraserebral hematoma	19 (12.5)	14 (11.8)	5 (15.2)	
Contusio cerebri	11 (7.2)	10 (8.4)	1 (3)	
Brain edema	8 (5.3)	8 (6.7)	0	

Values are expressed as the number of patients and percentage.

Table 5. *The multivariate logistic regression analysis results*

Variables	Odds Ratio	95% CI (min-max)	p-value
GCS score	1.475	1.154-1.885	**.002**
APACHE-II score	0.901	0.830-0.977	**.012**
Marshall score	0.669	0.386-1.160	.152
Rotterdam score	0.447	0.265-0.755	**.003**
Constant	388.6		.001

CI: Confidence interval (minimum-maximum), GCS: Glasgow Coma Scale, APACHE-II: Acute Physiology and Chronic Health Assessment-II

Table 6. *Prognostic performance of GCS, APACHE-II, Marshall and Rotterdam score for predicting mortality*

	Cut-off	Sensitivity	Specificity	AUC (95% CI)
GCS	6.5	0.909	0.731	0.851 (0.788-0.914)
APACHE-II	21.5	0.970	0.672	0.866 (0.806-0.926)
Marshall	3.5	0.848	0.697	0.827 (0.755-0.899)
Rotterdam	3.5	0.818	0.832	0.864 (0.799-0.929)

AUC: Area under curve, CI: Confidence interval (minimum-maximum), GCS: Glasgow coma scale, APACHE-II: Acute Physiology and Chronic Health Assessment-II

4. DISCUSSION

In this study, the values of physiological scoring systems and CT-based scoring systems in predicting mortality in patients with TBI followed in the ICU were investigated. It was determined that GCS, APACHE-II, Marshall, and Rotterdam scores helped predict mortality. In addition, GCS, APACHE-II, and Rotterdam scores were independent predictors of mortality. In the ROC analysis of the scores' prognostic values, the areas under the curve were close to each other and ranked from largest to smallest as APACHE-II (0.866), Rotterdam (0.864), GCS (0.851), and Marshall (0.827).

TBI affects people of all ages and genders in both developed and developing countries and is the leading cause of death and disability (16). Mortality rates as high as 30-40% have been reported in patients with severe TBI (17, 18). Gursoy et al. (19) from Turkiye reported that 57% of patients with

TBI were male, the mean age was 47±17 years, and the most common diagnoses were subarachnoid hemorrhage and subdural hematoma. The authors reported that the mortality rate was 34.6%. Goswami et al. (10) from India reported that 85% of patients with TBI were male and that gender did not have a significant effect on mortality. The authors reported that severe TBI was detected in 65% of patients, traffic accidents were the cause in 84% of patients, and the mortality rate was 32.3%. In our study, in line with the literature, the mean age in the entire population was 46±21 years, and 82.4% of the patients were male. Although the mean age was significantly higher in the nonsurvivor group, gender did not significantly affect mortality. The most common causes of TBI were falls (44.7%) and traffic accidents (38.2%). The most common primary diagnoses were acute subdural hematoma (30.9%) and subarachnoid hemorrhage (24.3%). Our 28-day mortality rate was found to be 20.7%. Our mortality rate, being lower than Gürsoy (19) and Goswami (10), may be affected by many parameters, such as the type of trauma, the presence of accompanying polytrauma, and the status of the operation, as well as the lower rate of serious TBI (55%) detected in our study. Although operations are frequently performed in TBI, it has been reported that the mortality of patients who undergo surgical procedures increases compared to patients who receive conservative treatment (20). In our study, although the rate of neurosurgery was high in the nonsurvivor group, no significant difference was observed (63% vs. 52%, p = .274). Intracranial hemorrhages and other traumatic brain injuries are the most important causes of brain death (21). In the current study, brain death was detected in 1.3% of the patients (n=2). We believe that early diagnosis and prediction of prognosis in patients with TBI are also crucial for organ transplantation.

Accurate and reliable scores are essential in assessing disease severity, predicting prognosis, and managing healthcare resources in critically ill TBI patients. Physiological scoring systems such as GCS and APACHE-II are frequently used in ICUs (5,11,12). Dalgiç et al. (20) reported that APACHE-II and GCS scores have sufficient sensitivity and specificity in predicting mortality in patients with head trauma accompanied by systemic trauma and that the APACHE-II (AUC=0.94) score is superior to GCS (0.87) in predicting mortality. Nik et al. (22) reported no significant difference between GCS and APACHE-II scores in predicting mortality in ICU patients with TBI. The authors stated that both scores had acceptable positive predictive value, but the APACHE-II (AUC=0.83) score performed better than the GCS (AUC=0.81). Gürsoy et al. (19) investigated the prognostic value of APACHE-II and INCNS (Infection, Nutrition, Consciousness, Neurological function, Systemic Condition) scores in patients with TBI. The authors reported that INCNS and APACHE-II scores had good prognostic performance, and the INCNS score was superior to APACHE-II in predicting TBI mortality. In our study, in line with the literature, the APACHE-II score was more successful in predicting mortality than GCS in patients with TBI (AUC=0.866 vs. 0.851). However, both GCS and APACHE-II scores were independent predictors of mortality. We believe that the APACHE-II score is superior to the GCS in predicting mortality because many factors can affect the GCS (use of sedatives and neuromuscular drugs, inability to assess verbal responses due to endotracheal intubation), and the 12 parameters evaluated in the APACHE-II score, as well as age and chronic health status, are effective.

In patients with head trauma, computed tomography provides a rapid and practical assessment of TBI severity using morphological characterization and diagnosis. For this purpose, CT-based scoring systems developed by Marshall (1995) and Rotterdam (2005) allow early prediction of clinical outcomes and prognosis in TBI patients (21). These CT-based scoring systems are repeatable, show minimal variability between evaluators, and are easy to use. Goswami et al. (10) reported that the Rotterdam and Marshall scores at the time of initial presentation of patients with TBI were significantly higher in the mortality group. The authors stated that the cut-off value of the Rotterdam score was >4, and the AUC was 0.827, and the cut-off value of the Marshall score was >3, and the AUC was 0.833. Asim et al. (23) stated that both scoring systems were independent predictors of mortality in patients with TBI. The authors stated that the Rotterdam score was superior to the Marshall score in predicting mortality. In another study, it was reported that both the Rotterdam and Marshall scores helped predict mortality in patients with TBI, and the AUC value was determined to be 0.85 (24). In our study, both scores were significantly higher in the mortality group, which aligns with the literature. However, while the Rotterdam score was an independent predictor of mortality, the Marshall score was not. While the cut-off value for both the Marshall and Rotterdam scores was determined as 3.5, the Rotterdam score was superior in predicting mortality (0.864 vs. 0.827). We compared the performance of physiologically based scoring systems and CT-based scoring systems in predicting mortality in patients with TBI, realizing that the literature lacks sufficient data to compare these scoring systems. We found that physiological scoring systems such as GCS and APACHE-II and CT-based scoring systems such as Rotterdam and Marshall scores have similar and acceptable prognostic values in critically ill patients with TBI.

Our study has some limitations. The main limitation is that it is retrospective and single-centered. In addition, Rotterdam and Marshall scores were obtained from cranial CT findings at the time of first admission to the emergency department, and CT findings during patient follow-up were not considered.

5. CONCLUSION

In conclusion, GCS, APACHE-II, Marshall, and Rotterdam scores are scoring systems with high sensitivity and specificity for predicting mortality in patients with TBI in the ICU. When the performances of physiological and CT-based scoring systems in predicting mortality were compared, it was determined that they were ranked as APACHE-II, Rotterdam, GCS, and Marshall, although they were similar. Using physiological and CT-based scores in patients with TBI will be helpful in

the early detection of patients with poor prognosis and in planning aggressive treatment.

Acknowledgements: *None.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by the Ethics Committee of the University of Health Sciences, Istanbul Kanuni Sultan Süleyman Training and Research Hospital Clinical Research Ethics Committee (Approval date: 11.11.2023; Number: KAEK/2023.10.142)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: KA, ÜT
Design of the study: KA, ÜT, ASŞ
Acquisition of data for the study: KA, ÜT
Analysis of data for the study: KA, ÜT
Interpretation of data for the study: KA, ÜT, ASŞ
Drafting the manuscript: KA, ÜT, ASŞ
Revising it critically for important intellectual content: KA, ÜT, ASŞ
Final approval of the version to be published: KA, ÜT, ASŞ

REFERENCES

[1] GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators: Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019,18(5):56-87. https://doi.org/10.1016/S1474-4422(18)30415-0

[2] Dang B, Chen W, He W, Chen G. Rehabilitation treatment and progress of traumatic brain injury dysfunction. Neural Plasticity 2017; 2017:1582182. https://doi.org/10.1155/2017/1582182

[3] Settervall CH, De Sousa RM, Fürbringer e Silva SC. In-hospital mortality and the Glasgow Coma Scale in the first 72 hours after traumatic brain injury. Rev Lat Am Enfermagem. 2011;19(6):1337-1343. https://doi.org/10.1590/s0104-11692011000600009

[4] Bledsoe BE, Casey MJ, Feldman J, Johnson L, Diel S, Forred W, Gorman C. Glasgow Coma Scale scoring is often inaccurate. Prehosp Disaster Med. 2015;30(1):46–53. https://doi.org/10.1017/S1049023X14001289

[5] Arslan K, Sahin AS, Yalcın N, Kaya E. Evaluation of trauma patients followed up and treated in intensive care unit: The sample of istanbul province training and research hospital. Turk J Intensive Care 2023;21(1):41-47. https://doi.org/10.4274/tybd.galenos.2022.20591

[6] Rea-Neto A, da Silva Junior ED, Hassler G, Dos Santos VB, Bernardelli RS, Kozesinski-Nakatani AC, Martins-Junior MJ, Reese FB, Cosentino MB, Oliveira MC, Teive HAG. Epidemiological and clinical characteristics predictive of ICU mortality of patients with traumatic brain injury treated at a trauma referral hospital - A cohort study. BMC Neurol. 2023;23(1):101. https://doi.org/10.1186/s12883-023-03145-2

[7] Arslan K, Sahin AS. Clinical characteristics of patients with methyl alcohol intoxication followed up in the intensive care unit and factors affecting mortality. Bagcilar Med Bull. 2023;8(3):222-229. https://doi.org/10.4274/BMB.galenos.2023.2022-12-106

[8] Arslan K, Arslan HC, Sahin AS. Evaluation of critically ill obstetric patients treated in an ıntensive care unit during the COVID-19 pandemic. Ann Saudi Med. 2023;43(1):10-16. https://doi.org/10.5144/0256-4947.2023.10

[9] Munakomi SA. Comparative study between Marshall and Rotterdam CT scores in predicting early deaths in patients with traumatic brain injury in a major tertiary care hospital in Nepal. Chin J Traumatol. 2016;19(1):25-27. https://doi.org/10.1016/j.cjtee.2015.12.005

[10] Goswami B, Nanda V, Kataria S, Kataria D. Prediction of in-hospital mortality in patients with traumatic brain injury using the rotterdam and marshall ct scores: A retrospective study from Western India. Cureus. 2023;15(7):e41548. https://doi.org/10.7759/cureus.41548

[11] Teasdale G, Jennett B. Assessment of coma and impaired consciousness: A practical scale. Lancet 1974;2(7872):81-84. https://doi.org/10.1016/s0140-6736(74)91639-0

[12] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: A severity of disease classification system. Crit Care Med. 1985;13(10):818-829.

[13] Steyerberg EW, Mushkudiani N, Perel P, Butcher I, Lu J, McHugh GS, Murray GD, Marmarou A, Roberts I, Habbema JD, Maas AI. Predicting outcome after traumatic brain injury: development and international validation of prognostic scores based on admission characteristics. PLoS Med. 2008;5(8):e165. https://doi.org/10.1371/journal.pmed.0050165 .

[14] Maas AI, Hukkelhoven CW, Marshall LF, Steyerberg EW. Prediction of outcome in traumatic brain injury with computed tomographic characteristics: A comparison between the computed tomographic classification and combinations of computed tomographic predictors. Neurosurgery 2005;57(6):1173-1182. https://doi.org/10.1227/01.neu.0000186013.63046.6b

[15] Marshall LF, Klauber MR, Van Berkum Clark M, Eisenberg HM, Jane JA, Luerssen TG, Marmarou A, Foulkes MA. A new classification of head injury based on computerized tomography. J Neurosurg. 1991;75(suppl):S14-S20. https://doi.org/10.3171/sup.1991.75.1s.0s14

[16] Garza N, Toussi A, Wilson M, Shahlaie K, Martin R. The increasing age of TBI patients at a single level 1 trauma center and the discordance between GCS and CT Rotterdam scores in the elderly. Front Neurol. 2020;11:112. https://doi.org/10.3389/fneur.2020.00112

[17] Unden J, Ingebrigtsen T, Romner B; Scandinavian Neurotrauma Committee (SNC). Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: An evidence and consensus-based update. BMC Med. 2013;25:11-50. https://doi.org/10.1186/1741-7015-11-50

[18] Maas AIR, Menon DK, Adelson PD, Andelic N, Bell MJ, Belli A, InTBIR Participants and Investigators. Traumatic brain injury: Integrated approaches to improve prevention, clinical care, and research. Lancet Neurol. 2017;16(2):987-1048. https://doi.org/10.1016/S1474-4422(17)30371-X

[19] Gürsoy G, Gursoy C, Kuscu Y, Gumus Demirbilek S. APACHE II or INCNS to predict mortality in traumatic brain injury: A retrospective cohort study. Ulus Travma Acil Cerrahi Derg. 2020;26(6):893-898. https://doi.org/10.14744/tjtes.2020.22654

[20] Dalgic A, Ergüngör FM, Becan T, Elhan A, Okay Ö, Yüksel BC. The revised Acute Physiology and Chronic Health Evaluation System (APACHE II) is more effective than the Glasgow Coma Scale for prediction of mortality in head-injured patients with systemic. Ulus Travma Acil Cerrahi Derg. 2009;15(5):453–458.

[21] Arslan K, Sahin AS. Evaluation of patients diagnosed with brain death in the intensive care unit: 10 years of tertiary center experience in Istanbul. North Clin Istanbul 2024;17;11(2):127-132. https://doi.org/10.14744/nci.2023.06937

[22] Nik A, Sheikh Andalibi MS, Ehsaei MR, Zarifian A, Ehsan Karimiani G, Bahadoorkhan G. The efficacy of Glasgow Coma Scale (GCS) Score and Acute Physiology and Chronic Health Evaluation (APACHE) II for predicting hospital mortality of icu patients with acute traumatic brain injury. Bull Emerg Trauma 2018;6(2):141-145. https://doi.org/10.29252/beat-060208

[23] Asim M, El-Menyar A, Parchani A, Nabir S, Ahmed MN, Ahmed Z, Ramzee AF, Al-Thani A, Al-Abdulmalek A, Al-Thani H. Rotterdam and Marshall Scores for Prediction of inhospital mortality in patients with traumatic brain injury: An observational study. Brain Inj. 2021;35(7):803-811. https://doi.org/10.1080/02699052.2021.1927181

[24] Mata-Mbemba D, Mugikura S, Nakagawa A, Murata T, Ishii K, Li L, Takase K, Kushimoto S, Takahashi S. Early CT findings to predict early death in patients with traumatic brain injury: Marshall and Rotterdam CT scoring systems compared in the major academic tertiary care hospital in northeastern Japan. Acad Radiol. 2014;21(5):605-611. https://doi.org/10.1016/j.acra.2014.01.017

How to cite this article: Arslan K, Topal Ü, Şahin AS. Computerized Tomography-Based Scoring Systems (Marshall and Rotterdam score) versus Physiological Scoring Systems (GCS and APACHE II score) in Predicting Mortality in Traumatic Brain Injury. Clin Exp Health Sci 2025; 15: 83-89. DOI: 10.33808/clinexphealthsci.1457777

Clinical and Experimental Health Sciences

First Molecular Investigation of VSSC-Linked Permethrin Resistance in Human Scabies in Türkiye

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Received: March 25, 2024 **Accepted:** December 4, 2024

ABSTRACT

Objective: Scabies, a longstanding public health concern, is a contagious and pruritic skin condition caused by the parasite *Sarcoptes scabiei*, affecting an estimated 300 million individuals worldwide annually. Recent increases in incidence can be attributed to challenges in accurate diagnosis and instances of treatment resistance. Permethrin, an insecticide belonging to the pyrethroid group, is the primary choice for scabies treatment. However, recent treatment failures suggest the emergence of permethrin resistance. Pyrethroids, widely employed as insecticides over the past three decades, have led to resistance development across various organisms. Pyrethroid acaricides like permethrin target the neuronal voltage-sensitive sodium channel (*VSSC*) protein, crucial for action potential generation in excitable cells. Specific mutations in the *VSSC* gene have been associated with pyrethroid resistance. Our objective is to elucidate the correlation between treatment failure and pyrethroid resistance stemming from *VSSC* gene mutations in *Sarcoptes scabiei* mites responsible for scabies cases in the Sivas region, Türkiye.

Methods: In this study, we analyzed 30 scabies cases where initial permethrin treatment proved ineffective. The *VSSC* gene of scabies mites was partially isolated from genomic DNA to identify potential mutations via DNA sequencing.

Results: Results yielded significant insights into the relationship between permethrin resistance and *VSSC* gene mutations. Notably, 43.3% of mites exhibited mutated *VSSC* genes.

Conclusion: This study represents the first investigation into Vssc-associated permethrin resistance in human scabies. The study highlights the importance of detecting genotypic resistance in 43.3% of phenotypically resistant cases.

Keywords: *Sarcoptes scabiei*, Membrane Transport Proteins, Single Nucleotide Polymorphism, Drug Resistance, Scabies.

1. INTRODUCTION

Sarcoptes scabiei is an obligate mammalian ectoparasitic arthropod responsible for causing scabies, a contagious, pruritic skin disease in humans, and mange in other mammals. This arthropod completes its life cycle within the stratum corneum layer of the skin, as it fulfills its oxygen requirement through skin respiration. Consequently, it remains confined to the stratum corneum, predominantly settling in body areas with higher temperatures and thinner stratum corneum layers (1). According to The Global Burden of Diseases, Injuries, and Risk Factors Study conducted in 2015 and 2017, the global prevalence of scabies was reported as 204,151,715 and 175,406,000, respectively (2,3). The annual prevalence of scabies remains approximately 300 million people worldwide, irrespective of race, age, or gender, persisting as a health concern for centuries (4). However, recent years have witnessed an increase in the disease's prevalence due to challenges in accurate diagnosis and the emergence of drug resistance.

The primary treatment for scabies is permethrin, a locally applied drug from the pyrethroid group (5). Pyrethroids, extensively utilized as insecticides worldwide over the past three decades, have led to the development of resistance in numerous organisms. Studies by Mazzatenta et al. (6), Balestri et al. (7), and Meyersburg et al. (8) have all reported decreased sensitivity to permethrin, suggesting treatment failure associated with permethrin resistance (9,10).

This resistance is attributed to mutations in the voltage-sensitive sodium channel (*VSSC*) protein, the target of pyrethroid acaricides like permethrin. Mutations in the *VSSC* gene directly contribute to resistance in various arthropod species (7,11). The voltage-sensitive sodium channel (*VSSC*) is

https://doi.org/10.33808/clinexphealthsci.1458615

a membrane protein composed of four homologous domains and six intermembrane domains. It plays a crucial role in generating action potentials in excitable cells and serves as the target for pyrethroid acaricides such as permethrin.

Pyrethroids disrupt the function of the *VSSC* protein by slowing the channel's activation and inactivation kinetics, leading to prolonged channel opening. This prolonged opening results in paralysis, ultimately causing the death of the organism. Studies have demonstrated that mutations in various positions of the *VSSC* gene, which encodes the channel protein, are directly associated with the development of resistance in many arthropod species. Pasay et al. investigated *Sarcoptes scabiei* var. *canis* mites that had undergone long-term permethrin therapy and exhibited resistance to treatment in vitro, showing reduced sensitivity to permethrin. Specifically, they identified a G → A mutation at position 1535 of the *Sarcoptes scabiei* var. *canis VSSC* gene, leading to a glycine to aspartic acid transition. This mutation has been implicated in conferring resistance to permethrin (11–13).

Despite the absence of reported scabies outbreaks in Türkiye, a notable increase in the number of cases has been observed since 2017. A study conducted across nine different provinces of Türkiye revealed a staggering 7-fold increase in cases from 2017 to 2018, followed by a 30-fold increase from 2018 to 2019. Furthermore, while no instances of resistance were noted until 2018, subsequent analyses indicated 20 cases (13.3%) and 87 cases (13.14%) of topical resistance in 2018 and 2019, respectively (14,15). Another study conducted in Erzurum province highlighted a nearly twofold increase in scabies cases observed in 2019 compared to those reported in the first quarter of 2020 (16). Additional findings from *Özçelik›s* study revealed that 57.6% of patients had undergone multiple treatments due to various factors including improper and inadequate drug application, reinfestation, or drug resistance. These data underscore the escalating burden of scabies in Türkiye and emphasize the importance of addressing challenges such as treatment efficacy, adherence, and resistance to effectively manage the disease (17).

Scabies has become increasingly prevalent in both the Sivas region and Türkiye overall in recent years, with permethrin proving ineffective in most cases. This study aims to investigate the presence of *VSSC* gene mutations associated with the *kdr* resistance phenotype, indicative of pyrethroid resistance, in *Sarcoptes scabiei* var *hominis* mites isolated from permethrin-treated scabies cases that failed treatment in the Sivas region, Türkiye.

2. METHODS

2.1. Ethics

This study was approved by the Sivas Cumhuriyet University Ethics Committee (decision no: 2020-12/04, Date 03/12/2020). The sample size for our study was determined based on practical considerations, including the prevalence of resistance observed in preliminary data related to the availability of cases during the study period. Before the sample collection, informed consent was obtained for the confidentiality of the information and its use in the study. When α (alpha) = 0.05, β (beta) = 0.20 and 1-β = 0.80 were taken, it was decided to include 30 individuals in the study and the strength of the test was found to be p = 0.80060..

2.2. Collecting the Sarcoptes scabiei samples

Sarcoptes scabiei samples were collected from volunteer patients diagnosed with scabies at the Sivas Cumhuriyet University, Faculty of Medicine, Department of Dermatology and Venereal Diseases. The study comprised 30 volunteer patients aged between 18 and 65 years. During sample collection, the skin was scraped multiple times along the tunnels without causing bleeding, using a scalpel and needle. In cases where typical tunnels were not visible, scraping samples were obtained from papules or 4-5 suspicious lesions. Mineralized oil was applied directly to the lesion or onto the scalpel to collect the *Sarcoptes scabiei* and its products in the scraping samples. Mites were positively identified in all skin scrapings. Skin scraping samples were collected both before the initial treatment and after the second treatment.

Subsequently, the mites were separated from the skin tissues under a stereo microscope and collected individually using a needle-tipped loop. DNA isolation was performed using multiple mites. Sarcoptes scabiei mite bodies were confirmed by examining the obtained material under a dry objective, and positive samples were stored at – 20°C for further analysis.

2.3. Genomic DNA Isolation

The genomic DNA (gDNA) samples were isolated utilizing the GeneJET Genomic DNA Purification Kit (Thermo ScientificTM/ catalog no K0721), following the manufacturer's protocol without any modifications. Subsequently, the quality of the isolated gDNA was assessed through agarose gel electrophoresis.

2.4. Partial isolation of VSSC gene

The conventional PCR method was used to amplify a 144 bp region of the *VSSC* gene containing the mutation. Gene-specific primers, VSSC_F_2 (5'-GAGCAGCCAGAGAAAGAAGTCAA-3') and VSSC_R_2 (5'-AGATCCGCCGGCTTTCTTT-3'), were utilized for this purpose. Each reaction was prepared in a total volume of 50 µL, consisting of 10 µL of 5X Phusion HF buffer, 0.5 µL of Phusion DNA Polymerase (2 U/µL) (Thermo Scientific™, Phusion™ High-Fidelity DNA Polymerase, catalog no. F530S), 10 mM dNTP mix, primers (0.5 µM each), and 5 µL of gDNA. The PCR conditions were set as follows: an initial denaturation at 96°C for 30 seconds; 27 cycles of 96°C for 15 seconds (denaturation), 58°C for 15 seconds (primer annealing), and 72°C for 30 seconds (extension); followed by a final extension

at 72°C for 5 minutes. PCR products were analyzed by agarose gel electrophoresis to confirm the expected band size and subsequently purified using the GeneJET PCR Purification Kit (Thermo Scientific™, catalog no. K0701), following the manufacturer's protocol without modifications.

2.5. DNA Sequencing

The purified PCR products were directly submitted for DNA sequencing without being transferred into a plasmid vector. The analysis was conducted at the Central Research Laboratory of Ankara Yıldırım Beyazıt University using gene-specific primers.

2.6. Multiple Sequence Analysis

The DNA sequencing results obtained from the forward and reverse directions of the *VSSC* gene, sampled from 30 individuals, were analyzed by aligning them using MEGAX software (18). All results were compared to the wild type *VSSC* mRNA (NCBI ID: DQ077149.2) to identify any mutations (19). Subsequently, the sequences obtained from multiple sequence alignment were translated into protein sequences and subjected to multiple sequence alignment again to identify amino acid mutations.

3. RESULTS

In the study, a point mutation resulting in a glycine to aspartic acid transition (GGC → GAC) in the *Sarcoptes scabiei VSSC* gene was identified in 13 out of 30 patients. All raw data and multiple sequence alignment of the DNA sequences are provided in the supplementary files (S1, S2 and S3). The multiple alignment of the protein sequences is illustrated in Figure I.

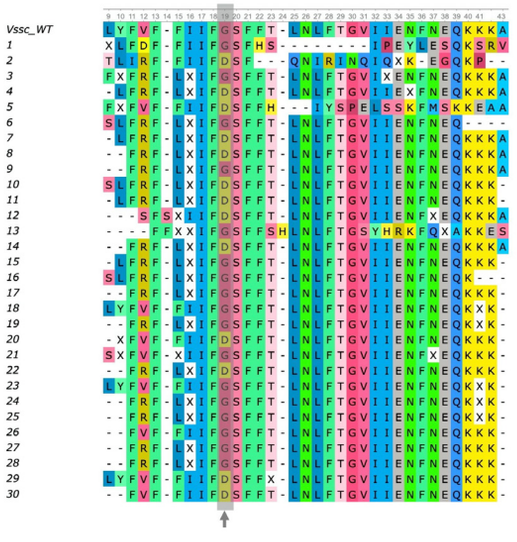

Figure 1. *The multiple sequence alignment of sequencing results with the reference gene (VSSC_WT). (A) Forward and (B) reverse orientation results of the DNA sequencing results.*

The patients who come to clinic who do not respond to the treatment are followed up by our clinic. All volunteers (30 cases) consisted of permethrin resistant scabies patients who did not respond to second or third permethrin treatment. Permethrin treatment was terminated in our patients (13 cases) who did not respond to the first three permethrin treatments and were treated with pomades containing sulfur and benzyl benzoate. 17 out of 30 cases, lesions were decreased after the second treatment, and a significant decrease in the number of parasites was observed in the skin scraping samples. Complete recovery was noted after the fourth drug application. However, 13 out of 30 cases, showed no response to permethrin treatment only. Therefore, we can associate the presence of mutation with the treatment response. However, we can consider patients who do not have a mutation and who do not respond to permethrin as not using the drug correctly or using it inadequately in the first treatment.

4. DISCUSSION

Scabies presents a significant global public health challenge, affecting individuals of all ages, races, genders, and socio-economic backgrounds, often leading to severe itching and diminishing quality of life. Particularly in recent years, there has been a notable increase in scabies cases worldwide. Permethrin application (5% topical) is typically recommended as the first-line treatment according to current guidelines for scabies management. However, despite the utilization of various treatment schedules, a decline in the efficacy of permethrin has been observed (6). Furthermore, two complementary studies have indicated decreased treatment effectiveness even with more aggressive treatment approaches suggested by guidelines and diverse treatment regimens (7,8).

Several potential reasons have been proposed for the failure of permethrin treatment. These include inadequate exposure time or amount of application, misuse of permethrin, failure to maintain short nails (which may harbor mites), insufficient treatment of hyperkeratosis, omission of permethrin application on the heads of children, failure to reapply permethrin after hand washing, reinfection from contact with infected individuals or contaminated items such as clothing, sheets, and towels, and the development of resistance to permethrin. Additionally, treatment failure may occur due to the inability to treat all family members simultaneously, particularly in communal living conditions (20).

Although not conclusively proven, it is hypothesized that permethrin resistance may result from various mechanisms, including the formation of mutations. Increased transcription rates of genes encoding glutathione-S-transferase, cytochrome p450, and monooxygenase in *Sarcoptes scabiei* mites have been suggested as potential mechanisms contributing to permethrin resistance (9,11). Pasay et al. identified a single point mutation at position 1535 of the *VSSC* gene in *Sarcoptes scabiei* mites resistant to permethrin (11).

This study identified a G→A single point mutation in the *VSSC* gene, resulting in a glycine → aspartic acid change in 13 out of 30 patients. This mutation causes a structural alteration in the protein, preventing permethrin binding and thereby conferring resistance (21). While a knockdown resistance (*kdr*) mutation associated with permethrin resistance has been detected in *Sarcoptes scabiei* var. *canis* mites and lice, it was not found in *Sarcoptes scabiei* var. *hominis* mites (22). Another study reported permethrin's effectiveness against *Sarcoptes scabiei* mites, attributing treatment failure to patient non-adherence (23).

In cases of permethrin resistance, sulfur ointments have proven to be an effective and safe alternative. Another viable option is the use of topical crotamiton (5% or 10%) applied for three to five consecutive days. Benzyl benzoate (25% for adults, 10% for children) applied topically for three consecutive days is also considered an effective alternative treatment. These options provide clinicians with multiple strategies to address resistance while tailoring treatment to individual patient needs (24).

Ivermectin is another treatment option for scabies, with potential mechanisms of action including genetic alterations and changes in the structure of the glutamate chloride channel and p-glycoprotein membrane transport protein. Ivermectin tablets (3 mg) are approved for the systemic treatment of scabies. The recommended dosage is 200 µg/kg of body weight for patients weighing 15 kg or more (24,25). Although recent meta-analyses suggest the efficacy of permethrin treatment, clinical observations indicate its diminishing effectiveness compared to previous years. Prospective genetic analyses are warranted to corroborate these findings (20).

5. CONCLUSION

Scabies has become increasingly prevalent in recent years, posing challenges in treatment efficacy. Our study reveals the presence of mutations previously documented in the literature in patient samples. Specifically, mutations were observed in 13 out of 30 samples collected. Significantly, our study highlights the importance of detecting genotypic resistance in 43.3% of phenotypically resistant cases, representing the first report from the Sivas region, Türkiye. Given the ineffectiveness of traditional scabies treatments in these cases, exploring alternative treatment options and developing novel therapeutic agents are imperative.

Funding
This work is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University under the project number T-2021-916.

Conflicts of interest
The authors declare that they have no conflict of interest.

Ethics Committee Approval: *This study was approved by Ethics Committee of Sivas Cumhuriyet University Clinic Ethics Committee (Approval date 03/12/2020; Number: 2020-12/04)*

Author Contributions:
Research idea: OK, MT, NO
Design of the study: OK, MT, NO
Acquisition of data for the study: OK, MT
Analysis of data for the study: OK
Interpretation of data for the study: OK, MT, NO
Drafting the manuscript: OK, MT, NO
Revising it critically for important intellectual content: OK, MT, NO
Final approval of the version to be published: OK, MT, NO

REFERENCES

[1] Mounsey KE. Molecular mechanisms of emerging ivermectin resistance in scabies mites from northern Australia. Charles Darwin University (Australia); 2007.

[2] Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: A systematic analysis for the Global Burden of Disease Study 2015. The lancet. 2016;388(10053):1545–602.

[3] James SL, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. The Lancet 2018;392(10159):1789–858.
https://linkinghub.elsevier.com/retrieve/pii/S0140673618322797

[4] Turan Ç, Metin N, Utlu Z. Epidemiological evaluation of scabies cases encountered in the last three years as a tertiary health center. Turkiye Parazitol Derg. 2020;44(2):77.

[5] Rosumeck S, Nast A, Dressler C. Ivermectin and permethrin for treating scabies. Cochrane Database of Systematic Reviews 1996;2018(4): CD012994.
https://doi.org/10.1002/14651858.CD012994

[6] Mazzatenta C, Piccolo V, Argenziano G, Bassi A. Is Scabies becoming less sensitive to permethrin therapy? Journal of the European Academy of Dermatology and Venereology 2021;35(9):e607–609. https://doi.org/10.1111/jdv.17339

[7] Balestri R, Magnano M, Infusino SD, Rizzoli L, Girardelli CR, Rech G. Scabies is becoming less sensitive to permethrin therapy. Journal of the European Academy of Dermatology and Venereology 2021;35(12):e889–91.
https://doi.org/10.1111/jdv.17538

[8] Meyersburg D, Kaiser A, Bauer JW. 'Loss of efficacy of topical 5% permethrin for treating scabies: an Austrian single-center study.' Journal of Dermatological Treatment 2022;33(2):774–777. https://doi.org/10.1080/09546634.2020.1774489

[9] Mounsey KE, Pasay CJ, Arlian LG, Morgan MS, Holt DC, Currie BJ. Increased transcription of Glutathione S-transferases in acaricide exposed scabies mites. Parasit Vectors. 2010;3(1):1–9.

[10] Pasay C, Arlian L, Morgan M, Gunning R, Rossiter L, Holt D. The effect of insecticide synergists on the response of scabies mites to pyrethroid acaricides. PLoS Negl Trop Dis. 2009;3(1):e354.

[11] Pasay C, Arlian L, Morgan M, Vyszenski-Moher D, Rose A, Holt D. High-resolution melt analysis for the detection of a mutation associated with permethrin resistance in a population of scabies mites. Med Vet Entomol. 2008 Mar;22(1):82–88.

[12] Miyazaki M, Ohyama K, Dunlap DY, Matsumura F. Cloning and sequencing of thepara-type sodium channel gene from susceptible and kdr-resistant German cockroaches (Blattella germanica) and house fly (Musca domestica). Mol Gen Genet.1996;252(1):61–68.

https://doi.org/10.1007/BF02173205

[13] Williamson MS, Martinez-Torres D, Hick CA, Devonshire AL. Identification of mutations in the houseflypara-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. Mol Gen Genet. 1996;252(1):51–60. https://doi.org/10.1007/BF02173204

[14] Özden MG, Ertürk K, Kartal SP, Yayli S, Göktay F, Doğramacı CA, et al. An extraordinary outbreak of scabies in Turkey. Journal of the European Academy of Dermatology and Venereology 2020;34(12):e818–820. https://doi.org/10.1111/jdv.16699

[15] Baykal C, Atci T, Kutlay A, Baykut B, Türkoğlu Z. Scabies outbreak in Turkey in 2018–2019. Journal of the European Academy of Dermatology and Venereology 2021;35(6):e384–385. https://doi.org/10.1111/jdv.17152

[16] Turan Ç, Metin N. Impact of Pandemic in the Frequency of Scabies: Possible Scabies Outbreak Scenario Aftermath COVID-19. Türkiye Parazitol Derg. 2021;45(3):190.

[17] Özçelik S. A neglected disease: Scabies a retrospective study on children. Türkiye Çocuk Hastalıkları Dergisi. 2021;121–6.

[18] Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6):1547–1549.

[19] Pasay C, Walton S, Fischer K, Holt D, McCarthy J. PCR-based assay to survey for knockdown resistance to pyrethroid acaricides in human scabies mites (Sarcoptes scabiei var hominis). Am J Trop Med Hyg. 2006 Apr;74(4):649–657.

[20] Sunderkötter C, Aebischer A, Neufeld M, Löser C, Kreuter A, Bialek R, et al. Increase of scabies in Germany and development of resistant mites? Evidence and consequences. Journal der Deutschen Dermatologischen Gesellschaft. 2019;17(1):15–23.

[21] Silver KS, Du Y, Nomura Y, Oliveira EE, Salgado VL, Zhorov BS, et al. Chapter Five - Voltage-Gated Sodium Channels as Insecticide Targets. In: Cohen E, editor. Advances in Insect Physiology [Internet]. Academic Press; 2014. p. 389–433.

[22] Andriantsoanirina V, Izri A, Botterel F, Foulet F, Chosidow O, Durand R. Molecular survey of knockdown resistance to pyrethroids in human scabies mites. Clinical Microbiology and Infection 2014;20(2):O139–41.

[23] Yürekli A. Is there a really resistance to scabies treatment with permethrin? In vitro killing activity of permethrin on Sarcoptes scabiei from patients with resistant scabies. Dermatol Ther. 2022;35(3):e15260.

[24] Currie BJ, Harumal P, McKinnon M, Walton SF. First documentation of in vivo and in vitro ivermectin resistance in Sarcoptes scabiei. Clinical Infectious Diseases. 2004;39(1):e8–12.

[25] Xu M, Molento M, Blackhall W, Ribeiro P, Beech R, Prichard R. Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. Mol Biochem Parasitol. 1998;91(2):327–35.

How to cite this article: Kılıçkaya O, Tosun M, Özpınar N. First Molecular Investigation of VSSC-Linked Permethrin Resistance in Human Scabies in Türkiye. Clin Exp Health Sci 2025; 15: 90-94. DOI: 10.33808/clinexphealthsci.1458615

Clinical and Experimental Health Sciences

Individualized Pelvic Floor Muscle Training with Single Session Versus Long Term Biofeedback for Treating Stress Urinary Incontinence: A Prospective Randomized Trial

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Received: April 1, 2024 **Accepted:** February 2, 2025

ABSTRACT

Objective: This study was planned to compare the effects of individualized pelvic floor muscle training (PFMT) in the treatment of stress urinary incontinence (SUI) applied with a single session versus long-term biofeedback (BF).

Methods: Thirty-three female patients with SUI were randomized into two groups. Sixteen patients in the first group were given an individualized PFMT program with BF, 2 days a week for 8 weeks, and a home exercise program on the other days. Seventeen patients in the second group were given a home exercise program after individualized PFMT with BF in a single session. After 8 weeks, both groups continued the exercises as a home program for another 4 weeks. Primary outcome parameters included a 3-day bladder diary, 1-hour pad test, maximum contraction pressure, duration of sustained contractions, King's Health Questionnaire, incontinence impact questionnaire, incontinence quality of life scale and Beck depression inventory. Patients were questioned in terms of fecal incontinence, sexual dysfunction and treatment satisfaction as a secondary outcome parameters.

Results: Thirty patients were able to complete the treatment. In the evaluations made at the 8th and 12th weeks, all of the primary outcome parameters improved in both groups ($p<.001$), and no statistically significant difference was found between the groups ($p>.05$). There was also improvement in secondary outcome parameters in both groups.

Conclusion: In the treatment of SUI, it was determined that individualized exercise program might be continued as a home program after BF was used as a single session to teach the exercises correctly in PFMT.

Keywords: Biofeedback, pelvic floor muscle training, stress urinary incontinence

1. INTRODUCTION

Urinary incontinence (UI), the involuntary loss of urine, is a common condition affecting women worldwide, impacting their quality of life (QoL) (1). Stress urinary incontinence (SUI), characterized by involuntary loss of urine during *physical exertion or effort* affects upto 37.5-53% in adult women with UI (2,3).

Initial management for all women with stress, urge and mixed UI is conservative management. The findings of the Cochrane review suggest that pelvic floor muscle training (PFMT) can be included in first-line conservative management for women with UI. Based on the data available, PFMT can cure or improve symptoms of SUI (4).

Many studies have shown that; even after individual training, more than 30% of women fail to contract PFMs correctly in their initial assessment (5,6). The most common mistake is contracting the hip adductors, abdominal and gluteal muscles (7). It has been determined that 25% of women use pushing force instead of lifting movement (6). Findings highlight the importance of instructing precise PFM contraction for successful treatment. Biofeedback (BF), can be used for the treatment of SUI to show PFMs activity at rest and during contraction. BF is particularly helpful for patients who have difficulty recognizing and isolating the correct muscles. The main contribution of the use of BF in PFMT is facilitating learning, improving contractions and encouraging the patients by providing exercise motivation. BF allows the patient to contract the PFMs voluntarily and accurately, provides the opportunity to correct and change the contractions to achieve better contraction; and also increases the patient's self-confidence and commitment to training in exercise performance (8).

https://doi.org/ 10.33808/clinexphealthsci.1462829

Considering that the most important benefit of BF is to teach the correct contraction of the PFMs, we aimed to investigate whether performing PFMT with an individually designed home program after teaching the correct contraction of the PFMs with a single session of BF in women with SUI could provide the same effectiveness as a long-term application of BF. As far as we know, this is the first study in the literature to compare the effectiveness of single session BF and long-term BF in PFMT in women with SUI. In case of similar efficacy, it may be recommended that women with SUI do PFMT at home, with an individually prepared home program after a one-time BF training, instead of going to the hospital for a long time.

2. METHODS

This prospective randomized study included 33 patients diagnosed with SUI admitted to the Ege University Medical Faculty, Obstetrics and Gynecology outpatient clinic between May 2019 and June 2020. The local ethics committee of Ege University Medical Faculty approved the study (date: 05.06.2018, no: 18-6/33). Patients were informed about the purpose and contents of the study and all women gave written consent to participate.

Female patients aged >18 years with mild to moderate symptoms of SUI and an increase in pad test measurement greater than 2 g were included in the study. Symptom severity was assessed by the 1-hour pad test, with a 1-10 g increase in pad weight indicating mild incontinence, an 11–50 g increase in moderate and a >50 g increase in severe incontinence (9). Patients with a previous history of genitourinary or SUI surgery, receiving pharmacological incontinence treatment, conservative treatment in the last 6 months, vaginal or urinary tract infection, genitourinary malignancy, overactive bladder, PFM strength <3/5 according to Modified Oxford Scale, stage 2 or more prolapse according to POP-Q, psychiatric or neurological disease that prevents feeling of PFM contractions, patients with poor perception that prevent to understand the verbal or visual instructions and patients who had known allergic response or sensitivity to the condom used with probe were not included in the study.

Thirty-three patients included in the study were divided into two groups according to the simple randomization scheme. All the participants were informed about the anatomical structure of PFMs and their importance in incontinence mechanism and treatment. Sixteen patients in the first group were given an individualized PFMT program with pressure – BF, lasting 20 minutes, 2 days a week for 8 weeks, and a home exercise program on other days. Seventeen patients in the second group were given a home exercise program after individualized PFMT with pressure-BF in a single session. BF was applied with Sonoplus 692, (Enraf-Nonius, Rotterdam, The Netherlands) device. An intravaginal pressure probe was used during the procedure. A disposable condom was placed on the pressure probe before each application. The probe was advanced 3-5 cm into the vagina while the patients were lying on their back with hip and knee flexion. They were trained how to contract the PFMs correctly without contracting abdominal, gluteal and hip adductor muscles. Individualized exercise program was arranged according to the baseline values determined by BF. The rapid maximal contraction exercises were continued as 1 second contraction and 2 seconds relaxation, and it was aimed to start with the number of fast contractions that the patient could do and reach a period of 10 repetitive fast contractions. Endurance exercises were started as long as the patient was able to maintain the contraction, and an endurance exercise program consisting of 10 repetitions was prepared with cycles followed by a resting period of twice the duration of the contraction. It was aimed to reach the target of 10 seconds of sustained maximal contractions and 20 seconds of relaxation in weekly increments. An individualized exercise program was planned in the form of 3 sets of 10 repetitions for each contraction type, in which rapid maximal contractions, sustained maximal contractions and then rapid maximal contractions were applied sequentially. A training session included 90 contractions in total, with 1-2 minutes of resting period between sets. Patients were asked to do the daily PFMT program twice a day for 8 weeks, to continue this until the 12th week, and to complete a regular exercise schedule.

Patients in the first group receiving regular BF training were instructed to repeat the exercises once more on the same day as their BF session, and to perform the exercises twice daily on the remaining days as part of their home program. Once the patients in the second group received accurate instruction in performing PFM exercises with BF during a single session, they were instructed to adhere to the home program, consisting of exercise sets similar in the first group, twice daily, every day.

Weekly exercise follow-up of the patients in the home exercise program was provided by phone calls. From the 4th week, the patients were told to do the exercise sets twice a day, one set in lying position, one set in sitting, and one set in standing position. From the end of the 8th week to the 12th week, all patients in both groups were told to continue the home exercise program consisting of 3 sets of exercises twice a day. At the end of the 8th week, all of the patients were taught the Knack maneuver and were asked to contract the PFMs just before and during activities that increase abdominal pressure such as coughing, sneezing, and laughing.

2.1. Evaluation Parameters

2.1.1. Bladder diary: The UI frequency and the number of pads used determined by the 3-day bladder diary.

2.1.2. One-hour pad test: A 1-hour pad test was performed to assess incontinence severity (9).

2.1.3. Maximum PFM Contraction Pressure (cmH_2O): Maximum contraction pressure of the PFMs was evaluated with intravaginal pressure probe of the BF. When the correct

contraction was achieved, the values of three consecutive contractions were recorded and the average value was used.

2.1.4. Sustained PFM contraction duration (sec.): The duration of the PFMs to sustain maximum or near-maximum contraction was measured with intravaginal pressure probe of the BF. The time to the point where the maximum contraction pressure is reduced to half was recorded. The average duration of three repeated contractions was used.

2.1.5. King's Health Questionnaire (KHQ): The KHQ is designed to measure the effects of UI symptoms on quality of life (QoL) and is used to evaluate improvement after treatment (10,11). The KHQ is composed of three sections. The first two sections of the KHQ, which consists of 21 items in nine areas, were used in this study. In these two sections, the results are scored between 0-100, lower scores indicate good health.

2.1.6. Incontinence Impact Questionnaire (IIQ-7): IIQ-7 is a 7-item quick questioning scale that shows the effects of incontinence on QoL (12,13). Lower scores indicate good health.

2.1.7. Incontinence Quality of Life Scale (I-QOL): I-QOL consists of 22 items that evaluate the effects of incontinence on QoL (14,15). Higher scores indicate better QoL.

2.1.8. Beck Depression* Inventory *(BDI) : BDI is a 21-item self-report questionnaire on which presence and severity of depressive symptoms are assessed (16,17).

Turkish validity and reliability studies of all the questionnaires used in this study were conducted (11,13,15,17).

2.1.9. Presence of sexual dysfunction and fecal incontinence: The presence of any of the symptoms of reluctance to have sexual intercourse, pain during intercourse, dissatisfaction, UI and lack of pleasure were stated as SD. Fecal incontinence was recorded as present or absent .

2.1.10. Visual Analog Scale (VAS): Treatment satisfaction is evaluated with a VAS. In the VAS evaluation, the far left of the 10 cm line was determined as "no improvement with treatment" and the far right as "much improvement with treatment".

All of them were recorded at the beginning of the treatment, at the end of the treatment (8th week), and at the end of the 12th week. As a secondary outcome of this study, patients were questioned in terms of flatus/fecal incontinence and sexual dysfunction (SD) at the beginning of the treatment and at the end of the 12th week. Treatment satisfaction was also evaluated as a secondary outcome by visual analog scale (VAS) at the end of treatment (8th week) and at the end of the 12th week.

2.2. Statistical Analysis

In this study, numerical data were summarized with mean, standard deviation, median, minimum and maximum values, and categorical data were summarized using frequency and percentage values with the help of IBM SPSS Statistics 25.0 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) package program. The significance level was determined as 0.05 in all analyzes (except for interaction, p< .1). The conformity of quantitative variables to normal distribution was evaluated with the Shapiro Wilk test. While demographic quantitative data were compared between groups with the Mann Whitney U test, the relationship between qualitative data and groups was evaluated with the Pearson – Chi-square test or Fisher's exact probability test. Time-dependent changes of measurements and questionnaire data in groups were analyzed with non-parametric Brunner-Langer model (F1-LD-F1 design), using R 3.5.2 software (R software, version 3.5.2, package: nparLD, R Foundation for Statistical Computing, Vienna, Austria; http://r-project.org). When significant differences were found between the times, pairwise comparisons were made under the same design and p-values were given with Bonferroni correction.

3. RESULTS

During the treatment period, one participant from the long-term BF group and two participants from the single-session BF group were excluded from the study. Two participants were excluded due to non-compliance with study requirements and follow-ups, while one participant was diagnosed with malignancy during treatment. At the end of the 12-week period, the outcomes of 30 participants were assessed (Figure 1). No adverse effects or incidents necessitating treatment discontinuation were noted during either the BF sessions or the home exercise programs.

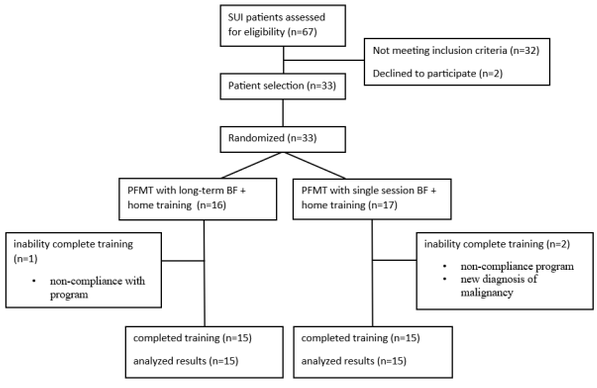

Figure 1. *Flow diagram. SUI, stress urinary incontinence; PFMT, pelvic floor muscle training; BF, biofeedback.*

Age, education level, symptom duration, body mass index (BMI), smoking habits, tea and coffee consumption, number of births, mode of delivery, history of episiotomy, menopausal status, and hormone replacement therapy history did not exhibit any statistically significant differences between the groups (Table 1).

Table 1. *Baseline comparison of demographic data*

	Group 1 (n= 15)	Group 2 (n= 15)	*p*
Age (years)			
mean ± SD	51.6 ± 8.92	53.5 ± 10.07	.617 *
(median; min-max)	(50; 41-73)	(51; 42-73)	
BMI (kg/height m^2)			
mean ± SD	28.3 ± 4.28	25.9 ± 8.18	.481 *
(median; min-max)	(28.9; 19.4-35.4)	(26.8; 20.1-37.9)	
Symptom time (years)			
mean ± SD	5.1 ± 4.55	5.7 ± 4.97	.722 *
(median; min-max)	(3.0; 0.5-15.0)	(4.0; 1.0-20.0)	
Education level, n, %			
literate	2 (13.3)	1 (6.7)	.482 **
primary school	5 (33.3)	9 (60.0)	
high school	4 (26.7)	2 (13.3)	
university	3 (20.0)	1 (6.7)	
Smoking, n, %			
yes	2 (13.3)	4 (26.7)	.651 ***
no	13 (86.7)	11 (73.3)	
Daily tea consumption,			
n, %	3 (20.0)	0 (0)	.156 **
0	2 (13.3)	4 (26.7)	
1-2 cup	10 (66.7)	11 (73.3)	
>3 cup			
Daily coffee			
consumption, n, %	5 (33.3)	1 (6.7)	.188 **
0	8 (53.3)	11 (73.3)	
1-2 cup	2 (13.3)	3 (20.0)	
>3 cup			
Childbirth, n, %			
0	2 (13.3)	0 (0)	.260 **
1-3	11 (73.3)	14 (93.3)	
>4	2 (13.3)	1 (6.7)	
Delivery, n, %	(n=13)	(n=15)	
vaginal	10 (76.9)	13 (86.7)	.746 **
cesarean section	1 (7.7)	1 (6.7)	
vaginal+ C/s	2 (15.4)	1 (6.7)	
Episiotomy, n, %	(n=13)	(n=14)	
yes	7 (53.8)	6 (42.9)	.568 **
no	6 (46.2)	8 (57.1)	
Menopause, n, %			
yes	6 (40)	6 (40)	1.000 **
no	9 (60)	9 (60)	
Hormone medication,			
n, %			
yes	1 (6.7)	2 (13.3)	.543 **
no	14 (93.3)	13 (86.7)	
Sexual dysfunction, n, %			
yes	10 (66.7)	8 (53.3)	.456 **
no	5 (33.3)	7 (46.7)	
Flatus/feces			
incontinence, n, %			
yes	5 (33.3)	7 (46.7)	.456 **
no	10 (66.7)	8 (53.3)	

Group1: Long-term BF+PFMT, Group 2: Single session BF+PFMT, PFMT: pelvic floor muscle training, BF: biofeedback, SD: Standart deviation, min: minimum, max: maximum, BMI: Body mass index (kg/m2)

** Mann Whitney U test was used for comparison between groups. ** Relationship between nominal data and groups was analyzed with Pearson – Chi-square test and *** Fisher's full probability test.*

In the evaluations conducted at the 8th and 12th weeks, both patient groups receiving PFMT, either via long-term BF or single-session BF, displayed significant improvements across primary outcome parameters. These improvements encompassed reductions in the number of daily UI episodes, daily pad usage, and urine volume measured during the 1-hour pad test. Additionally, measures of voluntary rapid maximum contraction pressure and duration of sustained contractions, as measured by BF, exhibited increases. Statistical analysis revealed notable enhancements in KHQ, IIQ-7, I-QOL, and BDI data, indicating significant improvement (p<.001). Temporal variations in the data were deemed statistically significant in both groups (p<.001). However, there was no statistical significance observed regarding group-time interaction concerning the changes over time. Consequently, the lack of difference in interaction suggests that the temporal change between groups was not statistically significant (p> .05). Furthermore, no statistically significant differences were observed between groups at any time point across all evaluation parameters (p>.05). These findings indicate that both the group-time effect and the intergroup effect were not statistically significant, and baseline values were comparable in both groups across all quantitative measures and questionnaire data (p>.05) (Table 2 and 3). Moreover, upon examining the evaluation criteria through pairwise comparisons at different time points, statistically significant improvements were evident in both groups at the 8th and 12th weeks compared to the commencement of treatment, across all quantitative data and questionnaire inquiries (p<.001). It was noted that this significant improvement persisted between the 8th and 12th weeks in all questionnaire data, except for the BDI, and in numerical data such as maximum contraction pressure, contraction duration, and daily pad use (p<.05).

The change in VAS scores assessing treatment satisfaction between the 8th and 12th weeks proved statistically significant in both groups (p<.05). However, there wasn't a statistically significant difference at any time point in the groups concerning group-time interaction (p>.05) (Table 2). In the initial assessment of patients queried for SD, symptoms of SD were identified in 10 (66.7%) and 8 (53.3%) individuals in group 1 and group 2, respectively. Upon reevaluation at the 12th week, it was noted that the number of patients reporting SD decreased to 5 in both groups. These results indicated a statistically significant temporal variation of SD within both groups (p<.05). However, no statistical significance was observed in the time-dependent variation between the groups (p>.05) (Table 4). Similarly, among patients queried for the presence of fecal incontinence, 5 (33.3%) in group 1 and 7 (46.7%) in group 2 initially reported complaints. Upon reevaluation at the 12th week, 2 patients in group 1 and 3 patients in group 2 reported fecal incontinence. The temporal change of fecal incontinence within both groups was statistically significant (p<.05). However, no statistical significance was found in the time-dependent variation of symptoms between the groups (p>.05) (Table 4).

Table 2. *Intergroup and time-dependent comparison of measurement results*

	Group 1 (n= 15) mean ± SD (median; min-max)			Group 2 (n= 15) mean ± SD (median; min-max)			P*	
Variables	Before treatment	8th week	12th week	Before treatment	8th week	12th week	group	interaction
Contraciton pressure (cmH2O)	14.4 ± 8.21 (14; 4-30)	21.7 ± 12.24 (20; 7.5-50) **	24.6 ± 12.41 (18; 8-40) **	16.8 ± 7.53 (15; 5-30)	22.8 ± 12.98 (20; 5-60) **	24.6 ± 13.55 (21; 12-65) **	.713	0.339
Contraction duration (s)	3.8 ± 1.01 (3.0; 3.0-5.0)	7.1 ± 2.30 (6.5; 5.0-10.0) **	8.6 ± 1.91 (10; 5-10) **	3.9 ± 1.03 (3.0; 3.0-5.0)	6.8 ± 2.26 (7; 3-10) **	7.3 ± 2.79 (7; 3-10) **	.446	0.110
Incontinence frequency	4.9 ± 4.52 (3.3; 0.6-10.0)	1.3 ± 1.61 (0.6; 0-5) **	0.7 ± 0.92 (0.3; 0-3) **	4.0 ± 2.11 (4.0; 1.0-8.0)	1.6 ± 1.50 (1; 0-4) **	1.0 ± 1.22 (1; 0-4) **	.802	0.774
Daily pad	2.8 ± 2.48 (2; 1-5)	0.9 ± 0.88 (1; 0-3) **	0.6 ± 0.91 (0; 0-3) **	2.0 ± 1.25 (2; 1-4)	1.1 ± 0.99 (1; 0-3) **	0.8 ± 0.86 (1; 0-2) **	.771	0.262
1 h pad test (g)	9.2 ± 10.85 (6; 2-39)	3.6 ± 5.00 (3; 0-20) **	2.7 ± 2.46 (2; 0-7) **	12.8 ± 15.38 (7; 2-50)	6.6 ± 10.59 (3; 0-40) **	6.1 ± 9.60 (2; 0-30) **	.496	0.650
Treatment satisfaction (VAS)	-	7.5 ± 1.72 (8; 3-10)	7.6 ± 2.02 (8; 3-10) ***	-	6.4 ± 2.03 (7; 2-10)	7.0 ± 1.98 (8; 3-10) ***	.208	0.345

Group1: Long-term BF+PFMT, Group 2: Single session BF+PFMT, PFMT: pelvic floor muscle training, BF: biofeedback, VAS: Visual Analogue Scale, h:hour, s:second, g:gram, SD: standart deviation, min:minimum, max: maximum

** The time-dependent change in the groups was similar (interaction p>.1) and time was found significant (p<.001) in all variables, among the group, time and group-time interaction effects were analyzed with the Brunner-Langer method, but only the group and interaction p values are given in the table.*

*** In the binary time comparison results with Bonferroni-corrected Brunner-Langer, the p value at 8 and 12 weeks compared to pretreatment: <.001*

**** Time effect p value calculated by Brunner-Langer method: < .05*

Table 3. *Intergroup and time-dependent comparison of patient-based questionnaire*

	Group 1 (n= 15) mean ± SD (median; min-max)			Group 2 (n= 15) mean ± SD (median; min-max)			p**	
	Before treatment	8th week	12th week	Before treatment	8th week	12th week	group	interaction
Incontinence Quality of Life Questionnaire (I-QOL)	42.5 ± 25.84 (39.7; 4.5-86.3)	69.3 ± 17.99 (67.0; 45.4-98.8) *	78.9 ± 15.21 (79.5; 47.7-98.8) *	41.5 ± 26.28 (45.4; 3.4-81.8)	64.5 ± 27.38 (65.9; 10.2-97.7) *	68.8 ± 26.90 (75; 10.2-98.8)*	.513	0.509
Incontinence Impact Questionnaire (IIQ-7)	69.2 ± 31.68 (85.7; 0-100)	37.1 ± 2.88 (38.0; 0-85.7) *	19.6 ± 16.87 (23.8; 0-52.3) *	62.8 ± 28.29 (66.6; 9.5-100)	39.0 ± 33.24 (33.3; 0-100) *	34.6 ± 30.93 (19.0; 0-95.2) *	.728	0.127
King Health Questionnaire (KHQ)								
General health	53.3 ± 24.76 (50; 25-100)	31.6 ± 11.44 (25; 25-50) *	25.0 ± 18.89 (25; 0-50) *	55.0 ± 19.36 (50; 25-75)	38.3 ± 15.99 (25; 25-75) *	33.3 ± 15.43 (25; 25-75) *	.267	0.757
Incontinence impact	84.4 ± 24.77 (100; 33.3-100)	53.3 ± 21.08 (66.6; 33.3-100) *	37.7 ± 17.21 (33.3; 0-66.6) *	77.2 ± 25.29 (66.6; 25-100)	46.6 ± 24.55 (33.3; 0-100) *	39.9 ± 31.37 (33.3; 0-100)*	.607	0.463
Role limitations	63.3 ± 34.61 (66.6; 0-100)	32.2 ± 18.32 (33.3; 0-66.6) *	18.8 ± 18.75 (16.6; 0-66.6) *	65.5 ± 31.15 (66.6; 0-100)	34.4 ± 32.40 (33.3; 0-100) *	22.2 ± 31.28 (16.6; 0-100) *	.986	0.901
Physical limitations	79.9 ± 28.31 (83.3; 0-100)	41.1 ± 21.69 (33.3; 0-66.6) *	22.1 ± 21.41 (16.6; 0-66.6) *	71.1 ± 28.49 (83.3; 0-100)	43.3 ± 32.61 (33.3; 0-100) *	31.1 ± 33.84 (16.6; 0-100) *	.932	0.206
Social limitations	57.0 ± 39.09 (66.6; 0-100)	24.4 ± 18.87 (22.2; 0-66.6) *	14.0 ± 16.48 (11.1; 0-44.4) *	57.3 ± 31.38 (66.0; 11.1-100)	33.3 ± 33.06 (33.3; 0-100) *	25.1 ± 33.91 (16.6; 0-100) *	.542	0.795
Personal relationship	46.4 ± 35.31 (33.3; 0-100)	14.2 ± 15.81 (8.3; 0-33.3) *	7.1 ± 14.19 (0; 0-33.3) *	47.4 ± 29.53 (66.6; 0-83.3)	26.3 ± 33.67 (16.6; 0-100) *	19.4 ± 32.43 (0; 0-100) *	.467	0.546
Emotions	58.3 ± 35.84 (55.5; 3-100)	30.7 ± 22.89 (33.3; 0-77.7) *	11.4 ± 12.67 (11.1; 0-33.3) *	66.2 ± 33.10 (66.6; 11.1-100)	34.07 ± 31.27 (33.3; 0-100) *	22.9 ± 32.65 0 (0-100) *	.479	0.583
Sleep/energy	47.7 ± 34.42 (33.3; 0-100)	27.4 ± 18.12 (33.3; 0-50) *	11.1 ± 13.60 (0; 0-33.3) *	47.4 ± 30.56 (33.3; 11.1-100)	22.2 ± 24.93 (16.6; 0-83.3) *	19.9 ± 29.68 (16.6; 0-100) *	.946	0.126
Incontinence severity measures	71.9 ± 24.19 (80; 20-100)	37.7 ± 19.70 (33.3; 6-80) *	19.5 ± 18.93 (13.3; 0-73.3) *	68.8 ± 21.77 (66.6; 26.6-100)	43.5 ± 28.82 (33.3; 6.6-100) *	29.3 ± 29.99 (13.3; 0-100) *	.638	0.295
Symptom severity	15.4 ± 5.97 (15; 7-30)	9.0 ± 2.75 (10; 4-14) *	5.6 ± 4.14 (5; 0-15) *	15.1 ± 4.8 (15; 7-23)	9.2 ± 4.63 (9; 3-18) *	6.7 ± 6.01 (5; 0-24) *	.917	0.896
Beck Depression Inventory (BDI)	19.4 ± 15.15 (19; 0-46)	11.0 ± 11.41 (6; 0-35) *	5.8 ± 8.37 (2; 0-29) *	15.8 ± 10.17 (12; 2-35)	9.5 ± 10.53 (6; 0-35) *	9.0 ± 9.61 (6; 0-32) *	.691	0.216

Group 1: Long-term BF+PFMT, Group 2: Single session BF+PFMT, PFMT: pelvic floor muscle training, BF: biofeedback, SD: standart deviation, min:minimum, max: maximum

** From the binary time comparison results with Bonferroni-corrected Brunner-Langer, the p value at weeks 8 and 12 compared to pretreatment: <.001*

*** The time-dependent change in the groups was similar (interaction p>.1) and time was found significant (p<.001) in all variables, among the group, time and group-time interaction effects tested with the Brunner-Langer method, but only the group and interaction p values are given in the table.*

Table 4. *Comparison of sexual dysfunction and flatus/feces incontinence between groups*

	Group 1 (n= 15) n (%)		Group 2 (n= 15) n (%)		*p**		
	Before treatment	12th week	Before treatment	12th week	group	time	interaction
Sexual dysfunction	10 (66.7)	5 (33.3)	8 (53.3)	5 (33.3)	0.646	0.013	0.535
Flatulence/ fecal incontinence	5 (33.3)	2 (13.3)	7 (46.7)	3 (20.0)	0.455	0.012	0.721

Group 1: Long-term BF+PFMT, Group 2: Single session BF+PFMT, PFMT: pelvic floor muscle training, BF: biofeedback

** Time-dependent change in groups was similar (interaction p>.1) and time was significant (p<.001) in all variables from group, time and group-time interaction effects tested with the Brunner-Langer method*

4. DISCUSSION

The PFMT program is considered the first-line treatment for stress, mixed, and urge UI (18). A Cochrane systematic review noted that the addition of BF to PFMT did not yield additional benefits in QoL scales, pad tests, PFM strength, or incontinence frequency compared to those exclusively following the PFMT program. However, patients reported a significant increase in recovery rates at the end of treatment. This subjective perception of recovery observed in patients may be attributed to variations in treatment program intensity, enhanced control during BF treatment, and increased face-to-face contact opportunities with the physiotherapist (8).

In a systematic review exploring the role of PFMT with BF in treating SUI, it was noted that the studies demonstrated low methodological quality and utilized varied treatment protocols (19). The review concluded that PFMT with BF did not yield additional benefits in terms of QoL scales and PFM strength compared to the PFMT alone. However, prior to commencing the PFMT program, it has been suggested that BF could serve as an initial training regimen to help women learn to contract their PFMs correctly. It has been emphasized that the primary clinical benefit of PFMT with BF in patients with SUI may be linked to the enhancement of PFM contraction perception rather than solely focusing on strengthening the PFMs. A recent systematic review found moderate evidence that PFME combined with BF were significantly more effective than PFME alone in managing UI (20).

In our study, significant improvements were found in both groups receiving regular BF and single-session BF treatment in terms of contraction strength, contraction duration, frequency of incontinence, pad test, QoL scales, and treatment satisfaction. However, no difference was observed between the groups. These findings suggest that when PFM exercises are taught accurately through single-session BF training and practiced consistently, similar results can be achieved compared to long-term BF training.

In our present study, patients were taught how to perform PFM exercises correctly through a single session of BF. Those who received a home program were monitored through weekly phone. When patient follow-up is conducted at regular intervals, it has been demonstrated that individuals who received PFMT with a single session of BF achieved comparable improvements in QoL scales, pad test measurements, PFM strength, and endurance results compared to those who underwent regular BF training by the end of the treatment period.

Ozlu et al. (21) conducted a randomized controlled study comparing the efficacy of three intervention groups: home exercise alone, home exercises combined with intravaginal pressure-BF, and home exercises combined with perineal electromyography-BF in patients with SUI. It was stated that the groups that continued the exercises with BF showed statistically significantly more improvement in PFM strength, treatment satisfaction, incontinence severity and pad test results compared to the home exercise group. The results of this study show that, BF is more effective than home exercise program in improving muscle function and incontinence symptoms. One of the issue that may have an effect on obtaining different results from our study in favor of BF may be that women in the BF group were given a more intense exercise program compared to our study. Women in both BF groups were received PFMT with BF, 3 times a week for 8 weeks in addition to the basic PFMT program given to the home exercise group. Another issue that may affect the results against the home exercise group is, women in the home exercise group were given PFMT with digital palpation only once, and no supervision or follow-up was made for 8 weeks.

In a meta-analysis performed by Cheng et al. (22), depression and anxiety levels of patients with UI are found to be high. Additionally, Yazdany et al. (23) reported that the prevalence of depression and anxiety tends to rise among patients who do not undergo treatment for UI. Moreover, in the presence of depression and anxiety, continuing PFMT is insufficient (24). A study by Weber-Rajek et al. (25) demonstrated a significant enhancement in BDI and KHQ scores following a PFMT program among SUI patients, compared to an untreated cohort. In our study, we observed significant improvements in BDI and KHQ scores in both groups. Screening and addressing depression in women with UI may not only ameliorate depressive symptoms but also potentially enhance adherence to exercise regimens and improve continence outcomes.

Women with SUI often experience SD as part of pelvic floor dysfunction (26). Liebergall et al. (27) found that improvement in SD post-PFMT correlated with better QoL scales and pad test values. Our study similarly noted a significant reduction in SD post-treatment across both groups. Additionally, PFMT is advocated by the ICS as the primary treatment for fecal incontinence, with significant symptom alleviation observed in our study (28).

To the best of our knowledge, our current study is the first study in the literature to compare the effectiveness of individually designed PFMT with long term BF and a single session BF in addition to home exercise in women with

SUI. In the treatment of SUI, it is essential to teach PFM exercises correctly, and if PFM exercises are taught correctly to the patient with a single session BF, similar results can be obtained with long-term BF.

There are some limitations in our study including the lack of a control group in which PFMT was given, for example, only with verbal training, the low number of participants and the lack of long-term follow-up after the treatment was completed. Although the recommended duration of treatment is at least 12 weeks, due to the difficulty of patients coming to treatment for a long time, the treatment was performed for 8 weeks and the patients were evaluated for control at the 12th week. In the future, it would be appropriate to plan studies with larger numbers of patients and control groups and long-term follow-up after treatment.

5. CONCLUSION

PFMT with BF is used to increase the patient's awareness of PFMs, to create effective contraction and to increase the patient's compliance with the exercise program. When correct PFM contraction is taught with single session BF, a similar level of improvement is achieved in SUI symptoms, muscle strength and quality of life with long term BF. In clinical practice, in patients who cannot participate in BF treatment regularly, it may be recommended that patients continue PFMT as a home program with close follow-up after training with a single session BF.

Acknowledgements: *We would like to thank Associate Professor Timur Köse, a faculty member at Ege University, Faculty of Medicine, Department of Biostatistics and Medical Informatics, who conducted the statistical analysis of the study.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Ege University, Faculty of Medicine (Approval date: 05.06.2018; Number: 18-6/33)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: YA
Design of the study: YA
Acquisition of data for the study: AK, YA
Analysis of data for the study: TK
Interpretation of data for the study: YA, AK, AÖY
Drafting the manuscript: AK, YA
Revising it critically for important intellectual content: YA, AÖY
Final approval of the version to be published: YA, AK, AÖY

REFERENCES

[1] Haylen B, de Ridder D, Freeman R, Swift S, Berghmans B, Lee J. An International Urogynecological Association (IUGA)/ International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Neurourol Urodyn. 2010;29(1):4–20. https://doi.org/10.1002/nau.20798

[2] Patel UJ, Godecker AL, Giles DL, Brown HW. Updated prevalence of urinary ıncontinence in women: 2015–2018 National Population-Based Survey Data. Female Pelvic Medicine & Reconstructive Surgery 2022; 28(4):181-187. https://doi.org/10.1097/SPV.000.000.0000001127

[3] Anand A, Khan SM, Khan AA. Stress urinary incontinence in females. Diagnosis and treatment modalities – past, present and the future. Journal of Clinical Urology 2023; 16(6): 622-630. https://doi.org/10.1177/205.141.58211044583

[4] Cacciari LP, Dumoulin C, Hay-Smith EJ. Pelvic floor muscle training versus no treatment, or inactive control treatments, for urinary incontinence in women: A cochrane systematic review abridged republication. Braz J Phys Ther. 2019; 23(2):93-107. https://doi.org/10.1016/j.bjpt.2019.01.002

[5] Kegel AH. Progressive resistance exercise in the functional restoration of the perineal muscles. Am J Obstet Gynecol. 1948; 56(2): 238-248.
https://doi.org/10.1016/0002-9378(48)90266-x

[6] Bump RC, Hurt WG, Fantl JA, Wyman JF. Assessment of Kegel pelvic muscle exercise performance after brief verbal instruction. Am J Obstet Gynecol. 1991; 165(2): 322-327. https://doi.org/10.1016/0002-9378(91)90085-6

[7] Bo K, Larsen S, Oseid S. Knowledge about and ability to correct pelvic floor muscle exercises in women with urinary stress incontinence. Neurourol Urodyn. 1988;7:261–262.

[8] Herderschee R, Hay-Smith E, Herbison G. Feedback or biofeedback to augment pelvic floor muscle training for urinary incontinence in women. Cochrane Database Syst Rev 2011 6:(7):CD009252. https://doi.org/10.1002/14651858.CD009252.

[9] Diaz DC, Robinson D, Bosch R, Costantini E, Cotterill N, Espuna-Pons M. Initial assesment of urinary incontinence in adult male and female patients. In: Abrams P, Cardozo L, Wagg A, Wein A, editors. International Consultation on Incontinence. 6th Ed. Tokyo: ICUD ICS; 2017. p. 500–35.

[10] Vij M, Srikrishna S, Robinson D, Cardozo L. Quality assurance in quality of life assessment measuring the validity of the King'sHealth Questionnaire. Int Urogynecol J. 2014;25(8):1133 – 1135. https://doi.org/10.1007/s00192.014.2370-5

[11] Kaya S, Akbayrak T, Çelenay T, Dolgun A, Ekici G, Beksaç S. Reliability and validity of the Turkish King's Health Questionnaire in women with urinary incontinence. Int Urogynecol J. 2015;26 (12):1853–1859. https://doi.org/10.1007/s00192.015.2786-6

[12] Uebersax J, Wyman J, Shumaker S, McClish D, Fantl J& the CP for WRG. Short forms to assess life quality and symptom distress for urinary incontinence in women: The incontinence impact questionnaire and the urogenital distress inventory. Neurourol Urodyn. 1995;14(2):131-139.
https://doi.org/10.1002/nau.193.014.0206.

[13] Cam C, Sakalli M, Ay P, Cam M, Karateke A. Validation of the Short Forms of the Incontinence Impact Questionnaire (IIQ-7) and the Urogenital Distress Inventory (UDI-6) in a Turkish Population. Neurourol Urodyn. 2007; 26 (1):129–133.
https://doi.org/10.1002/nau.20292

[14] Patrick D, Martin M, Bushnell D. Quality of life of women with urinary incontinence: Further development of the incontinence quality of life instrument (I-QOL. Urology 1999;53 (1):71–76. https://doi.org/10.1016/s0090-4295(98)00454-3

[15] Eyigor S, Karapolat H, Akkoç Y, Yeşil H, Ekmekci O. Quality of life in patients with multiple sclerosis and urinary disorders: Reliability and validity of Turkish-language version of Incontinence Quality of Life Scale. J Rehabil Res Dev. 2010; 47(1):67–71. https://doi.org/10.1682/jrrd.2009.08.0132

[16] Beck AT, Steer RA, Brown GK. 1996. Beck Depression Inventory-Second Edition Manual. San Antonio, TX: The Psychological Corporation.

[17] Kapci EG , Uslu R, Turkcapar H, Karaoglan A. Beck Depression Inventory II: Evaluation of the psychometric properties and cut-off points in a Turkish adult population. Depress Anxiety 2008;25(10):E104-110. https://doi.org/10.1002/da.20371

[18] Dumoulin C, Adewuyi T, Bradley C, Burgio K, Hagen S, Hunter K. Adult Conservative Management. In: Abrams P, Cardozo L, Wagg A, Wein A, editors. Incontinence. 6th Intern. Tokyo: ICUD ICS; 2016. p. 1446–537.

[19] Nunes E, Sampaio L, Biasotto-Gonzalez, DA, Nagano R dos R, Lucarelli P, Politti F. Biofeedback for pelvic floor muscle training in women with stress urinary incontinence: A systematic review with meta-analysis. Physiotherapy 2019;105(1):10–23. https://doi.org/10.1016/j.physio.2018.07.012

[20] Matsi AE, Billis E, Lampropoulou S, Xergia SA, Tsekoura M, Fousekis K. The Effectiveness of pelvic floor muscle exercise with biofeedback in women with urinary ıncontinence: A systematic review. Appl Sci. 2023;13(23):1-22.
https://doi.org/10.3390/app132312743

[21] Özlü A, Yıldız N, Öztekin Ö. Comparison of the efficacy of perineal and intravaginal biofeedback assisted pelvic floor muscle exercises in women with urodynamic stress urinary incontinence. Neurourol Urodyn. 2017; 36(8):2132-2141. https://doi.org/10.1002/nau.23257

[22] Cheng S, Lin D, Hu T, Cao L, Liao H, Mou X, Zhang Q,Liu J, Wu T. Association of urinary incontinence and depression or anxiety: A meta-analysis. J Int Med Res. 2020;48(6):1–12.
https://doi.org/10.1177/030.006.0520931348

[23] Yazdany T, Bhatia N, Reina A. Association of depression and anxiety in underserved women with and without urinary incontinence. Female Pelvic Med Reconstr Surg. 2014;20(6):349–353.
https://doi.org/10.1097/SPV.000.000.0000000071

[24] Khan Z, Whittal C, Mansol S, Osborne L, Reed P, Emery S. Effect of depression and anxiety on the success of pelvic floor muscle training for pelvic floor dysfunction. J Obstet Gynaecol (Lahore). 2013;33 (7):710–714.
https://doi.org/10.3109/01443.615.2013.813913

[25] Weber-Rajek M, Straczynska A, Strojek K, Piekorz Z, Pilarska B, Podhorecka M, Sobieralska-Michalak K, Goch A, Radziminska A. Assessment of the effectiveness of pelvic floor muscle training (pfmt) and extracorporeal magnetic innervation (exmı) in treatment of stress urinary incontinence in women: A randomized controlled trial. Biomed Res Int. 2020:1-7.
https://doi.org/10.1155/2020/1019872

[26] Brubaker L, Chiang S, Zyczynski H, Norton P, Kalinoski D, Stoddard A, Kusek JW, Steers W. The impact of stress incontinence surgery on female sexual function. Am J Obs Gynecol. 2009; 200(5): 562.e1–562.e7.
https://doi.org/10.1016/j.ajog.2008.11.017

[27] Liebergall-Wischnitzer M, Paltiel O, Celnikier D, Lavy Y, Manor O, Woloski Wruble A. Sexual function and quality of life of women with stress urinary ıncontinence: A randomized controlled trial comparing the paula method (circular muscle exercises) to pelvic floor muscle training (PFMT) exercises. Int Soc Sex Med. 2012; 9(6):1613–1623.
https://doi.org/10.1111/j.1743-6109.2012.02721.x

[28] Bliss D, Mimura T, Berghmans B, Bharucha A, Chiarioni G, Emmanuel A. Assessment and conservative management of faecal incontinence and quality of life in adults. In: Abrams P, Cardozo L, Wagg A, Wein A, editors. 6th International Consultation on Incontinence. 6th Intern. Tokyo: ICUD ICS; 2016. p. 2034–2047.

How to cite this article: Kılıçkap A, Akkoç Y, Yeniel AÖ. Individualized Pelvic Floor Muscle Training with Single Session Versus Long Term Biofeedback for Treating Stress Urinary Incontinence: A Prospective Randomized Trial. Clin Exp Health Sci 2025; 15: 95-102. DOI: 10.33808/clinexphealthsci.1462829

Clinical and Experimental Health Sciences

The Application of Analytical Period Six Sigma in Tumor Markers in Clinical Biochemistry Laboratory

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Received: April 6, 2022 **Accepted:** November 2, 2024

ABSTRACT

Objective: Six Sigma Methodology is a quality management methodology that provides information on process performance, focuses on variables in the process, and is based on statistical calculations.

Methods: In the present study, analytical period sigma scores in tumor markers were calculated by using monthly and cumulative quarterly data. The present study is the first study in which sigma scores calculated with different Total Allowable Error (TEa (%)) and bias (%) values are compared by using statistical tests and the frequency of six sigma application is discussed.

Results: When it was examined whether there was a statistically significant difference between the sigma scores that were calculated according to the biases obtained from the Internal Quality Control (IQC) and External Quality Control (EQC) data, although there was a significant difference in the Alpha-Fetoprotein (AFP) and Cancer Antigen 15.3 (CA 15.3) tests, no significant difference was found in the other tests. When the sigma scores that were calculated according to the TEa (%) values determined by the reference institutions were analyzed statistically, it was found that different TEa (%) values caused significant differences in sigma scores. When the sigma scores obtained by months and cumulatively were examined, it was found that there were significant differences between the examined periods.

Conclusion: As a conclusion, it is important to determine the optimal TEa (%) value in sigma score examinations and to monitor the quality by analyzing the sigma scores on a monthly basis in terms of the sustainability of the result quality of the tests and for the early detection of problems.

Keywords: Six sigma, tumor markers, quality control

1. INTRODUCTION

Clinical laboratories have important functions in the diagnosis and treatment of patients. Laboratory tests are applied to approximately 85% of patients applying to healthcare institutions (1). Clinical laboratories affect 60% – 70% of the diagnosis and treatment decisions (2). For this reason, the performance of laboratories affects the quality of healthcare institutions significantly. In clinical laboratories, the Total Testing Process (TTP) is divided into five periods; pre-preanalytical, preanalytical, analytical, postanalytical, and post-postanalytical (3). The performance of a laboratory test is evaluated by dividing the test process into periods based on quality indicators, sigma scores, and statistical criteria such as accuracy and repeatability (4,5).

Six Sigma Methodology is a quality management methodology that provides information on process performance, focuses on variables in the process, and is based on statistical calculations (6). This methodology, which has proven its benefits in the industrial field, has started to gain importance in healthcare and clinical laboratories. The analytical period sigma scores can be calculated with the following formula (7):'(TEa (%) – Bias (%)) / CV (%)'

The Total Allowable Error (TEa %) represents the maximum permissible error for ensuring the clinical reliability of a test. Values for TEa (%) are established by reference institutions such as the Clinical Laboratory Improvement Amendments (CLIA 88) and Richtlinien der Bundesärztekammer (RiliBÄK) and can be employed to calculate sigma scores. The coefficient of variation (CV (%)) is the ratio of the standard deviation to the mean, reflecting the extent of variability relative to the population mean. This statistic is particularly useful for comparing the variability between different data sets, even when their means differ significantly. A higher CV

https://doi.org/10.33808/clinexphealthsci.1098988

indicates greater distribution. Bias refers to the discrepancy between the true value of an analyte and its measured value, serving as an indicator of accuracy. The bias is determined by comparing the analyte values obtained through a test method with those from a reference method. Additionally, bias can be calculated using results from External Quality and Internal Quality Control assessments.

In the present study, analytical period sigma scores in tumor markers were calculated by using monthly and cumulative quarterly data. The purposes of the study were;

- Examining the relations between monthly and cumulative sigma scores,

- Examining the relations between the sigma scores calculated according to the TEa (%) values determined by the reference institutions,

- Examining the relations between sigma scores calculated according to the bias (%) values obtained from IQC and EQC materials,

- With the data obtained, providing perspective on which data should be used to calculate analytical period sigma scores and how often six sigma should be evaluated.

- The present study is the first study in which sigma scores calculated with different TEa (%) and bias (%) values are compared by using statistical tests and the frequency of six sigma application is discussed.

2. METHODS

In the present study, the IQC and EQC data of the tumor markers AFP, CA 15.3, Cancer Antigen 19.9 (CA 19.9), Cancer Antigen 125 (CA 125), Carcinoembryonic Antigen (CEA), and Total Prostate Specific Antigen (TPSA) tests were used. The IQC and EQC samples were examined by using the Electrochemiluminescent Method on the Cobas 8000 e602 (Roche Diagnostics GmbH, Mannheim, Germany) autoanalyzer in the Biochemistry Laboratory. Elecsys PC TM 1 (Lot: 297057) and PC TM 2 (Lot: 297059) were used as the IQC samples. The two-level IQC results examined between April and June 2019 were obtained retrospectively from the recordings of the Cobas 8000 e 602 device in our laboratory. Internal quality results outside the acceptable range due to random errors were not included in our study. Random error causes may be the using of wrong quality control material by the staff, incorrect dilution of the quality control material, using of control material that is not suitable for storage conditions, etc. Single-level Quality Systems Immunoassay (Bio Group Medical System, Italy) EQC materials were used as the EQC samples. The EQC data of each month examined in April-May-June were obtained retrospectively from the Quality Systems Website.

TEa (%) Sources: The TEa (%) values determined by EQA standards of China, RCPA standards, Biological variation, RiliBÄK, and CLIA for each of the AFP, CA 15-3, CA 19-9, CA 125, CEA and TPSA tests, which were used in the study are shown in Table 1 (8,9).

Table 1. *Total Allowable Error (TEa (%)) values of references institutions*

	Total Allowable Error (TEa (%)) VALUES of REFERENCES INSTITUTIONS				
TESTS	**EQA Standards of China**	**RCPA standards**	**Biologicalvariation**	**RiliBÄK**	**CLIA**
AFP	25	20	21.8	24	24
CA 125	25	15	35.4	24	24
CA 15-3	25	20	20.8	24	24
CA 19-9	25	15	39	24	24
CEA	25	20	24.7	24	24
TPSA	25	20	33.6	25	24

EQA standards of China: External quality assessment standards of China; RCPA: The Royal Collage of Pathologists of Australasia; RiliBÄK: Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations; CLIA: Clinical Laboratory Improvement Amendments; AFP: Alpha-Fetoprotein; CA 125: Cancer Antigen 125; CA 15.3: Cancer Antigen 15.3; CA 19.9: Cancer Antigen 19.9; CEA: Carcinoembryonic Antigen; TPSA: Total Prostate Specific Antigen

CV (%) was calculated from the IQC data over the three-month period using the equality:

'(Standard deviation × 100)/ laboratory mean (IQC)'

Two different levels of IQC samples were analyzed. CV (%) was calculated using monthly IQC results and cumulative 3-month IQC results.

Bias was calculated from the EQC data using the equality:

'(mean of all laboratories – our mean)/ (mean of all laboratories) × 100'

The arithmetic mean of the calculated biases was used as the bias in the cumulative sigma calculation.

Bias was calculated from the IQC results using the equality:

'(Our mean – target mean)/ (target mean) × 100'

Our mean was calculated using monthly IQC results and cumulative 3-month IQC results.

The sigma scores was calculated separately according to the quality control material from which the bias value was obtained and according to each TEa (%) value that was employed. The process sigma levels were calculated monthly and cumulatively 3-month for both internal quality control levels. The following formula was used to calculate the sigma score:

'(TEa (%) – bias (%)) / CV (%)'

The sigma scores of each test were examined in 4 groups according to the periodically as April, May, June, and cumulative, and in 2 groups according to the quality control material from which the bias value was obtained. Sigma scores that were calculated according to TEa (%) values

determined by reference institutions were examined in 5 groups.

The comparison of sigma scores between periods, between the quality control material from which the bias value was obtained, and according to the different TEa (%) values determined by the reference institutions were evaluated with statistical tests. The relations between the sigma scores that were calculated according to the biases obtained from the IQC and EQC data were evaluated statistically with correlation tests. Microsoft Office Excel program was used to calculate the mean, standard deviation (SD), CV (%), bias (%), and sigma score. Statistical analyses were conducted using SPSS version 25.0 (SPSS, Chicago, IL, USA). The normality of the data distribution within groups was assessed using both visual and analytical methods. Data following a normal distribution were presented as mean ± standard deviation (SD), while non-normally distributed data were reported as median (minimum-maximum). For comparisons of more than two independent groups with non-normally distributed data, the Kruskal-Wallis test was utilized. The One-Way ANOVA test was employed for comparing normally distributed data across more than two independent groups. When comparing two independent groups, the Mann-Whitney U Test was used for non-normally distributed data, and the Independent-Sample T Test was applied for normally distributed data. Correlations for non-normally distributed data were assessed using the Spearman Correlation Coefficient, while correlations for normally distributed data were evaluated using the Pearson Correlation Coefficient. A P value of <0.05 was considered statistically significant.

3. RESULTS

April, May, June, cumulative, and 3-month sigma scores of AFP, CA 15-3, CA 19-9, CA 125, CEA, and total PSA tests are given in the table (Table 2).

Table 2. *Sigma scores of tumor markers*

CA 125 PROCESS SIGMA SCORES									
REFERENCES INSTITUTIONS	CONTROL MATERIAL	APRIL		MAY		JUNE		CUMULATIVE	
		LEVEL1	LEVEL2	LEVEL1	LEVEL2	LEVEL1	LEVEL2	LEVEL1	LEVEL2
EQA Standards of China	INTERNAL	10.65	15.36	9.14	11.52	9.39	9.55	8.60	10.88
	EXTERNAL	13.46	16.50	9.69	10.11	10.81	9.82	10.04	10.84
RCPA	INTERNAL	7.61	11.64	6.61	8.88	7.07	7.45	6.27	8.36
	EXTERNAL	10.42	12.77	7.15	7.46	8.49	7.71	7.71	8.32
Biological variation	INTERNAL	16.96	23.10	14.41	17.02	14.21	13.94	13.45	16.12
	EXTERNAL	19.77	24.24	14.96	15.61	15.64	14.21	14.89	16.07
RiliBÄK	INTERNAL	10.04	14.62	8.63	11.00	8.92	9.13	8.13	10.38
	EXTERNAL	12.85	15.75	9.18	9.58	10.35	9.40	9.57	10.33
CLIA	INTERNAL	10.04	14.62	8.63	11.00	8.92	9.13	8.13	10.38
	EXTERNAL	12.85	15.75	9.18	9.58	10.35	9.40	9.57	10.33
AFP PROCESS SIGMA SCORES									
EQA Standards of China	INTERNAL	9.36	12.78	8.17	7.55	9.99	11.42	9.12	8.30
	EXTERNAL	10.16	10.81	8.84	7.39	7.30	7.39	8.80	6.83
RCPA	INTERNAL	6.94	10.21	6.06	5.79	7.46	8.86	6.77	6.48
	EXTERNAL	7.75	8.24	6.73	5.62	4.77	4.83	6.46	5.01
Biological variation	INTERNAL	7.81	11.13	6.82	6.42	8.37	9.78	7.62	7.14
	EXTERNAL	8.62	9.17	7.49	6.26	5.68	5.75	7.31	5.67
RiliBÄK	INTERNAL	8.87	12.26	7.75	7.20	9.49	10.91	8.65	7.94
	EXTERNAL	9.68	10.30	8.41	7.03	6.80	6.88	8.34	6.46
CLIA	INTERNAL	8.87	12.26	7.75	7.20	9.49	10.91	8.65	7.94
	EXTERNAL	9.68	10.30	8.41	7.03	6.80	6.88	8.34	6.46
CA 15.3 PROCESS SIGMA SCORES									
EQA Standards of China	INTERNAL	9.75	8.22	7.27	7.11	5.48	6.34	6.89	7.13
	EXTERNAL	7.43	7.50	2.78	2.99	5.89	6.96	4.95	5.76
RCPA	INTERNAL	5.78	4.22	4.24	3.85	2.99	3.40	4.02	3.79
	EXTERNAL	3.47	3.50	-0.25	-0.27	3.40	4.02	2.07	2.41
Biological variation	INTERNAL	8.08	6.54	6.00	5.75	4.43	5.11	5.69	5.73
	EXTERNAL	5.77	5.82	1.51	1.62	4.84	5.73	3.74	4.35
RiliBÄK	INTERNAL	9.35	7.82	6.97	6.79	5.23	6.05	6.61	6.80
	EXTERNAL	7.04	7.10	2.48	2.66	5.64	6.67	4.66	5.42
CLIA	INTERNAL	9.35	7.82	6.97	6.79	5.23	6.05	6.61	6.80
	EXTERNAL	7.04	7.10	2.48	2.66	5.64	6.67	4.66	5.42

CA 19.9 PROCESS SIGMA SCORES									
EQA Standards of China	INTERNAL	11.88	8.71	9.16	13.85	6.75	5.18	8.51	7.69
	EXTERNAL	11.45	10.38	8.18	12.91	7.00	6.12	8.20	8.41
RCPA	INTERNAL	7.11	4.38	5.13	7.51	3.81	2.61	4.90	3.99
	EXTERNAL	6.67	6.04	4.16	6.56	4.06	3.55	4.59	4.70
Biological variation	INTERNAL	18.58	14.78	14.79	22.74	10.86	8.78	13.57	12.87
	EXTERNAL	18.14	16.44	13.82	21.79	11.11	9.72	13.25	13.59
RiliBÄK	INTERNAL	11.41	8.28	8.75	13.22	6.46	4.92	8.15	7.32
	EXTERNAL	10.97	9.94	7.78	12.27	6.70	5.86	7.84	8.04
CLIA	INTERNAL	11.41	8.28	8.75	13.22	6.46	4.92	8.15	7.32
	EXTERNAL	10.97	9.94	7.78	12.27	6.70	5.86	7.84	8.04
CEA PROCESS SIGMA SCORES									
EQA Standards of China	INTERNAL	5.83	5.97	13.44	17.19	10.44	11.79	7.55	8.76
	EXTERNAL	4.44	3.86	13.05	13.49	13.88	11.97	7.54	6.95
RCPA	INTERNAL	4.42	4.74	10.11	13.74	7.54	9.28	5.64	7.00
	EXTERNAL	3.03	2.63	9.71	10.04	10.98	9.47	5.63	5.19
Biological variation	INTERNAL	5.75	5.89	13.24	16.99	10.27	11.64	7.44	8.66
	EXTERNAL	4.36	3.79	12.85	13.29	13.70	11.82	7.43	6.84
RiliBÄK	INTERNAL	5.55	5.72	12.78	16.50	9.86	11.29	7.17	8.41
	EXTERNAL	4.16	3.62	12.38	12.80	13.30	11.47	7.16	6.60
CLIA	INTERNAL	5.55	5.72	12.78	16.50	9.86	11.29	7.17	8.41
	EXTERNAL	4.16	3.62	12.38	12.80	13.30	11.47	7.16	6.60
TOTAL PSA PROCESS SIGMA SCORES									
EQA Standards of China	INTERNAL	14.47	13.00	11.97	12.37	8.41	7.38	11.09	10.18
	EXTERNAL	12.87	11.71	13.33	13.81	8.38	7.31	11.10	10.23
RCPA	INTERNAL	11.21	10.03	9.21	9.51	6.55	5.76	8.58	7.87
	EXTERNAL	9.61	8.74	10.57	10.94	6.52	5.69	8.59	7.92
Biological variation	INTERNAL	20.09	18.11	16.73	17.30	11.60	10.17	15.40	14.15
	EXTERNAL	18.48	16.82	18.09	18.73	11.58	10.09	15.42	14.20
RiliBÄK	INTERNAL	14.47	13.00	11.97	12.37	8.41	7.38	11.09	10.18
	EXTERNAL	12.87	11.71	13.33	13.81	8.38	7.31	11.10	10.23
CLIA	INTERNAL	13.82	12.41	11.42	11.80	8.03	7.06	10.58	9.71
	EXTERNAL	12.22	11.12	12.78	13.23	8.01	6.98	10.60	9.76

EQA standards of China: External quality assessment standards of China; RCPA: The Royal Collage of Pathologists of Australasia; RiliBÄK: Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations; CLIA: Clinical Laboratory Improvement Amendments; AFP: Alpha-Fetoprotein; CA 125: Cancer Antigen 125; CA 15.3: Cancer Antigen 15.3; CA 19.9: Cancer Antigen 19.9; CEA: Carcinoembryonic Antigen; TPSA: Total Prostate Specific Antigen

Table 3. *Correlation and comparison of the sigma scores that were calculated according to the biases obtained from the Internal Quality Control (IQC) and External Quality Control (EQC) data*

	N	IQC (n=40)	EQC (n=40)	P value (Comparison)	Pearson Correlation	Spearman's rho	P value (Correlation)
CA 125**	40	10.04 (6.27-23.10)	10.34 (7.15-24.24)	0.223		0.862	<.001
AFP*	40	8.66±1.77	7.51±1.57	.003	0.543		<.001
CA 15 3*	40	6.22±1.61	4.48±2.08	<.001	0.557		<.001
CA 19.9**	40	8.28 (2.61-22.74)	8.13 (3.55-21.79)	0.977		0.955	<.001
CEA**	40	8.71 (4.42-17.19)	8.51 (2.63-13.88)	0.450		0.871	<.001
TPSA*	40	11.37±3.26	11.5±3.24	0.982	0.951		<.001

**Values were expressed as means±SD.*

***Values were expressed as median (min-max)*

IQC, internal quality control; EQC, external quality control; AFP: Alpha-Fetoprotein; CA 125: Cancer Antigen 125; CA 15.3: Cancer Antigen 15.3; CA 19.9: Cancer Antigen 19.9; CEA: Carcinoembryonic Antigen; TPSA: Total Prostate Specific Antigen

Table 4. *Comparison of the sigma scores that were calculated according to Total Allowable Error (TEa (%)) values determined by the reference institutions*

	EQA Standards of China	RCPA	Biological variation	RİLİBAK	CLIA	P value
CA 125**	10.38 (8.60-16.50)	7.71 (6.27-12.77)	15.62 (3.45-24.24)	9.81 (8.13-15.75)	9.81 (8.13-15.75)	<.001
AFP*	9.01±1.67	6.74±1.49	7.56±1.55	8.56±1.63	8.56±1.63	.001
CA 15 3**	6.92 (2.78-9.75)	3.48 (-0.27-5.78)	5.71 (1.51-8.08)	6.64 (2.48-9.35)	6.64 (2.48-9.35)	<.001
CA 19.9*	9.02±2.45	4.98±1.40	14.67±4.00	8.61±2.35	8.61±2.35	<.001
CEA*	9.75±3.92	7.44±3.15	9.62±3.88	9.29±3.77	9.29±3.77	.402
TPSA*	11.10±2.27	8.58±1.76	15.43±3.18	11.10±2.27	10.59±2.17	<.001

**Values were expressed as means±SD.*

***Values were expressed as median (min-max)*

EQA standards of China: External quality assessment standards of China; RCPA: The Royal Collage of Pathologists of Australasia; RiliBÄK: Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations; CLIA: Clinical Laboratory Improvement Amendments; AFP: Alpha-Fetoprotein; CA 125: Cancer Antigen 125; CA 15.3: Cancer Antigen 15.3; CA 19.9: Cancer Antigen 19.9; CEA: Carcinoembryonic Antigen; TPSA: Total Prostate Specific Antigen

The sigma scores of each test were examined in two groups according to the quality control material from which the bias value was obtained.The correlation of the sigma scores that were calculated according to the biases obtained from the IQC and EQC data in CA 125, CA 19.9, and CEA tests with the Spearman Test, and in AFP, CA 15.3, and TPSA tests with the Pearson Test was examined. In the CA 125, CA 19.9, and CEA tests a strong positive correlation, AFP and CA 15.3 tests a weak-moderate positive correlation, and TPSA test a strong positive correlation was found (r=0.862-0.955-0.871-0.543-0.557-0.951, respectively) (Table 3). The distribution of the point graphs of the sigma scores that were calculated according to the biases obtained from the IQC and EQC data of each test are given in figure 1.

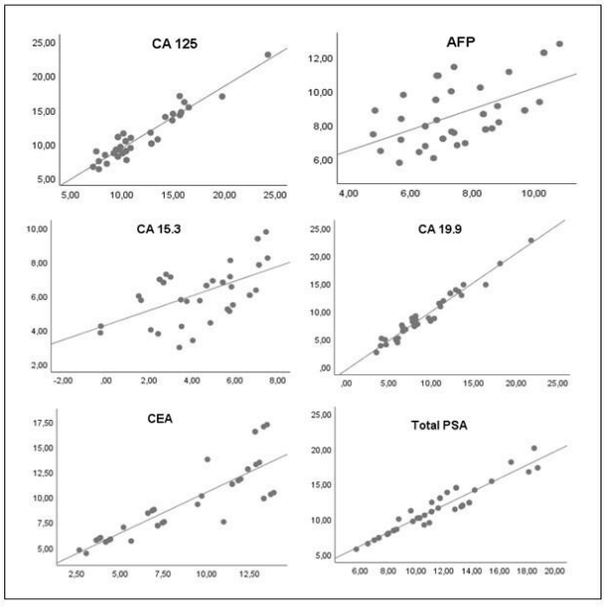

Figure 1. *Distribution of the point graphs of the sigma scores that were calculated according to the biases obtained from the Internal Quality Control (IQC) and External Quality Control (EQC) data (x-axis: sigma score determined according to the bias (%) value obtained from EQC data; y-axis: sigma score determined according to the bias (%) value obtained from IQC data)*

When it was evaluated whether there was a statistically significant difference between the sigma scores that were calculated according to the biases obtained from the IQC and EQC data, although there were significant differences in the AFP and CA 15.3 tests, no significant differences were detected in the other tests (Table 3).

Sigma scores that were calculated according to TEa (%) values determined by the reference institutions were examined in 5 groups. Only the CEA test sigma scores did not differ at significant levels according to different TEa (%) values determined by the reference institutions (Table 4).

The sigma scores of each test were examined in 4 groups according to the periodically as April, May, June, and cumulative. The each test, sigma scores were found to be significantly difference according to periodically (Table 5).

Table 5. *Comparison of sigma scores according to period*

	APRIL	MAY	JUNE	CUMULATIVE	P value
CA 125**	14.40 (7.61-24.24)	9.58 (6.61-17.02)	9.40 (7.07-15.64)	10.18 (6.27-16.12)	.001
AFP*	9.76±1.57	7.19±0.89	7.98±2.03	7.41±1.12	<.001
CA 15 3**	7.10 (3.47-9.75)	3.42 (-0.27-7.27)	5.56 (2.99-6.96)	5.42 (2.07-7.13)	<.001
CA 19.9**	10.67 (4.38-18.58)	10.71 (4.16-22.74)	6.29 (2.61-11.11)	8.04 (3.99-13.59)	<.001
CEA**	4.43 (2.63-5.97)	12.95 (9.71-17.19)	11.38 (7.54-13.88)	7.16 (5.19-8.76)	<.001
TPSA**	12.87 (8.74-20.09)	12.57 (9.21-18.73)	7.69 (5.69-11.60)	10.40 (7.87-15.42)	<.001

**Values were expressed as means±SD.*

***Values were expressed as median (min-max)*

AFP: Alpha-Fetoprotein; CA 125: Cancer Antigen 125; CA 15.3: Cancer Antigen 15.3; CA 19.9: Cancer Antigen 19.9; CEA: Carcinoembryonic Antigen; TPSA: Total Prostate Specific Antigen

4. DISCUSSION

Six Sigma shows the details necessary to improve the quality and efficiency of processes. The process begins with a clear understanding of what the required performance is. Then, with the help of Six Sigma, the root causes of problems are revealed, analyzed, and various statistical tools are applied to avoid them (10).

In the present study, two different sigma scores were calculated according to the bias values obtained from the IQC and EQC data. When it was examined whether there was a statistically significant difference between the sigma scores that were calculated according to the biases obtained from the IQC and EQC data, although there was a significant difference in the AFP and CA 15.3 tests, no significant difference was found in the other tests (Table 3). The significant difference detected in the two tests in our study shows that six sigma practitioners must consider the source of the bias data. The optimal method is to compare results obtained from fresh human specimens using the measurement procedure and a reference measurement procedure. In our literature review, it was found that the bias values obtained from the EQC data were generally used in sigma score calculations. In a study that was conducted by Aslan et al. (11), bias values that were obtained from IQC results were used in sigma score calculations. Some researchers recommend using the bias from EQC results (12). If bias calculation cannot be made with the optimal method, we recommend using the CV (%) value from the IQC data and the bias (%) value from the EQC data to ensure that the data of two quality control materials used in clinical biochemistry laboratories are included as a variable in the calculation of the sigma score.

When the sigma scores that were calculated according to the TEa (%) values determined by the reference institutions were analyzed statistically, it was found that different TEa (%) values caused significant differences in sigma scores (Table 4). Liu et al. calculated the sigma scores of tumor markers (AFP, CA 15-3, CA 19-9, CA 125, CEA, and TPSA) according to different TEa (%) sources, as was the case in the present study (8). There is no appropriate consensus to set a TEa (%) target for an assay. The choice of TEa (%) value can lead to significant differences in the evaluation of the sigma score and can also have a significant impact on laboratory operational routines. Choosing a high TEa (%) value leads to the possibility of missing errors while choosing a low TEa (%) value leads to false outliers. The optimal TEa (%) should be determined based on the requirements and conditions of the laboratory, and the suitability of the TEa (%) for clinical use should be evaluated.

The present study is the first in this field in which short and long-term sigma scores were compared. When the sigma scores obtained by months and cumulatively were examined, it was found that there were significant differences between the examined periods (Table 5). For this reason, we think that 3-month or longer-term sigma score calculations may be insufficient to reflect the problems occurring in the past. We suggest that laboratories must follow their sigma scores monthly and take corrective and preventive actions according to the data they obtain.

5. CONCLUSION

As a conclusion, it is important to determine the optimal TEa (%) value in sigma score examinations and to monitor the quality by analyzing the sigma scores on a monthly basis in terms of the sustainability of the result quality of the tests and for the early detection of problems.

Acknowledgements: *We all authors thank to our all laboratory staff for their great contribution during IQC proceses.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *Ethics committee approval is not required.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: BT, MS
Design of the study: BT, MS
Acquisition of data for the study:BT, MS
Analysis of data for the study: BT, MS
Interpretation of data for the study: BT, MS
Drafting the manuscript: BT, MS
Revising it critically for important intellectual content:BT, MS
Final approval of the version to be published: BT, MS

REFERENCES

[1] Taga Y, Aslan D, Güner G, Kutay ZF. Tıbbi Laboratuvarlarda Standardizasyon ve Kalite Yönetimi. TBD Yayınları Ankara. 2000.

[2] Plebani M. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med 2006;44(6):750–9. https://doi.org/10.1515/CCLM.2006.123

[3] Goldschmidt HM. A review of auto validation software in laboratory medicine. Accredict Qual Asur, 2002;7:431-40. https://doi.org/10.1007/s00769-002-0547-y

[4] Demir S. Süreç Performansının Ölçülmesi: Altı Sigma Yöntemi. Aslan D. ed. Klinik laboratuvarlarda Analitik Kalite Yönetimi Kursu Kitabı, Türk Biyokimya Derneği, İzmir, 2010;83-90. (Turkish)

[5] Miller G. Quality Control. McPherson R, Pincus M, eds. Henry's Clinical Diagnosis and Management by Laboratory Methods, 22nd ed. 2011;119-234.

[6] Aslan D, Demir S. Laboratuvar Tıbbında Altı Sigma Kalite Yönetimi. Turk J Biochem. 2005;30(4):272- 8.

[7] Lippi G, Banfi G, Buttarello M, Ceriotti F, Daves M, Dolci A, Caputo M, Giavarina D, Montagnana M, Miconi V, Milanesi B, Mosca A, Morandini M, Salvagno GL. Recommendations for detection and management of unsuitable samples in clinical laboratories. Clin Chem Lab Med. 2007;45(6):728-36. https://doi.org/10.1515/CCLM.2007.174

[8] Liu Q, Fu M, Yang F, Liang W, Yang C, Zhu W, Ma L, Zhao C. Application of Six Sigma for evaluating the analytical quality of tumor marker assays. J Clin Lab Anal. 2019;33(2):e22682. https://doi.org/10.1002/jcla.22682

[9] Wang W, Zhong K, Yuan S, He F, Du Y, Hu Z, Wang Z. National survey on internal quality control for tumour markers in clinical laboratories in China. Biochem Med (Zagreb). 2018;28(2):020702.
https://doi.org/10.11613/BM.2018.020702

[10] Woodard TD. Addressing variation in hospital quality: is Six Sigma the answer? Journal of Healthcare Management 2005;50(4):226-36.

[11] Aslan D, Sert S, Aybek H, Yılmaztürk G. Klinik laboratuvarlarda toplam laboratuvar performansının değerlendirilmesi: Normalize OPSpec Grafikleri, Altı Sigma ve Hasta Test Sonuçları. Turk J Biochem. 2005;30(4);296- 305.

[12] Kristiansen J. Description of a generally applicable model for the evaluation of uncertainty of measurement in clinical chemistry. Clin Chem Med Lab 2001;39:920-31.
https://doi.org/10.1515/CCLM.2001.148

How to cite this article: Tanrıverdi B, Seyithanoğlu M. The Application of Analytical Period Six Sigma in Tumor Markers in Clinical Biochemistry Laboratory. Clin Exp Health Sci 2025; 15: 103-109. https://doi.org/10.33808/clinexphealthsci.1098988

Clinical and Experimental Health Sciences

The Effect of Nursing Students' Emotional Intelligence Levels on Their Attitudes Toward the Nursing Profession

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Received: May 2, 2024 **Accepted:** February 12, 2025

ABSTRACT

Objective: To determine the effect of nursing students' emotional intelligence levels on their attitudes toward the nursing profession.

Methods: In this study involving 746 nursing students from the nursing department of a university's faculty of health sciences, a specific sample selection was not employed; instead, an attempt was made to reach the entire population. The study was conducted with 586 (78.55%) students. The data was collected between March and April 2021 using the information form, Emotional Intelligence Test, and Attitude Scale for Nursing Profession.

Results: The nursing students' Emotional Intelligence Test mean score was 140.08±18.06, and their Attitude Scale for Nursing Profession mean score was 4.24±0.30. Males (t=-2.856; p=.004<.01; d=0.324; η^2=0.014), those who chose the nursing profession willingly (t=5.272; p=.001<.01; d=0.436; η^2=0.045) and satisfied with being nursing students (t=2.851; p=.005<.01; d=0.343; η^2=0.014) had higher emotional intelligence total scores. Those who choose the profession willingly (t=13.099; p=.001<.01; d=1.084; η^2=0.227) and were satisfied with being a nursing student (t=10.691; p=.001<.01; d=1.286; η2=0.164) had higher attitude toward the profession total scores. Emotional intelligence levels explained their attitudes toward the nursing profession by 16.6% (R2=0.166; F=117.176; p=.001<.01).

Conclusion: The results showed that nursing students had average levels of emotional intelligence and a positive attitude toward the nursing profession. Those who chose the profession and became nursing students had higher emotional intelligence and more positive professional attitudes. Emotional intelligence increases the attitude level of nursing students toward the nursing profession overall. Therefore, to strengthen their positive attitudes towards the profession, nursing students should be encouraged to participate in scientific and social activities that will improve their emotional intelligence levels.

Keywords: Emotional intelligence, nursing, nursing students, attitude, occupations

1. INTRODUCTION

Emotional intelligence affects the quality of care provided by nurses (1). It is also a crucial attribute influencing the quality of nurses' tasks, such as clinical decision-making and critical thinking (2). Therefore, it is essential and important to impart and enhance emotional intelligence skills during the education of nursing students who are future practitioners of the nursing profession (3). Previous studies have emphasized that emotional intelligence facilitates learning in nursing students (4) and that cognitive skills should be balanced with emotional intelligence (5). Developing emotional intelligence in nursing students will support them in career planning and contribute to becoming more considerate healthcare providers in the future (6). Christianson (5) and Dugué et al. (7) found that students with good emotional intelligence skills were more successful and productive throughout their education and professional careers. Emotional intelligence also affects nursing students' teamwork (8), caring behaviours (1), personal and social responsibilities (9), empathy (10) and communicationskills (3).

Nursing students are expected to be able to cope with the emotional difficulties that arise during clinical training and to manage the emotional problems related to themselves, their peers, and patients before graduating and entering the profession. To ensure this, it has been proposed that emotional intelligence should be incorporated into the nursing curriculum to empower nursing students to develop positive professional attitudes (11) because nurses dissatisfied with their jobs with a negative attitude toward the profession are more likely to encounter psychological problems such as nervousness, stress, and anxiety. Nurses with a negative attitude toward the profession can negatively influence their colleagues and lower their job performance and motivation (12). Studies observed that nursing students' attitudes toward the profession affect their career choices

https//doi.org/10.33808/clinexphealthsci.1477183

(13), perceptions of the profession's image (14), peer support (15) and career choices and motivations (16) during student years. A study on the relationship between nursing students' thoughts about their profession and their emotional intelligence reported that students who love their profession, want to pursue it and would recommend it to others evaluate their emotions better and are more optimistic, and that this perspective reflects positively on their emotional intelligence levels (17). As this positive reflection may be the other way around, it was anticipated that students' emotional intelligence levels may also affect their attitudes towards the nursing profession. Therefore, the present study aimed to determine the relationship between nursing students' emotional intelligence levels and their attitudes toward the nursing profession.

2. METHODS

2.1. Aim and Type of Research: This correlational cross-sectional study was conducted to determine the relationship between nursing students' emotional intelligence levels and their attitudes toward the nursing profession.

2.2. Research Questions: What is the level of emotional intelligence of nursing students?

What is the level of attitude of nursing students toward the nursing profession?

Does the level of emotional intelligence in nursing students affect their attitudes toward the nursing profession?

2.3. Universe and Sample of the Research: The study population covered all students (N=746) in the nursing department of a health sciences faculty in the Black Sea Region of Turkey. An attempt was made to reach the entire population without sample selection. The research was conducted with 586 (78.55%) students who agreed to participate.

2.4. Data Collection Tools: An information form to collect students' sociodemographic data, the Emotional Intelligence Test, and the Attitude Scale for Nursing Profession were used.

Information Form: There are five questions about nursing students' gender, age, grade year, whether they choose nursing willingly, and their satisfaction with studying nursing.

Emotional Intelligence Test (EIT): It was developed by Hall in 1999, and its Turkish validity and reliability study was conducted by Ergin in 2000. It is a 30-item 6-point Likert-type scale with five subscales, each comprising six items: emotional awareness, managing emotions, self-motivation, empathy, and social skills. A score of 155 and above from the EIT indicates a high level (fairly strong) of emotional intelligence. A score between 130-150 is considered normal (some improvement is needed), and a score of 129 and below is regarded as a low level (definitely needs improvement) of emotional intelligence (18). The Cronbach Alpha value of the scale was found to be 0.84 in the original study and 0.92 in this study.

Attitude Scale for Nursing Profession (ASNP): Developed by Çoban and Kaşıkçı in 2011 (19), it is a five-point Likert-type scale with three subscales regarding nursing profession characteristics (18 items), preference for the nursing profession (13 items), and attitude toward the general state of the nursing profession (9 items), totalling 40 items. Eight items contain negative statements and are reverse-scored. Higher scores from the scale indicate a positive attitude toward the nursing profession. Individuals with an average score below three may be considered to have a negative attitude. In contrast, those with an average score of 3 and above may be deemed to have a positive attitude. The Cronbach Alpha value of the scale was found to be 0.91 in the original study and 0.85 in this study.

2.5. Data Collection: After obtaining institutional and ethics committee approval, data were collected through Google Forms using personal email addresses between March and April 2021. Students who volunteered to participate marked the checkbox stating 'I accept to participate in the study' at the beginning of the data collection tool and proceeded to answer the questions, submitting their responses to the researchers.

2.6.Data Analysis: Frequency and percentage analyses were used to determine the participating students' descriptive characteristics and mean and standard deviation statistics were used to examine the scale. The relationships between subscales determining the students' scale scores were analysed through Pearson correlation and linear regression analyses. To investigate variations in scale levels based on the descriptive characteristics of students, t-tests, one-way analysis of variance (ANOVA), post hoc tests (Tukey, LSD), and effect size calculations using Cohen's d and Eta squared (η^2) coefficients were employed. The results were evaluated at a 95% confidence interval and a 5% significance level.

2.7. Ethical Consideration: To conduct the research, written permission from the dean's office of the faculty of health sciences to which the nursing department is affiliated (Date: 29.01.2021, Number: E-63582098.299.1009) and approval from the university's ethics committee (Date: 24.02.2021, Number: 24237859-207) were obtained.

2.8. Limitations of the Research: The research is limited to the opinions of students from the nursing department of a university's faculty of health sciences.

3. RESULTS

84.3% of the students were female, and 50.5% were under the age of 21. 53.1% chose the nursing profession willingly. 27% were first-, 24.1% were second-, 20.1% were third-, and 28.8% were fourth-year students. 86.3% were satisfied with studying nursing.

Their Emotional Intelligence Test total mean score was 140.08±18.06, and the Attitude Scale for Nursing Profession total mean score was 4.24±0.30. The mean scores for the subscales are given in Table 1.

Table 1. *Students' mean scores on the Emotional Intelligence Test and Attitude Scale for Nursing Profession (n=586)*

Emotional Intelligence Test Subscales	Min.	Max.	Mean	SD
Emotional awareness	18.00	36.00	30.04	3.57
Managing emotions	11.00	36.00	25.70	5.34
Self-motivation	13.00	36.00	28.01	4.48
Empathy	15.00	36.00	28.96	4.24
Social skills	9.00	36.00	27.35	4.57
Emotional Intelligence Test Total	93.00	180.00	140.08	18.06
Attitude Scale for Nursing Profession Subscales				
Nursing profession characteristics	3.61	5.00	4.62	0.30
Preference for the nursing profession	1.85	5.00	3.93	0.61
Attitude toward the general state of the nursing profession	3.00	5.00	3.92	0.28
Attitude scale for the nursing profession total	3.38	5.00	4.24	0.30

A weak positive correlation was found between students' total scores on ASNP and EIT (r=0.409; p=.001<.01). Weak but significant positive correlations were found between the subscales (r=0.363-0.170, p<.01) (Table 2).

The regression analysis to determine the cause-and-effect relationship between the Emotional Intelligence total and Attitude Scale for the Nursing Profession total yielded significance (F=117.176; p=.001<.01). Emotional Intelligence total explains the total change in the Attitude Scale for Nursing Profession total by 16.6% (R^2=0.166). Emotional intelligence increases nursing students' overall attitude toward nursing (ß=0.409) (Table 3).

The emotional intelligence subscales, including emotional awareness, managing emotions, self-motivation, empathy, and social skills, explained 17% of the nursing students' total attitude toward the nursing profession. The model was significant (R^2=0.17; F=24.961; p=.001<.01). Students' emotional awareness (ß=0.170) and empathy (ß=0.161) levels, respectively, affect their attitude levels toward the nursing profession positively (p<.01) (Table 4).

Table 2. *Correlation Analysis Between Emotional Intelligence and Attitude Towards Nursing Profession Scores*

		Emotional Intelligence Test Total	Emotional awareness	Managing emotions	Self-motivation	Empathy	Social skills
Attitude scale for the nursing profession total	r	0.409*	0.363*	0.318*	0.352*	0.348*	0.290*
	p	.001	.001	.001	.001	.001	.001
Nursing profession characteristics	r	0.296*	0.332*	0.170*	0.212*	0.286*	0.236*
	p	.001	.001	.001	.001	.001	.001
Preference for the nursing profession	r	0.341*	0.247*	0.318*	0.334*	0.256*	0.217*
	p	.001	.001	.001	.001	.001	.001
Attitude toward the general state of the nursing profession	r	0.289*	0.282*	0.189*	0.214*	0.279*	0.229*
	p	.001	.001	.001	.001	.001	.001

**<0.01; Pearson Correlation Analysis*

Table 3. *The effect of emotional intelligence on attitudes toward the nursing profession*

Independent Variable	Non-Standardised Coefficients		Standardised Coefficients	t	P	95% Confidence Interval	
	B	SE	ß			Alt	Üst
Constant	3.263	0.091		35.756	.001	3.083	3.442
Emotional Intelligence Total	0.007	0.001	0.409	10.825	**.001**	0.006	0.008

**Dependent Variable=Attitude Scale for Nursing Profession Total, R=0.409; R2=0.166; F=117.176; p=.001; Durbin Watson Value=1.717*

Table 4. *The effect of emotional intelligence subscales on attitudes toward the nursing profession*

Independent Variable	Non-Standardised Coefficients		Standardised Coefficients	t	P	95% Confidence Interval	
	B	SE	ß			Lower	Upper
Constant	3.153	0.104		30.428	.001	2.949	3.356
Emotional awareness	0.015	0.004	0.170	3.324	**.001**	0.006	0.023
Managing emotions	0.004	0.004	0.063	1.033	.302	-0.003	0.011
Self-motivation	0.008	0.005	0.113	1.702	.089	-0.001	0.017
Empathy	0.012	0.004	0.161	2.746	**.006**	0.003	0.020
Social skills	0.000	0.004	-0.002	-0.030	.976	-0.008	0.007

**Dependent Variable=Attitude Scale for Nursing Profession Total, R=0.421; R2=0.170; F=24.961; p=.001; Durbin Watson Value=1.702*

Nursing students who were male (t=-2.856; p=.004<.01; d=0.324; η^2=0.014) chose the nursing profession willingly (t=5.272; p=.001<.01; d=0.436; η^2=0.045), and were satisfied with studying nursing (t=2.851; p=.005<.01; d=0.343; η^2=0.014) had statistically significantly higher total emotional intelligence scores (Table 5).

In the nursing profession characteristics subscale, for female students (t=2.474; p=.014<.05; d=0.281; η^2=0.010), those who chose the profession willingly (t=13.099; p=.001<.01; d=1.084; η^2=0.227) and were satisfied with studying nursing had statistically significantly higher attitude toward the nursing profession total mean scores (t=10.691; p=.001<.01; d=1.286; η^2=0.164) (Table 6).

Table 5. *Differentiation of emotional intelligence scores according to descriptive characteristics*

Demographic Features	n	Emotional Intelligence Total	Emotional awareness	Managing emotions	Self-motivation	Empathy	Social skills
Gender		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Female	494	139.166±17.924	30.024±3.517	25.302±5.278	27.660±4.448	28.964±4.203	27.217±4.614
Male	92	144.989±18.112	30.141±3.913	27.902±5.221	29.902±4.209	28.967±4.512	28.076±4.310
t=		-2.856	-0.288	-4.346	-4.476	-0.008	-1.657
p=		**.004**	.774	**.001**	**.001**	.994	.098
Age		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
17-20	296	140.439±17.901	30.203±3.534	25.980±5.006	28.071±4.183	29.020±4.445	27.166±4.904
21 and above	290	139.714±18.250	29.879±3.623	25.435±5.674	27.952±4.776	28.907±4.045	27.541±4.212
t=		0.486	1.094	1.234	0.322	0.323	-0.994
p=		.627	.274	.218	.748	.747	.320
Grade year		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	158	141.076±18.412	30.076±3.681	26.329±4.879	28.538±4.254	28.975±4.597	27.158±4.821
2	141	141.028±17.523	30.411±3.289	25.773±5.540	27.993±4.439	29.177±4.358	27.674±4.647
3	118	138.254±17.590	29.805±3.731	25.119±5.176	27.788±4.228	28.703±3.971	26.839±4.326
4	169	139.633±18.539	29.870±3.611	25.491±5.701	27.692±4.877	28.959±4.027	27.621±4.443
F=		0.725	0.807	1.289	1.111	0.266	1.017
p=		.537	.490	.277	.344	.850	.385
Choosing the nursing profession willingly		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Yes	311	143.698±17.528	30.508±3.492	26.820±5.217	28.839±4.166	29.633±4.199	27.897±4.462
No	275	135.989±17.814	29.516±3.609	24.455±5.227	27.076±4.649	28.207±4.184	26.735±4.630
t=		5.272	3.377	5.473	4.841	4.110	3.093
p=		**.001**	**.001**	**.001**	**.001**	**.001**	**.002**
Satisfaction with studying nursing		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Yes	506	140.921±17.916	30.150±3.592	26.010±5.198	28.277±4.332	29.036±4.349	27.449±4.574
No	80	134.763±18.198	29.363±3.439	23.813±5.913	26.338±5.054	28.513±3.544	26.738±4.561
t=		2.851	1.833	3.446	3.633	1.023	1.293
p=		**.005**	.067	**.002**	**.002**	.238	.197

F: ANOVA Test; t: Independent Groups T-Test; Post Hoc: Tukey, LSD

Table 6. *Comparison of nursing students' attitude scores toward the nursing profession according to their descriptive characteristics*

Demographic Features	n	Attitude Scale for Nursing Profession Total	Nursing Profession Characteristics	Preference for the Nursing Profession	Attitude Toward the General State of the Nursing Profession
Gender		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Female	494	4.250±0.307	4.638±0.294	3.938±0.618	3.923±0.280
Male	92	4.203±0.319	4.554±0.339	3.911±0.582	3.924±0.288
t=		1.329	2.474	0.391	-0.040
p=		.184	**.014**	.696	.968
Age		Mean±SD	Mean±SD	Mean±SD	Mean±SD
17-20	296	4.240±0.301	4.608±0.304	3.957±0.592	3.916±0.290
21 and above	290	4.244±0.317	4.643±0.300	3.910±0.632	3.930±0.271
t=		-0.151	-1.417	0.925	-0.602
p=		.880	.157	.355	.547
Grade year		Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	158	4.232±0.315	4.582±0.314	3.969±0.568	3.911±0.319
2	141	4.260±0.268	4.654±0.269	3.954±0.604	3.913±0.256
3	118	4.240±0.331	4.609±0.329	3.955±0.639	3.915±0.278
4	169	4.239±0.321	4.653±0.296	3.869±0.640	3.947±0.265
F=		0.221	2.124	0.910	0.612
p=		.882	.096	.436	.608
Choosing the nursing profession willingly		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Yes	311	4.381±0.264	4.673±0.296	4.254±0.479	3.978±0.264
No	275	4.086±0.281	4.571±0.300	3.571±0.540	3.860±0.286
t=		13.099	4.147	16.238	5.191
p=		**.001**	**.001**	**.001**	**.001**
Satisfaction with studying nursing		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Yes	506	4.292±0.290	4.635±0.303	4.065±0.523	3.934±0.273
No	80	3.928±0.231	4.563±0.294	3.099±0.462	3.856±0.319
t=		10.691	1.974	15.600	2.316
p=		**.001**	**.049**	**.001**	**.041**

F: ANOVA Test; t: Independent Groups T-Test; Post Hoc: Tukey, LSD

4. DISCUSSION

Emotional intelligence is a professional skill considered essential for a career in the nursing profession. Nursing students' capacity to understand, express and manage their own and others' emotions is important in developing positive perceptions, beliefs and behaviours related to the profession (7,20). This study examining nursing students' emotional intelligence levels and attitudes towards nursing demonstrated a positive relationship between emotional intelligence and attitudes towards nursing. As students' level of emotional intelligence increased, their attitudes towards the profession also increased positively. A study further suggested that adopting nursing values is related to students' emotional intelligence skills (21). However, in this study, emotional intelligence subscales also positively affected students' attitudes towards the nursing profession. This is because nursing students' levels of emotional intelligence positively influence their problem-solving skills (22), their transitioning to the profession and competency (23), their resilience, as well as their ethical, critical, and empathetic thinking styles (24), and their communication in clinical settings (25). These factors may have positively influenced the professional attitudes of nursing students.

If the development of emotional intelligence is compromised, it can lead to failures in interpersonal relationships and professional life. Therefore, nurses are particularly expected to have higher emotional intelligence (26). In the current study, however, the nursing students had moderate levels of emotional intelligence. The nursing students were aware of their emotions but seemed to struggle with managing them. Supporting these results, nursing students had a medium level of emotional intelligence (8,22), with their lowest score being from managing emotions subscale (10). However, according to national and international studies, nursing students are expected to have high levels of emotional intelligence (27,28). On the other hand, this study found that students, especially male students, who were satisfied with studying in the department of nursing had high levels of emotional intelligence, mainly in managing their emotions and self-motivation. It has been previously mentioned that male students generally had higher levels of emotional intelligence and were better than female students in expressing and utilizing emotions (29). The differing results of studies may be attributed to cultural differences (24). Additionally, nursing students who voluntarily chose the profession had higher emotional intelligence levels across all subscales compared to others. This finding may be due to the positive impact of self-sensitivity on emotional intelligence among nursing students (27).

Attitude towards the profession is a determinant of professional success and satisfaction in nursing (30). In this study, the nursing students were found to have a positive attitude toward the profession, which is similar to the results of some other studies (14,15,31-33). Professional attitudes of nursing students can be improved in educational processes and clinical practices. Finally, consistent with previous studies, this study found that students who are female (16,31), voluntarily chose nursing (13,15,31), and are satisfied with studying in the nursing program (14,15) have more positive attitudes toward the profession. Nurses' attitudes toward the profession are thought to be associated with their professional attitudes (34). Therefore, being satisfied with studying nursing may foster a positive attitude in this context.

5. CONCLUSIONS

It was concluded that the nursing students had average levels of emotional intelligence and positive attitudes toward the nursing profession. A positive relationship was found between emotional intelligence and their attitudes toward the nursing profession, and it was inferred that emotional intelligence increased nursing students' attitudes toward the profession.

We suggest improving the emotional intelligence levels of students who have problems managing their emotions despite being aware of them, incorporating emotional intelligence in the nursing curriculum to offer students who have not chosen the profession willingly or are not satisfied with being a nursing student support in the components of emotional intelligence and guiding students to scientific activities such as seminars, courses, etc. on these topics. This may increase their overall performance, and they can be empowered in emotional awareness and management, empathy, self-motivation, and social skills. Developing emotional intelligence can lead to more positive attitudes toward the profession among students, contributing to the overall enhancement of the quality of the nursing profession.

Acknowledgement: *Thank you for supporting the participants in this study. The abstract of this paper was presented as oral presentation at the International Health Services Congress, Mersin, Turkey, 21-22 November 2023.*
Funding: *The authors received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Scientific Research Ethics Committee of Karadeniz Technical University Medicine Faculty, (approval date 24.02.2021 and number 24237859-207)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: KÖK
Design of the study: KÖK, ÇTK, HÖ
Acquisition of data for the study: ÇTK
Analysis of data for the study: KÖK
Interpretation of data for the study: KÖK, ÇTK, HÖ
Drafting the manuscript: KÖK, ÇTK, HÖ
Revising it critically for important intellectual content: KÖK, ÇTK, HÖ
Final approval of the version to be published: KÖK, ÇTK, HÖ

REFERENCES

[1] Çolak Okumuş D, Uğur E. Hemşirelerin duygusal zeka düzeylerinin bakım davranışlarına etkisi. ACU Sağlık Bil Derg. 2017; 2:104-109. (Turkish)

[2] Barkhordarı M, Rostambeygı P. Emotional intelligence in nursing students. Journal Of Advances in Medical Education & Professionalism. 2013;1(2):46-50.

[3] Erigüç G, Eriş H, Kabalcıoğlu F. Emotional intelligence and communication skills of nursing students: Example of Harran University School of Health. International Online Journal of Educational Sciences. 2014;6(2):398-412. https//doi.org/10.15345/iojes.2014.02.013

[4] Fernandez R, Salamonson Y, Griffiths R. Emotional intelligence as a predictor of academic performance in first-year accelerated graduate entry nursing students. J Clin Nurs. 2012;21(23–24):3485–3492. https//doi.org/10.1111/j.1365-2702.2012.04199.x

[5] Christianson KL. Emotional intelligence and critical thinking in nursing students: Integrative review of literature. Nurse Educator. 2020;45(6):E62-E65. https//doi.org/10.1097/NNE.0000000000000801

[6] AkbariLakeh M, Naderi A, Arbabisarjou A. Critical thinking and emotional intelligence skills and relationship with students' academic achievement. Prensa Med Argent. 2018;104(2):1-5. https//doi.org/10.4172/0032-745X.1000280

[7] Dugué M, Sirost O, Dosseville F. A literature review of emotional intelligence and nursing education. Nurse Education in Practice. 2021; 54:1-10. https//doi.org/10.1016/j.nepr.2021.103124

[8] Önler E, Yıldız T, Süzen ER, Aydınyılmaz H, Urcanoğlu ÖB, Kılıç Ö, Malak A. Hemşirelik bölümü öğrencilerinin ekip çalışması tutumları ile duygusal zeka düzeyleri arasındaki ilişkinin değerlendirilmesi. International Anatolia Academic Online Journal Health Sciences. 2014;2(2):19-29. (Turkish)

[9] Bayrak B, Çelik A, Sevinç M, Alev B. Hemşirelik öğrencilerinin duygusal zeka ile kişisel ve sosyal sorumlulukları arasındaki ilişki. Sağlık ve Hemşirelik Yönetimi Dergisi. 2023;10(3):310-318. https//doi.org/10.54304/SHYD.2023.57475 (Turkish)

[10] Duman D, Acaroğlu R. Hemşirelik yüksekokulu birinci sınıf öğrencilerinin duygusal zekâ düzeyleri ile empati becerileri arasındaki ilişki. Florence Nightingale Journal of Nursing. 2014;22(1):25-32. (Turkish)

[11] Strickland HP, Cheshire MH. Exploring the correlation between nontraditional variables and student success: A longitudinal study. J Nurs Educ. 2017;56(6):351–355. https//doi.org/10.3928/01484834-20170518-06

[12] Coban GI, Kirca N, Yurttas A. Analysis of nurses' attitudes about the nursing profession in southern Turkey. International Journal of Caring Sciences. 2015;8(3):665-672.

[13] Zencir G, Eşer İ. Hemşirelik öğrencilerinin hemşirelik mesleğine yönelik tutumları ile hemşirelik tercihi arasındaki ilişki: Türkiye örneği. Dokuz Eylül Üniversitesi Hemşirelik Fakültesi Elektronik Dergisi. 2016;9(2):30-37. (Turkish)

[14] Sümen A, Teskereci G, Aksoy S, Ergen Z, Ala MM, Üzüm F. Hemşirelik öğrencilerinin hemşirelik mesleğinin imajına yönelik algılarının ve tutumlarının incelenmesi. Ordu Üniversitesi Hemşirelik Çalışmaları Dergisi, 2022;5(1):75-83. https//doi.org/10.38108/ouhcd.852072 (Turkish)

[15] Ayaz-Alkaya S, Terzi H. Predictors of attitudes towards nursing profession and peer caring behaviors of the nursing students: A cross-sectional study. Nurse Education Today. 2022; 116:1-7. https//doi.org/10.1016/j.nedt.2022.105467

[16] Suluhan D, Gezginci E, Ergin ME. The relationship between nursing students' attitudes toward the nursing profession concerning career choice and motivation. European Archives of Medical Research. 2020;36(4):251-257. https//doi.org/10.4274/eamr.galenos.2020.50469

[17] Ceylantekin Y, Öcalan D. Hemşirelik öğrencilerinin mesleği ile ilgili düşünceleri ve duygusal zekâ arasındaki ilişki. Yükseköğretim ve Bilim Dergisi. 2020;10(3):531-538. https//doi.org/10.5961/jhes.2020.413 (Turkish)

[18] Ergin EF. Üniversite öğrencilerinin sahip oldukları duygusal zeka düzeyi ile 16 kişilik özelliği arasındaki ilişki üzerine bir araştırma. [Yüksek Lisans Tezi]. Selçuk Üniversitesi; 2000. (Turkish)

[19] Coban GI, Kasikci M. Development of the attitude scale for nursing profession. International Journal of Nursing Practice. 2011;17(5):518-524. https//doi.org/10.1111/j.1440-172X.2011.01961.x

[20] Alghamdi AA, Alyousef SM, Alyahya NM, Bahari G, Qadhi OA, Hudays A. Emotional intelligence and implications for nursing education. Journal of Coastal Life Medicine. 2023;11(2):920-937.

[21] Culha Y, Acaroglu R. The relationship amongst student nurses' values, emotional intelligence and individualised care perceptions. Nursing Ethics. 2019;26(7-8):2373-2383. https//doi.org/10.1177/096973301879668

[22] Köşgeroğlu N, Alparslan GB, Babadağ B, Öztürk B, Ünver G. Hemşirelik öğrencilerinin duygusal zekâ düzeyleri ve problem çözme becerileri. International Journal of Social and Humanities Sciences Research (JSHSR). 2020;7(56):1969-1977. https//doi.org/10.26450/jshsr.1793 (Turkish)

[23] Chae, HJ. Relationships between nursing competency and emotional intelligence and nurse role transition of nursing students. Journal of Digital Convergence 2019;17(9):221-229. https//doi.org/10.14400/JDC.2019.17.9.221

[24] Cleary M, Visentin D, West S, Lopez V, Kornhaber R. Promoting emotional intelligence and resilience in undergraduate nursing students: An integrative review. Nurse Education Today. 2018; 68:112-120. https//doi.org/10.1016/j.nedt.2018.05.018

[25] Kong L, Liu Y, Li G, Fang Y, Kang X, Li P. Resilience moderates the relationship between emotional intelligence and clinical communication ability among Chinese practice nursing students: A structural equation model analysis. Nurse Education Today. 2016; 46:64-68. https//doi.org/10.1016/j.nedt.2016.08.028

[26] McNulty JP, Politis Y. Empathy, emotional intelligence and interprofessional skills in healthcare education. Journal of Medical Imaging and Radiation Sciences. 2023;54(2):238-246. https//doi.org/10.1016/j.jmir.2023.02.014

[27] Gümüştekin Ö, Kaya F. Hemşirelik öğrencilerinde duygusal zekâ ile öz duyarlık arasındaki ilişki. Kırşehir Ahi Evran Üniversitesi Sağlık Bilimleri Dergisi. 2022;6(3):195-205. (Turkish)

[28] Khodabakhshi Koolaee A, Chaeichi Tehrani N, Sanagoo A. The relationship between spiritual intelligence and emotional intelligence with self-compassion of nursing students. Iranian Journal of Medical Education. 2019;19(5):44-53.

[29] Tambağ H, Kaykunoğlu M, Gündüz Z, Demir Y. Hemşirelik öğrencilerinin duygusal zeka düzeyleri ve etkileyen faktörler. Hemşirelikte Eğitim ve Araştırma Dergisi. 2014;11(1):41-46. (Turkish)

[30] Yılmaz MÇ, Kurtgöz A. Hemşirelik öğrencilerinin kişilik özellikleri ile hemşirelik mesleğine yönelik tutum ve algıları arasındaki ilişki. Artuklu International Journal of Health Sciences. 2023;3(2):188-195. https//doi.org/10.58252/artukluder.1285920 (Turkish)

[31] Çalışkan E, Kargın M, Ersöğütçü F. Hemşirelik öğrencilerinde covid-19 korkusu ile hemşirelik mesleğine yönelik tutum arasındaki ilişki. Sürekli Tıp Eğitimi Dergisi. 2021;30(3):170-180. https//doi.org/10.17942/sted.880773 (Turkish)

[32] Mai BH, Ho TMY, Nguyen TTT, Hoang TH, Phuong NTA. Attitudes and perceptions towards nursing profession among nursing students at Hue University of Medicine and Pharmacy. Journal of Problem-Based Learning. 2018;5(2):55-62. https//doi.org/10.24313/jpbl.2018.5.2.55

[33] Shohani M, Abedi L, Rasouli M. Professional attitude in Iranian nursing students. Journal of Clinical and Diagnostic Research. 2018;12(6): JC10-JC13. https//doi.org/10.7860/JCDR/2018/27804.11649

[34] Tarhan G, Kılıç D, Yıldız E. Hemşirelerin mesleğe yönelik tutumları ile mesleki profesyonellikleri arasındaki ilişkinin incelenmesi. Gülhane Tıp Derg. 2016;58(4):411-416. https//doi.org/10.5455/gulhane.176909 (Turkish)

How to cite this article: Özkol Kılınç K, Torun Kılıç Ç, Öztürk H. The Effect of Nursing Students' Emotional Intelligence Levels on Their Attitudes Toward the Nursing Profession. Clin Exp Health Sci 2025; 15: 110-116. https//doi.org/10.33808/clinexphealthsci.1477183

Clinical and Experimental Health Sciences

Pharmacopoeia Studies on Zingiber Officinale Roscoe (Ginger) Samples Purchased from a Pharmacy and Herbalists in İzmir and İstanbul

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Received: May 12, 2024 **Accepted:** March 13, 2025

ABSTRACT

Objective: The aim of this study is to discover if the samples of *Zingiber officinale* were compatible with the criteria written in the European Pharmacopoeia, as quality control tests, which the analyses were done with the samples supplied from a pharmacy and the herbal stores. Also gathering knowledge from the scientifical literature of the drug's activities a contribution was made to the phytotherapy.

Methods: One pharmacy and five herbalist samples were examined and the quality control tests were done due to the monograph in the European Pharmacopoeia. According to the monograph macroscopic and microscopic assays, thin layer chromatography with methanolic extract, loss on drying, total ash and essential oil determination assays accomplished.

Results: With the macroscopic analysis and the microscopic analysis, the pharmacy sample were compatible with the criteria stated in Pharmacopoeia but one sample from herbal store had some unwanted and unidentified particles. The results of the loss on drying test which is repeated three times for each sample showed that the pharmacy sample and one from herbal store has more moisture than stated in the Pharmacopoeia. Also, total ash determination test was done three times and found that all the samples met the criteria stated in the Pharmacopoeia 7.0.

Conclusion: The results of the essential oil determination tests showed us that the sample obtained from pharmacy and three of the herbal stores were meeting the criteria but the EO's of the other samples were under the amount stated in the Pharmacopoeia.

Keyword: Antioxidant, European Pharmacopoeia, *Zingiber officinale* Roscoe, Zingiberis rhizoma

1. INTRODUCTION

Zingiber officinale Roscoe is not a naturally growing plant in Türkiye, it has only one name and known as "zencefil." The plant is a member of Zingiberaceae family. Its name comes from two different thoughts. First one is the name comes from the Sanskrit name "singabera" (srngaveram–srngam) meaning horn and (vera) meaning body as it looks like a deer's horns (1). And the second comes from an antique language, Dravidian (Tamil and Malayalam), a spice called "inchi/inji" and afterwards addition of the word "ver" that means tuber (2).

There were detected 115 compounds in *Z. officinale* when a gas chromatography mass spectrometry is done (3). Literature search of *Z. officinale* showed that the terpenic compounds are responsible for the main activity; oleoresins that are responsible for the smell of it; gingerols that gives the hot sense on tongue when consumed; and starch with a very high ratio of %60 (4,5). In addition to these, protein; minerals like calcium, potassium, phosphate and magnesium (6); vitamins like A, B3, B5 and B6; folic acid; waxes; lipids; lecitine (6,7); musilage and resin (8); carbonhydrate and proteolitic enzymes like zingibaine (9); and aminoacids like arginine (10).

The Zingiberis rhizoma has oleoresins with the ratio of %4.7-7.5 in which quarter compound of it consists of gingerdiol, zingerone, diterpenes, diarilheptanes and diarilheptanoides which are responsible with the sharp taste of plant; non-volatile phenilpropaoide compounds like gingerols and shogaols which are responsible with the characteristic sharp taste of plant (3,7,9).

Gingerols are responsible with the anti-oxidant, anti-inflammatory, anti-microbial and enzyme-regulation metabolism activities (4,11,12). They can transform to first shogaol and zingeron and then to paradol with the hydrogenation of shogaol (9).

The rhizomes which are preserved and stored for a while offer more active ingredients than fresh ones. This can be seen with shogaols. (13).

Essential oil has approximately 24 compounds like monoterpenic and sesquiterpenic hydrocarbons, alcohols

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and aldehyde compounds like neral and geraniale. The main compounds are camphene, β-fellandrene, 1,8-cineole, geraniale and neral. As monoterpenes there are compounds like limonene, myrsene, α – pinen, borneol, sitronellol, geraniol, geranyl acetate. Sesquiterpenes exist which takes the %30-70 of volatile oil like β-bisabolene, (-)-zingiberene, (+)-*ar*-curcumene, (-)-β-sesquifellandrene. Zingiberen amount was calculated %31.08 in the essential oil (3,14,15,16)

When we look to the fatty oil it has a ratio of %46 of saturated oils like palmitic acid and a ratio of %53 of unsaturated oils like oleic and linoleic acid (3).

Tannen compounds play the role of hemorrhoid and burn healing activity. Zinc, chromium and manganese are the heavy metal ingredients and it can be called with the safe levels of them and with the absence of lead and cadmium (17).

The plant is used for many different biological activities. Some of common aim of these uses are anti-emetic, carminative, anti-oxidant, anti-microbial as anti-viral, anti – bacterial and anti-fungal activities and anti-inflammatory as anti-ulcerative and anti-arthritis activities (18).

Today, drug treatment is preferred as the efficacy of synthetic drugs has been proven in the treatment of diseases and the exact treatment results can be achieved with appropriate dosage and treatment methods with these synthetic drugs. However, both herbal and animal drugs are also used for the treatment of diseases because of their activities proven by both traditional information and scientific data. Especially, because of the side effects and unwanted effects of synthetic medicines, herbal products have become popular nowadays. And ginger is also considered as a good supplement with many biological activities. Besides these information, great attention must be paid to the side effects especially related to teratogenicity. Apart from the side effects, the reliabilities of these herbal drugs are also important due to the possibility of mixing them with other herbal drugs both intentionally or unconsciously. For this purpose, ginger is selected as the subject which has ethnopharmacological data and has been the subject of many scientific research with the information that is coming from old times about the usage in the public medicine.

The aim of the study was achieving the information that if the herbal drugs which are sold in pharmacies and the herbal stores in Turkey fulfill the properties stated in the European Pharmacopoeia. For this reason, samples bought from Istanbul and Izmir herbal stores and bought from a pharmacy is used in the pharmacognostic analysis and quality control tests according to European Pharmacopoeia 7.0.

2. METHODS

2.1. Plant Materials

The plant material samples were bought from 5 herbalists that were randomly chosen that three of them were in Izmir and two of them were in Istanbul. Unfortunately, one sample was obtained from pharmacy because of the absence of another GMP packaged sample. All the samples were coded. (Pharmacy sample: E and the herbal store samples: T1, T2, T3, T4 and T5) All the samples except the pharmacy sample were powdered. Pharmacy sample was powdered manually by a cutting mill (Retsch SM100) in laboratory.

2.2. Macroscopic Analysis

Specifications that were stated in the European Pharmacopoeia 7.0 was morphologically examined with all the six plant material. Only the pharmacy sample was examined for shape, length, color and texture specifications as it was the only rhizome drug. After powdering the pharmacy sample all of the six samples were examined under the loope.

2.3. Microscopic Analyses

All the plant material must be in the powdered form before examination under microscope. Each sample was examined as preparations that were prepared by using chloralhydrate. Under chloralhydrate characteristic brown oleoresins, brown cork and groups of large, thin-walled, septate fibers; fragments that are containing vessels often accompanied by narrow, thin-walled cells containing brown pigment and amyliferous parenchyma.

2.4. Thin Layer Chromatography (TLC)

For the test solution 1g of each powdered plant material were dissolved in methanol (Merck 106009). For the reference solution 10 µL of citral (Sigma C83007) and 10 mg of resorcinol (Merck 107593) were dissolved in 10 mL methanol. The reference solution must be freshly prepared. Mobile phase was hexane/ether with the ratio of 40/60 (V/V). After a development of 15 cm path in an unsaturated tank and drying in air, 10 g/L solution of vanillin in sulfuric acid was sprayed as reagent. The silica gel plates were heated at 100-105 °C for 10 minutes and examined in daylight.

2.5. Loss on Drying

For this test gravimetrical method was chosen. Due to this path glass crucibles were made constant weight by keeping in the 105 °C drying oven and after by cooling in a desiccator. Empty crucibles were weighted and 4 grams of sample were weighted and put in each of them. Subsequent to drying in the 105 °C oven for 2 hours, each crucible was cooled in the desiccator and reweighed. For every sample this procedure was repeated 3 times, and the weight loss percentage was calculated.

2.6. Total Ash

Porcelain crucibles were kept 30 minutes at 105 °C in the heating oven and then kept in the desiccator to cool down and get to constant weight. 1 gram of each sample were put

into a crucible after crucibles were weighted empty. After drying at 100 – 105 °C for an hour, they were burned by slowly increasing temperature from in ashing furnace (Protherm PC442T, Protherm Furnaces, Ankara, Turkey) till 600 °C until white ashes were seen. Flaming was not permitted. Then crucibles were cooled to constant weight in desiccator and then weighed again to find the percentage of ash to 1 gram of sample.

2.7. Determination of Essential Oils

20 grams of each coarsely powdered fresh sample were weighted and put into 1000 mL round-bottomed flask. Then 10 drops of liquid paraffin and 500 mL water were added for distillation. 0.5 mL of xylene was added into the graduated tube. At the end of the distillation the volume of collected essential oils were measured on the graduated tube. Minimum 15mL/kg essential oil is expected.

3. RESULTS

3.1. Macroscopic Analysis

Pharmacy sample was bought in sealed packages as rhizome drug. Colour was between light brown to golden yellow. It was covered with the hard cork with evident, narrow, longitudinal and transverse ridges and occasional loose fibres. Inner surface yellowish – light brown parts were seen. After powdering in the laboratory when looked under the loope pharmacy sample, it showed light yellowish – brown colour. The pharmacy drug was shown in Figure 1. Herbal store samples were bought in powdered form. They also showed light yellowish – brown colour. But there were some unwanted and unidentified particles in samples which didn't belong to ginger. In conclusion pharmacy sample was more reliable than herbal store samples according to European Pharmacopeia criteria. For more detailed information microscopical analyses were done.

***Figure 1**. Pharmacy sample before powdering and after powdering*

3.2. Microscopic Analyses

Powdered samples were examined under microscope with the mounting solution chloral hydrate. There were seen large, thin walled and septate fibers; and vessels brown colored and narrow and thin-walled cells and with amyliferous parenchyma; brown to yellow-colored cells containing oleoresins; brown cork fragments. For only one herbal store sample (T3) the unwanted and unidentified particles which were seen on macroscopic analyses, also seen on microscopical analyses. Some specific microscopic images were taken and can be seen in Figure 2.

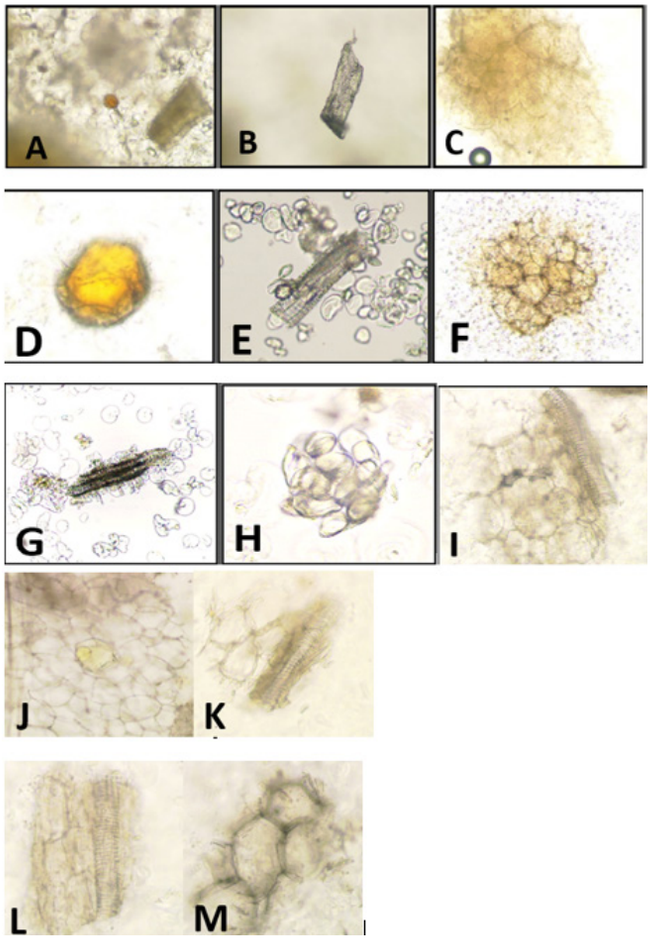

***Figure 2**. Microscopical images of the samples. Oleoresin (A), Reticulate vessel (B) and starch (C);*

T1 sample: Oleoresin (D), Reticulate vessel (E) and starch (F); T2 sample: Reticulate vessel (G) and starch (H); T3 sample: Reticulate vessel and starch (I); T4 sample: Oleoresin and starch (J) and Reticulate vessel and parenchyma (K); T5 sample: Reticulate vessel and parenchyma (L) and starch (M)

3.3. Thin Layer Chromatography

The results of the thin layer chromatography analyses were compatible with the criteria written in the European Pharmacopoeia 7.0 for all the samples.

The reference solution, that citral and resorcinol in methanol, gave us an intense red zone in the lower parts of the chromatogram because of resorcinol and two purple zones in the upper part because of citral.

The test solution, that sample in the methanol, applied chromatogram gave us two purple zones because of gingerols under the area of red zones because of the resorcinol in reference solution. In the middle parts there were two less intense purple zones caused by shogaols, between the two zones caused by resorcinol and citral that exists in the reference solution. Photos of two samples of the test results can be seen in Figure 3.

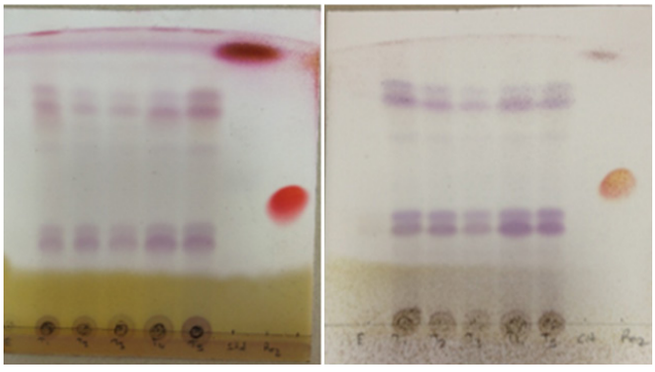

Figure 3. *TLC plates show zones of gingerols, resorcinol, shogaols and citral*

3.4. Loss on Drying and Total Ash

According to the monograph of *Zingiber officinale* in the European Pharmacopoeia 7.0 maximum 100 mL/kg of water is permitted from 20 grams of the powdered drug. Maximum %6 of total ash is indicated as criteria for each plant sample. The results of the tests can be seen on the Table 1. Results showed us that for all the samples total ash amounts remained under the criteria of maximum %6. For the water loss assay E and T2 samples were having more moisture than the criteria stated in the Pharmacopoeia. The other samples T1, T3, T4 and T5 remained under the maximum criteria.

Table 1. *Loss on drying test results*

Sample	Water Loss (g)	Total Ash (g)
E	0.42	0.04
T1	0.37	0.03
T2	0.43	0.04
T3	0.39	0.03
T4	0.39	0.04
T5	0.38	0.03

3.5. Essential Oil Test

Samples were tested for the amount stated in the pharmacopoeia of minimum 15 mL/kg essential oil. The essential oil accumulated in the graduated tube was measured. After measuring all the samples' essential oils, they were put to glass tubes to compare them relatively. The assay results can be seen on the table below. According to the results, pharmacy sample (E) and herbal store samples (T1, T2 and T5) had enough amount of essential oil as the criteria stated in the Pharmacopoeia. Herbal store samples T3 and T4 did not fulfill the same criteria.

Table 2. *Essential oil test results*

Sample	Result
T1	0.35 mL
T2	0.45 mL
T3	0.25 mL
T4	0.20 mL
T5	0.30 mL
E	0.40 mL

4. DISCUSSION

In this study, the *Zingiber officinale* drug samples which were bought from herbal stores and a pharmacy were controlled for the conformity of them to the specifications of monograph written in European Pharmacopoeia 7.0. Macroscopic and microscopic studies, thin layer chromatography, water test, total ash amount test and essential oil test on drug samples were done. The come outs were compared between each other for all the tests done for every test heading; and compared with the Pharmacopoeia data.

Zingiber officinale rhizomes which usually known as ginger have been used as a spice since ancient times. With scientifical researches on it the drug reveals a very great role in medicine with its active ingredients and so activities. And with the guide of these researches the drug serves a wide range of therapeutical activities like mostly known as carminative, antimicrobial and antioxidant effects.

The drug is being used since the Chinese and Indian traditional medicine. So, the outcomes of these knowledge since ancient times, today gives us many ways of usage of the drug. Anti-emetic activity and usage in the upper respiratory system infections are some examples for this situation (8, 14). As a traditional medicine in Turkey the drug is also used for antiemetic and antinausea effects.

The mature rhizomes, are harvested in the fall and have brownish-yellow color. It can be used as dried and then powdered drug or with making slices of the rhizome which is often used with honey together.

The main ingredients are active ingredients like gingiberol, gingerol, shogaol, citral, geranial and neral; curcuminoids and starch with the ratio of approximately half of it. The main aroma of the plant is because of the active ingredient gingiberol (19).

In human body free radicals that are generated throughout oxidation pathway, can cause temporary or permanent damage especially in the nervous system, reproductive system, and liver leading to significant problems. So many researches were done about the antioxidant activity of the drug. When calculated with the terms of quercetin it showed a very high activity (20). In another research, with

the higher dose of ginger, the MDA level which is related to the increasing free radical levels, decreased and the SOD and GSH levels which are the sign of the body defense, exhibited a significant increase. This antioxidant activity is maybe due to the high polyphenolic active ingredients of ginger that shogaol, 6-gingerol and 6-paradol (21). With its high antioxidant activity properties ginger is also a good antioxidant for reproductive system (22,23).

Highly alleviating vomiting and nausea as effective as vitamin B6 and having any teratogenic effect, make ginger more important for pregnant women (24). When compared with dimenhydrinate in a study, ginger extract showed similar effect with lower side effects, except one heartburn case in the study (25).

Although according to the British Herbal Compendium (BHC) usage in pregnancy related nausea is advised that ginger has very little side effects and this increases its reliability, the German Commission E and ESCOP approach cautiously and do not recommend its use in this kind of usage (26).

Studies made about post-operative patient care have shown that ginger can have anti-emetic activity as high as metoclopramide (27,28).

Besides these main activities ginger has activities like being an agent against migraine, obesity and diabetes, anti-thrombotic and hepatoprotective effects and protective effects against radioactivity. Regulating menstrual bleeding and angiogenesis are the other benefits of ginger.

The drug is registered in the European Pharmacopoeia, French Pharmacopoeia, British Pharmacopoeia, Swiss Pharmacopoeia, ESCOP, Commission E and WHO Monographs.

According to German Commission E the daily dosage must be between 2-4 grams (7). The high dose of the drug may cause gastrointestinal disturbances and burning, diarrhea, contact dermatitis, cardiac arrhythmia, central nervous system depression and allergic reactions related to immunoglobulin E through inhalation (29,30,31,32).

The results of the macroscopic and microscopic studies showed that the sample bought from pharmacy is suitable to the specifications of Pharmacopoeia and the herbal store samples (T1-T5) were also suitable to the criteria but they had some other unwanted and unidentified particles that must not be in the plant sample obtained from herbal stores.

The shogaols and the gingerols were seen within thin layer chromatography analysis which was shown in the Pharmacopoeia.

According to the results of the loss on drying test, pharmacy sample (E) and one sample of herbal stores (T2) were having higher moist loss values (0.42 and 0.43 g respectively) than the values stated in Pharmacopoeia. The other samples bought from herbal stores (T1, T3, T4 and T5) were having values between 0.37-0.39 g which were under the stated value which is 100 mL/kg and was 0.4 g for this study. All the samples met the criteria of Pharmacopoeia as they had %3-4 total ash.

The essential oils obtained from the samples were calculated in the range of 0.25-0.45 mL. The samples bought from herbal stores (T3 and T4) were under the criteria and the samples bought from pharmacy (E) and the herbal stores (T1, T2 and T5) were appropriate for the criteria while having values under 0.3 mL. According to the ecological conditions of a plant's production area and the production circumstances, plant's essential oil constituents and the yield changes. The other factors that affect the yield are harvesting date, the time passed since the plant material reaches laboratory where studied and the storage conditions. So, the differences of the studied samples' result values may be referred to these.

So, at the end of the study, it was observed that all the herbal store samples meet almost all the requirements of the European Pharmacopoeia 7.0. But, considering the production and packaging pathways and storage conditions, and taking into account that the use of herbal products under the supervision of a pharmacist would be more appropriate for patient health, it was concluded that the pharmacy drug would be more reliable. Drugs with medicinal properties should be made available to public health through pharmacies, in compliance with pharmacopeial standards and properly packaged.

5. CONCLUSION

This is the first study in Türkiye about *Zingiber officinale* plant material samples' compliance with the criteria written in the monograph in the European Pharmacopoeia 7.0.

Starting from the folk usage about the drug, the scientifically proven effects were collected so that a guide-like source about *Zingiber officinale* was aimed and prepared for the ones who want to use the drug for medicinal purpose or start a scientifical research.

Considering the scientifical studies are being continued about the Zingiberis rhizoma drug, hoping that *in-vivo* studies then will increase and with the *in-vitro* studies, more reliable knowledge will be known about the plant and the drugs of it.

Acknowledgement: *We would like to thank Istanbul University for permission to access academic literature.*
Funding: *This study was supported by Istanbul University Research Project Unit (BAPSIS). Project number: 23383*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study in this article was completed before the year 2020 (25.12.2018), and since ethical committee approval was not an obligation at that time, there is no ethical committee approval.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: KD, ÇGÜ
Design of the study: KD

Acquisition of data for the study: KD, ÇGÜ
Analysis of data for the study: KD
Interpretation of data for the study: KD
Drafting the manuscript: KD, ÇGÜ
Revising it critically for important intellectual content: KD, ÇGÜ
Final approval of the version to be published: KD

REFERENCES

[1] Jansen PCM. Species, condiments and medicinal plants in Ethiopia, their taxonomy and agricultural significance. Wageningen: Centre for Agricultural Publishing and Documentation; 1981.

[2] Caldwell RA. Comparative Grammer of Dravidian or South Indian Family of Languages (3rd ed.). Madras: Asian Educational Services; 1998.

[3] ESCOP. Zingiberis rhizoma the scientific foundation for herbal medicinal products. European Scientific Cooperative on Phytotherapy (2th ed.). New York; 2003.

[4] Rahmani AH, Al Shabrmi FM, Aly SM. Review article: Active ingredients of Ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. *International Journal of Physiology Pathophysiology* and *Pharmacology 2014;* 6: 125-136.

[5] Aly UI, Abbas MS, Taha HS, Gaber ESI. Characterization of 6-Gingerol for *in-vivo* and *in-vitro* Ginger (*Zingiber officinale*) using high performance liquid chromatography. Global Journal of Botanical Science 2013; 1: 9-17.
https://doi.org/10.12974/2311-858X.2013.01.01.2

[6] Morakinyo AO, Achema PU, Adegoke OA. Effect of *Zingiber officinale* (Ginger) on sodium arsenite-induced reproductive toxicity in male rats. African Journal of Biomedical Research 2010; 13: 39–45.

[7] Blumenthal M, Busse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW, Rister RS. The Complete German Commission E Monographs. Therapeutic Guide to Herbal Medicines. American Botanical Council. Austin; 1998.

[8] Çubukçu B, Meriçli AH, Sarıyar G, Mat A, Sütlüpınar N, Meriçli F. Fitoterapi Yardımcı Ders Kitabı. İstanbul: İstanbul Üniveristesi Basım ve Yayınevi; 2002.(Turkish)

[9] Shakya SB. Medicinal uses of Ginger (*Zingiber officinale* Roscoe) improves growth and enhances immunity in aquaculture. International Journal of Chemical Studies 2015; 3(2): 83-87.

[10] Qin F, Xu H-L. High-performance liquid chromatography–electrospray mass spectrometric analysis of pungent constituents of Ginger. Medicinal and Aromatic Plant Science and Biotechnology 2008; 2(29): 72-78.
https://doi.org/10.1016/S0021-9673(97)01013-3

[11] McKay DL, Chen CY, Zampariello CA, Blumberg JB. Flavonoids and phenolic acids from cranberry juice are bioavailable and bioacitve in healthy older adults. Food Chemistry 2015; 168: 233-240. https://doi.org/10.1016/j.foodchem.2014.07.062

[12] Ojewole JAO. Analgesic, antiinflammatory and hypoglicaemic effects of ethanol extract of *Zingiber officinale* (Roscoe) rhizomes (Zingiberaceae) in mice and rats. Phytotherapy Research 2006; 20: 764-772. https://doi.org/10.1002/ptr.1952

[13] Narasimhan S, Govindarajan VS. Evalution of spices and oleoresin: VI. Pungency of the ginger components gingerol and shogaol and their quality. Journal of Food Technology 1978; 13: 31-36.
https://doi.org/10.1111/j.1365-2621.1978.tb00773.x

[14] Chrubasik S, Pittler MH, Roufogalis BD. Zingiberis rhizoma: A comprehensive review on the Ginger effect and efficacy profiles. Phytomedicine 2005; 12: 684-701.
https://doi.org/10.1016/j.phymed.2004.07.009

[15] Young H-Y, Liao J-C, Chang Y-S, Luo Y-L, Lu M-C, Peng W-H. Synergistic effect of Ginger and Nifedipine on human platelet aggregation: A study in hypertensive patients and normal volunteers. The American Journal of Chinese Medicine 2006; 34(4): 545–551.
https://doi.org/10.1142/S0192415X06004089

[16] Jeena K, Liju VB, Kuttan R. A preliminary 13-week oral toxicity study of Ginger oil in male and female wistar rats. International Journal of Toxicology 2011; 30(6): 662-670.
https://doi.org/10.1177/1091581811419023

[17] Ladipo MK, Doherty VF, Kanife UC. Heavy metal analysis and phytochemical screening of two indigenous species (*Zingiber officinale* and *Centrosema Pubescens*) from Nigeria. International Journal of Current Research 2011; 33(4): 95-99.

[18] Kemper JK. Ginger. Longwood Herbal Task Force, Rev: 09.11, 2; 1999.

[19] Ghayur MN, Gilani AH, Mehmood MH, Aziz N. Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. Digestive Diseases Sciences 2005; 50(10): 1889-1897. https://doi.org/10.1007/s10620-005-2957-2

[20] Oluwatoyin A. Physicochemical characterisation, and antioxidant properties of the seeds and oils of Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*). Science Journal of Chemistry 2014; 2(6): 44-50.
https://doi.org/10.11648/j.sjc.20140206.11

[21] Al-Kushi AG, El-Boshy ME, ElSawy NA, Omar OAS, Header EA. Pathological comparative studies on aqueous and ethanolic extracts of *Zingiber officinale* on antioxidants and hypolipidemic effects in rats. Life Science Journal 2013; 10(2): 2393-2403.

[22] Sakr SA, Shalaby SY. Ginger extract protects metalaxyl-induced histomorphological and histochemical alterations in testes of albino mice. Journal of Applied Pharmaceutical Science 2011; 1(10): 36-42.

[23] Ramadan MM, El-Shershaby EM, Ismail MF, Farag SAM. The protective effect of Ginger on ovotoxicity induced by 7,12-Dimethylbenz[A]Anthracene (DMBA) in rat. Egyptian Journal of Experimental Biology (Zoology) 2009; 5: 227 – 233. https://doi.org/10.12816/0027817

[24] Ensiyeh J, Sakineh MA.Comparing Ginger and vitamin B6 for the treatment of nausea and vomiting in pregnancy: a randomised controlled trial Midwifery 2009; 25: 649–653. https://doi.org/10.1016/j.midw.2007.10.013

[25] Pongrojpaw D, Somprasit C, Chanthasenanont A. A randomized comparison of Ginger and dimenhydrinate in the treatment of nausea and vomiting in pregnancy. Journal of the Medical Association of Thailand 2007; 90(9): 1703-1709.

[26] Abascal K, Yarnell E. Clinical uses of *Zingiber officinale* (Ginger). Alternative and complementary therapies 2009; 15(5): 231-237. https://doi.org/10.1089/act.2009.15501

[27] Phillips S, Ruggier R, Hutchinson SE. *Zingiber officinale* (Ginger)-an antiemetic for day case surgery. Anaesthesia 1993; 48: 715-717.
https://doi.org/10.1111/j.1365-2044.1993.tb07188.x

[28] Bone ME, Wilkinson DJ, Young JR, McNeil J, Charlton S. Ginger root-a new antiemetic. The effect of Ginger root on postoperative nausea and vomiting after major gynaecological surgery. Anaesthesia 1990; 45: 669-671. https://doi.org/10.1111/j.1365-2044.1990.tb14395.x

[29] Kapalka GM. Nutritional and Herbal Therapies for Children and Adolescents: A Handbook for Mental Health Clinicians. Londra. Academic Press. 2010; 250. https://doi.org/10.1016/C2009-0-01890-X

[30] Van Toorenenbergen AW, Dieges PH. Immunoglobulin-E antibodies against coriander and other spices. Journal of Allergy and Clinical Immunology 1985; 76: 477–481. https://doi.org/10.1016/0091-6749(85)90730-4

[31] Desai HG, Kalro RH, Choksi AP. Effect of ginger & garlic on DNA content of gastric aspirate. Indian Journal of Medicinal Research. 1990; 92: 139–141.

[32] Gruenwald J, Brendler T, Jaenicke C. PDR for Herbal Medicines. (4th ed.). Florence: Thompson Healthcare Library Products. 2007.

How to cite this article: Demir K, Gürer ÇÜ. Pharmacopoeia studies on Zingiber officinale Roscoe (ginger) samples purchased from a pharmacy and herbalists in İzmir and İstanbul. Clin Exp Health Sci 2025; 15: 117-123. https//doi.org/10.33808/clinexphealthsci.1482709

Clinical and Experimental Health Sciences

Evaluation of Medication and Herbal Product Usage Habits in the Geriatric Population: A Pilot Study From Trabzon Province, Türkiye

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Received: October 29, 2024 **Accepted:** March 5, 2025

ABSTRACT

Objective: Polypharmacy and the uncontrolled use of herbal products increase drug interactions and adverse drug reactions, leading to possible risks for the geriatric population. This study aimed to investigate the medications and herbal products used by geriatric individuals who presented to a family health center in Trabzon province, Türkiye, as well as their related habits and knowledge and information sources.

Methods: The study was conducted through face-to-face interviews with geriatric patients who presented to the Yomra Family Health Center in Trabzon between February and May 2019. The questionnaire forms obtained from a total of 236 individuals with appropriate data quality were included in the study.

Results: Of the participants, 137 (58.1%) were primary school graduates, whose mean age was 69.23 ± 4.81 years. It was found that 212 (89.9%) of the participants used medications regularly, and 143 (60.5%) of the regular medication users did not remember the name or purpose of at least one medication. The participants used a total of 646 medications, of which 63 (9.7%) were kept under unsuitable storage conditions. It was also determined that 76 (32.2%) of the participants used herbal products, with the most commonly used herbal products being linden and lemon-and-mint tea.

Conclusion: Pharmacists can improve the treatment adherence and outcomes of geriatric patients by monitoring their treatment regimens in terms of dose, duration, and drug interactions, while also providing counseling on medications and herbal products, including necessary warnings and instructions. Furthermore, healthcare professionals must be fully aware of the potential health complications arising from the combined intake of herbs and drugs to ensure optimal patient care..

Keywords: Aging, drug-related side effects and adverse reactions, drug therapy, geriatrics, herbal, polypharmacy

1. INTRODUCTION

Cognitive and physical functions decline with advancing chronological age, and the incidence of chronic diseases and complications is increasing (1). The World Health Organization (WHO) defines individuals aged 65 years and over as the geriatric population (2). With developments in health technologies and public health services, as well as increased accessibility to health services, the average life expectancy has increased, resulting in a gradual increase in the geriatric population all over the world, including Türkiye. According to data from the Turkish Statistical Institute, the proportion of the elderly population among the total population was 8.8% in 2018, which increased to 10.2% by 2023. According to population projections, this percentage is estimated to reach 16.3% in 2040, 22.6% in 2060, and 25.6% in 2080 (3).

Physiological changes such as reduction in the size and perfusion of organs, in the level of albumin and in the body water-fat ratio, as well as biochemical alterations in the frequency of receptors and signal transduction systems that occur with age in the geriatric population, affect the efficacy and safety of treatment by altering the pharmacokinetic and pharmacodynamic parameters of drugs (4-6). Moreover, polypharmacy and uncontrolled use of herbal products

https//doi.org/*10.33808/clinexphealthsci.1575123*

in the geriatric population increase the incidence of adverse drug reactions and potential risks associated with pharmacotherapy (7).

The term of "herbal product" defined by WHO corresponds to raw drugs, teas, and pharmaceutical-formulated products obtained from plants (8). Herbal products on the Türkiye market are classified as food supplements or traditional herbal medicinal products in compliance with European Union regulations. The herbal product that is used for therapeutic administration, registration application, evaluation, and approval procedures of this product are performed by the Republic of Türkiye Ministry of Health (7). Furthermore, dietary supplements that are identified as "the products for which the daily intake dose is determined by being prepared alone or in mixtures, in capsules, tablets, drops, disposable powder packs, liquid ampoules, dropper bottles, and other similar liquid or powder forms consisting of nutrients such as vitamins, minerals, proteins, carbohydrates, fibers, fatty acids, amino acids, or concentrates or extracts of plants, animal-originated substances, bioactive substances, and similar substances that have nutritional or physiological effects to supplement the normal diet" are crucial components of the market (9).

Studies examining the use of drugs and herbal products in the Turkish geriatric population are limited in the literature (10-13). Currently, due to the increasing variety of herbal products, easy access to these products, and their widespread use (often influenced by social media) without the recommendation of a physician/pharmacist, there is a need for new studies that examine the geriatric population's habits concerning medical/herbal product use. Therefore, the aim of this study was to investigate medical/herbal products used by the geriatric population, as well as the geriatric population's related habits, level of knowledge, and information sources regarding these products. This was accomplished by administering a face-to-face questionnaire to geriatric individuals who presented to a family health center (FHC) in Trabzon province, Türkiye.

2. METHODS

This descriptive study, which was conducted through a face-to-face questionnaire method, involved geriatric individuals who presented to the Yomra Family Health Center in Trabzon province, Türkiye, between February and May 2019, agreed to participate in the study, and signed an informed consent form. It was determined that the number of patients over the age of 65 years who applied to the family health center where the study was conducted for 4 months was approximately 660. No sample selection was performed in this study, but the method for calculating the minimum sample size was used to determine the sample size. In the power analysis conducted using OpenEpi® (*version 3.01*), the design effect was assumed to be 1 when the prevalence of herbal drug use in the geriatric population was 18% (14), and the minimum sample size was calculated as at least 169 individuals with a 5% error level, 95% confidence interval, and 80% power (10, 14).

Individuals with dementia and hearing, vision, and speech difficulties, as well as those under 65 years of age, were not included in the study. The collected data were checked by the researchers, and questionnaires from participants who gave incomplete, contradictory, or inappropriate responses were excluded (Figure 1).

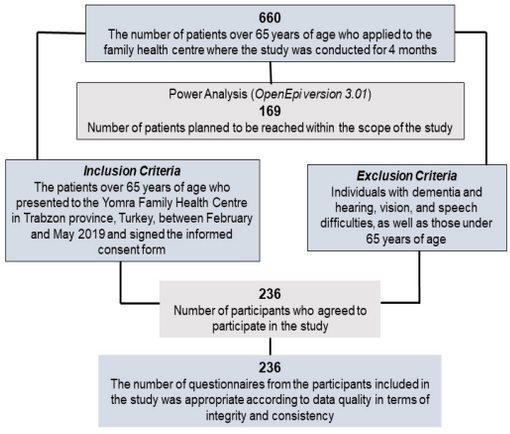

Figure 1. *Flow diagram of study population*

The questions directed at geriatric individuals aimed to evaluate the medical and herbal product usage habits of the participants. The questionnaire used for this purpose was developed by the researchers in light of previous studies in the literature. Before collecting the data, the questionnaire was pre-tested by administering it to a sample of 10 individuals, and it was finalized by correcting incomprehensible expressions in line with the feedback received. After all participants were informed about the content and purpose of the study, their informed consent was obtained. The questionnaire interviews lasted an average of 15 minutes. The questionnaire form consisted of 14 questions presented under three main sections: "sociodemographic and personal characteristics," "chronic disease status and medications used," and "characteristics related to medication/herbal product use." There were both multiple-choice and open-ended questions. Patient declarations formed the basis of this study. No plant or herbal product samples were taken from the participants, and no botanical identifications were made. Before the study, permission was obtained from the Public Health Services Directorate of the Trabzon Provincial Health Directorate and the Scientific Research Ethics Committee of the Karadeniz Technical University Faculty of Medicine (2019/4).

The IBM Statistical Package for Social Sciences Version 23.0 statistical program was used for the data analysis. The chi-squared test ($\chi 2$ and Fisher's exact tests) was used to compare categorical variables in independent groups. The statistical significance level was accepted as $p < .05$.

3. RESULTS

Of the 236 participants who agreed to participate in the study, 98 (41.5%) were female and 138 (58.5%) were male. The mean age of the participants was 69.23 ± 4.81 years. While 224 (94.9%) of the geriatric individuals participating in the study had social security, 12 (5.1%) did not. The educational status of the participants is given in Table 1.

Table 1. *Educational status of the participants*

Education	Number (percentage)
Illiterate	43 (18.2%)
Primary school	137 (58.1%)
Secondary education	42 (17.8%)
University	14 (5.9%)

The diagnosed chronic diseases of the participants and their distribution according to gender are given in Table 2. The rates of hypertension, dyslipidemia, thyroid diseases, osteoarthritis, and mood disorders were higher in women than in men (Table 2). Twenty-four (10.1%) of the participants stated that they did not use any medications, while 212 (89.9%) used an average of 3.17 medications per person. Of the participants who reported using medications, 143 (60.5%) could not remember the names of the medications they were taking. The participants' medication usage, including the types of medications and frequency of use, are given in Table 3. Of the participants, 191 (80.9%) followed a once-a-day pharmaceutical regimen, whereas two (0.8%) followed a two-or-three-times-a-day regimen, and 14 (5.9%) took their medications four times a day.

Table 2. *Diagnosed chronic diseases of the participants*

Chronic disease	Gender		
	Female	*Male*	*p value*
Hypertension	69 (70.4%)*	67 (48.6%)	.001
Congestive heart failure	14 (14.2%)	25 (18.1%)	.435
Atrial fibrillation	5 (5.1%)	10 (7.2%)	506
Dyslipidemia	28 (28.6%)*	22 (15.9%)	.019
Diabetes mellitus	25 (25.5%)	38 (27.5%)	.729
Hypothyroidism/hyperthyroidism	12 (12.2%)*	5 (3.6%)	.012
Osteoarthritis	15 (15.3%)*	3 (2.1%)	.0001
GERD Peptic/duodenal ulcer	25 (25.5%)	25 (18.1%)	.171
Mood disorder	20 (20.%)*	11 (7.9%)	.005
Prostatic disease	-	39 (28.2%)	-
Asthma	16 (16.3%)	5 (3.6%)	.001
Chronic obstructive pulmonary disease	1 (1%)	5 (3.6%)	.405

GERD: gastroesophageal reflux disease

Of the medications used by the participants, 68 (10.1%) were kept in the refrigerator, 54 (8%) in the medicine cabinet, 14 (2.1%) in the patient's bag, and 534 (79.3%) in other areas within their homes. It was determined that 43 (6.4%) of these medications were stored in refrigerators, and 63 (9.7%) were stored under unsuitable conditions.

Table 3. *Types of medications used by the participants (total medicine number=646)*

Medication type	Number of medicine (percentage)
Analgesic	38 (5.9%)
Antianginal	35 (5.4%)
Antiarrhythmic	4 (0.6%)
Antidepressant	34 (5.3%)
Antidiabetic	83 (12.8%)
Antihyperlipidemic	35 (5.4%)
Antihypertensive	144 (22.2%)
Antirheumatic	15 (2.3%)
Antithrombotic	43 (6.6%)
Diuretic	2 (0.3%)
Hypothyroidism/hyperthyroidism medication	23 (3.6%)
Inhalers (for the treatment of asthma and COPD)	32 (4.9%)
Parkinson's medication	3 (0.5%)
Peptic/duodenal ulcer and GERD medication	54 (8.3%)
Peripheral vasodilator	1 (0.1%)
Prostatic hypertrophy medication	28 (4.3%)
Urinary incontinence/retention medication	3 (0.5%)
Vestibular disorder medication	13 (2%)
Vitamin supplementation	16 (2.5%)
Other	16 (2.5%)

COPD: chronic obstructive pulmonary disease; GERD: gastroesophageal reflux disease

**more than one option was checked*

Of the participants, 231 (97.9%) were informed about the instructions for use of their medications, with 206 (87.3%) stating that they had received this information from a physician, 94 (39.8%) from a pharmacist, five (2.1%) from a nurse, and nine (3.8%) from a relative. The remaining five (2.1%) participants reported that they had not been informed about the medication they were taking (Table 4).

Table 4. *Distribution of participants according to their information status concerning medication use instructions (n=236)*

Informed about	Number (percentage)
Dosage	102 (43.2%)
Schedule	231 (97.9%)
Method of administration	220 (93.2%)
Drug–drug interactions	3 (1.3%)
Drug–nutrient interactions	6 (2.5%)
Side effects	14 (5.9%)
*More then one option has been thicked	

It was determined that 207 (87.7%) of the participants self-monitored their medication use, while 29 (12.3%) had their medication use monitored by a caregiver. When medication use was controlled by the patient, the rate of regular medication use was found to be higher (p = .013). To ensure regular medication use, 178 (75.4%) of the participants kept their medications within sight, 53 (22.5%) carried their medications with them, eight (3.4%) set an alarm or reminder, five (2.1%) used a medicine box, and one (0.4%) kept a chart. The medication usage habits of the participants are detailed in Table 5.

Table 5. *Participants' medication usage habits*

	Always *number (percentage)*	Frequently *number (percentage)*	Rarely *number (percentage)*	Never *number (percentage)*
I take my medications regularly.	178 (75.4%)	45 (19.1%)	9 (3.8%)	4 (1.7%)
I forget to take my medications.	1 (0.4%)	5 (2.1%)	126 (53.4%)	104 (44.1%)
I cannot read the labels of my medications.	38 (16.1%)	11 (4.7%)	21 (8.9%)	166 (70.3%)
I mix up my medications.	2 (0.8%)	1 (0.4%)	9 (3.8%)	224 (94.9%)
I do not take my medications because I do not find them useful.	12 (5.1%)	18 (7.6%)	50 (21.2%)	156 (66.1%)
I do not take my medications because of the inconvenience of keeping to the schedule, their unpleasant taste, or their large size.	4 (1.7%)	8 (3.4%)	27 (11.4%)	197 (83.5%)
I consult healthcare professionals when I forget to take my medications.	27 (11.4%)	15 (6.4%)	31 (13.1%)	163 (69.1%)
When I forget to take my medications, I take two doses the next time.	6 (2.5%)	-	7 (3%)	223 (94.5%)
I read the instructions for the use of medications.	72 (30.5%)	48 (20.3%)	29 (12.3%)	87 (36.9%)
I take medications based on the recommendation of my relatives.	7 (3%)	8 (3.4%)	28 (11.9%)	193 (81.8%)
I recommend medications to others.	3 (1.3%)	6 (2.5%)	36 (15.3%)	191 (80.9%)
I keep leftover/unused medications.	42 (17.8%)	13 (5.5%)	11 (4.6%)	170 (72%)
I throw away leftover/unused medications.	45 (19%)	17 (7.2%)	12 (5%)	162 (68%)
I give leftover/unused medications to others.	64 (27%)	1 (0.4%)	8 (3.3%)	163 (69%)

The frequency of herbal product use was reported to be "always" by 40 (16.95%) of the participants, "frequently" by 36 (15.25%), "rarely" by 75 (31.78%), and "never" by 85 (36.02). The rate of herbal product use was found to be significantly higher among women (p = .364).

The majority of participants (n = 175, 74.15%) stated that they used linden for "colds, stress relief, stomach ailments, shortness of breath, cough, sore throats, and high blood pressure." While 135 (57.20%) of the individuals used lemon-and-mint tea for "colds, stress relief, high blood pressure, stomach diseases, flu, headaches, sore throats, fever, weakness, abdominal pain," 90 (38.13%) used rosehip for "colds, stress relief, stomach diseases, boosting the immune system, constipation, vitamin C supplementation, weakness, cough, and diabetes." Seventy (29.66%) participants used parsley for the "treatment of inflammation and rheumatic conditions, reducing edema, diarrhea, weight loss, urinary tract infections, colds and sore throats, arteriosclerosis, blood thinning, gastrointestinal diseases, vitamin D supplementation, high blood pressure, prostatic hypertrophy, and cancer prevention." In addition, 70 (29.66%) participants used garlic for "inflammatory conditions, infection, cancer prevention, heart diseases, blood thinning, diabetes, intestinal regulation, colds, headaches, and immune protection." Finally, 38 (16.10%) of the participants reported using nettle for "rheumatic conditions, reducing edema, cardiovascular diseases, diabetes, blood purification, kidney diseases, strengthening the immune system, facilitating breathing, bronchitis, regulating bowel movements, cancer prevention, and hair loss." Some of the survey participants expressed their preference for pharmaceutical products. Six of the participants used Fito® five used Daflon®, three used Hametan®, three used Pharmaton®, one used Passiflora®, and one used Tebokan®. These products were found to be used by patients in accordance with their clinical indications.

It was determined that 30 (12.71%) of the participants used walnuts for "vitamin–mineral supplementation, lowering cholesterol, boosting the immune system, improving brain function, and regulating bowel movements," while 27 (11.44%) used green tea for "calming, gastrointestinal and digestive regulation, weight loss, pain relief, reducing edema, cold symptoms, and facilitating breathing." In addition, 21 (8.89%) participants reported using chamomile for "calming, regulating sleep, colds and sore throats, suppressing cough, asthma, and regulating bowel movements" and cinnamon for "diabetes, boosting the immune system, colds, facilitating breathing, asthma and painful conditions, and weight loss." Finally, six (2.54%) participants used fennel for "colds, coughs, and sleep disorders," four (1.69%) used flaxseed for "weight loss and prostate diseases," four (1.69%) used sycamore leaf for "rheumatic diseases and migraine," and two (0.84%) used black cumin seeds for "painful conditions and weight loss."

4. DISCUSSION

In this study, the medication and herbal product use of geriatric patients who presented to an FHC in Trabzon province was analyzed. The geriatric population experiences a steady decrease in physical and cognitive functions. In addition, the incidence of chronic diseases in the geriatric population is gradually increasing. According to the findings obtained in the current study, the most common diseases in the geriatric population were hypertension (females: 70.4%; males: 48.6%), dyslipidemia (females: 28.6%; males: 15.9%), and diabetes (females: 25.5%; males: 27.5%). Hypertension,

dyslipidemia, thyroid diseases, osteoarthritis, and mood disorders were significantly common among women.

A cross-sectional study performed with 241 geriatric individuals in Bursa province (Türkiye) revealed that 86.3 % of the participants were diagnosed with at least one chronic disease by a physician, while the most common chronic diseases of the participants were hypertension (62.6 %), coronary artery disease (26.1 %), and diabetes mellitus (21.1 %) (10). In a study based on one-to-one interviews, pharmacists stated that hypertension, diabetes, cardiovascular diseases, asthma, and mood disorders were the most common diseases in the geriatric population (15). In another study from Türkiye conducted by Demirbag and Timur (2012), it was found that 67.8% of the geriatric patients who presented to an FHC used antihypertensives, 62.4% used antirheumatics, 46% used diuretics, 26.6% used antidiabetics, 7.8% used antipsychotics, and 5.4% used hypnosedatives (16). In another study conducted in Türkiye, it was shown that 64.5% of individuals in the geriatric population used antihypertensive drugs (17). In line with the data from the literature, our findings showed that the most commonly used medications in the geriatric population were antihypertensives. In a study conducted by Brown et al. (2011), it was found that 85% of patients aged over 60 had moderate–severe pain, while 28% had chronic pain. Therefore, paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) were frequently used by the geriatric population (18). In another study from Türkiye, the frequency of NSAID use among geriatric individuals in nursing homes was found to be 20.8% (19). It was reported that analgesic drug use was significantly higher in geriatric individuals living in the community compared with those living in nursing homes (17). In the present study, the rate of analgesic use was found to be 5.9%. In the literature, the most commonly used medications without prescription among geriatric individuals (40–60%) were reported to be analgesics, laxatives, and vitamins (20).

According to previous research, 48% of geriatric individuals who present to healthcare services have more than three chronic diseases, while 21% have more than five chronic diseases (21). Comorbidities resulting from a low quality of life, a sedentary lifestyle, and adverse drug reactions are common among geriatric patients. Known diseases present with different clinical symptoms in geriatric individuals, and these patients may attribute symptoms such as hearing loss, confusion, incontinence, and constipation to normal age-related changes and thus not disclose them to their physicians. This makes it difficult to diagnose their disease and prevents effective and safe treatment planning (20). Consequently, the pathogenesis of the disease worsens, and the need for medication increases due to reasons such as the specificity of treatment guidelines, the focus on a single disease in treatment planning, low treatment compliance, and multiple and complex treatment regimens (22).

In our study, the mean number of medications used by the participants from the geriatric population was found to be 3.17. This was partially consistent with the findings obtained from previous studies conducted in Türkiye. In a study evaluating geriatric individuals in nursing homes in Türkiye, it was determined that the mean number of medications used was 3.59 among women and 2.39 among men. The authors reported that 60.6% of the geriatric individuals staying in nursing homes used an average of four medications. On completion of the study, it was concluded that the use of multiple medications was significantly higher among individuals living in care homes compared to those living in the community (23). In a study conducted with geriatric individuals presenting to an FHC, 52.9% of the participants used three medications on average (16). Another study carried out in nursing homes located in 23 different cities in Türkiye reported that 11.7% of the participants used four medications, while 17.3% used five medications (19). In another study involving 1,433 participants from 12 different cities in Türkiye, it was found that 38.2% of the individuals used four medications (20). In a cross-sectional study conducted by Bahat-Öztürk et al. (2017) with geriatric individuals, the number of medications used was determined to be five in 62.3% of the participants, five to nine in 52.7%, and 10 in 9.7% (24). In a cross-sectional study undertaken by Golchin et al. (2015) involving 59 geriatric individuals, it was shown that 35.6% of the participants had polypharmacy, while 20.3% used drug combinations that were contraindicated (25).

In our study, the majority of the participants (97.9%) stated that they were informed about the warnings and instructions pertaining to their drug treatment. Of the participants, 87.3% reported receiving this information from a physician, 39.8% from a pharmacist, 2.1% from a nurse, and 3.8% from a relative, while the remaining 2.1% indicated that they were not informed about the medications they used. According to a study by Demirbag et al. (2012), 86% of the geriatric individuals who presented to an FHC stated that they did not use their medications regularly, while 61% did not receive information about the medications they were using (16). In a descriptive-type questionnaire study conducted with geriatric patients who presented to an FHC, it was found that 85.5% of the participants did not use their medications regularly, while 61.1% did not have information about the medications they used. The authors also determined that 61.3% of the participants knew the intended uses of the medications they used; however, 58.7% were not aware of their potential side effects (16). In another geriatric study, it was observed that 39% of the participants were unaware of the potential adverse effects of the medications they used, while all the participants knew the drug indications (26). In the current study, only 5.9% of the participants stated that they received information about the possible side effects of their medications.

In our study, it was found that 3.8% of the geriatric individuals recommended medications to other patients. In addition, previous research indicated that geriatric individuals alter their drug treatment without consulting a physician (24), leading to inappropriate and irrational drug use and

increasing the frequency of drug interactions and adverse effects in this population.

In our study, we determined that most of the participants (82.7%) stored their medications in various locations in their homes, 8.4% in the medicine cabinet, 6.8% in the refrigerator, and 2.2% in their bags, while 5.5% of the medications were stored under unsuitable conditions. In a 2010 descriptive survey study conducted with geriatric individuals who presented to an FHC, it was found that 47.3% of the patients kept their medications in the refrigerator (16). The storage of medicines under appropriate conditions is one of the most important factors for ensuring the efficacy and safety of drug treatments. It is the responsibility of pharmacists to inform patients about the storage conditions of medications while conveying instructions for the use of medications within the scope of pharmaceutical counseling. In addition, for medical products such as insulin derivatives that need to be kept in the cold chain or require a higher level of care in terms of storage conditions, detailed instructions should be given in accordance with the patient's sociocultural level and cognitive functions. For this purpose, pharmacists should closely follow developing technologies (e.g., the use of portable mini refrigerators while traveling with medical products and biosimilars), as they can make a significant contribution to effective and safe drug treatment through appropriate recommendations tailored to individual patients.

In our study, the medication use habits of geriatric patients presenting to an FHC were evaluated, and the data obtained were found to be comparable to those reported in existing studies. A study evaluating geriatric individuals who presented to an FHC revealed that 82% of the participants forgot to take their medications, while only 19.3% of geriatric individuals rarely forgot to take their medications (24). The authors also noted that 21.2% of the participants experienced confusion over their medication schedule, while 24% forgot to take their medications. In another study, it was found that 24% of geriatric individuals forgot to take their medications at least once (26). Polypharmacy increases the frequency of forgetting medication (24). According to a previous study, 67.9% of geriatric individuals who presented to an FHC self-monitored their medication use (9). As a result, they used various precautions to ensure the regular use of their medications. In a study conducted by Taskin-Sayir et al. (2014), it was found that 61% of the patients placed their medications where they could see them to prevent forgetting to take them, while 15% used a medicine box and 3% used a chart for the same purpose (26). It is important for pharmacists to create a medication use plan suitable for the daily routine of each patient while filling prescriptions.

In this study, we also evaluated the herbal product usage habits of the geriatric individuals, determining that 63.9% used herbal products. In a study conducted by Varli et al. (2017), 74.21% of the non-prescription products used by patients presenting to the geriatric outpatient clinic were herbal products (27). In another descriptive survey study, it was reported that 59% of geriatric individuals who presented to an FHC used herbal products (26).

A cross-sectional survey by Golden et al. (2023) found that the rate of complementary and alternative medicine (CAM) use in the geriatric population in the past year was 60.1% (among 104 participants). Herbal products/dietary supplements were the most common form of CAM used by 37% of participants (28). Another cross-sectional study conducted by Taneri et. al. (2021) in Bursa (Türkiye) announced that herbal product usage rate in geriatrics was 18.3% as well as the most common sources of information about herbs were television and radio (10).

In our study, the herbal products frequently used by the participants were linden, lemon-and-mint tea, rosehip, parsley, garlic, nettle, walnut, green tea, cinnamon, chamomile, and sage tea. It is well established that many of the herbal products interact with cytochrome P450 enzymes as well as with the cellular transporters. The concomitant use of herbal products with drugs such as anticoagulants, antivirals, antihypertensives, antidiabetics, and antineoplastics, which are metabolized by these enzymes, increases the risk of adverse drug reactions. Furthermore, some herbal products such as St John's-wort, cranberry juice and goji berry can lead to increased bleeding risk in the warfarin using patients, as well as some of those such as sage and Prickly pear cactus can lead to hypoglycemia in patients treated with sulfonylurea derivatives (29). Patient declarations form the basis of this study, no plant samples were taken from the participants, and botanical descriptions of the plant species used were not made, so it is not possible to draw conclusions about a specific plant species or product. In addition, the methods of preparation of the products prepared from raw herbs were not questioned, as they were outside the scope and purpose of the study. However, in general, the frequency of use of herbal products was found to be comparable to that in previous studies. In a study undertaken by Taskin-Sayir et al. (2014), the herbal products frequently used by geriatric individuals were found to be linden, sage, green tea, lemon-and-mint tea, olive leaf tea, chamomile, garlic, flaxseed, nettle, senna, and lemon balm (26). It was ruled out that the most prevalent herbal products used by geriatric individuals in Bursa (Türkiye) were plane tree leaves (*Platanus orientalis*), black cumin oil (*Nigella sativa*), cinnamon (*Cinnamomum verum*), sage (*Salvia officinalis*) and plantain (*Plantago lanceolata*) (10).

The most common health conditions in which complementary products were used in the United States were arthritis, pain syndrome, dyslipidemia, and neuropsychiatric conditions, such as mood disorders, insomnia, and migraine (30). Taneri et. al. (2021) announced that the most common reason for using herbal products from geriatrics in Bursa (Türkiye) was the 'failure of previous treatments' (10).

It is also important where the herbs used for therapeutic purposes are obtained. This is because factors that may occur during the use of herbal products, such as improper plant use, and herbal drug quality that is unsuitable for pharmaceutical

use may also adversely affect the treatment of the patient. The possible herbal-drug interaction was detected by Taneri et al. al. (2021) in 3 of 40 (16.6% of participants) geriatric individuals who used herbal products on their own initiative. Moreover, in this study, only 34.1% of herbal product users reported their herbal product use to their physician (10).

According to the results of a cross-sectional study conducted in Ankara in 2011, 50% of the patients who visited the pharmacy preferred to obtain herbal products from the pharmacy (31). In addition, only 8.8% of the patients who visited the pharmacy deemed the counseling provided by pharmacists about herbal products to be sufficient (31). In a randomized controlled clinical trial, patients with polypharmacy were divided into experimental groups, and the effect of pharmacist-delivered drug counseling on treatment adherence was evaluated. It was reported that drug counseling provided by the pharmacist increased treatment compliance and decreased mortality and morbidity (32). In the hypertension monitoring program implemented by pharmacists in the geriatric population, an 80% improvement was found in drug treatment and blood pressure control (33).

5. CONCLUSION

When we evaluated the findings of our study in light of the literature, we concluded that the drug and herbal product usage habits of the geriatric population should be improved. Healthcare professionals must be fully aware of the potential health complications arising from the combined intake of herbs and drugs to ensure optimal patient care, while considering the treatment regimen of patients in terms of dose, duration, and drug interactions. They should also provide medication and herbal product counseling to geriatric patients, including the necessary warnings and instructions, and assess patients' levels of knowledge about drug therapy, specifically in terms of the therapeutical and potential side effects of drugs.

Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of* the Karadeniz Technical University Faculty of Medicine *(Approval date: 2019; Number: 4) and* Public Health Services Directorate of the Trabzon Provincial Health Directorate
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: YKY, GR, SFS
Design of the study: YKY, GR
Acquisition of data for the study: YAA, EK, EAE
Analysis of data for the study: YAA, GC
Interpretation of data for the study: YKY, GR
Drafting the manuscript: YKY
Revising it critically for important intellectual content: GR, SFS
Final approval of the version to be published: SFS

REFERENCES

[1] Kojima G, Liljas AEM, Iliffe S. Frailty syndrome: implications and challenges for health care policy. Risk Manag Healthc Policy. 2019;14 (12): 23-30. https://doi.org/10.2147/RMHP.S168750

[2] World Health Organization. Global strategy and action plan on ageing and health [e-book]. CC BY-NC-SA 3.0 IGO Publication 2017. [Internet]. Available from:https://iris.who.int/bitstream/handle/10665/329960/9789241513500-eng.pdf?sequence=1 Accessed: 20.02.2024. ISBN: 978-92-4-151350-0.

[3] Turkish Statistical Institute. Elderly Statistics, 2023 [press release] 2023. [Internet] Available from: https://data.tuik.gov.tr/Bulten/Index?p=Elderly-Statistics-2023-53710&dil=2. Accessed: 19.02.2025. ID number: 53710.

[4] Oktay S, Akici A. Drug use and desicion making process for rational pharmacotherapy in elderly. Turkish Journal of Geriatrics 2001; 4(Suppl 3): 127-133. (in Turkish)

[5] Potempa KM, Folta A. Drug use and effects in older adults in the United States. International Journal of Nursing Studies 1992; 29(1):17-26. https://doi.org/10.1016/0020-7489(92)90057-n

[6] BG Katzung. Special Aspects of Geriatric Pharmacology, In: Bertram G. Katzung , Anthony J. Trevor (Eds) Basic and Clinical Pharmacology. 13th Edition, Lange, Mc Graw Hill, USA 2015, pp 1024-1032.

[7] Dişli M, Yeşilada E. Herbal medicinal products in Turkey (Standardization, production and adulteration of herbal products in Turkey). J Biotechnol and Strategic Health Res 2019; 3(Special Issue):13-21. (in Turkish) https://doi.org/10.34084/bshr.545498

[8] WHO Expert Committee on Specifications for Pharmaceutical Preparations, Fifty-second report. https://apps.who.int/iris/bitstream/handle/10665/272452/9789241210195-eng.pdf?sequence=1&isAllowed=y (accessed on 12 August 2024).

[9] Turkish Food Codex Communiqué on Dietary Supplements. https://www.resmigazete.gov.tr/eskiler/2013/08/20130816-16.htm Sayı: 28737, 16.08.2013 (accessed on 12 August 2024).

[10] Taneri PE, Akis N, Karaalp A. Herbal product use patterns and possible herb-drug interactions among older adults in Turkey. Journal of Herbal Medicine 2021; 29: 100487. https://doi.org/10.1016/j.hermed.2021.100487

[11] Canan Demirbag B, Timur M. Knowledge, attitudes and behaviours of a group of elderly people about drug use. Ankara Journal of Health Services 2012; 11 (1): 1-8. https://doi.org/10.1501/Ashd_0000000070

[12] Orhan HG, Erdem SR. Use and Potential Risks of Herbal Products & Supplements in the Elderly. Turkiye Klinikleri Pharmacology-Special Topics; 2023. 11(2) p.31-35.

[13] Turkmenoglu FP, Kutsal YG, Dolgun AB, Diker Y, Baydar T. Evaluation of herbal product use and possible herb-drug interactions in Turkish elderly. Complement Ther Clin Pract. 2016; 23: 46-51. https://doi.org/10.1016/j.ctcp.2016.03.004

[14] OpenEpi. 2019. [cited 2019 3 March]. Available from: https://www.openepi.com/SampleSize/SSPropor.htm.

[15] Yegenoglu S, Baydar T. Information and Observations of Community Pharmacists on Geriatric Patients: A Qualitative Study in Ankara City. Turkish Journal of Geriatrics 2011; 14 (4): 344-351.

[16] Canan Demirbag B, Timur M. Knowledge, attitudes and behaviours of a group of elderly people about drug use. Ankara Journal of Health Services 2012; 11 (1): 1-8. https://doi.org/10.1501/Ashd_0000000070

[17] Discigil G, Gemalmaz A, Basak O. Depression in geriatric age group in primary care. Turkish Journal of Geriatrics 2005; 8(3):129-133. (in Turkish)

[18] Brown ST, Kirkpatrick MK, Swanson MS, McKenzie IL. Pain Experience of the Elderly. Pain Management Nursing 2011; 12(4): 190-196. https://doi.org/10.1016/j.pmn.2010.05.004

[19] Arslan S, Atalay A, Gokce-Kutsal Y. Drug use in the elderly. Journal of the American Geriatrics Society 2002; 50(6): 1163-1164. https://doi.org/10.1046/j.1532-5415.2002.50279.x

[20] Gokce Kutsal Y. Aging World. Turkish Journal of Physical Medicine and Rehabilitation 2006; 52 (Special Appendix A): A6-A11. (in Turkish)

[21] Boyd CM, Darer JD, Boult C, Fried LP, Boult L, Wu AW. Clinical practice guidelines and quality of care for older patients with multiple comorbid diseases, pay-for-performance outcomes. JAMA 2005; 294(6): 716-724. https://doi.org/10.1001/jama.294.6.716

[22] Mortazavi SS, Shati M, Keshtkar A, Malakouti SK, Bazargan M, Assari S. Defining polypharmacy in the elderly: a systematic review protocol. BMJ Open 2016;6: e010989. https://doi.org/10.1136/bmjopen-2015-010989

[23] Discigil G, Tekinc N, Anadol Z, Bozkaya AO. Polypharmacy in nursing home and community-dwelling elderly. Turkish Journal of Geriatrics 2006; 9(3):117-121. (in Turkish)

[24] Christopher C, Kc B, Shrestha S, Blebil AQ, Alex D, Ibrahim MIM, Ismail N. Medication use problems among older adults at a primary care: A narrative of literature review. Aging Med (Milton) 2022; 5(2):126-137. https://doi.org/10.1002/agm2.12203

[25] Golchin N, Frank SH, Vince A, Isham L, Meropol SB. Polypharmacy in the elderly. Journal of Research in Pharmacy Practice 2015; 4(2): 85-88. https://doi.org/10.4103/2279-042x.155755

[26] Taskin-Sayir C, Aslan-Karaoglu S, Evcik-Toprak D. Evaluation of polypharmacy and complementary therapy use in patients>=65 years, attending to Family Medicine Outpatient Clinic of Sisli Etfal Training and Research Hospital. The Turkish Journal of Family Practice 2014; 18(1): 35-41. https://doi.org/10.2399/tahd.14.35220

[27] Varli M, Bahsi R, Dogan S, Uysal H, Subaşı Ş, Toper M, Peksarı S, Keskin S, Sürmeli DM, Turgut T, Öztorun HS, Aras S. Nonprescription Product Use Among Geriatric Outpatients. Ankara Medical Journal 2017; 17 (4): 226-234. https://doi.org/10.17098/amj.364163

[28] Golden J, Kenyon-Pesce L, Robison J, Grady J, Guerrera M.P. 2023. Disclosure of Complementary and Alternative Medicine Use Among Older Adults: A Cross-Sectional Study, Gerontology & Geriatric Medicine, Volume 9: 1–7. https://doi.org/10.1177/23337214231179839

[29] Awortwe C, Makiwane M, Reuter H, Muller C, Louw J, Rosenkranz B. Critical evaluation of causality assessment of herb-drug interactions in patients. Br J Clin Pharmacol 2018; Apr; 84(4): 679-693. https://doi.org/10.1111/bcp.13490

[30] Hassen G, Belete G, Carrera K G, Iriowen RO, Araya H, Alemu T, Solomon N, Bam DS, Nicola SM, Araya ME, Debele T, Zouetr M, Jain N. Clinical Implications of Herbal Supplements in Conventional Medical Practice: A US Perspective. Cureus, 2022; 14(7): e26893. https://doi.org/10.7759/cureus.26893

[31] Sarinca Y, Doganli B, Senol FS, Orhan İE. Assessment of Consumer Perceptions in Ankara (Turkey) Toward Herbal Medicinal Products: A Survey Analysis in the Etimesgut District. Clin Exp Health Sci 2017; 9 (1): 7-13. https://doi.org/10.5152/clinexphealthsci.2017.774

[32] Wu JYF, Leung WYS, Chang S, Lee B, Zee B, Tong PCY, Chan JCN. Effectiveness of telephone counselling by a pharmacist in reducing mortality in patients receiving polypharmacy: A randomised controlled trial. BMJ 2006; 333(7567): 522-527. https://doi.org/10.1136/bmj.38905.447118.2F

[33] Lam AY. Assessing medication consultations, hypertension control, awareness, and treatment among elderly Asian community dwellers. Consult Pharm 2008; 23 (10): 795-803. https://doi.org/10.4140/tcp.n.2008.795

How to cite this article: Kaya Yaşar Y, Altun Ali Y, Kara E, Eroğlu EA, Renda G, Çan G, Sezen SF. Evaluation of medication and herbal product usage habits in the geriatric population: A pilot study from Trabzon Province, Türkiye. Clin Exp Health Sci 2025; 15: 124-131. https//doi.org/10.33808/clinexphealthsci.1575123

Clinical and Experimental Health Sciences

Evaluation of Clinical Findings and Visual Prognosis in Chemical Eye Injuries

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Received: May 17, 2024 **Accepted:** February 26, 2025

ABSTRACT

Objective: Chemical eye injuries are ophthalmic emergencies that require rapid intervention. It can cause extensive damage to the ocular surface and anterior segment leading to severe visual impairment. In this study, we aimed to report the clinical findings and visual outcomes of patients with chemical eye injuries.

Methods: A total of 59 eyes of 50 patients who were followed up and treated with the diagnosis of chemical eye injury in Marmara University Ophthalmology Department between 2013 and 2020 were included in the study. Demographic and clinical data of the patients were analyzed.

Results: Mean age of patients was 31.9±12.5 (1-55 years). The mean follow-up period of the patients was 21.3±40.9 (median: 13; range: 3-310) days. 74.6% of the injuries occurred in the workplace. The exposed agent was acidic in 47.5%, alkaline in 39.0%, and the agent in 13.5% of eyes was unknown. The mean initial and final BCVA were 0.51±0.44 and 0.09±0.42 LogMAR, respectively. The observed improvement in BCVA values was statistically significant (p<.001). According to the Roper-Hall classification, 62.7% (37) of the eyes were grade I, and according to the Dua classification, 54.9% (43) of the eyes were grade I and II. The two classifications were correlated with each other (p<.001). While medical treatment was sufficient in 94.9% of the eyes, additional amniotic membrane transplantation (AMT) was applied in 3 eyes. conjunctival limbal autograft transplantation combined with AMT was performed in a patient with a grade IV chemical eye injury.

Conclusion: Adequate and timely treatment helps to of paramount importance to mitigate complications. Additionally, staging of ocular surface burns is crucial to determine prognosis and management plan. Chemical eye injuries are often associated with occupational accidents; therefore, preventive measures and workers' education are imperative.

Keywords: Chemical eye injury, Limbal ischemia, amniotic membrane transplantation, Roper-Hall classification, Dua classification

1. INTRODUCTION

Chemical eye injuries are ophthalmic emergencies that require rapid intervention. It can cause extensive damage to the ocular surface and anterior segment leading to severe visual impairment. Approximately 8% – 21% of all ocular traumas were produced by chemical agents (1). In most cases, chemical eye injuries are work-related, due to home accidents, or may be associated with a criminal assault and are overwhelmingly encountered by 20 – 40-year-old males (1). The offending chemical agent can be acidic, alkaline, or neutral. Nonetheless, alkali injuries are the most common and associated with worse consequences. It penetrates deeper tissues due to the lipophilic nature of alkaline agents. On the other hand, acid injuries cause coagulation necrosis, wherein tissue damage is limited (2). The injury's extent and severity depend on the agent type, contact degree, and exposure duration. Various classification schemes for ocular surface burns were developed, which aid in predicting the prognosis of a chemical injury according to the degree of ocular damage. The Roper-Hall classification system is one of the major classification systems based on the degree of corneal haze and limbal ischemia, albeit conjunctival involvement is disregarded (3). This is a crucial drawback of the Roper-Hall classification system since conjunctival injury was shown to effectively predict progression to corneal melting and symblepharon formation, making it a crucial determinant of prognosis (4, 5). Another shortcoming of this classification method is that all injuries with >50% limbal ischemia are lumped together and categorized as grade IV (6). Conversely, Dua et al. 2001 showed that not all burns with 50% – 100% limbal involvement have the same prognosis as indicated

https//doi.org/*10.33808/clinexphealthsci.1485598*

previously by Roper Halls classification (grade IV), rather patients with total (100%) limbal ischemia were associated with a much worse prognosis than those with just over 50% limbal ischemia. Consequently, the Dua classification was introduced, where Roper Hall's grade IV (>50% limbal ischemia) is stratified into three distinct groups according to the extent of conjunctival injury (5). Factoring in conjunctival involvement is important as this factor is of great prognostic and predictive value in the setting of severe ocular burns (5). Treating chemical eye injuries aims to restore epithelial integrity, control inflammation, and prevent complications. This study reports the clinical findings and visual outcomes of patients with chemical eye injuries.

2. METHODS

This retrospective study reviewed the records of all participants admitted to Marmara University Pendik Educational and Research Hospital with a chemical eye injury between 2013 and 2020. A total of 59 eyes of 50 patients were included in the study. The follow-up time of patients, the offending agent they were exposed to, initial best-corrected visual acuity (BCVA), final BCVA, treatment modalities, and presence of accompanying limbal ischemia and/or corneal haze were recorded. The severity of the chemical injury was determined according to both Roper-Hall and Dua classifications (3, 5). All patients were irrigated at the time of admission. Morgan therapeutic lenses were used in certain cases. In addition to medical therapy, amniotic membrane transplantation (AMT) and conjunctival limbal autograft transplantation (CLAT) were used for patients who did not heal with medical treatment.

2.1. Statistical analysis

The extracted data were analyzed with SPSS (version 22.0, Chicago, USA). Continuous data were expressed as a mean ± standard deviation (SD), while categorical data were described using numbers and percentages. Wilcoxon signed rank test was applied to compare initial and final visual acuities. A p-value of 0.05 or less was considered statistically significant.

3. RESULTS

The mean age of patients was 31.9±12.5 years (range:1–55), and a male predominance was evident (79.6%). The average follow-up duration of patients with acute ocular burns was 21.3±40.9 days (range: 3–310). Work-related trauma (44 [74.6%] eyes) was the most common cause of injury, followed by home accidents (15 [25.4%] eyes). The injuries were right-sided in 20 (40%) eyes, left-sided in 20 (40%) eyes, and bilateral in 10 (20%) eyes. The offending chemical substance was acidic in 47.5% and alkaline in 39.0% of the patients; however, the offending agent was unknown in the remaining 13.5% (Table I). None of patients wore safety glasses at the time of injury.

Table 1. *Prevalence of ocular chemical injuries*

Age (years)	31.88±12.52
Genders (%)	
Male	79.59%
Female	20.41%
Laterality (%)	
Right eye	40%
Left eye	40%
Bilateral involvement	20%
Location (%)	
Workplace	74.6%
Home accident	25.4%
Agent	
Acid	47.5%
Alkali	39.0%
Unknown	13.5%

The mean initial and final BCVA were 0.51±0.44 LogMAR and 0.09±0.42 LogMAR, respectively. The observed improvement in BCVA values was statistically significant ($p < .001$). The mean intraocular pressure at the presentation and final visit were 15.30±2.81 and 14.57±2.09 mmHg, respectively. The healing time of the epithelial defect was 9.40±13.07 days (median: 11, range: 1-90). At first examination, limbal ischemia was observed in 24 (40.6%) eyes (Table 2). After treatment, corneal haze developed in 14 (23.7%) eyes. No correlation was observed between limbal ischemia and corneal haze ($p= .08$). No significant association was found between limbal ischemia and final visual outcome ($p= .37$). Additionally, no significant difference in final visual acuity was observed between the acid and base exposure groups ($p= .62$). There was also no correlation between the disease stage and final visual acuity ($p= .83$). While 56 (94.9%) eyes received only medical therapy, AMT was needed for 3 (5.08%) eyes. A symblepharon ring was used in 6 (10.2%) of the eyes. Autologous serum eye drops were added to the treatment in 16 (27.1%) eyes. Moreover, limbal deficiency developed in patients at an advanced stage despite all treatment options (medical therapy + AMT + CLAT). According to the Roper-Hall classification, 37 (62.7%) eyes were grade I, 14 (23.7%) eyes were grade II, 7 (11.9%) eyes were grade III, and 1 (1.7%) eye was grade IV. Meanwhile, according to the Dua classification, 17 (28.8%) of the eyes were grade I, 26 (44.1%) of the eyes were grade II, 15 (24.4%) of the eyes were grade III, and 1 (1.7%) eye was grade V (Figure I). The two classification schemes correlated with each other ($p < .001$).

Table 2. *Clinical findings of ocular chemical injuries*

Mean Visual acuity (LogMAR)	**Baseline 0.51±0.44**	**Final 0.09±0.42**
Mean Intraocular Pressure (mmHg)	**Baseline 15.30±2.81**	**Final 14.57±2.09**
Mean Healing time of the epithelial defect (day)	9.40±13.07	
Prevalence of Limbal ischemia	24 (40.6%)	

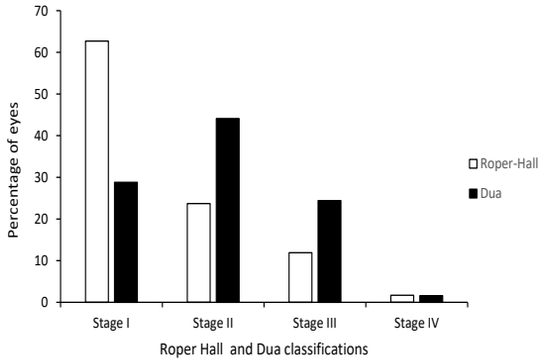

Figure 1. *Distribution of patients by Roper Hall and Dua classification.*

4. DISCUSSION

This study described the characteristics and outcomes of 50 patients treated with ocular chemical injuries. Most of the patients were males, and most of the accidents occurred at the workplace. Acidic agents caused the majority of injuries. According to the Roper-Hall classification, 37 (62.7%) eyes were at grade I. On the other hand, according to the Dua classification, 43 (72.9%) eyes were at grades I and II. Medical treatment was sufficient in 93.2% of the eyes.

Ocular chemical injuries are ophthalmic emergencies that require immediate treatment as they could have devastating effects on the patient's vision, potentially leading to blindness. Adequate management on time of each stage of the disease is necessary to achieve better visual outcome and to prevent complications. Young men (20-40 years old) are reported to be most susceptible to ocular chemical injuries (1). Ghosh et al. examined 110 eyes of 98 patients (average age: 36.5 ±17.1 years) and reported a male prevalence of 60% with 50% of the accidents occurring at work (7). In consistent with previous literature, the average age of patients included in our studied was 31.9±12.5 years (7). Kuckelkorn et al., in their study including 171 patients, revealed that industrial accidents caused 61% of the ocular surface burns, while 37% occurred at home. The cause of the accident was unknown in 3 patients (8). Likewise, our current study also concluded that the workplace was the most common location of accident. Haring et al. reported that alkaline injuries (53.6%) were more common than acid injuries (46.4%) (9). Although alkali injuries occur more frequently than acidic injuries, most injuries in this study were acid-related. This may be ascribed to the fact that 13.6% of the agents were of unknown nature. Prognostic factors reported to be associated with good final BCVA in chemical eye injuries include older age, poor initial BCVA, and irrigation after more than 24 hours (10). Safety glasses and worker education are of paramount importance (10). In present study, none of our patients wore protective goggles at the time of injury. A significant improvement between initial and final BCVA was detected, as reported by previous literature (10). Immediate irrigation to remove chemical agents is crucial to reduce damage (11). All patients in our study immediately irrigated their eyes after injury.

Treatment of ocular chemical injuries begins with adequate and timely irrigation. Irrigation reduces burn severity the need for surgery and improves the visual acuity (12). Medical treatment is mostly sufficient in patients with mild-to-moderate chemical burns, who have a good prognosis. Mainstay therapy controls acute inflammation and facilitates ocular surface restoration. Topical corticosteroid is the primary therapy to control the critical inflammatory reaction. The common choices include dexamethasone 0.1% and prednisolone acetate 1%. It should be preservative-free to reduce additional stress on the damaged ocular surface. Anti-protease therapy such as tetracyclines and ethylenediaminetetraacetic acid helps reduce and prevent corneal ulcers. In addition to its anti-inflammatory effect, AMT accelerates the healing of the corneal epithelium and reduces pain. Once the acute inflammatory reaction is controlled, the goal is to facilitate the recovery of the ocular surface. Preservative-free artificial tears, ascorbic acid, autologous serum, regenerating agent, bandage contact lens, AMT, and free conjunctival autograft are frequently used to heal the ocular surface. Although the ascorbic acid level is higher in aqueous humor, it decreases after chemical trauma. Oral and topical ascorbic acid increases aqueous humor concentration and reduces collagen degradation and corneal ulceration. Cycloplegic agents are added to the therapy to prevent synechiae and control iridocyclitis. Although fluoroquinolones are often preferred for prophylaxis, culture-sensitive agents are added to the treatment in case of infection (11, 13).

Complications of chemical injuries include poor vision, dry eye disease, glaucoma, limbal stem cell deficiency, and corneal scarring. Direct chemical damage to the conjunctiva can lead to scarring, forniceal shortening, symblepharon formation, and cicatricial entropion or ectropion (1). Various techniques have been reported for limbal stem cell transplantation (LSCT), including conjunctival limbal autograft (CLAU), cultured limbal stem cell transplantation (CLET), simple limbal epithelial transplantation (SLET), and limbal allograft, including keratolimbal allografts (KLAL) and living-related conjunctival allograft (LR-CLAL) (14). According to the Dua classification, limbal deficiency developed in only one patient in this study who was at grade V despite medical treatments, AMT, and CLAT. Glaucoma after chemical burns represents secondary, posttraumatic, and open-angle glaucoma. Lin et al. revealed that eyes with high-grade ocular chemical burns are more likely to have glaucoma and require glaucoma surgery (15). None of our patients developed glaucoma. Tenonplasty is one of the surgical options for severe chemical injuries, corneal scleral ulceration, and melting (16). Free conjunctival autografts may be another option when the other eye is not involved (17). The last stage in treating chemical injuries is a comprehensive restoration treatment that includes the treatment of valve, adnexal reconstruction, glaucoma, and cataract (11). However, these complications were not observed in our patients.

Ghosh et al. showed that four patients with Roper-Hall grade IV undergoing early AMT developed limbal stem cell deficiency (7). Eslani et al., in a study involving 60 grade IV patients, showed that adding early AMT to medical therapy did not accelerate corneal epithelialization. No difference was reported between the two groups in terms of final BCVA (18). In our study, while medical treatment was applied to all patients, AMT was applied in cases with delayed epithelial healing. The number of our patients classified as having an advanced stage was low. A total of three eyes underwent AMT surgery; AMT was applied in combination with CLAT in one patient. The remaining 55 eyes were treated conservatively with medical treatments and AMT.

Gupta et al. compared the Dua and Roper-Hall classification schemes (4). They showed that post-treatment corneal clarity was better in grade IV burns than in grades V-VI, and corneal vascularization was more prominent in grades V-VI than grade IV. Moreover, the formation of symblepharon positively correlated with the extent of conjunctival involvement. Therefore, they reported that the Dua classification has a superior prognostic value than the Roper-Hall classification in the setting of severe ocular burns (19). In present study, both classifications correlated with each other. However, there was no correlation between limbal ischemia and corneal haze. This may be related to fewer limbal ischemia areas in most of our patients.

This study had several limitations which were the retrospective nature of the study, the small number of patients studied and the short follow-up time.

5. CONCLUSION

As a result, adequate and timely treatment helps to of paramount importance to mitigate complications. Additionally, staging of ocular surface burns is crucial to determine prognosis and management plan. Chemical eye injuries are often associated with occupational accidents; therefore, preventive measures and workers' education are imperative. Better visual results can be obtained with medical treatment and appropriate surgical options.

Funding: *The author received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Marmara University, Noninvasive Clinic Ethics Committee (Approval date:* 05.02.2021*; Number:* 09.2021,229*)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: EBÇ
Design of the study: EBÇ, SAT
Acquisition of data for the study: EBÇ
Analysis of data for the study: EBÇ, SAT
Interpretation of data for the study: EBÇ, SAT, AET
Drafting the manuscript: EBÇ
Revising it critically for important intellectual content: EBÇ, SAT, AET, ÖŞ
Final approval of the version to be published: EBÇ, SAT, AET, ÖŞ

REFERENCES

[1] Singh P, Tyagi M, Kumar Y, Gupta K, Sharma P. Ocular chemical injuries and their management. Oman Journal of Ophthalmology 2013;6(2):83. https://doi.org/10.4103/0974-620X.116624

[2] Wagoner MD. Chemical injuries of the eye: current concepts in pathophysiology and therapy. Surv Ophthalmol. 1997;41(4):275-313. https://doi.org/10.1016/s0039-6257(96)00007-0

[3] Roper-Hall MJ. Thermal and chemical burns. Trans Ophthalmol Soc U K (1962). 1965;85:631-653.

[4] Gupta N, Kalaivani M, Tandon R. Comparison of prognostic value of Roper Hall and Dua classification systems in acute ocular burns. Br J Ophthalmol. 2011;95(2):194-198. https://doi.org/10.1136/bjo.2009.173724

[5] Dua HS, King AJ, Joseph A. A new classification of ocular surface burns. Br J Ophthalmol. 2001;85(11):1379-1383. https://doi.org/10.1136/bjo.85.11.1379

[6] Bizrah M, Yusuf A, Ahmad S. An update on chemical eye burns. Eye (Lond). 2019;33(9):1362-1377. https://doi.org/10.1038/s41433-019-0456-5

[7] Ghosh S, Salvador-Culla B, Kotagiri A, Pushpoth S, Tey A, Johnson ZK, Figueiredo FC. Acute chemical eye injury and limbal stem cell deficiency. A prospective study in the United Kingdom. Cornea 2019;38(1):8-12. https://doi.org/10.1097/ICO.0000000000001739

[8] Kuckelkorn R, Luft I, Kottek AA, Schrage NF, Makropoulos W, Reim M. Chemical and thermal eye burns in the residential area of RWTH Aachen. Analysis of accidents in 1 year using a new automated documentation of findings. Klin Monbl Augenheilkd. 1993;203(1):34-42. https://doi.org/10.1055/s-2008-1045646

[9] Haring RS, Sheffield ID, Channa R, Canner JK, Schneider EB. Epidemiologic trends of chemical ocular burns in the United States. JAMA Ophthalmology 2016;134(10):1119-1124. https://doi.org/10.1001/jamaophthalmol.2016.2645

[10] Li T, Jiang B, Zhou X. Clinical characteristics of patients hospitalized for ocular chemical injuries in Shanghai from 2012 to 2017. Int Ophthalmol. 2020;40(4):909-916. https://doi.org/10.1007/s10792-019-01263-w

[11] Dua HS, Ting DSJ, Al Saadi A, Said DG. Chemical eye injury: Pathophysiology, assessment and management. Eye 2020;34(11):2001-2019. https://doi.org/10.1038/s41433-020-1026-6

[12] Kuckelkorn R, Kottek A, Schrage N, Reim M. Poor prognosis of severe chemical and thermal eye burns: the need for adequate emergency care and primary prevention. Int Arch Occup Environ. Health 1995;67(4):281-284. https://doi.org/10.1007/BF00409410

[13] Salman İA, Gündoğdu C. Epithelial healing in experimental corneal alkali wounds with nondiluted autologous serum eye drops. Cutan Ocul Toxicol. 2010;29(2):116-121. https://doi.org/10.3109/15569521003709558

[14] Yin J, Jurkunas U, editors. Limbal stem cell transplantation and complications. Semin Ophthalmol. 2018;33(1):134-141. https://doi.org/10.1080/08820538.2017.1353834

[15] Lin MP, Ekşioğlu Ü, Mudumbai RC, Slabaugh MA, Chen PP. Glaucoma in patients with ocular chemical burns. Am J Ophthalmol. 2012;154(3):481-485.

https://doi.org/10.1016/j.ajo.2012.03.026

[16] Kuckelkorn R, Redbrake C, Reim M. Tenonplasty: A new surgical approach for the treatment of severe eye burns. SLACK Incorporated Thorofare, NJ; 1997. p. 105-110.

[17] Dua HS, Miri A, Faraj LA, Said DG. Free autologous conjunctival grafts. Ophthalmology 2012;119(10):2189-e2. https://doi.org/10.1016/j.ophtha.2012.06.004

[18] Eslani M, Baradaran-Rafii A, Cheung AY, Kurji KH, Hasani H, Djalilian AR, Holland EJ. Amniotic membrane transplantation in acute severe ocular chemical injury: A randomized clinical trial. Am J Ophthalmol. 2019;199: 209-215. https://doi.org/10.1016/j.ajo.2018.11.001

[19] Gupta N, Kalaivani M, Tandon R. Comparison of prognostic value of Roper Hall and Dua classification systems in acute ocular burns. Br J Ophthalmol. 2011;95(2):194-198. https://doi.org/10.1136/bjo.2009.173724

How to cite this article: Biberoğlu Çelik E, Akkaya Turhan S, Şahin, Ö, Toker AE. Evaluation of Clinical Findings and Visual Prognosis in Chemical Eye Injuries. Clin Exp Health Sci 2025; 15: 132-136. https//doi.org/10.33808/clinexphealthsci.1485598

Clinical and Experimental Health Sciences

Validity and Reliability of the Turkish Version of Team Effectiveness Questionnaires: Patient and Provider Perceptions

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Received: May 13, 2024 **Accepted:** February 11, 2025

ABSTRACT

Objective: To adapt the Patient/Family-Perfections of Team Effectiveness (Patient-PTE) Questionnaire and Provider-Perceptions of Team Effectiveness Questionnaire (Provider-PTE) into Turkish and to test their validity and reliability.

Methods: The sample of this methodological study included patients receiving inpatient treatment and care at a training and research hospital and their families (n1=230), and nurses and physicians (n2=260). After the questionnaires' language and content validity were confirmed, their construct validity was examined by test-retest and internal consistency.

Results: The original structure of the questionnaires was preserved in their Turkish versions. In construct validity, significant differences were determined according to the characteristics of the participants in both questionnaires. A statistically significant difference was detected when the mean scores of low and high functioning teams were compared for the sensitivity of the questionnaires.

Conclusion: Turkish versions of the questionnaires were considered valid and reliable. The Patient-PTE can be used to assess team processes and perceived outcomes of care from the patients' and their families' perspectives. The Provider-PTE can be used to assess interdisciplinary team processes and perceived care outcomes.

Keywords: Teamwork, perception of team effectiveness, patient, provider, validity, reliability.

1. INTRODUCTION

In the provision of health services, healthcare professionals have to work together to ensure that patients receive the care they need (1). The transition from professional care to patient-centered care challenges healthcare professionals' traditional roles and boundaries (2). Especially since the care of critically ill patients is complex, teamwork becomes even more important in the provision of quality and safe care (3).

There is growing interest in how to improve teams' functioning and performance in healthcare around the world because poor teamwork is considered as the key factor in adverse events occurring in patient safety (4). Team performance is assessed through system-based indicators including physical examination rates, waiting times and access to care. However, team-based processes are required to understand patient outcomes better. The processes here are defined as the level of interactions between patients and service providers. Processes that include teamwork, communication and patient participation are considered as cornerstones in the provision of effective care (5).

Teamwork is the process in which team members try to achieve the common goals of the organization by combining their knowledge, experience and skills with each other (6). Teamwork in health services is the provision of quality health care by at least two healthcare professionals in cooperation and coordination with patients and their relatives in line with the common goals in the care plan (7). A number of processes such as communication, harmony, coordination, decision-making, problem solving, and focusing on the needs of patients and their families affect team functioning (8).

Differences in healthcare professionals' education levels, attitudes and expectations, fields they work in, and working hours suggest that teamwork in health is difficult. Turnover rates of healthcare professionals can be high, and they may not know each other's authority (9). Shift work, patients'

https//doi.org/*10.33808/clinexphealthsci.1483360*

transfer to another service/institution, or frequent change of healthcare personnel due to human resources procedures may negatively affect team functions (10).

In a study, it was emphasized that in a self-assessment tool developed to measure interdisciplinary team effectiveness, only healthcare professionals' perspectives were addressed, and that patients' opinions should be considered to assess team effectiveness as well (11). Kash et al. reviewed 22 articles in which team effectiveness was measured and emphasized that for healthcare settings, tools which are more valid should be developed. They also stated that patient outcomes should be considered more comprehensively when team effectiveness is measured (12). Assessing the team's effectiveness as perceived by patients encourages active patient participation and prioritizes patient needs, allowing for personalized, holistic care. It also improves the patient-caregiver relationship and allows for standardized monitoring of patient outcomes and improved quality of care (13).

Although measurement tools used to assess healthcare professionals' evaluations of teamwork in health care are available in Turkish literature, there is no measurement tool used to assess patients and families evaluate the team effectiveness of healthcare professionals who provide care to them (14,15,16,17). In this context, the aim of this study was to adapt the Patient/Family-Perceptions of Team Effectiveness Questionnaire (Patient-PTE) developed by Kilpatrick et al. to assess the team effectiveness of healthcare providers as perceived by patients or their families and the Provider-Perceptions of Team Effectiveness Questionnaire (Provider-PTE) to assess the team effectiveness as perceived by healthcare providers into Turkish and to conduct the psychometric analyses of these questionnaires (5,18).

2. METHODS

2.1. Study Aim and Design

The aim of this study, which is within the scope of methodological research, is to adapt the Patients/Family Perception of Team Effectiveness Questionnaire and the Providers-Perception of Team Effectiveness Questionnaire into Turkish and to conduct validity and reliability analyses.

2.2. Sample Population and Sampling

Patients/families receiving inpatient treatment and care in a training and research hospital, and physicians and nurses working in this hospital comprised the population of the present study. The hospital has been operating since 2013. It is a tertiary hospital with a capacity of 800 beds. There were 522 physicians and nurses working at the hospital during the period the study was conducted. Since the study was conducted during the pandemic, the researchers were not informed about the number of monthly inpatients. To calculate the sample size, it was planned to reach 10-fold the number of the items in both questionnaires, as stated in the literature (19). Thus, 230 patients or patient relatives and 260 nurses and physicians determined by convenience sampling method constituted the sample of the study. The inclusion criteria for the patient/family sample were as follows: receiving inpatient treatment and care in the hospital for at least two nights, being literate in Turkish, and not having any psychiatric diagnosis. The Patient-PTE Questionnaire was responded by the patients, but if they had hearing or vision problems, or when they were asleep or were referred to the consultation or radiological imaging unit when the questionnaire was administered, their families who stayed with the patient for 2 days answered. The Provider-PTE Questionnaire was applied to nurses and physicians who treat and care for these patients.

2.3. Data Collection

The study data were collected with the Patient/Family Descriptive Information Form, Healthcare Professionals Descriptive Information Form, Patient-PTE Questionnaire and Provider PTE Questionnaire in March 2021 and April 2021.

2.3.1. Patient/Family Descriptive Information Form

The form prepared by the researchers includes eight items questioning the demographic characteristics of the patient/family.

2.3.2. Patient-PTE Questionnaire

The Patient-PTE Questionnaire developed by Kilpatrick et al. consists of total 23 items. The questionnaire consists of the sub-dimensions "Perception of Team Effectiveness Scale" (17 items, 1-17) and "Outcomes" (6 items, 18-23). The "Perception of Team Effectiveness Scale " consists of three sub-dimensions: "Trust" (item 5), "Role Clarity" (items 1,2) and "Team Processes" (items 3,4,6-17). The "Team Processes" sub-dimension is divided into seven sub-dimensions: "Perception of Team Effectiveness" (item 6), "Decision Making" (items 7,14), "Communication" (items 8-10), "Coordination" (items 12,13,17), "Cohesion" (item 11), "Problem Solving" (item 15) and "Patient/Family Focus" (items 3,4,16). Although the questionnaire was used as a 7-point Likert scale in the original study (5), it was later revised as a 6-point Likert scale. In this study, the items of the questionnaire were evaluated as a 6-point Likert scale (1: Strongly disagree, 6: Strongly agree). Items 10, 16, and 20 are reverse scored. Higher scores indicate that patients/families have a higher perception of team effectiveness. The Cronbach's Alpha internal consistency coefficient was 0.94 for the "PTE-Overall", 0.92 for the "Team Processes" and 0.76 for the "Outcomes" dimensions (5).

2.3.3. Healthcare Professionals Descriptive Information Form

The form prepared by the researchers includes 10 items which question the demographic characteristics of healthcare professionals.

2.3.4. Provider-PTE

The Provider-PTE Questionnaire developed by Kilpatrick et al. consists of total 26 items. The questionnaire consists of the sub-dimensions "Perception of Team Effectiveness Scale" (19 items, 1-19) and "Outcomes" (7 items, 20-26). "Perception of Team Effectiveness Scale" consists of four sub-dimensions: "Trust" (item 4), "Role Clarity" (items 1,2), "Team Meeting" (items 7,12) and "Team Processes" (items 3,5,6,8-11,13-19). The "Team Processes" sub-dimension is divided into seven sub-dimensions: "Perception of Team Effectiveness" (item 5), "Decision Making" (items 14,16), "Communication" (items 8-10), "Coordination" (items 18,19), "Cohesion" (item 11), "Problem Solving" (items 15,16) and "Patient/Family Focused" (items 3,13,17). The items of the questionnaire were evaluated as a 6-point Likert scale (1: Strongly disagree, 6: Strongly agree). Items 10, 17, and 23 are reverse scored. Higher scores indicate that healthcare providers have a higher perception of team effectiveness. The Cronbach's Alpha internal consistency coefficient was 0.91 for the "PTE-Overall", 0.88 for the "Team Processes" and 0.72 for the "Outcomes" dimensions (18).

2.4. Ethical Considerations

Permission to adapt the questionnaires were obtained from the author who developed them via e-mail. Ethics committee approval was received from Istanbul University-Cerrahpaşa Social and Humanities Research Ethics Committee (Date: September 13, 2020, Number: 134). Institutional permission was obtained from the hospital where the study was to be conducted. Written informed consent was obtained from the participants.

2.5. Cultural Adaptation Procedure

2.5.1. Language Validity

The questionnaires were adapted according to the ISPOR (International Society for Pharmacoeconomics and Outcome Research) Guidelines (20). The original questionnaires were translated into Turkish by three people with a good command of both English and Turkish independently of each other. The three translations were evaluated by the researchers and the first Turkish versions were created. The first Turkish versions were translated back to English by a translator. The original versions were compared with the back-translated versions by the researchers, and necessary revisions were made. Then the back-translated questionnaires were sent to the first author who developed both questionnaires via e-mail. The author approved the questionnaires without any need for correction.

2.5.2. Content Validity

In order to evaluate the content validity of the questionnaires, the opinions of 10 healthcare professionals specialized in nursing (three nursing academicians, two staff nurses) and medicine (two physicians, three academicians) were obtained. They rated the items of the questionnaires using the Davis method as follows: 1=not relevant, 2=somewhat relevant, 3=quite relevant, 4=highly relevant. The content validity index (CVI) was calculated by dividing the number of experts who ticked 3 and 4 for each item by the total number of experts.

2.5.3. Pilot Study

The patients to whom the questionnaire was first applied stated that the questionnaire items were understandable. The nurses and physicians among the professionals who received expert opinions stated that there was no incomprehensible item in the questionnaire. A separate pilot study could not be conducted because minimal contact between people was required due to the pandemic.

2.6. Data Analysis

Data were analyzed using the SPSS version 21.0. The content validity of the data was determined with the CVI. Mean, standard deviation, minimum and maximum values were used to analyze the descriptive data. While the intra-class correlation coefficient (ICC) was used for test-retest analysis, the Cronbach's Alpha coefficient was used for reliability. In establishing construct validity, factor analysis was not performed because the criterion that the dimensions should include at least three items to conduct factor analysis was not met (22,23). As in the original study, the known-groups validity method was used for both questionnaires (5,18). In this method, a type of construct validity, the measurement tool's ability to distinguish between different known groups is measured (21). In order to examine the known group validity, the following hypotheses were made, similar to the hypotheses in the original questionnaires development study (5,18). It was assumed that there would not be a significant difference between the scores obtained from the PTE-Overall and Outcomes dimensions by the patients according to the degree of kinship, sex and marital status variables, but that there would be a significant difference according to education level, clinic and reason for hospitalization variables. It was assumed that there would not be a significant difference between the scores obtained from the PTE-Overall and Outcomes dimensions by the healthcare providers according to age, sex and marital status variables, but that there would be significant differences according to education level, type of profession, unit they work in, and length of service in the profession and institution variables.

Dimensions for sensitivity analysis of the questionnaires were coded as follows; Scores 1-4 (Strongly Disagree, Disagree, Slightly Disagree, Slightly Agree) "low-functioning teams", and scores 5-6 (Agree, Strongly Disagree) "high-functioning teams". While the Independent Samples t-test was used to compare two groups, the one-way ANOVA was used to compare more than two groups. In cases where the assumption of homogeneity of variances was not met in the Levene test ($p < .05$), Welch and Brown-Forsythe tests were used instead of ANOVA. For the p value in the analysis, as stated by Kilpatrick et al., the binary combination was taken into account and α value was calculated by dividing by the number of groups (5).

3. RESULTS

3.1. The patient/families' sociodemographic characteristics

The participants' mean age was 33.04 (SD=14.98) years. Of them 71.7 were women, 51.3% were patients, 52.2% were married and 23.5% had an associate degree. Of the patients, 66.1% were hospitalized in surgical wards due to surgical diseases (58.3%). Their mean length of hospitalization was 6.98 (SD=7.58) (min=2, max=49) days.

3.2. The nurses and physicians' sociodemographic characteristics

The participants' mean age was 37.15 (SD=7.49) (min=21, max=65) years. Of them, 46.9% were in the age group of 36-45 years, 73.8% were women, 70% were married, 49.6% had an undergraduate degree, 77.3% were nurses, 22.7% physicians, 52.3% worked in surgical units, 52.3% had a length of service in the profession ranging between 11 and 25 years, 73.5% had a length of service in the institution ranging between 1 and 10 years, and 53.1% worked in shifts. Their average weekly working time was 44.09 hours (SD=6.919) (min=10, max=64).

3.3. Content Validity

By calculating the scores given by each health professional to the items in the questionnaires, the item CVI values for both of the overall questionnaires and its items were determined as 0.98 and > 0.80. The questionnaires were finalized according to the health professionals' suggestions regarding the understandability of the items.

3.4. Construct Validity

3.4.1. Findings on the Construct Validity of the Patient-PTE

The comparison of the scores obtained from the PTE-Overall and Outcomes dimensions of the Patient-PTE according to the degree of kinship variable revealed a significant difference only in the trust sub-dimension (t=-1.973; p = .05). There was a significant difference between the participants' trust and outcomes dimensions scores in terms of the marital status variable ($p < .05$). There were statistically significant differences between PTE-Overall and Outcomes scores in all the sub-dimensions according to education level and diagnosis at the hospitalization variables. There were statistically significant differences between the participants' PTE-Overall and Outcomes scores in terms of all the variables except for the clinic they were hospitalized in and role clarity variables ($p < .05$) (Table 1). However, there was no statistically significant difference between the participants' age, sex, number of hospitalization days, and their PTE-Overall and Outcomes scores ($p > .05$).

Table 1. *Findings for known group comparison of Patient-PTE scores*

	Sub-Dimensions		Trust	Team Processes	Role Clarity	PTE-Overall	Outcomes
Patient-PTE	Degree of Kinship	t	-1.973	-0.352	-0.292	-0.565	-0.796
		p	**.050***	.725	.770	.572	.427
	Marital Status	t	4.125	1.125	-0.568	1.261	2.610
		p	**< .001***	.262	.571	.208	**.010***
	Education Level	F	13.294	7.161	5.821	9.339	7.731
		p	**.001***	**.001***	**.001***	**.001***	**.001***
	Reason for Hospitalization	F	3.908	4.729	2.472	4.934	61.741
		p	.000	.000	.011	.000	.000
	Clinic	F	2.382	2.619	a	2.797	3.771
		p	**.025***	**.013***		**.009***	**.001***

**p < .05; t, Independent Samples T Test; F, ANOVA; a Because at least one group has 0 variance, robust equality of means tests cannot be performed for role salience.*

Table 2. *Findings for known group comparison of Provider-PTE scores*

	Sub-Dimensions		Trust	Team Processes	Role Clarity	Team Meeting	PTE-Overall	Outcomes
Provider-PTE	Age	t	0.127	1.587	2.627	1.123	1.642	4.971
		p	.881	.207	.077	.327	.196	**.008***
	Marital Status	t	-1.894	-2.692	-2.158	-2.505	-2.283	-0.433
		p	.061	**.005***	**.032***	**.008***	**.002***	.665
	Education Level	F	4.704	1.486	1.163	4.649	1.977	2.884
		p	**.006***	.219	.324	**.007***	.118	**.036***
	Type of Profession	t	0.190	0.370	2.165	-0.790	0.553	-0.402
		p	.850	.712	**.032***	.430	.581	.688
	Unit They Work	F	1.366	2.395	0.699	5.685	2.243	2.304
		p	.254	.069	.572	**.001***	.084	.089

**p < .05; t, Independent Samples T Test; F, ANOVA*

3.4.2. Findings on the Construct Validity of the Provider-PTE

The comparison of the scores obtained from the PTE-Overall and Outcomes dimensions of the Provider-PTE according to the age variable revealed a significant difference only in the Outcomes dimension ($p < .05$). The comparison of the scores obtained from the PTE-Overall and Outcomes dimensions in terms of the marital status variable revealed that there were significant differences in the role clarity, team meeting and team processes sub-dimensions. The comparison of the scores obtained from the PTE-Overall and Outcomes dimensions in terms of the educational status variable revealed that there were significant differences in the trust, team meeting and outcomes sub-dimensions. The comparison of the scores obtained from the PTE-Overall and Outcomes dimensions in terms of the participants' professions variable revealed that there were significant differences only in the role clarity sub-dimension. The comparison of the scores obtained from the PTE-Overall and Outcomes dimensions in terms of the unit they work in variable revealed that there were significant differences only in the team meeting sub-dimension. There was no significant relationship between the scores obtained from the PTE-Overall and Outcomes dimensions and the variables such as sex, length of service in the profession, length of service in the institution and weekly working hours ($p > .05$).

3.5. Comparison of PTE-Overall and Outcomes Scores with PTE Scores in Low and High Functioning Teams

The sensitivity of the questionnaires was assessed with the PTE scores of the high (5-6) and low (1-4) functioning teams. According to the analysis results, there was a significant difference between the low and high functioning teams in terms of their mean scores for all the sub-dimensions ($p < .05$) (Table 3).

3.6. Reliability

To determine the internal reliability of the questionnaires, Cronbach's Alpha coefficients were calculated. Cronbach's Alpha coefficients were 0.77 for the team processes, 0.83 for the PTE-Overall and 0.81 for the Outcomes dimensions of the Patient-PTE, whereas they were 0.86 for the team processes, 0.92 for the PTE-Overall and 0.79 for the Outcomes dimensions of the Provider-PTE.

Test-retest analysis was performed to determine the invariance of the questionnaires. The questionnaires were re-administered to 25% of the patients/families (n_1=58) one week after the first application. The analysis results demonstrated that the dimensions' ICC values ranged between 0.75 and 0.95 ($p < .05$). The questionnaires were re-administered to 25% of the healthcare professionals (n_2=65) 15 days after the first application. The analysis results demonstrated that the dimensions' ICC values ranged between 0.86 and 0.95 ($p < .05$) (Table 4).

3.7. Findings on Team Effectiveness in Healthcare

The mean scores the participants obtained from the PTE-Overall (4.47±0.61) and Outcomes (4.76±0.85) dimensions of the Patient-PTE were above the average. They obtained the highest mean score from the trust sub-dimension (4.87±1.20), and the lowest mean score from the team processes sub-dimension (4.39±0.59). The mean scores the obtained from team processes sub-dimension, the highest mean score from the perception of team effectiveness (4.97±0.95) and the lowest from the patient-family focus (3.60±0.92). The mean scores the participants obtained from the PTE-Overall (4.40±0.71) and Outcomes (4.61±0.69) dimensions of the Provider-PTE were above the average. They obtained the highest mean score from the trust sub-dimension (4.66±1.08), and the lowest mean score from the role clarity sub-dimension (3.62±1.36). The mean scores the obtained from team processes sub-dimension, the highest mean score from the coordination (4.94±0.82) and the lowest from the communication (4.10±0.72) (Table 5).

Table 3. *Findings for comparison of PTE-overall and outcomes scores with team effectiveness perception questionnaire scores in low and high functioning teams*

	Sub-Dimensions	PTE	n	M	SD	t	p
Patient-PTE	Trust	Low (1-4)	51	3.78	1.189	-8.364	**< .001***
		High (5-6)	179	5.18	1.014		
	Role Clarity	Low (1-4)	51	4.10	1.166	-5.296	**< .001***
		High (5-6)	179	5.03	0.882		
	Team Processes	Low (1-4)	51	3.64	0.596	-10.898	**< .001***
		High (5-6)	179	4.61	0.393		
	Outcomes	Low (1-4)	51	3.78	0.873	-9.657	**< .001***
		High (5-6)	179	5.04	0.605		
Provider-PTE	Role Clarity	Low (1-4)	58	2.81	1.154	-10.033	**< .001***
		High (5-6)	202	3.85	1.338		
	Trust	Low (1-4)	58	3.52	1.013	-5.366	**< .001***
		High (5-6)	202	4.99	0.867		
	Team Meeting	Low (1-4)	58	3.28	0.983	-7.359	**< .001***
		High (5-6)	202	4.48	1.114		
	Team Processes	Low (1-4)	58	3.77	0.782	-9.067	**< .001***
		High (5-6)	202	4.74	0.446		
	Outcomes	Low (1-4)	58	4.03	0.796	-7.703	**< .001***
		High (5-6)	202	4.88	0.525		

**p < .05; t, Independent Samples T Test; PTE, Perceptions of Team Effectiveness; M, mean; n, number; SD, standard deviation*

Table 4. *Test-retest results of questionnaires*

	Sub-Dimensions	Test	Retest	ICC	95% Confidence Interval	p
		M ± SD	M ± SD		Lower Limit-Upper Limit	
Patient-PTE	Trust	5.12 ±1.18	5.08 ±1.01	0.91	0.855-0.949	**< .001***
	Team Processes	4.39 ±0.66	4.46 ±0.56	0.96	0.932-0.976	**< .001***
	Role Clarity	4.84±1.14	5.06±0.66	0.75	0.580-0.853	**< .001***
	PTE-Overall	4.48±0.67	4.57±0.55	0.95	0.930-0.976	**< .001***
	Outcomes	4.83±0.95	4.81±0.71	0.91	0.862-0.952	**< .001***
Provider-PTE	Trust	4.92±0.853	4.87±0.718	0.91	0.856-0.946	**< .001***
	Team Processes	4.68±0.510	4.70±0.362	0.93	0.890-0.959	**< .001***
	Role Clarity	3.71±1.457	3.78±1.325	0.95	0.924-0.972	**< .001***
	Team Meeting	4.29±1.030	4.20±0.804	0.95	0.919-0.970	**< .001***
	PTE-Overall	4.55±0.573	4.56±0.418	0.95	0.917-0.969	**< .001***
	Outcomes	4.85±0.488	4.90±0.311	0.86	0.781-0.918	**< .001***

**p < .05; ICC, Intraclass correlation coefficient; M, mean; n, number; SD, standard deviation*

Table 5. *Findings on team processes in healthcare*

	Dimensions	Sub-Dimensions		M ± SD	Min-Max
Patient-PTE (n=230)	PTE-Overall	Trust		4.87 ±1.20	1-6
		Team Processes		4.39 ±0.59	2-6
			PTE	4.97 ±0.95	1-6
			Decision making	4.71 ±0.97	1-6
			Communication	4.26 ±0.69	1-6
			Coordination	4.74 ±0.85	1-6
			Cohesion	4.93 ±0.98	1-6
			Problem solving	4.42 ±1,05	1-6
			Patient/family focus	3,60 ±0.92	1-6
		Role Clarity		4.82±1.02	1-6
		PTE-Overall		4.47±0.61	1-6
	Outcomes	Outcomes		4.76±0.85	2-6
Provider-PTE (n=260)	PTE-Overall	Trust		4.66±1.08	1-6
		Team Processes		4.53±0.67	2-6
			PTE	4,88±0,92	1-6
			Decision making	4,63±0,95	1-6
			Communication	4,10±0,72	2-6
			Coordination	4,94±0,82	1-6
			Cohesion	4,76±1,09	1-6
			Problem solving	4,19±1,08	1-6
			Patient/family focus	4,64±0,79	2-6
		Role Clarity		3.62±1.36	1-6
		Team Meeting		4.21±1.19	1-6
		PTE-Overall		4.40±0.71	2-6
	Outcomes	Outcomes		4.61±0.61	2-6

**p < .05; M, mean; n, number; SD, standard deviation; min., minimum; max., maximum; PTE, perception of team effectiveness*

4. DISCUSSION

In the present study, two questionnaires developed in English to determine the team effectiveness of patients/families and healthcare professionals were adapted to Turkish, and their validity and reliability were tested. The results of the Turkish language validity study of the questionnaires conducted in line with the ISPOR Guidelines demonstrated that the Turkish versions were linguistically valid (20). The content validity study of the questionnaires was performed based on the scores given to the items in the questionnaires by 10 experts. CVI values of the questionnaires and the items were above 0.80, indicating that the Turkish versions provided content validity (24).

Since the structure of the questionnaires did not meet the criterion that the dimensions should include at least three items, the known groups validity method was used instead of factor analysis, as in the original questionnaires (5). In this regard, the scores obtained by the patients and healthcare professionals from the PTE-Overall and Outcomes dimensions, which constitute the PTE questionnaire, were compared according to the characteristics of the patients and healthcare professionals. Compared with the PTE-Overall and outcomes scores, a significant difference was found between the kinship degree variable of the patients and only the trust sub-dimension, and between the marital status variable and the trust and outcomes sub-dimensions. In this context, these hypotheses were partially accepted. As predicted, no difference was found between the patients' sex variable and the Team Effectiveness Perception Scale and Outcomes scores. A significant difference was found between the Team Effectiveness Perception Scale and Outcomes scores and the patients' educational status, reason for hospitalization and clinic they were hospitalized in. According to the results, the Patient-PTE questionnaire was mostly accepted except for the degree of kinship and marital status variables. Similarly, no significant difference was determined between the male and female participants in the construct validity of the original questionnaire for patients. Unlike the results of the present study, in other studies, the marital status of the patients/families did not lead to a significant difference. However, variables such as clinical expertise, educational status and reason for consultation led to a difference (5).

A statistically significant difference was also found between the scores of healthcare providers from the PTE-Overall and Outcomes sub-dimensions, and it was observed that variables such as age, marital status, education level, type of profession and unit worked in caused differences. However,

variables related to the characteristics of the participants such as sex, years of work in the profession and institution did not cause a statistically significant difference in any dimension. In the construct validity study of the original questionnaire, the sex variable did not cause a statistically significant difference, while variables such as type of profession and time spent in the team caused a statistically significant difference (18). According to the results, the hypothesis determined for the Provider-PTE questionnaire was accepted only for sex, education level, type of profession and unit worked in healthcare workers. By evaluating these results, it was accepted that both questionnaires had known group validity.

In original questionnaires, sensitivity is defined as the ability of the tool to detect a meaningful change. Although there is no method on which a consensus has been reached to assess responsiveness, in questionnaires, it is hypothesized that there will be differences between low – and high-functioning teams.

In both questionnaires, low – and high-functioning teams were evaluated with their PTE scores. Significant differences have been determined between the low – and high-functioning teams regarding their scores for the trust, team processes, role clarity and outcomes sub-dimensions (5,18). In the present study, in the comparison made for the sensitivity of the questionnaires, a statistically significant difference was determined between the low – and high-functioning teams in all the sub-dimensions. This result, together with the findings of content and known group validity, shows that the scale is valid.

The reliability of the questionnaires is determined by the test-retest method and Cronbach's Alpha internal consistency coefficient. In the present study, the ICC values of the sub-dimensions of the questionnaires ranged between 0.75 and 0.95 for the Patient-PTE, between 0.86 and 0.95 for the Provider-PTE. Since the values were above 0.75, it was concluded that there was a good level of correlation between the two applications. While the Cronbach's Alpha coefficients of the subscales of the Patient-PTE ranged between 0.72 and 0.84 in the original study of the questionnaires, they ranged between 0.77 and 0.83 in the present study (5). As for the Cronbach's Alpha coefficients of the sub-dimensions of the Provider-PTE, they ranged between 0.79 and 0.92 both in the present study and in the original study of the questionnaires (18). Accordingly, the Cronbach's Alpha values of the questionnaires were determined as good and very good, suggesting that the questionnaires were reliable.

In the present study, the mean scores obtained from the PTE-Overall and Outcomes dimensions of the Patient-PTE and Provider-PTE were slightly above the average and close to each other. While the highest score was obtained from the trust sub-dimension of both the Patient-PTE and Provider-PTE, the lowest score obtained from the role clarity sub-dimension.

In Kilpatrick et al.'s study, while the Patient-PTE scores were similar to those in the present study, in the Provider-PTE, the lowest scores were obtained from the role clarity and team processes sub-dimensions (5,18). Trust is important in the relationship between patients and healthcare professionals. Patients who highly trust in healthcare professionals comply with the recommended care and treatment better, take their medications regularly, their chronic disease management improves, and they make better use of health services, including sticking to their appointments (25). Presence of trust between healthcare professionals and patients also affects patients' trust in the healthcare system. Trust between healthcare professionals is an important element for teamwork and interdisciplinary cooperation (26). Trust positively affects effective team communication, performance, job satisfaction and sense of citizenship (27,28). Trust reduces burnout levels, stress levels, absenteeism and staff turnover in employees. Trust helps employees understand their and other employees' roles within the team and helps individuals develop their roles. It also improves team processes (28). Role ambiguity whose is lower than other dimensions' creates uncertainty about what the job descriptions of healthcare professionals are, how they will achieve their performance targets and how their performance will be evaluated, which affects healthcare professionals' motivation, work commitment and job satisfaction (29). The results of the present study show that there is a need for initiatives to improve team processes and outcomes in healthcare, especially role ambiguity.

5. CONCLUSION

In the present study, the Patient-PTE and Provider-PTE were adapted into Turkish and determined as valid and reliable measurement tools. While the Patient-PTE can be used to assess patients' and their families' perspectives of team processes and perceived care outcomes, the Provider-PTE is used to assess interdisciplinary team processes and perceived care outcomes. Patients' and healthcare professionals' PTE should be assessed periodically, and the effect of the improvements made in line with the results obtained on the teamwork effectiveness should be measured and monitored as well. It is recommended that team effectiveness and outcomes should be monitored as the team structure and members change. By evaluating patients' PTE, its relationship with quality indicators such as patient safety, health outcomes, patient experience, patient satisfaction and loyalty can be investigated. By evaluating healthcare professionals' PTE, its relationship with variables such as performance, job satisfaction, intention to stay and organizational commitment can be investigated.

Acknowledgements: *This research was produced from a section of the doctoral thesis titled "Determination of the Effectiveness of Interdisciplinary Teamwork in Health Care Services" supervised by Assoc. Prof. Dr. Betül SÖNMEZ.*
Funding: *The authors received no spesific funding for this work.*
Conflicts of interest: *The authors report no actual or potential competing interest*

Ethics Committee Approval: *Permission was received via e-mail from the author who developed the surveys to adapt the surveys to Turkish. Ethics committee approval for the research was received from (Istanbul University-Cerrahpaşa) University Social Sciences and Humanities Research Ethics Committee (Date: 13.10.2020, Number: 134). The study was conducted in accordance with the Declaration of Helsinki. Written permission was obtained from the hospital where the research would be conducted. After the healthcare professionals and patients/families included in the sample of the study were informed about the purpose of the research, the participants who wanted to participate voluntarily were signed an Informed Consent Form.*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: DGH, BS
Design of the study: DGH, BS
Acquisition of data for the study: DGH
Analysis of data for the study: DGH, BS
Interpretation of data for the study: DGH, BS
Drafting the manuscript: DGH, BS
Revising it critically for important intellectual content: DGH, BS
Final approval of the version to be published: DGH, BS

REFERENCES

[1] Agha L, Ericson KM, Geissler KH, Rebitzer JB. Team relationships and performance: Evidence from healthcare referral networks. Management Science 2021;68(5):3735-3754. https://doi.org/10.1287/mnsc.2021.4091

[2] Walton V, Hogden A, Long JC, Johnson J, Greenfield D. Exploring interdisciplinary teamwork to support effective ward rounds. International Journal of Health Care Quality Assurance 2020,33(4/5):373-387. https://doi.org/10.1108/IJHCQA-10-2019-0178

[3] Aghamohammadi D, Dadkhah B, Aghamohammadi M. Nurse–physician collaboration and the professional autonomy of intensive care units nurses. Indian journal of critical care medicine 2019,23(4):178-181. https://doi.org/10.5005/jp-journals-10071-23149

[4] Kilpatrick K, Paquette L, Jabbour M, Tchouaket E, Fernandez N, Al Hakim G, Landry V, Gauthier N, Beaulieu MD, Dubois CA. Systematic review of the characteristics of brief team interventionsto clarify roles and improve functioningin healthcare teams. Plos One 2020,15(6):e0234416. https://doi.org/10.1371/journal.pone.0234416

[5] Kilpatrick K, Tchouaket É, Paquette L, Guillemette C, Jabbour M, Desmeules F, Landry V, Fernandez N. Measuring patient and family perceptions of team processes and outcomes in healthcare teams: Questionnaire development and psychometric evaluation. BMC Health Services Research 2019a,19(9): 1-16. https://doi.org/10.1186/s12913-018-3808-0

[6] Berber N, Slavić A, Aleksić M. Relationship between perceived teamwork effectiveness and team performance in banking sector of Serbia. Sustainability 2020,12(20):8753. https://doi.org/10.3390/su12208753

[7] Saygılı M, Özer Ö. The examnatıon of teamwork attıtudes ın healthcare professıonals . Süleyman Demirel Üniversitesi Vizyoner Dergisi 2020, 11(27):444-454. (Turkish)

[8] Kilpatrick K, Tchouaket E, Fernandez N, Jabbour M, Dubois CA, Paquette L, Landry V, Gauthier N, Beaulieu MD. Patient and family views of team functioning in primary healthcare teams with nurse practitioners: A survey of patient-reported experience and outcomes. BMC Fam Pract 2021,22(76): 1-14. https://doi.org/10.1186/s12875-021-01406-y

[9] Anderson JE, Lavelle M, Reedy G. Understanding adaptive teamwork in health care: Progress and future directions. Journal of Health Services Research & Policy 2021,26(3):208-214. https://doi.org/10.1177/1355819620978436

[10] Petit dit Dariel O, Cristofalo, P. A meta-ethnographic review of interprofessional teamwork in hospitals: What it is and why it doesn't happen more often. Journal of Health Services Research & Policy 2018,23(4):272-279. https://doi.org/10.1177/135581961878

[11] Van Dijk-de Vries AN, Duimel-Peeters IG, Muris JW, Wesseling GJ, Beusmans GH, Vrijhoef HJ. Effectiveness of teamwork in an integrated care setting for patients with COPD: Development and testing of a self-evaluation instrument for interprofessional teams. International Journal of Integrated Care 2016,16(1):1-10. https://doi.org/10.5334/ijic.2454

[12] Kash BA, Cheon O, Halzack NM, Miller TR. Measuring team effectiveness in the health care setting: An inventory of survey tools. Health Services Insights 2018,11:1-18. https://doi.org/10.1177/1178632918796230

[13] Campbell R, Ju, A, King MT, Rutherford, C. Perceived benefits and limitations of using patient-reported outcome measures in clinical practice with individual patients: a systematic review of qualitative studies. Quality of Life Research 2021, 1-24.

[14] Özsoy S, Bayık A, Uysal A, Ergül Ş, Özer M. Sağlık çalışanlarının sağlık hizmetlerinde ekip kavramına ilişkin görüşlerini incelenmesi. Sağlık ve Toplum Dergisi 2003,13(4):24-31. (Turkish)

[15] Öğüt A, Çetin D. Sağlık kurumlarında ekip çalışması. Selçuk Üniversitesi Sosyal ve Teknik Araştırmalar Dergisi 2011,1(1):87-96. (Turkish)

[16] Çıraklı Ü, Çelik Y, Beylik U. Etkili ekip çalışmasının sağlıktaki önemi ve faydaları: bir literatür çalışması. Sağlık Akademisyenleri Dergisi 2015,2(3):140-146 (Turkish). https://doi.org/10.5455/sad.2015131452108477

[17] Yardımcı F, Başbakkal Z, Beytut D, Muslu G, Ersun A. Ekip çalışması tutumları ölçeğinin geçerlilik ve güvenilirlik çalışması. Anadolu Psikiyatri Dergisi 2012,13(2):131-137. (Turkish)

[18] Kilpatrick K, Paquette L, Bird M, Jabbour M, Carter N, Tchouaket É. Team functioning and beliefs about team effectiveness in interprofessional teams: Questionnaire development and validation. Journal of Multidisciplinary Healthcare 2019b,12:827-839. https://doi.org/10.2147/JMDH.S218540

[19] Costello AB, Osborne J. Best practices in exploratory factor analysis: Four recommendations for getting the most from your analysis. Practical assessment, research, and evaluation 2019,10(1):7. https://doi.org/10.7275/jyj1-4868

[20] Wild D, Grove A, Martin M, Eremenco S, McElroy S, Verjee-Lorenz A, Ericson P. Principles of good practice for the translation and cultural adaptation process for Patient-Reported Outcomes (PRO) measures: Report of the ISPOR Task Force for Translationand Cultural Adaptation. Value in Health 2005,8:94-104. https://doi.org/10.1111/j.1524-4733.2005.04054.x

[21] Bolat E, Özmen D. Hemşirelikte Nezaketsizlik Ölçeği'nin Türkçe geçerlik ve güvenirlik çalışması. Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi 2019,22(4):250-260. https://doi.org/10.17049/ataunihem.454253 (Turkish)

[22] Tabachnick BG, Fidell LS. Using Multivariate Statistics (6nd ed.). Pearson; 2013.

[23] Byrne BM. Structural equation modeling with AMOS. Basic concepts, applications, and programming (2nd ed.).New York: Routledge; 2010.

[24] Erdoğan S, Nahcivan N, Esin MN. Hemşirelikte Araştırma Süreç, Uygulama ve Kritik. (2nd ed.). Nobel tıp Kitapevleri, İstanbul 2015. (Turkish)

[25] Greene J, Ramos C. A mixed methods examination of health care provider behaviors that build patients' trust. Patient Education and Counseling 2021,104(5):1222-1228. https://doi.org/10.1016/j.pec.2020.09.003

[26] Sutherland BL, Pecanac K, LaBorde TM, Bartels CM, Brennan MB. Good working relationships: How healthcare system proximity influences trust between healthcare workers. J Interprof Care 2022,36(3):331-339. https://doi.org/10.1080/13561820.2021.1920897

[27] Sifaki-Pistolla D, Melidoniotis E, Dey N, Chatzea VE. How trust affects performance of interprofessional health-care teams. Journal of Interprofessional Care 2020,34(2):218-224. https://doi.org/10.1080/13561820.2019.1631763

[28] Hamilton AL, Layden EA, Storrar N, Skinner J, Harden J, Wood M. Definition, measurement, precursors, and outcomes of trust within health care teams: A scoping review. Academic Medicine 2024,99(1):106-117. https://doi.org/10.1097/ACM.0000000000005320

[29] Chiara P, Luca C, Annalisa P, Chiara R. Emotional exhaustion among healthcare professionals: the effects of role ambiguity, work engagement and professional commitment. Acta Bio Med 2019,90 (6):60-67. https://doi.org/10.23750/abm.v90i6-S.8481

How to cite this article: Gündüz Hoşgör D, Sönmez B. Validity and Reliability of the Turkish Version of Team Effectiveness Questionnaires: Patient and Provider Perceptions. Clin Exp Health Sci 2025; 15: 137-146. https//doi.org/10.33808/clinexphealthsci.1483360

Clinical and Experimental Health Sciences

Experiences of Elderly Living Alone During the COVID-19 Pandemic: A Phenomenological Research

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Received: June 30, 2024 **Accepted:** February 26, 2025

ABSTRACT

Objective: While one of the groups most affected by the COVID-19 pandemic is elderly individuals, those living alone are more disadvantaged. This study aimed to explore the experiences of elderly individuals living alone during the COVID-19 pandemic and to address this process and the problems they experience with all aspects of health..

Methods: This study adopted a phenomenological approach and was conducted with 14 elderly people living alone. Individual in-depth face-to-face interviews were conducted using a semi-structured interview form. Content analysis was used for the data analysis.

Results: The main themes identified were i) the effects of pandemic restrictions, ii) the view of the elderly on pandemic rules, iii) physical, iv) mental and v) social health problems, vi) coping strategies of the elderly, vii) support received in problem-solving, viii) and solution suggestions from the elderly.

Conclusion: Pandemic restrictions in elderly individuals living alone have disrupted health checks and changes in daily life. The elderly experienced physical, mental and social health problems during the pandemic and developed coping strategies to combat the process. The experiences of the elderly and their suggestions should be taken into account in being prepared for any crisis that may affect the whole society, especially the elderly living alone.

Keywords: COVID-19, elderly, experience, living alone

1. INTRODUCTION

The elderly were one of the groups most affected by COVID-19 with high mortality and morbidity rates (1). In February 2021, fatal cases were 86% among people aged 65 and over (2). Therefore, strict restrictions were imposed on the elderly in Turkey, as in the rest of the world. The Ministry of Interior issued a circular on March 21, 2020, prohibiting individuals aged 65 and over from going out (3). Elderly individuals observed social distancing, minimized face-to-face interactions, avoided crowded areas, stayed at home, and faced travel restrictions (4).

During the COVID-19 pandemic, elderly individuals living alone felt social isolation more deeply and were more disadvantaged than those living with their families, children or grandchildren (5,6,7). In 2022, 26% of elderly individuals lived alone in Türkiye (8). Those living alone faced challenges during the lockdown, such as grocery shopping, accessing healthcare, and obtaining medicine, and were at risk of physical and psychological problems due to prolonged isolation (5,6). A study showed that elderly individuals living alone were the most affected by the pandemic, and that they experienced worsening orthopedic problems due to inactivity and not being able to go to periodic check-ups and taking their medications at the same dose for a long time. However, their religious beliefs helped them cope better (7). A study conducted in the USA revealed that elderly people living alone communicate less with others and are less likely to receive help and support (6). However, those living alone communicate more with their friends via phone and social media. It has been stated that those who communicate face-to-face have better psychosocial health and therefore those living alone are disadvantaged (6,9).

In one study, more than 70% of older adults practiced social distancing early in the pandemic and experienced challenges mostly due to limited social interactions and activities. They also reported psychosocial, health, financial, global environmental, and death anxiety concerns (10). Elderly individuals, especially those living alone, should be supported individually to address the feelings of loneliness, exclusion, uncertainty, anxiety, and stress caused by the pandemic (11).

https//doi.org/*10.33808/clinexphealthsci.1507625*

In future outbreaks, as seen during COVID-19, it is very important to understand the experiences and needs of elderly people living alone and to offer solutions. Studies have been conducted on elderly individuals during the COVID-19 pandemic in the literature (7,10), and two studies focused on those living alone (6,12). But no research has examined their experiences and health in a holistic, detailed and comprehensive manner.

1.1. Aim of The Study

This study aimed to explore the experiences of elderly individuals living alone during the COVID-19 pandemic and to address this process in all its aspects in terms of health.

1.2. Research Questions

- What are the problems experienced by elderly individuals living alone during the COVID-19 pandemic?
- In which area of health did elderly individuals living alone experience the most problems during the COVID-19 pandemic?

2. METHODS

2.1. Design and Setting

The study, conducted from November 2021 to March 2022, included elderly individuals registered at a Family Health Center in a province, living alone, and coming for various reasons (e.g. medication, examination, injection). This study was prepared according to the COREQ (Consolidated Criteria for Reporting Qualitative Research) (13) (Supplementary File 1) checklist. Ethical approval was obtained from Akdeniz University Clinical Research Ethics Committee (Date: 18.08.2021, Number: KAEK-568) and institutional permission from the Family Health Center. The female nurse researcher, who has a master's degree in Public Health Nursing, explained the purpose of the study to the participants before the interview and obtained written consent. Participants had no previous relationship with the researcher to avoid bias. Personal information forms were filled out by those who gave their consent and the interview schedule was determined. In-depth, semi-structured interviews were recorded with a voice recorder with the consent of the participants; those who did not accept the voice recording were recorded in writing. Content, functionality and questions were checked with a pre-test conducted with two suitable individuals not in the sample. Repeat interviews were not conducted with the participants and the Declaration of Helsinki was adhered to throughout the study.

2.2. Sample

In phenomenological studies, the focus and the amount of data are important in determining the sample size. Lincoln and Guba (1985) suggest that 12 participants may be sufficient if selected appropriately (14). The sample of this study consists of 14 elderly individuals (66-89 years old) living alone during the COVID-19 pandemic period and data saturation has been reached. Purposive sampling was used. Inclusion criteria were people aged 65 years and over living alone at home; Exclusion criteria were psychological disorders and conditions that hinder communication.

2.3. Data Collection and Instruments

Individual interviews with elderly individuals living alone were conducted in a quiet and well-lit room of the Family Health Center, in accordance with social distancing, masks and hygiene rules. The interviews lasted 45-60 minutes. Observational interviews, recommended as a data collection method in phenomenological studies, were conducted together with personal information form, semi-structured interview form and observation notes.

Personal Information Form: It consists of questions that inquire about the socio-demographic characteristics of the individual (age, gender, education level, perception of income level), presence of chronic disease, and proximity to the children who help him/her (4,6,7).

Semi-Structured Interview Form: It was prepared by the authors based on previous literature to determine the experiences of elderly individuals living alone during the COVID-19 pandemic and to investigate all aspects of health by focusing on them (7). Opinions of geriatrics and qualitative research experts were obtained. A preliminary application was made with two individuals who met the inclusion criteria (not in the sample) and the questions were finalized.

2.4. Data Analysis

Content analysis, based on the approach by Graneheim and Lundman (15), was used to explore the experiences of older people living alone during the pandemic. The interviews were transcribed verbatim and the transcripts were reviewed several times to gain insight. After two researchers independently coded the transcripts, they came together to compare codes, group them into subthemes, and develop themes. Two other researchers then independently assessed the codes (kappa analysis). Finally, all researchers met to finalize the themes and subthemes.

2.5. Validity and Reliability

Lincoln and Guba's criteria of reliability, confirmability, and transferability (16) were used to assess data accuracy. Validity and reliability were established according to Guba and Lincoln's criteria (17). After data saturation, interviews were recorded and stored on Google Drive. Purposive sampling ensured diversity in age, gender, education, income, and support networks. The research process, data collection, and analysis were described in terms of reproducibility. Semistructured questions were pretested and reviewed by the research team. In-depth interviews took place in a

quiet environment where participants could speak freely. Recordings were reviewed, codes were compared with raw data, and participants verified their statements to reduce bias. To verify external validity, two faculty members reviewed the code categories. Internal consistency was assessed using kappa analysis to strengthen reliability. Participants' statements were included to ensure transferability.

3. RESULTS

The average age of the participants was 76.1±7.1 years and 71.4% (n=10) were female. 57.2% had primary school education or less (n=8) and 71.4% had income that covered their expenses (n=10). 71.4% had chronic diseases, mostly hypertension, diabetes and cardiovascular diseases. 50% (n=7) had children who helped them, but none of them lived in the same apartment building. 14.3% (n=2) had children living in the same neighborhood.

Eight main themes emerged: i) the effects of pandemic restrictions, ii) elderly people's perspective on pandemic rules, iii) physical, iv) mental, v) social health problems, vi) coping strategies of the elderly, vii) support received in problem-solving, viii) solution suggestions from the elderly. Main themes and subthemes are shown in Figure 1, and explanatory quotes are shown in Table 1.

3.1. Main Theme 1: The Effects of Pandemic Restrictions

The effects of the pandemic restrictions were determined as i) disruption of health checks, ii) change of daily life, iii) lack of social support, iv) awareness/learning, v) insufficiency in physical activity, vi) financial inadequacy. Health checks were disrupted and daily life changed during the pandemic. Meetings were canceled due to the curfew, individuals were confined to their homes, physical activity decreased, they could not visit their neighbors or friends, and they had to shop in bulk. Lack of social support and not being able to get help from relatives for grocery shopping were another effect. Financial problems arose due to inflation, rent increases, unemployment, and low wages. However, the pandemic restrictions also had positive effects because individuals became more aware of their health and learned about issues such as nutrition, hygiene, and social distancing (Table 1).

3.2. Main Theme 2: Elderly People's Perspective on Pandemic Rules

Three sub-themes were generated: i) uncertainty perception regarding COVID-19, ii) supportive approach to restrictions, iii) critical approach to constraints. Participants expressed uncertainty about the outlook and lethal potential of the virus. While some believed that the pandemic restrictions were necessary, others felt that society was not complying with the Ministry of Health guidelines. Some criticized the restrictions, stating that the first 2 to 4 hours given to the elderly to go out were insufficient and too many restrictions were imposed on the elderly (Table 1).

3.3. Main Theme 3: Physical Health Problems

The subthemes under physical health problems were as follows: i) pain, ii) weight change, iii) weakness, iv) heart disease, v) forgetfulness. Elderly participants reported problems such as migraines, back pain, and leg pain. They became inactive, which led to weight gain. Many experienced fatigue/weakness after the COVID-19 vaccine. Forgetfulness also increased, and some had difficulty remembering whether they took their medications (Table 1).

3.4. Main Theme 4: Mental Health Problems

Five subthemes emerged under mental health issues: i) loneliness, ii) depression, iii) anxiety/stress, iv) fear, v) sadness. Elderly individuals felt lonely due to the lack of visitors and curfews, and loneliness was their biggest challenge. The pandemic disrupted their lives, leading to home confinement and depression. While some participants were worried about infecting their children and grandchildren when they were sick, others were afraid of being infected. Many were afraid of catching the virus, especially given its higher impact on the elderly and the increasing mortality rate. Participants also felt sad due to the unemployment of their loved ones and economic difficulties during the pandemic. One participant who lost her son expressed sadness at not being able to attend his funeral (Table 1).

3.5. Main Theme 5: Social Health Problems

The subthemes of social isolation and exclusion were determined as social health problems in elderly people living alone. Participants were confined to their homes, could not attend sociocultural activities, and this ended their social lives. Many also distanced themselves from society to avoid the virus. In addition, the perception among young people that "elderly individuals caught the virus" emerged, which led to their exclusion. Elderly people felt that society treated them as if they were spreading the virus (Table 1).

3.6. Main Theme 6: Coping Strategies of The Elderly

Five subthemes emerged: i) becoming a solution developer, ii) taking refuge in faith/spirituality, iii) use of mass media and social media, iv) home remedies, v) recreational activities. Some participants avoided public transportation to avoid exposure to the virus, preferred taxis, did physical activities on roofs, and carried spare masks. Most of them engaged in religious activities to cope with fear of death, depression, and stress, believed that God was the greatest healer, and prayed. Participants stayed in touch with friends and relatives through phone calls to combat loneliness. Some learned about their health from television, while others used home remedies for their health problems. Elderly individuals also engaged in leisure activities such as listening to Turkish classical music, attending courses, and traveling to address their psychosocial problems (Table 1).

Table 1. *Main themes and subthemes with illustrative quotations*

Main theme 1: The effects of pandemic restrictions	
Subthemes	**Illustrative quotations**
Disruption of health checks	"...it became a problem when my hospital work started. I already knew that I had cancer. I couldn't go for my check-ups due to the pandemic (P5)" "I did not have my sugar and cholesterol checked during this period. It's off, I don't know what my blood pressure is or anything. (P11)"
Change of daily life	"…. There were meetings and negotiations before. Every day, people were coming to me, and I was leaving. Now they are gone, they are gone. My travels and meetings were blocked (P7)" "Of course, I was affected a little badly during the period when the markets were closed and we could not go out... I could not go to my neighbors or friends... Living alone made everything difficult for me. We couldn't go out.'(P10)"
Lack of social support	"I was angry at my sister. I asked them to do the shopping for me, so I wouldn't go out too much, but they didn't come because there was a curfew. (P4)" "No one from my family helped me with the grocery shopping. It didn't help much. (P6)"
Insufficiency in physical activity	"I used to walk, now I can't walk (P3)" "I am a person who walks regularly, but I couldn't do them. (P11)" "Yes, the biggest challenge for me is the reduced range of motion.,... (P12)"
Financial inadequacy	"Economically, our economy was restricted, be it the house rent or my son's unemployment, and we suffered a lot from that. We are still shooting. (P10)" "No matter how much you cut your expenses, the salary you receive is not enough to live on. With the effect of the pandemic and inflation, we cannot buy anything, this is the truth. (P12)"
Awareness/Learning	"We learned to clean, we learned to eat healthier, we learned to take care of ourselves. (P11)" "... The positive contribution of the pandemic is that we are more careful. We need to stay away from dangers, pay attention to our distance, and be a little more conscious. If they said to wash your hands 20 times, I washed them 40 times. (P13)"
Main theme 2: Elderly people's perspective on pandemic rules	
Subthemes	**Illustrative quotations**
Uncertainty perception regarding COVID-19	"It is clear that this virus is neither a fly, worm, or insect. we don't know (P8)" "At first we didn't know how it was transmitted, they told us later, but I still can't understand it. 10 viruses knock down and kill a man weighing 150 kg... I cannot understand it, I mean I cannot understand the power of this virus. (P11)"
Supportive approach to restrictions	"I think whatever the Ministry of Health thought about the restrictions was good, but not everyone followed these restrictions. (P6)" "I swear, you know that the Scientific Board takes those rules. I don't think they did anything wrong... In some periods, curfews were increased, bans were imposed on entering crowded places such as cinemas, theaters and shopping malls, and markets were closed. Of course it was good to bring these.(P11)"
Critical approach to constraints	"Of course you can't go where you want, you can't run and get what you want. Let the time come. They gave me two or three hours at first. You're old, two hours will be over before you walk and get something and come back. I could never get over my fear of him. Time is short. (P3)" "In other words, it was limited to three or four hours and many restrictions were imposed on people over the age of 65. (P4)"
Main theme 3: Physical health problems	
Subthemes	**Illustrative quotations**
Pain	"She suffers from pain everywhere, from not being able to walk, from sitting all the time, and of course when she cannot move. (P3)" "Physically, my migraines increased, back pain and hernia increased when I sat too much. My legs cramped. (P14)"
Weight change	"I gained weight due to staying at home and sitting all the time. (P7)" "Since I couldn't walk... I gained 10 kilos during the closure period and I still can't lose those 10 kilos. Even though I'm careful about eating so much, I can't lose weight due to inactivity.... (P9)"
Weakness	"We are given this health injection, but it makes us a little sick. I can't get up, I can't help myself, I'm lying down (P3)" "Inactivity, diabetes, blood pressure, stress, vaccinations, I don't know, I have to lie down because of these, I have weakness and weakness. (P5)"
Heart disease	"I'm stuck, my heart is stuck. I thought I had a heart problem. (P4)" "After the vaccination, I felt stuck, my heart felt tight. It's just the tremors and stuff. (P6)"

Forgetfulness	"My forgetfulness increased during the pandemic (P7)" "I started to become forgetful and couldn't remember whether I had taken some of my medications or not.. I became very forgetful during the pandemic (P10)
Mian theme 4: Mental health problems	
Subthemes	**Illustrative quotations**
Loneliness	"The biggest challenge was loneliness. (P6)" "Living alone made everything difficult for me. We couldn't go out... That's just my problem, I'm overwhelmed with loneliness and I have to go out once a day..(P10)"
Depression/overwhelm	"We encountered so many negativities. This virus has turned our lives upside down and I still haven't gotten over the stress of it, I've fallen into depression. (P4)" "For the first three months, I felt depressed, unable to fit into the house, stuck, and imprisoned. I experienced that kind of depression. (P9)" "Since I cannot go out, mentally; 'There was a frequent state of depression and boredom (P14)"
Anxiety/stress	"Of course, you panic a little... I didn't go around young people much to worry about infecting them. I wonder if it can be transmitted or something? You know, we are old so it would be difficult for that reason. I wondered if the grandchildren would get sick? I didn't go to their house to protect them (P3)" "I was very stressed. I am always worried about whether I will be infected with the disease. (P9)"
Fear	"As he got older, he became afraid of death. 'I was scared because this disease affects the elderly more. (P6)" "I heard that the neighbor upstairs was sick with corona. I didn't go to his house. How should I go? If I leave, it will infect me too. I was afraid that something would happen to me. He became afraid of death because there was death on the horizon. (P7)"
Sadness	"My grandson's shop was closed during the pandemic and he was unemployed. I felt sorry for him. I feel sorry for people who are having financial difficulties. (P3)" "I lost a son in the pandemic. When my child died, I couldn't go to his funeral due to corona. I am really sad. It comes to my mind often and I feel sad (P8)"
Main theme 5: Social health problems	
Subthemes	**Illustrative quotations**
Social isolation	"The biggest challenge, of course, is being confined at home... I used to go to the choir, but I don't have a choir, I used to participate in events. I couldn't even play baglama. I couldn't go to social environments... (P4)" "Theaters, cinemas etc. have already been closed. I never went to public places, events, or shopping malls in fear of getting infected. For example, I stayed away from society. (P6)" ""I can never go out and meet my friends. My social life is over, I have nothing... When I was socially bored, I didn't do anything anymore, I had to get them, my friends, out of my mind. I was just calling. I had to sacrifice my friends because it was no longer possible for us to get together. (P9)"
Social exclusion	"At first, when restrictions were placed on people over the age of 65, everyone acted as if the elderly were carrying diseases. Everyone thought so. I was ostracized by society (P1)" "Unfortunately, when young and old people got on the bus, they acted as if they were transmitting the virus. We were excluded (P3)"
Main theme 6: Coping strategies of the elderly	
Subthemes	**Illustrative quotations**
Becoming a solution developer	"For example, I could not take public transportation or buses. I go everywhere by taxi... I went everywhere by taxi whenever I needed. (P5)" "I went up to the roof and walked there for an hour. (P11)" "I always have a spare clean mask in my pocket... (P13)"
Taking refuge in faith/ spirituality	"I thought that Allah is the greatest doctor... I constantly read prayers and surahs to get rid of the fear of death (P7)" "Before, ablution and prayer were few, now they are many. I fasted, read more Quran, and relaxed a little spiritually. My faith increased, I slowed down my soul, my resistance increased... 'I also prayed and read the Quran for spiritual depression and boredom (P14)"
Use of mass media and social media	"Since my family was abroad, we were constantly talking via video during the pandemic... I followed the special programs on television for my mental health. I was careful about my health by following the information given there. (P6)" "I took care of the phone in my hand, and that's how I established relationships with my friends... Phones became a tool for socialization (P12)"
Home remedies	"Sage tea is good for solving my physical problems. Apple cider vinegar and garlic are also very good. 'I treated myself with these (P7)" "I didn't go to the doctor when my arm hurt,... I tried to find a solution on my own by applying vixen and thyme oil. (P8)"
Recreational activities	"The pandemic still continues. Now I'm going to courses to keep myself busy. I'm going to Turkish classical music. When permission was given, I went to courses and trips. (P6)" "I embroider at home myself. I got through that period by doing things for the house... I talk and chat with my flowers (P9)" "If I have free time, I solve the riddles of newspapers... I have small sports equipment at home. I continue some physical movements.. (P12)"
Main theme 7: Support received in problem-solving	

Subthemes	Illustrative quotations
Getting support from family	"My son would call and ask. I also have a brother, I turn to them when I need something, if there is something I cannot do. They take care of it... When I couldn't get the medicine, my children bought it and brought it to me. (P1)" "My daughter was bringing a car full of my needs. Thanks to them, I didn't have much difficulty (P7)"
Getting support from friends/neighbors	"It's good that they were, my friends helped. For example, I could order from them and they brought what I wanted. When my heart sank, I informed my friends. They took me to the hospital (P4)" "My friends called me, my neighbors in Korkuteli also brought food. They helped me. (P7)"
Getting support from health professionals	"My own pharmacist brought my medications. At that time, he always brought pharmacists (P2)" "The nurse has now made an appointment for my waist. My family doctor helped me a lot with my health problems. (P10)"
Getting support from sellers	"When there was a curfew, the vendors brought bread to our door. Our milkman has arrived. We bought our milk, eggs and cheese from him... When I needed groceries, I made do with what I bought from vendors in the neighborhood such as fruits, vegetables and milk (P5)" "During the pandemic curfew, vendors in the neighborhood brought vegetables and fruits to our front door. (P8)"
Main theme 8: Solution suggestions from the elderly	
Subthemes	**Illustrative quotations**
Doing physical activity	"I think walking should not be abandoned (P1)" "I recommend Zumba for inactivity. It is important to move with music and exercise. (P9)"
Doing recreational activities	"I can recommend this. People can relieve their loneliness by keeping a few flowers at home. (P9)" 'He/she should definitely make use of that gap with activities such as reading books, newspapers, solving puzzles, doing sports, going to the sea. (P12)"
Complying with the recommendations of health professionals	"The world should be looked at, what the world is doing should be done, and the discourses of the Turkish Medical Association should be listened to (P4)" "We need to take the precautions told by healthcare professionals. We need to wear our masks and get our vaccines (P 5)"
Providing food aid	"...food distribution should be made by cars to people living alone (P4)" "As people living alone, we cannot go to the market. I wish they would distribute food. (P13)"
Providing home health care/creating elderly homes	"I would like home health care because I can't afford it... If only they would distribute panties and diapers to the elderly for urination incontinence. If home care could visit us during this process (P3)" "There are elderly homes abroad. In those old people's homes, everyone has their own room. A doctor or health services are provided every day. If they need to be washed, they are washed. If they have a health problem, they are listened to and a solution is found. I wish it were so. (P4)" "But I thought it would be nice to have something like home health care if I were to become paralyzed, if something happened, if I needed it. (P5)"

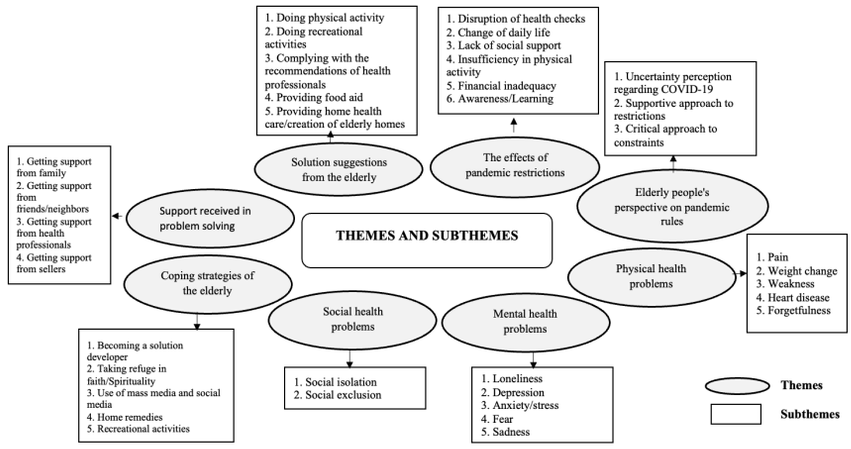

Figure 1. *Main themes and subthemes of the study*

3.7. Main Theme 7: Support Received in Problem-Solving

Participants received support for problem solving from i) family, ii) friends/neighbours, iii) healthcare professionals and iv) sellers. Elderly individuals reported that their children provided psychosocial support and helped them get medicine. Friends and neighbors helped them in emergencies and shopping. Healthcare professionals also supported them; pharmacists distributed medicines, nurses arranged appointments, and family doctors dealt with health problems. Due to the curfews, vendors solved shopping problems by delivering foods such as vegetables, fruits, milk, eggs, and cheese to the homes of the elderly (Table 1).

3.8. Main Theme 8: Solution Suggestions From The Elderly

Five sub-themes were identified: i) doing physical activity, ii) doing recreational activities, iii) complying with the recommendations of health professionals, iv) providing food aid, v) providing home health care/creation of elderly homes. The elderly suggested walking, Zumba and staying active. Participants suggested activities such as taking care of plants, reading, and solving puzzles in their free time. Following the recommendations of health professionals was another solution, and participants insisted on following global guidelines and the recommendations of the Ministry of Health. They also suggested distributing food, financial support such as underwear and diapers, and to establish home care services and elderly care homes (Table 1).

4. DISCUSSION

COVID-19 and social distancing have affected all segments of society, with older individuals being the most affected. During the pandemic, those living alone faced greater challenges (18). Using a phenomenological approach, we investigated the experiences of older individuals living alone, focusing on the effects of restrictions, their views on the rules, the health problems they faced, and their coping strategies. We also examined the support they received and the solutions they proposed. The discussion section is presented in accordance with the main themes.

4.1. The Effects of Pandemic Restrictions

Elderly individuals were allowed to go out only two days a week, which disrupted their health checks and social activities. Sometimes the curfews also applied to adults, leaving the elderly without support, especially for grocery shopping. Physical activities were limited and economic difficulties arose due to inflation. The only benefit of the restrictions was increased awareness of health protection. Similar to our findings, interruptions in health services during the pandemic affected elderly people with chronic diseases (19), and it was stated that elderly people needed help shopping and their physical activities decreased (12,20). A study conducted in the USA found financial difficulties that harmed mental health due to reduced income (21). Based on the literature and our findings, conditions should be created to ensure that health checks and physical activities continue, and support systems should assist with grocery shopping and medication purchases.

4.2. Elderly People's Perspective on Pandemic Rules

The elderly experienced uncertainty and confusion regarding the coronavirus. While some supported the pandemic rules, others stated that the restrictions were excessive. It was emphasized that the pandemic was an uncertain situation and that each elderly person reacted differently to it (22). Intolerance to uncertainty was high during the quarantine and worsened loneliness (23). Restrictive measures led to feelings of stigma and being seen as a source of disease (24). Social restrictions negatively affected the elderly physiologically, psychologically and economically, it was observed that they did not receive sufficient support and felt lonely (25). In one study, most elderly people found social isolation beneficial during the pandemic (26). Although there were studies with positive perceptions (26,27), there is more research showing the negative effects of the restrictions (22,25,28-30). In this regard, home care services should be provided and psychosocial and economic support programs should be created for the elderly to reduce the negative effects of restrictions and to raise awareness.

4.3. Physical Health Problems

Elderly individuals living alone have experienced physical problems such as pain, weight gain, fatigue, heart disease, and forgetfulness during the pandemic. Studies show that the pandemic restricts physical activity, encourages sedentary lifestyle, increases insomnia, and causes headaches and stomach aches (12,31,32). Pain, weight gain, and fatigue are linked to limited mobility, and pandemic stress may contribute to heart problems and forgetfulness. During the pandemic period, restrictions should be adjusted to encourage physical activity of older individuals and take initiatives to improve their mental health.

4.4. Mental Health Problems

Participants experienced mental health issues such as loneliness, depression, fear, and anxiety during the pandemic. Older adults experienced anxiety disorders, obsessive-compulsive disorder, and most importantly, loneliness (12,32,33). Restrictions on visits and lack of social contact increased isolation. This study suggests that loneliness, a major concern for older adults living alone, worsened during the pandemic. In addition, staying home increased depression, anxiety, and stress (34). Interventions are needed to reduce loneliness and depression and improve mental health.

4.5. Social Health Problems

During the pandemic, participants faced social isolation and exclusion. Elderly individuals have not been able to participate in sociocultural activities and have distanced themselves from society to prevent virus transmission. Due to concerns about the virus and curfew measures, their communication with their social environment has significantly decreased (12). Social distancing has become a form of isolation for the elderly (35,36,37). One study found that elderly people need socialization and have difficulty living alone (12). They have preferred to use their limited outdoor time more actively (34). Although curfews and distancing are essential to control the pandemic, they have separated elderly individuals from their families and friends and caused social problems. Initially, the restrictions created a false belief that elderly people could spread the virus, which led to their exclusion. Reliable sources (e.g., the Minister of Health, health workers) should correct these misconceptions.

4.6. Coping Strategies of The Elderly

Older people coped with the pandemic through religious beliefs, mass media, home remedies, and leisure activities. One study found that listening to the Quran helped their mental health despite a lower quality of life (38). Another study found that older people walked alone for their own and others' health (34). Home remedies were used for the prevention and treatment of COVID-19 (39), and leisure activities supported mental health (40). Encouraging social media use and phone/video connections can reduce isolation and increase cognitive stimulation (41). Religious activities benefit mental health, and complementary therapies can be beneficial alongside chronic disease medications with physician approval. Leisure activities also improve psychosocial health. Older people often get their health information from television and should be encouraged to consult with their health care providers. In future pandemics, reliable information from trusted sources should be made available to the public.

4.7. Support Received in Problem-Solving

During public health measures, basic needs such as food, water, and clothing were scarce, and many older people were deprived of routine medical care, medicines, and prescriptions (42). They received support for food, medicine, and psychosocial support from family, friends, healthcare professionals, and retailers. A study in the UK found that older people, especially those with dementia, were unable to access social support services, increasing anxiety and reducing quality of life (43). Another study found that their needs were mostly met by family and neighbors (12). In this study, older people did not receive support from local government or NGOs. Local government services are crucial to addressing the impacts of COVID-19, ensuring older people have access to healthcare, and improving quality of life.

4.8. Solution Suggestions From The Elderly

Participants recommended that older adults engage in physical and leisure activities during the pandemic and follow the advice of healthcare professionals, and emphasized the need for food assistance and home care services. It was suggested that information systems and technology should be used in future pandemics (44). Home remedies and professional health advice were seen as key to improving health (45). Another recommendation was to provide online videos, apps, and telehealth services to increase physical activity, cognitive activity, and quality of life (46). These recommendations should be prioritized for older individuals living alone, who are a vulnerable group in future pandemics.

4.9. Strength and Limitations

Individual face-to-face interviews during the pandemic provided detailed data. Although the study's strength is that it included individuals from all age groups, the majority of women may not fully represent the perspectives of both genders. Another limitation is that the study was conducted in only one region.

5. CONCLUSION

COVID-19 has become a global pandemic, and has made elderly people living alone more vulnerable. Restrictions disrupted health checks and routines, deteriorated their physical and psychosocial health, but they developed coping

strategies. Support came mostly from close circles and community resources were used very little.

Restrictions harmed the health of older people, highlighting the need for measures to protect and improve their well-being. These findings are important for understanding the experiences of older people living alone during the pandemic. Healthcare professionals should address the challenges faced by older people and provide home care and social services in future pandemics. Information/support programs using technology should be developed and post-pandemic older people's health should be reassessed.

Acknowledgements: *The authors would want to express gratitude to the health professionals at the Family Health Center as well as the elderly people who live alone. This study was presented as an oral presentation at the 16th EPH Conference in Dublin, Ireland held on 8-11 November 2023.*
Funding: *The authors received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Akdeniz University Clinical Research (Approval date: 18.08.2021; Number: KAEK-568)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: AI, ES, SG, SO
Design of the study: AI, ES, SG, SO
Acquisition of data for the study: ES
Analysis of data for the study: AI, ES, SG, SO
Interpretation of data for the study: AI, ES, SG, SO
Drafting the manuscript: AI, ES
Revising it critically for important intellectual content: AI, SG, SO
Final approval of the version to be published: AI

REFERENCES

[1] World Health Organization. Coronavirus disease (COVID-19): Risks and safety for older people. Published [01 December 2020]. Accessed [01 January 2024]. https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-risks-and-safety-for-older-people

[2] World Health Organization. COVID-19 weekly surveillance report. Published [05 March 2021]. Accessed [01January 2024]. https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/weekly-surveillance-report/weeklysurveillance-report-alt

[3] Ministry of Internal Affairs. Curfew circular for those aged 65 and over and those with chronic diseases. Published [01 December 2020]. Accessed [20 February 2021]. https://www.icisleri.gov.tr/65-yas-ve-ustu-ile-kronik-rahatsizligi-olanlara-sokaga-cikma-yasagi-genelgesi

[4] Tufan İ, Koç O, Barkın D, Gürdal FY, Ayan FS, Özgür Ö, Başıbüyük GÖ, Başıbüyük HH. The perspective of older people on "COVID-19 curfew": A phone survey. Journal of Geriatric Science 2020;3(2):51-59. https://doi.org/10.47141/geriatrik.755856

[5] Ek S, İlhanlı H, Kahraman SÖ. The weak ring of COVID-19: Elderly populationTurkish Geographical Review 2020;(76):33-44. https://doi.org/10.17211/tcd.809688

[6] Fingerman KL, Ng YT, Zhang S, Britt K, Colera G, Birditt KS, Charles ST. Living alone during COVID-19: Social contact and emotional well-being among older adults. The Journals of Gerontology: Series B. 2021;76(3):e116-e121. https://doi.org/0.1093/geronb/gbaa200

[7] Ercan M, Arıcı A. Bio-psycho-social effects of covid-19 pandemia process on elderly. JADEM. 2020;1(3):5-22.

[8] Turkish Statistical Institute. Seniors with statistics. Published [01 September 2022]. Accessed [01 January 2024]. https://data.tuik.gov.tr/Bulten/Index?p=Istatistiklerle-Yaslilar-2022-49667

[9] Teo AR, Choi H, Andrea SB, Valenstein M, Newsom JT, Dobscha SK, Zivin K. Does mode of contact with different types of social relationships predict depression in older adults? Evidence from a nationally representative survey. J Am Geriatr Soc. 2015;63(10):2014-2022, https://doi.org/10.1111/jgs.13667

[10] Heid A, Cartwright F, Wilson-Genderson M, Pruchno R. Challenges experienced by older people during the initial months of the COVID-19 pandemic. The Gerontologist 2021;61(1):48-58. https://doi.org/10.1093/geront/gnaa138

[11] Gencer N. Being elderly in Covid-19 process: Evaluations on curfew for 65-year-old and over citizens and spiritual social work. Turkish Journal of Social Work Research 2020;4(1):35-42.

[12] Tamkoç B, Kök H, Atalay Z. The experiences of the elderly living alone in Türkiye in the Covid-19 Process: The case of Ankara. Journal of Abant Social Sciences 2023;23(3):1315-1332. https://doi.org/10.11616/asbi.1265973

[13] Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. International Journal for Quality in Health Care 2007;19(6):349 – 357. https://doi.org/10.1093/intqhc/mzm042

[14] De Chesnay M. Nursing Research Using Phenomenology: Qualitative Designs And Methods in Nursing. USA: Springer Publishing Company; 2015.

[15] Graneheim UH, Lundman B. Qualitative content analysis in nursing research: Concepts, procedures and measures to achieve trustworthiness. Nurse Educ Today 2004;24(2):105-112, https://doi.org/10.1016/j.nedt.2003.10.001

[16] Lincoln Y, Guba E. Naturalistic inquiry. Lincoln and Guba's evaluative criteria. New York: Sage Publications; 1985.

[17] Guba EG, Lincoln YS. Epistemological and methodological bases of naturalistic inquiry. Educational Communication and Technology 1982;30(4):233–252. https://doi.org/10.1007/BF02765185

[18] Armitage R, Nellums LB. COVID-19 and the consequences of isolating the elderly. The Lancet Public Health 2020;5(5):e256. https://doi.org/10.1016/S2468-2667(20)30061-X

[19] Mistry SK, Ali AM, Yadav UN, Ghimire S, Hossain MB, Das Shuvo S, Saha M, Sarwar S, Nirob MH, Sekaran VC, Harris MF. Older adults with non-communicable chronic conditions and their health care access amid COVID-19 pandemic in Bangladesh: Findings from a cross-sectional study. PLoS One 2021;16(7):e0255534. https://doi.org/10.1371/journal.pone.0255534

[20] Oliveira MR, Sudati IP, Konzen VM, de Campos AC, Wibelinger LM, Correa C, Miguel FM, Silva RN, Borgi-Silva A. Covid-19 and the impact on the physical activity level of elderly people: A systematic review. Exp Gerontol. 2022;159:111675. https://doi.org/10.1016/j.exger.2021.111675

[21] Samuel LJ, Dwivedi P, Hladek M, Cudjoe TK, Drazich BF, Li Q, Szanton SL. The effect of COVID-19 pandemic-related financial challenges on mental health and well-being among

US older adults. Journal of the American Geriatrics Society. 2022;70(6):1629-1641. https://doi.org/10.1111/jgs.17808

[22] Del Valle MV, Andrés ML, Urquijo S, Yerro-Avincetto M, López-Morales H, Canet-Juric L. Intolerance of uncertainty over COVID-19 pandemic and its effect on anxiety and depressive symptoms. Interamerican Journal of Psychology 2020;54(2):e1335-e1335. https://doi.org/10.30849/ripijp.v54i2.1335

[23] Parlapani E, Holeva V, Nikopoulou VA, Sereslis K, Athanasiadou M, Godosidis A, Stephanou T, Diakogiannis I. Intolerance of uncertainty and loneliness in older adults during the COVID-19 pandemic. Frontiers in Psychiatry 2020;11:842. https://doi.org/10.3389/fpsyt.2020.00842

[24] Şimşek N, Albayrak E, Selvi Y, İzgi NG, Bektas O. Experiences of older people in the COVID-19 process towards stigmatization and anxiety: A qualitative study. Current Approaches in Psychiatry 2022;14(1):11-20. https://doi.org/10.18863/pgy.1005892

[25] Akkuş Y, Parlak AG, Karacan Y, Karatay G. Perceptions and experiences of older people regarding the COVID-19 pandemic process: a phenomenological study. Turkish Journal of Geriatrics. 2021;24(4):546-556.

[26] Kahraman B, Uğur TD, Girgin D, Koçak AB. Being old during COVID-19 era: problems experienced by individuals aged 65 and over during the pandemic process. Hacettepe University Journal of Faculty of Letters 2021;39(1):124-143. https://doi.org/10.32600/huefd.988467

[27] Magdy Mohammed Abd Elsalam R, El-Sakkar MAE-H. COVID-19 pandemic: The positives and negatives from older adults' perspective. Egyptian Journal of Health Care 2022;13(1):977-995.

[28] Banerjee D, Rao TS. The graying minority: Lived experiences and psychosocial challenges of older transgender adults during the COVID-19 pandemic in India, a qualitative exploration. Frontiers in Psychiatry 2021;11:604472. https://doi.org/10.3389/fpsyt.2020.604472

[29] McKinlay AR, Fancourt D, Burton A. A qualitative study about the mental health and wellbeing of older adults in the UK during the COVID-19 pandemic. BMC Geriatrics 2021;21:1-10. https://doi.org/10.1186/s12877-021-02367-8

[30] Derrer-Merk E, Ferson S, Mannis A, Bentall R, Bennett KM. Older people's family relationships in disequilibrium during the COVID-19 pandemic. What really matters? Ageing & Society 2022; 44(3):721-738. https://doi.org/10.1017/S0144686X22000435

[31] Yamada K, Yamaguchi S, Sato K, Fuji T, Ohe T. The COVID-19 outbreak limits physical activities and increases sedentary behavior: A possible secondary public health crisis for the elderly. J Orthop Sci. 2020;25(6):1093-1094. https://doi.org/10.1016/j.jos.2020.08.004

[32] Chakrawarty A, Ranjan P, Klanidhi KB, Kaur D, Sarkar S, Sahu A, Bhavesh M, Baitha U, Kumar A, Wig N. Psycho-social and behavioral impact of COVID-19 on middle-aged and elderly individuals: A qualitative study. J Educ Health Promot. 2021;10:269. https://doi.org/10.4103/jehp.jehp_1458_20

[33] Girdhar R, Srivastava V, Sethi S. Managing mental health issues among elderly during COVID-19 pandemic. Journal of Geriatric Care and Research 2020;7(1), 32-35.

[34] Kaplan K, Demir D. A qualitative study on elderly individuals participating in physical activity in limited free time during the Covid-19 pandemic. Journal of National Sport Sciences 2021;5(2):122-137.

[35] Altın Z. Elderly people in Covid-19 outbreak. Tepecik Training and Research Hospital Journal 2020;30(2):49-57.

[36] Pant S, Subedi M. Impact of COVID-19 on the elderly. Journal of Patan Academy of Health Sciences 2020;7(2):32-38. https://doi.org/10.3126/jpahs.v7i2.31104

[37] Cocuzzo B, Wrench A, O'Malley C. Effects of COVID-19 on older adults: Physical, mental, emotional, social, and financial problems seen and unseen. Cureus 2022;14(9):e29493. https://doi.org/10.7759/cureus.29493

[38] Fattah O, Zureigat A. The effect of holy Quran voice on the quality of life among the elderly during Coronavirus outbreak. International Journal of Psychosocial Rehabilitation 2020;24(8):4984-4993.

[39] Malapela RG, Thupayagale-Tshweneagae G, Baratedi WM. Use of home remedies for the treatment and prevention of coronavirus disease: An integrative review. Health Sci Rep. 2023;6(1):e900. https://doi.org/10.1002/hsr2.900

[40] Rivera-Torres S, Mpofu E, Jean Keller M, Ingman S. Older adults' mental health through leisure activities during COVID-19: A scoping review. Gerontology and Geriatric Medicine 2021;7:23337214211036776. https://doi.org/10.1177/23337214211036776

[41] İlgili Ö, Kutsal YG. Impact of Covid-19 among the elderly population. Turkish Journal of Geriatrics 2020;23(4):419-423. https://doi.org/10.31086/tjgeri.2020.179

[42] Brooks SK, Webster RK, Smith LE, Woodland L, Wessely S, Greenberg N, Rubin GJ. The psychological impact of quarantine and how to reduce it: Rapid review of the evidence. The Lancet 2020;395(10227):912-920. https://doi.org/10.1016/S0140-6736(20)30460-8

[43] Giebel C, Lord K, Cooper C, Shenton J, Cannon J, Pulford D, Shaw L, Gaughan A, Tetlow H, Butchard S, Limbert S, Callaghan S, Whittington R, Rogers C, Komuravelli A, Rajagopal M, Eley R, Watkins C, Downs M, Reilly S, Ward K, Corcoran R, Bennet K, Gabbay M. A UK survey of COVID-19 related social support closures and their effects on older people, people with dementia, and carers. Int J Geriatr Psychiatry 2021;36(3):393-402, https://doi.org/10.1002/gps.5434

[44] He W, Zhang Z, Li W. Information technology solutions, challenges, and suggestions for tackling the COVID-19 pandemic. International Journal of Information Management 2021;57:102287. https://doi.org/10.1016/j.ijinfomgt.2020.102287

[45] Banerjee D. Age and ageism in COVID-19: Elderly mental health-care vulnerabilities and needs. Asian J Psychiatr. 2020;51:102154. https://doi.org/10.1016/j.ajp.2020.102154

[46] Sepúlveda-Loyola W, Rodríguez-Sánchez I, Pérez-Rodríguez P, Ganz F, Torralba R, Oliveira DV, Rodríguez-Manas L. Impact of social isolation due to COVID-19 on health in older people: Mental and physical effects and recommendations. The Journal of Nutrition, Health and Aging 2020;24(9):938-947. https://doi.org/10.1007/s12603-020-1500-7

How to cite this article: Ilgaz A, Sert EN, Gözüm S, Öncel S. Experiences of Elderly Living Alone During the COVID-19 Pandemic: A Phenomenological Research. Clin Exp Health Sci 2025; 15: 147-156. https//doi.org/10.33808/clinexphealthsci.1507625

Clinical and Experimental Health Sciences

Turkish Validity and Reliability Study of the Trust in the Health Care Team Scale

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Received: June 3, 2024 **Accepted:** February 18, 2025

ABSTRACT

Objective: This study aimed to conduct the Turkish validity and reliability study of the Trust in Health Care Team Scale and to contribute to the literature by proposing a valid, reliable and easy-to-use measurement tool for researchers

Methods: This methodological study was conducted between March and May 2022, using the survey method, among 1013 people over the age of 18 who live in Konya and can read and write Turkish. The research was designed within the scope of the quantitative research model in terms of method. The first part consists of demographic characteristics and the second part consists of adapted version of the items. The data were analyzed using the SPSS and AMOS programs. First, the construct validity of the scale was performed followed by the content validity.

Results: As a result of the construct validity, it was found that the fit indices of the scale were at an acceptable level (X2/df=2.215; GFI=0.998; AGFI=0.997; NFI=0.998; RFI=0.997; RMR=0.043; SRMR=0.029). As a result of the reliability analysis, the Cronbach's alpha value was 0.975.

Conclusion: The results indicated that the Trust in the Health Care Team Scale has an adequate level of validity and reliability in Turkish culture to measure the level of trust in the health care team.

Keywords: Health Communication; Health Professional-Patient Relationship; Trust; Validation Studies

1. INTRODUCTION

Trust in health system and health care professionals is considered an important component of high-quality care, as well as achieving desired health outcomes (1-4). Trust is an optimistic acceptance of one's vulnerability, accompanied by a belief that one's own interests will be taken care of by physicians, health institutions or the system (5,6). There are multiple aspects of trust regarding the concept of health care in the literature, the aspects associated with trust are as follows: fidelity, honesty, confidentiality, competency, and general trust (6,7). Patients' trust in health professionals has rarely been examined in the literature. However, with the developments in information and communication technology, and the increase in the use of the Internet and social media, it has become easier for individuals to access information. The news and information published in the media, and in various platforms about issues such as physician negligence, incorrect treatment and practices, and medical errors attract the attention of the society, cause public to question the trust in health care providers (8).

In the last 20 years, research has proved that patients who trust more in their physicians and health service providers are more interested in their own care process, more likely to follow the recommended care and medications and generally are abler to control chronic conditions (9-13). An individual's trust in a health care provider is also related to better use of health care, including greater continuity of care with a provider, not delaying care, and keeping track of appointments (14). Conceptually, distrust in the health care system and health care professionals causes a delay in health care – seeking, which complicates the care process and often adversely affects patient outcomes (15). Delayed access to healthcare services causes patients to seek treatment at advanced stages, necessitating complex and costly treatments; this puts economic strain on the healthcare system as well as individuals. Chronic diseases such as cancer or diabetes, which can be prevented with early diagnosis, in particular, require intensive care and long-term treatment processes in their advanced stages. These additional costs consume more of the healthcare system's

https//doi.org/*10.33808/clinexphealthsci.1495337*

resources and put an extra burden on the budget. At the same time, when treatment is started late, the likelihood of the disease spreading to other organs increases, which reduces the quality of life of patients and creates a need for more social support. In order to reduce such risks, the importance of early diagnosis should be emphasized, regular health check-ups should be encouraged, and strategies that increase access to healthcare should be implemented. (15).

Many factors affect patients' trust in health professionals. These are as follows: health care workers allocating time for patient and relatives, listening to them, giving opportunity to ask questions, answering questions, providing information about diseases or conditions, as well as adapting a polite, patient, empathetic, and honest approach towards them, and attaching importance to their privacy (6,16,17). In addition, factors such as physician's gender, appearance, educational status, competencies, professional experience, communication skills, reputation, being a media figure, and social circle have also influence on patients' trust in professionals (18). Although it primarily depends on the attitude and behavior exhibited by the physician, trust regarding the physician-patient relationship is influenced by many factors. Various factors such as the situation patients are in, their expectations from treatment, physical and technological condition of the institution from which they purchase service, approaching policies toward patients, requirements for health care services, sociodemographic characteristics of patients, and expectations of physicians towards their relationship with patients may influence the level of trust.

Recent systematic reviews emphasize concerns about the number of measurement tools used in order to assess the level of trust and distrust in health care institutions and professionals, in addition to quantitative and qualitative validity of existing instruments (19-21). A separate concern is that current measures do not adequately capture all relevant dimensions of trust. Based on a systematic review of the criteria used to assess trust in a health system, the Health Systems Trust Content Domain Framework was developed to conceptualize trust (20). The review concluded that some key constructs, such as fairness, are rarely included in confidence measures. Similarly, recent literature suggests that patient-provider communication is a critical component of trust, but communication is rarely the focus of current measures (22). Also, most existing scales measure trust as a one-dimensional construct, but previous research shows that trust is complex and consists of multiple dimensions (20,22). Therefore, current measures do not allow researchers to assess certain dimensions of trust. For example, in studies focused on health equity, it is crucial to evaluate aspects such as fairness and impartiality in the treatment of patients, which may not be adequately captured by existing measurement tools. This scale, on the other hand, includes the most comprehensive trust measures ever developed, based on the Health Systems Trust Content Analysis created by Ozawa and Sripad (20). It is seen that trust studies conducted in the field of health in Turkey are very limited. It is essential to better understand what constitutes patients' trust in health care providers, and to take proper measures based on the outcomes. Since no valid and reliable studies have been found to measure individuals' level of trust in health care professionals and factors affecting the formation of trust, the current study is considered important in terms of filling a significant gap in the literature. In this study, it was aimed to perform Turkish validity and reliability of the Trust in Health Care Team Scale, developed by Richmond et al., and to contribute to the literature through suggesting a valid, reliable and easy to use measure instrument for researchers (23).

2. METHOD

2.1. Study Design

This methodological study is a cross-sectional study and was conducted between March and May 2022 in Konya province with individuals over the age of 18 who can read and write Turkish through online platforms. The research was designed within the scope of the quantitative research model in terms of method. Methodological studies are defined as research on data editing and analysis methods designed to evaluate and validate research instruments and techniques (24). The survey prepared at docs.Google.com/forms was sent to individuals over the age of 18 via online tools (e-mail, WhatsApp, Facebook and Instagram). The survey application time was approximately 10 minutes. A detailed information letter was written before the online survey was prepared. An explanation was made about the research and the surveys were given to them. The inclusion criteria for the research were individuals over the age of 18 who can read and write Turkish, live in Konya and have used healthcare services in the last six months. The exclusion criteria are, first of all, individuals under the age of 18 are excluded from the scope of the research. In addition, individuals who have difficulty reading or writing Turkish are also excluded, because this situation may prevent them from reaching the level of communication and understanding required by the research. Individuals who have not received healthcare services in the last six months are also not included in the research, because the target of the study is individuals with healthcare experience.

2.2. Participants

In validity and reliability studies, 10 times the number of items is considered adequate to determine the sample size (25). In this context, considering that the scale consists of 29 items, at least 290 people are considered to constitute an adequate sample size. Individuals who are over the age of 18 and can read and write in Turkish were included in the study. Within the scope of the research, the convenience sampling method was used, and 1013 people were reached.

2.3. Instrument

Personal information form (eight questions about socio-demographic characteristics such as age, gender, marital status, educational status, health institution preference, income status), and the "Trust in the Health Care Team Scale" were used to collect the data. The scale consists of 29 items and 7 subscales. The subscales are as follows: Communication Competency (items 1,2,3,4, and 5), Fidelity (item 6,7,8,9, and 10), Systems Trust (items 11,12, and 13), Confidentiality (items 14, 15, and 16), Fairness (items 17, 18, 19, 20, 21, 22, and 23), Stigma-Based Discrimination (items 24, 25 and 26), and Global Trust (items 27, 28, and 29). The participants were asked to mark the most suitable option ranging from "1-strongly disagree, 2-Disagree, 3-Neutral, 4 – Agree to 5-strongly agree".

2.4. Procedure and Statistical Analyses

The data were analyzed using package programs of SPSS 26.0 and AMOS 24.0. For quantitative variables, mean, standard deviation, and median were calculated, while number and percentage were used for qualitative variables. In the study, language validity, content validity, and construct validity were conducted regarding the scale validity.

In order to test the language validity of the scale, the translation-reverse translation method was used. The language validity was achieved by comparing the items of the original scale and the adaptation, thus ensuring semantic equality. The scale was translated into Turkish without establishing any changes by six different academicians who were experts in the field. A common text in target language was created after the evaluation of all translations. The translated version was retranslated into English by a different expert independently of the previous translators, who had a command of the culture of the country where the scale was developed. The original scale and Turkish translation were assessed for language equivalence, and the final form of the scale was determined in order to submit for expert opinion. After it was decided that there was no difference in meaning in the expressions, a preliminary application was made to 10 people in order to test the intelligibility of the items.

After the necessary corrections were made through the pilot application, the content validity was established. To test whether a scale is suitable for a new language, construct and content validity should be ensured (26). Therefore, the content validity of the scale items was calculated using the Kendall W Test. Academician who are experts in their fields were asked to evaluate the suitability of each scale item in terms of content and language on a range from 1 to 4 (1 point: inappropriate; 2 points: somewhat appropriate/requires revision; 3 points: right but requires small changes; 4 points: very reasonable). The percentage value of the answers reporting the appropriateness of each item was calculated with the scores given by each expert to the statements. When the responses of six experts were analyzed with Kendall's W test for the comprehensive items' comprehensibility, simplicity, and correlational validity, there was no statistical difference between the scale items and expert opinions (Kendall's W= 0.060; p= 0.526 > 0.05).

Construct validity analysis was performed in the second phase of the study. Exploratory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA) were used to determine the construct validity. Kaiser Meyer Olkin (KMO) and Bartlett's test were used for factor analysis suitability. The KMO test is used to assess the validity of factor analysis and measures the partial correlation between variables. The closer the KMO value is to 1.0, the higher the suitability of the data set for factor analysis. Bartlett's test tests the significance of the correlation matrix and determines whether there is a significant relationship between the variables. These two tests are important to assess the suitability of the data prior to factor analysis. Acceptable factor values for EFA should be 0.60 and above (27). The value obtained by dividing the chi-square into degree of freedom below the value of 3, RMSEA value of 0.08 or lower, NNFI, CFI and NFI values over 0.90, RMR value close to zero, and GFI and AGFI values close to 1 shows that the model is strong (28,29). Divergent and convergent validity were assessed using the average variance extracted (AVE), construct reliability (CR), and the square of correlation. In order to determine the reliability, the Cronbach's alpha coefficient and item-total score correlation were calculated to test the internal consistency. A p value of < 0.05 was set for the analyses. The Cronbach's alpha coefficient was used to evaluate the internal consistency. Values above 0.70 are considered acceptable, while those above 0.80 indicate good internal consistency (30).

2.5. Ethics

Permission was obtained via email from the authors in order to perform Turkish adaptation of the Trust in the Health Care Team Scale, which was originally developed in English. The participants were informed about the research and the volunteer application form was provided on the online system, and the data of those who approved the form were included in the study. In addition, ethical approval was granted by Non-Interventional Clinical Research Ethics Committee of Selçuk University, Faculty of Health Sciences, dated 31/03/2022 and numbered 2022/275.

3. RESULTS

Descriptive findings of the participants, exploratory factor analysis, confirmatory factor analysis, reliability analysis and correlation findings of the scales were given in this part.

As given in Table 1, the average age of the participants was 34.76±13.59 years. Of the participants, 605 (59.7%) are female and 408 (40.3%) are male. 493 (48.7%) are married, 481 (47.5%) are single, and 395 (39.0%) have a bachelor's degree. Of the participants, 495 (48.9%) prefer public hospitals, 572 (56.5%) have moderate income status, 650 (64.2%) have insurance, and 443 (43.7%) have applied to the doctor 1 to 2 times in the last 6 months.

The adjusted item-total correlation values were examined prior to performing EFA for the Trust in the Health Care Team Scale, and it was determined that the lowest value was 0.551, and the analyses were continued without removing any items. EFA analyses were performed using principal component analysis and varimax rotation methods.

Table 1. *Descriptive Findings Regarding the Demographic Characteristics of the Participants*

		n	%
Age	Avg ±SD (34.76±13.59)		
Gender	Male	408	40.3
	Female	605	59.7
Marital Status	Married	493	48.7
	Single	481	47.5
	Living separately from his/her spouse' or 'spouse deceased	39	3.8
Educational Status	Primary	51	5.0
	High School	271	26.8
	Associate Degree	154	15.2
	Bachelor's Degree	395	39.0
	Postgraduate	142	14.0
Preferred Health Care Institution	Family physician	66	6.5
	Private hospital	246	24.3
	Public hospital	495	48.9
	University hospital	206	20.3
Income Status	Very low	19	1.9
	Low	69	6.8
	Middle	572	56.5
	Good	294	29.0
	Very good	59	5.8
Insurance Status	Yes	650	64.2
	No	363	35.8
Frequency of Visits to the Doctor in the Last 6 Months	Never	135	13.3
	1 to 2 times	443	43.7
	3 to 6 times	275	27.1
	More than 6 times	160	15.8
	Total	**1013**	**100**

When the KMO (0.965) and Bartlett's sphericity test values ($\chi 2(300) = 28978.409$; $p < .01$) were examined according to the EFA results of the Trust in the Health Care Team Scale, the data were found to be suitable for analysis. It was seen that the items "people working in health services can conduct experiments (scientific research) on patients without their knowledge", and "people working in health services respect patients' confidentiality" were in different subscales, and were removed from the analysis, respectively. As a result of the analysis conducted with 27 items, the items "people working in health services keep medical records confidential" and "people working in health services use secure systems to store medical records" were found to be overlapping. Both items were excluded from the analysis since "Privacy" subscale would not be available in case of excluding either of the items. The final version of the scale consists of 6 subscales and 25 items. The Justice subscale explained 21.26% of the variance, while Communication Competency explained 17.26%, Fidelity explained 13.78%, Stamping-Based Discrimination explained 11.80%, System Trust explained 10.97%, Global Trust explained 7.95%, and the total explained variance was 83.03% (Table 2).

According to the scale structure that emerged after EFA, second level CFA was performed. When the Figure 1, including CFA results of the Trust in the Health Care Team Scale was examined, it was found that the fit indices were at an acceptable level ($X^2/df=2.215$; GFI=0.998; AGFI=0.997; NFI=0.998; RFI=0.997; RMR=0.043; SRMR=0.029). Factor loads were statistically significant ($p < .05$). These findings indicate that the scale is a valid instrument compatible with the data.

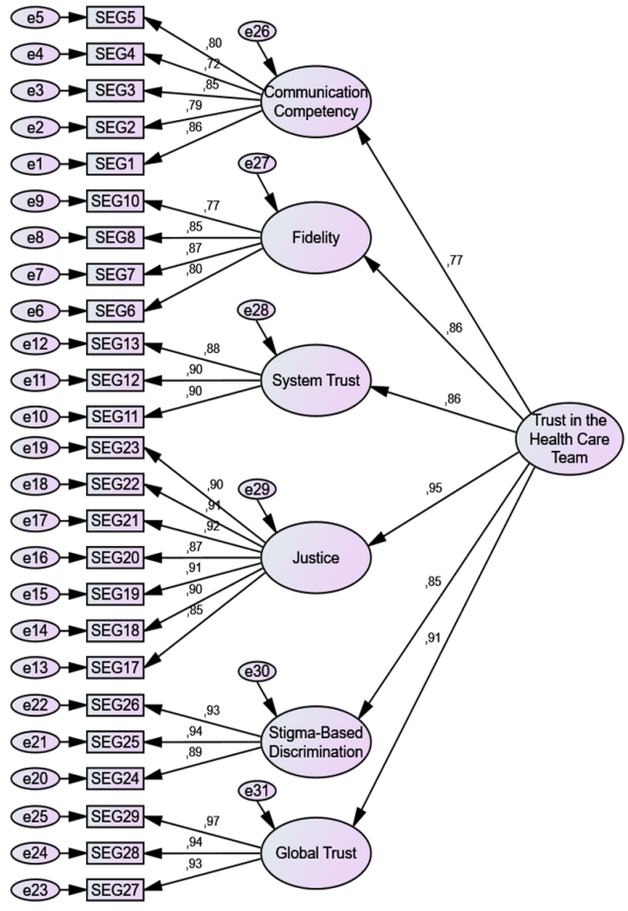

Figure 1. *CFA Model of the Trust in the Health Care Team Scale*

Results of descriptive analysis, correlation analysis, reliability, and convergent and divergent validity related to the Trust in the Health Care Team Scale were given in Table 3. The AVE value of the scale dimensions of Trust in the Health Care Team greater than 0.500, and the CR value greater than 0.700 indicate convergent validity. When the results were examined, it was seen that the AVE value for any of the two subscales was greater than the square of their correlation, and it was determined that the scale had divergent validity (30). According to the CA values, the scale and subscales were found to be reliable.

Table 2. *Descriptive Factor Analysis Results of the Trust in the Health Care Team Scale*

The Trust in the Health Care Team Scale	F1	F2	F3	F4	F5	F6
People working in health services treat patients fairly regardless of their sexual orientation	**0.740**	0.237	0.289	0.216	0.265	0.151
People working in health services treat patients fairly regardless of their gender	**0.740**	0.270	0.235	0.303	0.231	0.241
People working in health services treat each patient fairly regardless of their race and ethnicity	**0.717**	0.368	0.307	0.218	0.207	0.140
People working in health services treat patients fairly regardless of their religious beliefs	**0.717**	0.234	0.210	0.356	0.317	0.203
People working in the health services treat patients fairly regardless of their educational status	**0.715**	0.304	0.287	0.255	0.244	0.176
People working in health services treat patients fairly regardless of their weight	**0.714**	0.286	0.196	0.324	0.250	0.287
People working in health services treat patients fairly regardless of their financial status	**0.593**	0.348	0.366	0.122	0.273	0.223
People working in health care believe patients when they say something is wrong	0.141	**0.787**	0.214	0.055	0.216	-0.013
People working in health services explain the benefits and risks of treatments to patients	0.196	**0.787**	0.133	0.196	0.072	0.182
People working in health services listen to patients	0.240	**0.776**	0.204	0.138	0.092	0.225
People working in health services have common sense	0.284	**0.723**	0.201	0.219	0.029	0.222
People working in health services follow up patients when necessary	0.272	**0.704**	0.096	0.098	0.324	0.084
People working in health care recommend expensive treatments to earn money	0.327	0.190	**0.726**	0.252	0.150	0.241
People working in health services keep appointment durations short	0.183	0.270	**0.713**	0.230	0.291	0.016
For people working in healthcare, making money is more important than patients' needs	0.273	0.146	**0.712**	0.254	0.113	0.291
People working in health services hide their mistakes	0.284	0.290	**0.708**	0.235	0.232	0.082
People working in health services treat patients diagnosed with HIV (AIDS) unfairly	0.358	0.204	0.311	**0.764**	0.246	0.122
People working in health services treat patients with a mental health history unfairly	0.303	0.185	0.307	**0.753**	0.202	0.187
People working in health services treat patients with substance use unfairly	0.320	0.237	0.322	**0.752**	0.199	0.172
People working in health services are held responsible if they do not treat patients fairly	0.324	0.227	0.277	0.217	**0.770**	0.173
People working in health services are held responsible for discriminating against patients	0.373	0.227	0.280	0.170	**0.736**	0.142
People working in health care services are held responsible in case of mistakes	0.346	0.200	0.199	0.352	**0.660**	0.263
I believe that I can trust people working in health care	0.422	0.335	0.272	0.246	0.273	**0.653**
When I think about everything, I trust people who work in health care	0.423	0.324	0.267	0.237	0.279	**0.652**
People providing health care services are reliable	0.459	0.365	0.282	0.256	0.278	**0.585**
Self-values	15.739	1.679	1.103	0.865	0.760	0.612
Variance explained (%)	21.26	17.26	13.78	11.80	10.97	7.95
The total variance explained (%)	21.26	38.52	52.30	64.10	75.08	83.03

Table 3. *Correlational Findings of the Subscales*

Subscales	1	2	3	4	5	6	7
1. CC	-	0.352	0.336	0.470	0.305	0.471	0.592
2. F	0,594**	-	0.465	0.555	0.534	0.505	0.744
3. ST	0,580**	0,682**	-	0.611	0.470	0.541	0.748
4. J	0,686**	0,745**	0,782**	-	0.579	0.710	0.863
5. SBD	0,553**	0,731**	0,686**	0,761**	-	0.504	0.741
6. GT	0,687**	0,711**	0,736**	0,843**	0,710**	-	0.815
7. THCT	0,770**	0,863**	0,865**	0,929**	0,861**	0,903**	-
Average	3.630	3.145	3.491	3.563	3.595	3.586	3.502
Std Deviation	0.865	1.148	1.173	1.133	1.167	1.147	0.961
AVE	0.645	0.681	0.801	0.800	0.851	0.900	0.768
CR	0.900	0.895	0.923	0.965	0.945	0.964	0.988
CA	0.899	0.894	0.923	0.965	0.945	0.964	0.975

CC = Communication Competency; F= Fidelity; ST = System Trust; J= Justice; SBD = Stigma-Based Discrimination; GT = Global Trust; THCT = Trust in the Health Care Team; AVE = Average Variance Extracted; CR = Construct Reliability; CA = Cronbach's Alpha

The values below the diagonal show the correlation between the factors, while the values above show the square of the correlation.

***p < .01*

4. DISCUSSION

The study was carried out in order to examine the Turkish validity and reliability of the "Trust in the Health Care Team Scale", developed by Richmond et al. (23), and to measure adult individuals' level of trust in the health care team in health institutions that provide patient-centered care and work environment for large number of health care professionals working in cooperation. Given that the internal consistency level was required to be greater than 0.80 for measurement instruments, the reliability of the Turkish adaptation (CA= 0.975) was provided (31). Additionally, as a result of the item analysis, it was found that the item-total correlation coefficients of the scale met the minimum criterion of 0.50. The scale was found to be adequate in terms of item-test correlations (32). In the construct validity analysis of the scale, the model fit, which was found using the descriptive analysis, was tested with the confirmatory factor analysis. Although no conclusion has been established on which fit indices are considered in the statistics calculated for model-data fit with confirmatory factor analysis, the index values of X2/df, RMSEA, SRMR, GFI, TLI, CFI, etc. are provided (32). The original scale fit index values were X2/df= 1.613; RMSEA= 0.065; SRMR= 0.03 and CFI= 0.98, while the fit index values in the current study were found X2/df=2.215; GFI=0.998; AGFI=0.997; NFI=0.998; RFI=0.997; RMR=0.043; SRMR=0.029. When the fit indices were examined, it was found that the six-dimensional model provided acceptable fit, and the original factor structure of the scale was found to be compatible with the Turkish adaptation, except items 9, 14, 15 and 16. Therefore, the model consisting of 25 items and

six subscales was found to be theoretically and statistically significant, and according to the results of the reliability and validity studies, the Trust in the Health Care Team Scale is an applicable instrument.

5. CONCLUSION

Today, it is observed that patients' trust towards healthcare providers has decreased. Insecurity can cause loss of time and money by going from hospital to hospital. In medical applications where not harming the patient and providing the patient with the highest benefit is a priority, the patient's trust in the healthcare team is important in terms of commitment to the healthcare provider or institution. In determining the quality of the health service received, studies should be carried out to evaluate the trust of the patients in the health care team. It can be used as a criterion in the evaluation of health services. Periodically repeated evaluations will be an important topic for both hospital administrators and policy makers in the future. It is extremely important to determine the reasons that affect the trust levels of the patients in the health care team in health institutions and to make improvements for these reasons in order for the hospitals to provide sustainable and quality services.

There are many measurement tools in the literature that measure trust in the health system, health institution and health professionals. Most of the existing scales measure trust as a single-dimensional construct; however, previous studies have shown that trust is complex and consists of multiple dimensions. Therefore, the existing measures do not provide researchers with the opportunity to assess specific dimensions of trust that may be particularly relevant to the research questions (e.g., justice in health equity studies). This scale, whose validity and reliability we measured, was designed to comprehensively assess various dimensions of trust. As a result of the validity and reliability analyses, it was revealed that the scale is an important tool in understanding the relationship between health professionals and patients. The strength of the study is that it provides researchers with the opportunity to measure trust in all its dimensions.

However, among the limitations of the study is that the focus of trust relationships may differ according to the health service delivery model. Various health systems have distinct structures, practices, and cultural contexts that can influence how trust is established and perceived. For instance, in systems where patient-centered care is prioritized, trust dynamics may be more pronounced compared to models with a more hierarchical approach. Additionally, since this study was conducted in Turkey, it only measures trust in health teams working within the Turkish health system. This geographical and contextual specificity means that the findings may not be generalizable to other countries or health care systems, where different socio-economic, political, and cultural factors might affect trust levels. Consequently, the level of trust may vary significantly in different health service delivery systems, and further research in diverse settings is necessary to understand these variations comprehensively.

Funding: *The author received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Selcuk University, Noninvasive Clinic Ethics Committee (Approval date:31/03/2022.; Number: 2022/275)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: EF, HE, SÜ, ŞK
Design of the study: EF, HE, SÜ, ŞK
Acquisition of data for the study: EF, HE, SÜ, ŞK
Analysis of data for the study: EF, HE, SÜ, ŞK
Interpretation of data for the study: EF, HE, SÜ, ŞK
Drafting the manuscript: EF, HE, SÜ, ŞK
Revising it critically for important intellectual content: EF, HE, SÜ, ŞK
Final approval of the version to be published: EF, HE, SÜ, ŞK

REFERENCES

[1] Gilson L. Trust and the development of health care as a social institution. Soc Sci Med. 2003;56(7):1453-1468. https://doi.org/10.1016/S0277-9536(02)00142-9

[2] Brennan N, Barnes R, Calnan M, Corrigan O, Dieppe P, Entwistle V. Trust in the health-care provider–patient relationship: A systematic mapping review of the evidence base. Int J Qual Health Care. 2013;25(6):682-688. https://doi.org/10.1093/intqhc/mzt063

[3] Lee YY, Lin JL. How much does trust really matter? A study of the longitudinal effects of trust and decision-making preferences on diabetic patient outcomes. Patient Educ Couns. 2011;85(3):406-412. https://doi.org/10.1016/j.pec.2010.12.005

[4] Kelley JM, Kraft-Todd G, Schapira L, Kossowsky J, Riess H. The influence of the patient-clinician relationship on healthcare outcomes: A systematic review and meta-analysis of randomized controlled trials. PLoS One. 2014;9(6). https://doi.org/10.1371/journal.pone.0094207

[5] Giordano GN, Lindström M. Trust and health: Testing the reverse causality hypothesis. J Epidemiol Community Health. 2016;70(1):10-16. https://doi.org/10.1136/jech-2015-205822

[6] Hall MA, Zheng B, Dugan E, Camacho F, Kidd KE, Mishra A, Balkrishnan R. Measuring patients' trust in their primary care providers. Med Care Res Rev. 2002;59(3):293-318. https://doi.org/10.1177/1077558702059003004

[7] Nikodem K, Ćurković M, Borovečki A. Trust in the healthcare system and physicians in Croatia: A survey of the general population. Int J Environ Res Public Health. 2022;19(2):993. https://doi.org/10.3390/ijerph19020993

[8] Gülcemal E, Keklik B. A study on the investigation of factors affecting patients' confidence in physicians: The case of Isparta Province. Mehmet Akif Ersoy University Journal of Social Sciences Institute. 2016;8(14):64-87. https://doi.org/10.20875/sb.66346

[9] Braksmajer A, Fedor TM, Chen SR, Corales R, Holt S, Valenti W, McMahon JM. Willingness to take PrEP for HIV prevention: The combined effects of race/ethnicity and provider trust. AIDS Educ Prev. 2018;30(1):1-12. https://doi.org/10.1521/aeap.2018.30.1.1

[10] Gupta S, Brenner AT, Ratanawongsa N, Inadomi JM. Patient trust in physician influences colorectal cancer screening in

low-income patients. Am J Prev Med. 2014;47(4):417-423. https://doi.org/10.1016/j.amepre.2014.04.020

[11] Ratanawongsa N, Karter AJ, Parker MM, Lyles CR, Heisler M, Moffet HH, Schillinger D. Communication and medication refill adherence: The diabetes study of Northern California. JAMA Intern Med. 2013;173(3):210-218. https://doi.org/10.1001/jamainternmed.2013.1216

[12] Schoenthaler A, Montague E, Baier Manwell R, Brown R, Schwartz MD, Linzer M. Patient–physician racial/ethnic concordance and blood pressure control: The role of trust and medication adherence. Ethnicity & Health. 2014;19(5):565-578. https://doi.org/10.1080/13557858.2013.857764

[13] Fernandez A, Seligman H, Quan J, Stern RJ, Jacobs EA. Associations between aspects of culturally competent care and clinical outcomes among patients with diabetes. Med Care. 2012;74-79. https://doi.org/10.1097/MLR.0b013e3182641110

[14] Hillen MA, Butow PN, Tattersall MH, Hruby G, Boyle FM, Vardy J, Smets EM. Validation of the English version of the Trust in Oncologist Scale (TIOS). Patient Educ Couns. 2013;91(1):25-28. https://doi.org/10.1016/j.pec.2012.11.004

[15] LaVeist TA, Isaac LA, Williams KP. Mistrust of health care organizations is associated with underutilization of health services. Health Serv Res. 2009;44(6):2093-2105. https://doi.org/10.1111/j.1475-6773.2009.01017.x

[16] Gezergün A, Şahin B, Tengilimoğlu D, Demir C, Bayer E. Physician-patient relationship and communication from the perspective of patients: Example of a teaching hospital. Anadolu University Journal of Social Sciences 2006;6(1):129-144.

[17] Mazinani A. Building trust between patients and physicians. Multidisciplinary Cancer Investigation. 2017;1(3):25-26. https://doi.org/10.21859/mci-01033

[18] Shaya B, Al Homsi, Eid K, Haidar Z, Khalil A, Merheb K, Akl EA. Factors associated with the public's trust in physicians in the context of the Lebanese healthcare system: A qualitative study. BMC Health Serv Res. 2019;19(1):1-9. https://doi.org/10.1186/s12913-019-4354-0

[19] Müller E, Zill JM, Dirmaier J, Härter M, Scholl I. Assessment of trust in physician: A systematic review of measures. PLoS One. 2014;9(9). https://doi.org/10.1371/journal.pone.0106844

[20] Ozawa S, Sripad P. How do you measure trust in the health system? A systematic review of literature. Soc Sci Med. 2013;91:10-14. https://doi.org/10.1016/j.socscimed.2013.05.005

[21] Williamson LD, Bigman CA. A systematic review of medical mistrust measures. Patient Educ Couns. 2018;101(10):1786-1794. https://doi.org/10.1016/j.pec.2018.05.007

[22] Greene J, Ramos C. A mixed methods examination of health care provider behaviors that build patients' trust. Patient Educ Couns. 2021;104(5):1222-1228. https://doi.org/10.1016/j.pec.2020.09.003

[23] Richmond J, Boynton MH, Ozawa S, Muessig KE, Cykert S, Ribisl KM. Development and validation of the Trust in My Doctor, Trust in Doctors in General, and Trust in the Health Care Team Scales. Soc Sci Med. 2022; 298:114827. https://doi.org/10.1016/j.socscimed.2022.114827

[24] Wood GL, Haber J. Desenhos não-experimentais. In: Wood GL, Haber J, editors. Pesquisa em enfermagem: Métodos, avaliação crítica e utilização. Rio de Janeiro (RJ): Guanabara Koogan; 2001. p. 110-21.

[25] Alpar R. Spor, sağlık ve eğitim bilimlerinden örneklerle uygulamalı istatistik ve geçerlik-güvenirlik. Ankara: Detay Yayıncılık; 2016. (Turkish)

[26] Karakoç A, Dönmez P. Basic principles of scale development. World of Medical Education 2014;13(40):39–49. https://doi.org/10.25282/ted.228738

[27] Hooper D, Coughlan J, Mullen MR. Structural equation modelling: Guidelines for determining model fit. Electron J Business Res Methods. 2008;6(1):53-60.

[28] Karagöz Y. SPSS and AMOS applied quantitative-qualitative-mixed scientific research methods and publication ethics. Istanbul: Nobel Akademik Yayıncılık; 2017. (Turkish)

[29] Hair JF, Black WC, Babin BJ, Anderson RE. Multivariate data analysis. Upper Saddle River, NJ: Pearson; 2014.

[30] Özdamar K. Ölçek ve test geliştirme yapısal eşitlik modelleme IBM SPSS, IBM SPSS AMOS ve MINITAB uygulamalı. Eskişehir: Nisan Kitabevi; 2017. (Turkish)

[31] Halıcı M, Soyuk S, Gün İ. Organizational trust in healthcare professionals. J Manage Econ. 2015;13(3):180-198.

[32] Cortina JM. What is coefficient alpha? An examination of theory and applications. J Appl Psychol. 1993;78(1):98. https://doi.org/10.1037/0021-9010.78.1.98

How to cite this article: Filiz E, Ertaş H, Ünal SD, Kahveci Ş. Turkish Validity and Reliability Study of the Trust in the Health Care Team Scale. Clin Exp Health Sci 2025; 15: 157-163. https//doi.org/10.33808/clinexphealthsci.1495337

Clinical and Experimental Health Sciences

Determining the Relationship Between Health-Related University Students' Attitudes Towards Gender Roles and Their Attitudes Towards Domestic Violence

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Received: September 5, 2024 **Accepted:** Febtuary 26, 2025

ABSTRACT

Objective: The aim of the study is to determine the relationship between health-related university students' attitudes towards domestic violence and their attitudes towards gender roles.

Methods: This descriptive study was conducted with 500 students studying at a private university in Ankara. "Personal Information Form," "Domestic Violence Attitude Scale (DVAS)" and "The Gender Roles Attitude Scale (GRAS)" were used for data collection.

Results: A negative and weak correlation was found between the total mean scores of DVAS and GRAS among the students participating in the study ($p<.01$). Additionally, it was determined that 24% of the total variation in students' attitudes towards domestic violence was explained by their attitudes towards gender roles.

Conclusion: The results of the study show that students' attitudes towards gender roles affect their attitudes towards domestic violence. Students with an egalitarian gender perspective exhibit an attitude against domestic violence. Therefore, approaches to develop an egalitarian gender perspective in individuals can be effective in preventing domestic violence, which is an important problem in all societies.

Keywords: Domestic violence, gender roles, university students, attitude.

1. INTRODUCTION

Domestic violence is a term encompassing all forms of violence that can occur among family members. However, those most frequently exposed to domestic violence are often women, children, and the elderly (1-3). A study conducted in Turkiye reported that 35.5% of women had been subjected to physical violence by family members at some point in their lives (4). Considering that this statistic only includes physical violence, the proportion of individuals exposed to all forms of violence within the family is likely much higher (2). While many factors influence the occurrence, frequency, type, and magnitude of domestic violence, it can be said that the primary source is fundamentally the asymmetrical power relations and gender inequality brought about by patriarchal society (5,6).

Gender inequality refers to discrimination between sexes in the use of opportunities, rights, and resources due to these roles assigned to men and women. This deprivation of rights, discrimination, and inequality restricts or completely eliminates individuals' ability to exercise their rights and freedoms in health, economic, political, cultural, and social domains (7-9). Policies aimed at preventing gender inequality and domestic violence, both in Turkiye and globally, are structured within the framework of legal regulations, awareness-raising initiatives, and educational strategies. In Turkiye, the Law No. 6284 on the Protection of the Family and Prevention of Violence against Women serves as a critical legal instrument for safeguarding women from violence and promoting gender equality (10). Internationally, key frameworks include the Council of Europe Convention on Preventing and Combating Violence against Women and Domestic Violence (Istanbul Convention), the United Nations Convention on the Elimination of All Forms of Discrimination against Women (CEDAW), and the 2030 Sustainable Development Goals (5,11). Efforts to advance gender equality are further supported by programs aimed at enhancing women's participation in the workforce, broadening access to education, and addressing gender-based discrimination. These initiatives are often implemented through collaborative efforts involving civil society organizations, local governments, and the private sector. Despite these measures, gender inequality and domestic violence remain pressing social challenges, underscoring the need for inclusive and transformative approaches to address their root causes. The first area where these gender roles, inequalities, and

https://doi.org/10.33808/clinexphealthsci.1544128

gender identities, which cause such discrimination among individuals, are produced and developed is the family, the smallest and strongest building block of society. The family emerges as a social institution where the hierarchy between men and women is first recognized and structured (6). One of the most basic problems brought about by inequality in these families is domestic violence. It is believed that individuals' attitudes towards domestic violence will be negative if they have negative attitudes towards gender roles and adopt traditional roles. Some studies in the literature report that students studying in the health field have attitudes towards gender roles that align with traditional roles, which negatively affects their attitudes towards domestic violence (12-15).

In the literature, both qualitative (6) and quantitative studies (8, 12-16) aim to evaluate university students' attitudes towards domestic violence and gender roles. It is believed that attitudes towards domestic violence and gender roles will vary across different segments of society, different age groups, and varying socioeconomic levels. Determining the attitudes of young individuals towards domestic violence is considered an important step in preventing domestic violence in the future (8-17).

Based on this premise, the aim of this study is to determine the relationship between health-related university students' attitudes towards domestic violence and their attitudes towards gender roles and to contribute quantitative and concrete findings to the literature on this relationship.

2. METHODS

2.1. Design and sample

This descriptive study was conducted between January 15 and July 31, 2023, at a private university in Ankara. The study population consisted of all students enrolled at the university, totaling 2922 students. The sample size was calculated using the sample size formula for known populations, resulting in a target sample of 340 students. The study was completed with 500 students. Post hoc analysis conducted after the completion of the study indicated that with an error margin of .05 and an effect size of .29, the power of the sample was found to be 99%. The analyses were performed using the G Power 3.1.9.2 software package.

2.2. Data collection tools

Personal Information Form: This form was developed by the researchers by reviewing the literature and consists of 5 questions regarding the sociodemographic characteristics of the university students (7,12).

Domestic Violence Attitude Scale (DVAS): Developed by Şahin and Dişsiz (2009), this five-point Likert scale ranges from 13 to 65 points. Higher scores indicate a more positive attitude towards domestic violence. The scale comprises four sub-dimensions: "normalization of violence," "generalization of violence," "rationalization of violence," and "concealment of violence," with a total of 13 items. The original Cronbach's alpha reliability coefficient of the scale was .71 (17). In this study, the Cronbach's alpha reliability coefficient was found to be .82.

The Gender Roles Attitude Scale (GRAS): Developed by Zeyneloğlu and Terzioğlu (2011) to determine university students' attitudes towards gender roles, this five-point Likert scale consists of five sub-dimensions: "egalitarian gender role," "female gender role," "gender role in marriage," "traditional gender role," and "male gender role," with a total of 38 items. Higher scores indicate a more egalitarian attitude towards gender roles, while lower scores indicate a more traditional attitude. The original Cronbach's alpha reliability coefficient of the scale was .92 (18). In this study, the Cronbach's alpha reliability coefficient was found to be .93.

2.3. Ethical aspect of the research

Ethical approval was obtained from the scientific researchs ethics committee of Lokman Hekim University (Number: 2023/09), and institutional permission was obtained from the deans and directors of all faculties and schools of the university where the study was conducted. The individuals who agreed to participate in the study did so with the requisite informed consent. Throughout all stages, the principles of research and publication ethics and the Helsinki Declaration Principles were adhered to.

2.4. Data collection

Data were collected through face-to-face interviews after informing participants about the study.

2.5. Data analysis

Data were analyzed using the IBM SPSS 22.0 statistical software. Frequency and percentage analyses were used to determine descriptive characteristics, and mean and standard deviation statistics were used to evaluate scale scores. Kurtosis and Skewness values were examined to determine the homogeneity of the variables, indicating that the variables followed a normal distribution, and parametric tests were used in the analysis. Relationships between scales were determined using Pearson correlation and linear regression analysis.

3. RESULTS

The gender of 82.6% of the students was female, 76.6% were studying at the Faculty of Health Sciences, and 44.0% were in the first grade. Additionally, 33.8% of the students' mothers and 49.4% of the students' fathers have a university education (Table 1).

According to correlation analysis results, there was found negative and weak correlation between the mean total score of GRAS and the mean total score of DVAS of the students ($p<0.01$) (Table 2).

The regression analysis conducted to determine the cause and effect relationship between GRAS and DVAS was found significant ($F=158.413$; $p<.001$-$p< .05$). The total change in students' attitudes towards domestic violence is explained by their attitudes towards gender roles at a rate of 24% ($R^2= .240$). The level of attitude towards gender roles decreases the level of attitude towards domestic violence ($\beta= – .491$) (Table 3).

Table 1. *Distribution of students by descriptive characteristics and scale mean scores*

Characteristics	n	%
Gender		
Male	87	17.4
Female	413	82.6
Faculty/School		
Faculty of Health Sciences	383	76.6
Faculty of Pharmacy	47	9.4
Faculty of Medicine	47	9.4
Other*	23	4.6
Class		
Class 1	220	44.0
Class 2	84	16.8
Class 3	99	19.8
Class 4	78	15.6
Class 5	19	3.8
Mother's Education Status		
Primary School and Below	97	19.4
Middle School	78	15.6
High School	156	31.2
University	169	33.8
Father's Education Status		
Primary School and Below	37	7.4
Middle School	61	12.2
High School	155	31.0
University	247	49.4
	Mean	**Sd**
DVAS Total Score	18.78	5.89
GRAS Total Score	171.17	18.63

**Other: Faculty of Dentistry, Faculty of Sports Sciences, Vocational School of Health Services*

Table 2. *Correlation analysis between total and subscale scores of DVAS and GRAS*

		DVAS Total Score	Normalizing Violence	Generalizing Violence	Causalizing Violence	Hiding Violence
GRAS Total Score	r	-.49*	-.48*	-.42*	-.22*	-.40*
	p	.000	.000	.000	.000	.000
Egalitarian Gender Role	r	-.336*	-.332*	-.378*	-.130*	-.228*
	p	.000	.000	.000	.004	.000
Female Gender Role	r	-.439*	-.439*	-.315*	-.207*	-.400*
	p	.000	.000	.000	.000	.000
Gender Role in Marriage	r	-.418*	-.433*	-.382*	-.161*	-.328*
	p	.000	.000	.000	.000	.000
Traditional Gender Role	r	-.455*	-.428*	-.345*	-.233*	-.416*
	p	.000	.000	.000	.000	.000
Male Gender Role	r	-035*	-.342*	-.314*	-.161*	-.280*
	p	.000	.000	.000	.000	.000

**p<.001; Pearson Correlation Analysis*

Table 3. *The effect of the Gender Roles Attitude Scale on the Domestic Violence Attitude Scale*

Independent Variable	Unstandardized Coefficients		Standardized Coefficients	t	p	95% Confidence Interval	
	B	SE	β			Lower	Upper
Constant	45.361	2.124		21.354	.000	41.187	49.534
GRAS Total Score	-.155	.012	-.491	-12.586	**.000***	-.180	-.131
Dependent Variable =DVAS Total Score. *R=.491; R^2=.240; F=158.413; p=.000; Durbin Watson Value=1.947;* *p<.001							
Constant	45.525	2.515		18.102	.000	40.584	50.466
Egalitarian Gender Role	-.077	.060	-.065	-1.297	.195	-.194	.040
Female Gender Role	-.186	.073	-.161	-2.549	**.011****	-.329	-.043
Gender Role in Marriage	-.284	.096	-.170	-2.945	**.003***	-.473	-.094
Traditional Gender Role	-.259	.067	-.240	-3.877	**.000***	-.390	-.128
Male Gender Role	.082	.094	.051	.877	.381	-.102	.267
*Dependent Variable = DVAS Total Score. R=.505; R2=.247; F=33.823; p=.000; Durbin Watson Value=1.942; *p<.001; *p<.05*							

The regression analysis conducted to determine the cause and effect relationship between the sub-dimensions of "egalitarian gender role", "female gender role", "gender role in marriage", "traditional gender role", "male gender role" and the level of attitude towards domestic violence was found significant (F=33.823; p<.001-p< .05). The total change in students' attitudes towards domestic violence was explained by "egalitarian gender role", "female gender role", "gender role in marriage", "traditional gender role", "male gender role" at a rate of 24.7% (R^2=.247). While "egalitarian gender role" and "male gender role" do not affect the level of attitudes towards domestic violence (p>.05); "female gender role", "gender role in marriage" and "traditional gender role" sub-dimensions decrease the level of attitudes towards domestic violence (Table 3).

4. DISCUSSION

This study aimed to determine the relationship between health-related university students' attitudes towards domestic violence and their attitudes towards gender roles. The average score for students on DVAS was found to be 18.78 (Table 1). Considering that the scale ranges from a minimum of 13 to a maximum of 65, students generally exhibited a positive attitude against domestic violence (17). When examining the subscales, it was observed that students scored below average on normalizing violence, generalizing violence, rationalizing violence, and concealing violence, indicating a desired stance against domestic violence in these aspects as well. Similar findings were reported by Çal and Aydın Avcı (2020) and Arslan and Şahin (2019), who found that university students generally scored below average on the DVAS, suggesting a rejection of domestic violence (15,19). This finding in the current study may be attributed to the fact that students attend a health-related university and take various courses related to the topic. Additionally, living in a metropolitan city where they are frequently exposed to campaigns against violence and having parents with higher education levels could also contribute to their positive attitudes towards domestic violence prevention (20).

In this study, the average score for students on the GRAS was found to be 171.17, indicating that students generally hold egalitarian attitudes towards gender roles (Table 1). Upon examining the subscales of the scale, it was found that students had the highest attitude scores in marital gender roles and egalitarian gender roles, while their scores for male gender role, female gender role, and traditional gender role were above average. Similarly, Çal and Aydın Avcı (2020) reported that students held egalitarian attitudes, with the highest scores in marital and egalitarian gender roles (19). Likewise, Güven and Altay (2020) found in their study with midwifery students that students had egalitarian attitudes, with the highest scores in marital and egalitarian gender roles among the scale's subscales (21). It is noted in the literature that educational interventions on gender during university education increase students' motivation to identify and address gender-related issues (22). In the study, it is thought that the reason for the high level of gender roles attitudes and egalitarian attitudes of the students is that the gender course is taught as a common elective course at the university where the students study. In addition, the fact that the education level of the parents of most of the students is at the undergraduate level and the gender of more than eighty per cent of the students is female also affects this situation (23). The results of the studies in the literature also indicate that women adopt a more egalitarian gender role (15, 19). The reason for this situation may be the gender roles adopted in the society. An understanding of education that will develop an egalitarian attitude towards traditional male and female roles and transfer this attitude to the society constitutes the key point to prevent this situation. The status and roles of women in a society are very important for the level of development of that society. With a qualified education, the status of women and thus the level of development of the society can be increased (24).

As a result of the study, similar to findings in the literature (19,24) a negative relationship was identified between the total score of GRAS and the total and subscale scores of DVAS (Table 2). This indicates that as students' scores on the GRAS increase, their scores on the DVAS decrease, suggesting that students exhibiting egalitarian attitudes towards gender roles tend to have more desirable attitudes towards domestic violence. Regression analysis conducted to determine the cause-effect relationship between GRAS and DVAS was found to be significant, indicating that 24% of the total variance in students' attitudes towards domestic violence can be explained by their attitudes towards gender roles (Table 3). This finding suggests that higher levels of egalitarian attitudes towards gender roles contribute to lower levels of acceptance of domestic violence attitudes (25, 26).

4.1. Limitations

There are several limitations to this study. The research was conducted at a foundation university located in Ankara. Therefore, the results are limited to the university where the study was conducted and may not be generalized to the broader population. It is important to conduct further research with larger sample sizes to enhance the generalizability of the findings. The study results provide information about the current status of the participants. The data collection tools used in the study relied on self-reporting by students, which could introduce bias into the responses. The students included in the sample are studying at a health-related university. In addition, most of the students have taken elective courses on gender and most of them are female. All these characteristics can be counted among the limitations as they are thought to affect the results of the study.

5. CONCLUSION

The research findings indicate that students' attitudes towards gender roles significantly influence their perspectives on domestic violence. Students with an egalitarian gender perspective tend to exhibit an attitude against domestic violence. Therefore, approaches aimed at fostering individuals' development of a perspective supportive of gender equality could also be effective in preventing a significant societal issue like domestic violence. To this end, integrating topics or courses in university curricula that address various dimensions of gender equality and aim to raise awareness among students is considered crucial. Such initiatives could play a vital role in preventing societal issues such as domestic violence. Additionally, it is recommended to plan projects and studies throughout university education aimed at raising awareness among students about preventing violence, understanding and internalizing the concept of gender equality. Encouraging students to participate in these activities could also be beneficial.

Acknowledgements: *We would like to thank the students who participated in our study.*
Funding: *This research was supported within the scope of TÜBİTAK 2209-A University Students Research Projects Support Program.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Lokman Hekim University Scientific Researchs ethics committee (Approval date:10/01/2023; Number: 2023/09).*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: FND
Design of the study: FND, ZG
Acquisition of data for the study: SA
Analysis of data for the study: FND
Interpretation of data for the study: FND, ZG
Drafting the manuscript: FND, SA
Revising it critically for important intellectual content: FND, ZG
Final approval of the version to be published: ZG

REFERENCES

[1] World Health Organization. The World Health Report 2002: Reducing Risks, Promoting Healty Life. Published [11 September 2002]. Accessed [19 August 2024]. https://www.who.int/whr/2002/en/summary_riskfactors_chp4.pdf?Ua=1

[2] Mshweshwe L. Understanding domestic violence: masculinity, culture, traditions. Heliyon 2020;6(10): 1-5. https://doi.org/10.1016/j.heliyon.2020.e05334

[3] Kahraman MS, Çokamay G. Domestic violence and its effects on children: Basic concepts, safety plan preparation and alternative treatment model examples. Curr. Approaches Psychiatry. 2016;8(4): 321-336. https://doi.org/10.18863/pgy.253438

[4] Dinçer F, Yüksel M. Aile içi şiddete şahit olan çocuklar: Bir alanyazın derlemesi. MUKATCAD. 2018;2(2):130-141. https://doi.org/10.26695/mukatcad.2018.22 (Turkish).

[5] Wilcox T, Greenwood M, Pullen A, O'Leary Kelly A, Jones D. Interfaces of domestic violence and organization: Gendered violence and inequality. Gend. Work Organ. 2021;28(2): 701-721. https://doi.org/10.1111/gwao.12515

[6] Beebeejaun-Muslum ZN. Gender relation, patriarchal control, and domestic violence: A qualitative study in mauritius. EJ-SOCIAL. 2024;4(3):9-19. https://doi.org/10.24018/ejsocial.2024.4.3.40

[7] Kimmel M, Gordon KM. Defining Gender. Ryan JM, editor. Core Concepts in Sociology. Oxford: John Wiley & Sons; 2018.p.118-122.

[8] Yilmaz EB, Öz F. Assessing The relation between attitudes towards gender roles and domestic violence of nursing and paramedic students. Clin. Exp. Health Sci. 2018;8(3):160-165. https://doi.org/10.5152/clinexphealthsci.2017.459

[9] Kuskoff E, Parsell C. Striving for gender equality: Representations of gender in "progressive" domestic violence policy. Violence Against Women 2021;27(3-4): 470-488. https://doi.org/10.1177/1077801220909892

[10] Dalkılıç RA. Kadına yönelik şiddetle mücadele: Kamu politikası süreç analizi. Reflektif Sos. Bilim. Derg. 2021;2(1): 61-82. https://doi.org/10.47613/reflektif.2021.15 (Turkish)

[11] Chinkin C, Yoshida K. The CEDAW Committee: Global leader in tackling violence against women and girls. EHRL. 2020(4):347-358.

[12] Kanbay Y, Işık E, Yavuzaslan M, Keleş S. Hemşirelik öğrencilerinin kadına yönelik aile içi şiddetle ilgili görüş ve tutumlarının belirlenmesi. Gümüşhane Univ. Sağlık Bilim. Derg. 2012;1:107-119 (Turkish).

[13] Karabulutlu Ö. Experiences and attitudes of nursing students regarding domestic violence against women. Cumhuriyet Hem Der. 2015;4:27- 34 (Turkish).

[14] Coleman JU, Stith SM. Nursing students' attitudes toward victims of domestic violence as predicted by selected individual and relationship variables. J Fam Viol. 1997;12:113-138. https://doi.org/10.1023/a:1022838226658

[15] Arslan K, Şahin H. Aile içi şiddetin toplumsal cinsiyet rolü üzerindeki etkisi: Üniversite öğrencilerine yönelik bir uygulama. Soc. Ment. Res. Think. J. 2019; 5(15):45-52. https://doi.org/10.31576/smryj.195 (Turkish).

[16] Tanrıverdi D, Özgüç S. Comparison of attitudes towards violence and aggression behaviors of children/adolescents with fragmented and whole families. J. Psychiatr. Nurs. 2019;10(3):165-172. https://doi.org/10.14744/phd.2019.47450

[17] Şahin N, Dişsiz M. Sağlık çalışanlarında aile içi şiddete yönelik tutum ölçeği geliştirme çalışması. Ulus. İnsan Bilim. Derg. 2009;6(2):263-274 (Turkish).

[18] Zeyneloğlu S, Terzioglu F. Toplumsal cinsiyet rolleri tutum ölçeği geliştirilmesi ve psikometrik özellikleri. Hacet. Egit. Derg. 2011;40: 409- 420 (Turkish).

[19] Çal A, Aydın Avcı İ. Hemşire ve ebe öğrencilerin toplumsal cinsiyet rol tutumları ile aile içi şiddete yönelik tutumları ve yaşam değerleri arasındaki ilişkinin incelenmesi. Samsun Sağ Bil Der. 2020;5(2):103-112. https://doi.org/10.47115/jshs.787564 (Turkish).

[20] Ribeiro Cardoso P, Jólluskin G, Paz L, Fonseca MJ, Silva I. Effects of awareness campaigns against domestic violence: perceived efficacy, adopted behavior and word of mouth. J. Crim. Res. Policy Pract. 2023;9(3):177-192. https://doi.org/10.1108/JCRPP-11-2022-0057

[21] Güven E, Altay B. Ebelik öğrencilerinin kadına uygulanan şiddete ve toplumsal cinsiyet rollerine ilişkin tutumlarının belirlenmesi. Samsun Sağ Bil Der. 2020;5(2):191-200. https://doi.org/10.47115/jshs.764948 (Turkish).

[22] Ben Natan M. Interest in nursing among academic degree holders in Israel: A cross-sectional quantitative study. Nurse Educ Today. 2016;38:150-153. https://doi.org/10.1016/j.nedt.2015.11.025

[23] Koç G, Özçırpan ÇY, Terzioğlu F, Çetinkaya ŞŞ, Uslu-Şahan F, Işık RA, Başkaya E. The effect of a gender course on the gender attitudes, critical thinking dispositions, and media literacy skills of university students. J. High. Educ. 2021;11(2):387-400. https://doi.org/10.2399/yod.20.640377

[24] Aşkun V, Erkoyuncu M. Toplumsal cinsiyet algısı ve demografik farklılıkların esenlik üzerindeki karmaşık etkisi: Türkiye örneği. Eskişehir Osmangazi Univ. İktisadi ve İdari Bilimler Derg. 2023;18(3), 834-855. https://doi.org/10.17153/oguiibf.1283016 (Turkish).

[25] Karakurt G, Cumbie T. The relationship between egalitarianism, dominance, and violence in intimate relationships. J. Fam. Violence. 2012;27(1):115-122. https://doi.org/10.1007/s10896-011-9408-y

[26] Dogan Gangal A, Yigit Y, Ali Y. Generational differences in attitudes towards gender roles and violence against women. Int J Caring Sciences. 2024;17(1):198–208.

How to cite this article: Duman NF, Akyüz S, Gölbaşı Z. Determining the Relationship Between Health-Related University Students' Attitudes Towards Gender Roles and Their Attitudes Towards Domestic Violence. Clin Exp Health Sci 2025; 15: 164-169. DOI: 10.33808/clinexphealthsci.1544128

Clinical and Experimental Health Sciences

Synthesis and Anti-Biofilm Activity Studies on Novel Quinazolinone-Thiadiazole Hybrids

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Received: September 10, 2024 **Accepted:** January 23, 2025

ABSTRACT

Objective: In this study it was aimed to synthesize novel 1,3,4-thiadizole bearing 4(3H)-quinazolinone compounds, elucidate their structure and evaluate their anti-biofilm activity.

Methods: Four novel 4(3H)-quinazolinone compounds (1-4) were synthesized with a two step reaction starting from 5-bromoanthranilic acid. Their anti-biofilm activity was investigated.

Results: The final compounds' structures were clarified by elemental analysis and spectroscopic methods (IR, 1H-NMR, 13C-NMR and MS). In the result of anti-biofilm activity studies, they possessed 26.0-30.0% biofilm formation inhibition.

Conclusion: Among the tested compounds, 6-bromo-3-{4-[5-(4-nitrophenylamino)-1,3,4-thiadiazol-2-yl]phenyl}-2-methylquinazolin-4(3H)-one formulated compound 3 was found as the most active one with 30.0% biofilm formation inhibition.

Keywords: 4(3H)-Quinazolinones, 1,3,4-thiadiazoles, anti-biofilm activity, P. aeruginosa

1. INTRODUCTION

P. aeruginosa is a gram negative opportunistic pathogen which is known as the reason of many chronic infections ending with morbidity and mortality. It perfoms pathogenity by forming biofilms and biofilm-associated *P. aeruginosa* infections could hardly be treated because of their strong resistance to antibiotics. Biofilm is the structural aggregation of the bacteria by adhesion over the living or non-living hosts. The Quorum sensing (QS) system is known as a cell-to-cell communication system controlling the biofilm formation in Gram (-) and Gram (+) bacteria. By blocking QS system, pathogenic host effects produced by infections could be reduced (1-3).

Quinazolinones are important pharmacophoric groups which are known for several years. Owing to their significant bioactivity, quinazolinone ring is also located in many commercially available drugs. As examples to them; raltitrexed is used against colorectal cancer (4,5), ketanserin as anti-hypertensive (6), albaconazole as antifungal (7-9), fenquizone as diuretic (10), febrifugine as antimalarial (11) and afloqualone as muscle relaxant (12), etc (Figure 1). Especially, 4(3*H*)-quinazolinones get attention for their diverse range of biological activities as antihypertensive (13), anticancer (14,15), anticonvulsant (16), antioxidant (17), anti-inflammatory (18), antimalarial (19), anthelmintic (20) and antiviral (21,22) activities. And also, there are many studies informing their effect on a variety of enzymes like monoamine oxidase, a-glucosidase and acetylcholinesterase (23-26). Besides, according to the recent studies, 4(3*H*)-quinazolinones have attracted great attention for their remarkable antibacterial, antifungal and anti-biofilm activities (27-31).

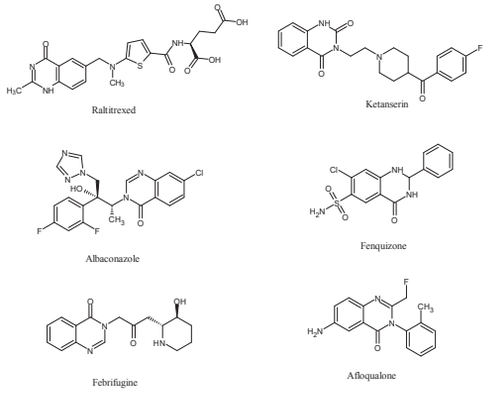


Figure 1. *4(3H)-quinazolinone compounds used as active drug substances.*

Clin Exp Health Sci 2025; 15: 170-174
ISSN:2459-1459

https://doi.org/10.33808/clinexphealthsci.1548035

1,3,4-Thiadiazoles are well known five membered heterocyclic structures for their various biological activities. They are commonly known for their carbonic anhydrase (CA) inhibitory effects by being located in important CA inhibitors like acetazolamide, methazolamide etc. Moreover, they arouse interest with their notable antituberculosis and antimicrobial activities (32,33).

In our previous study we have synthesized 4(3*H*)-Quinazolinones with the reaction of anthranilic acids and sulfonamides. The obtained thiadiazole bearing compounds showed remarkable potential at biofilm formation inhibition (34). Inspired by the therapeutic potential of 4(3*H*)-quinazolinones, 1,3,4-thiadiazoles and depending on the results we have previously obtained; in this study we targeted to assemble these two active groups and discover novel potent anti-biofilm agents.

2. METHODS

All of the chemicals, reagents and solvents were purchased from Sigma Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Melting points were determined by Schmelzpunktbestimmer SMP II apparatus. The IR spectra were recorded on a Schimadzu FTIR 8400 S Spectrometer. The NMR spectra were recorded (in DMSO-d_6) with a Varian Mercury Agilent spectrometer (Palo Alto, CA, USA) and Bruker AV spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, decoupled). The chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by Electron Spray Ionization (ES) method on LC-MS-Agilent 1100. Elemental analysis was performed on Leco 215 CHNS-932 analyzer.

2.1. Chemistry

General Synthesis Procedure of 4(3*H*)-Quinazolinone Derivatives (1-4): At first, 5-(4-aminophenyl)-*N*-substituted-1,3,4-thiadiazol-2-amines were synthesized according to the literature method (35,36). And then, 4(3*H*)-quinazolinone compounds (1-4) were achieved by a double-step reaction. At the first step, 5-bromoanthranilic acid (0.003 mol) was refluxed with 0.9 mL acetic anhydride to obtain the intermediate product, which was common for all derivatives. The completion of the reaction was checked with TLC and the excess of the acetic acid was evaporated under reduced pressure. At the final step, the intermediate product was reacted with equimolar moles of various 5-(4-aminophenyl)-*N*-substituted-1,3,4-thiadiazol-2-amine compounds at acetic acid media (34). At the end of the reaction, (1-4) compounds were precipitated by adding cold water and crushed ice in reaction media. The obtained solid was filtered, dried and purified with methanol.

6-Bromo-3-{4-[5-(ethylamino)-1,3,4-thiadiazol-2-yl] phenyl}-2-methylquinazolin-4(3*H*)-one (1): Yellow solid. Yield 95%; m.p. 261-262 °C; MW: 442.33 g/mol; FT-IR $u_{max.}$ (cm^{-1}): 3173 (N-H), 1690 (C=O), 1090 (Ar-Br). ^{1}H-NMR (DMSO-d_6/TMS) d (ppm): 1.22 (3H, t, – $CH_2C\mathbf{H}_3$), 2.16 (3H, s, Ar-CH_3), 3.36-3.39 (2H, m, – $C\mathbf{H}_2CH_3$), 7.57 (2H, d, *J:* 8.40 Hz, Ar-H), 7.64 (1H, d, *J:* 9.20 Hz, Ar-H), 7.94 (2H, d, *J:* 8.80 Hz, Ar-H), 7.99-8.01 (1H, dd, *J:* 8.60 Hz, *J:* 2.20 Hz, Ar-H), 8.03 (1H, t, – NH-), 8.18 (1H, d, *J:* 2.40 Hz, Ar-H). ^{13}C-NMR (DMSO-d_6/TMS) d (ppm): 5.62, 14.72, 24.59, 119.21, 122.59, 127.85, 128.80, 129.62, 129.76, 132.02, 137.95, 138.84, 146.81, 155.11, 155.51, 160.74, 169.23 **(**C=O**)**. MS (ES m/z): 442.00 (M^+). Elemental analysis for $C_{19}H_{16}BrN_5OS$ Calculated/Found (%): C: 51.59/51.44, H: 3.65/3.47, N: 15.83/15.69, S: 7.25/7.39.

6-Bromo-3-{4-[5-(phenethylamino)-1,3,4-thiadiazol-2-yl] phenyl}-2-methylquinazolin-4(3*H*)-one (2): Brown solid. Yield 93%; m.p. 218 °C; MW: 518.43 g/mol; FT-IR $u_{max.}$ (cm^{-1}): 3337 (N-H), 1670 (C=O), 1090 (Ar-Br). ^{1}H-NMR (DMSO-d_6/TMS) d (ppm): 2.17 (3H, s, Ar-CH_3), 2.94 (2H, t, – NH-CH_2-C$\mathbf{H}_2$-), 3.57-3.62 (2H, q, – NH-C$\mathbf{H}_2$-CH_2-), 7.23-7.34 (5H, m, Ar-H), 7.57 (2H, d, *J:* 8.40 Hz, Ar-H), 7.64 (1H, d, *J:* 8.80 Hz, Ar-H), 7.94 (2H, d, *J:* 8.40 Hz, Ar-H), 7.99-8.02 (1H, dd, *J:* 9.00 Hz, *J:* 2.60 Hz, Ar-H), 8.15 (1H, t, – NH-), 8.19 (1H, d, *J:* 2.40 Hz, Ar-H). ^{13}C-NMR (DMSO-d_6/TMS) d (ppm): 24.59, 34.95, 46.65, 119.22, 122.58, 126.71, 127.88, 128.81, 128.87, 129.23, 129.62, 129.76, 131.99, 137.94, 138.87, 139.64, 146.80, 155.31, 155.50, 160.74, 169.17 **(**C=O**)**. MS (ES m/z): 518.07 (M^+). Elemental analysis for $C_{25}H_{20}BrN_5OS$ Calculated/Found (%): C: 57.92/57.72, H: 3.89/3.92, N: 13.51/13.31, S: 6.19/6.41.

6-Bromo-3-{4-[5-(4-nitrophenylamino)-1,3,4-thiadiazol-2-yl]phenyl}-2-methylquinazolin-4(3*H*)-one (3): Yellow solid. Yield 90%; m.p. 218-220 °C; MW: 535.37 g/mol; FT-IR $u_{max.}$ (cm^{-1}): 3320 (N-H), 1711 (C=O), 1096 (Ar-Br). ^{1}H-NMR (DMSO-d_6/TMS) d (ppm): 2.16 (3H, s, Ar-CH_3), 7.61-7.91 (5H, m, Ar-H), 7.97-8.00 (1H, dd, *J:* 9.00 Hz, *J:* 2.20 Hz, Ar-H), 8.09 (2H, d, *J:* 8.40 Hz, Ar-H), 8.17 (1H, d, *J:* 2.40 Hz, Ar-H), 8.28 (2H, d, *J:* 8.80 Hz, Ar-H), 11.35 (1H, s, – NH-). ^{13}C-NMR (DMSO-d_6/TMS) d (ppm): 25.42, 114.38, 117.41, 119.16, 119.59, 122.56, 126.00, 128.18, 128.69, 129.99, 133.51, 136.90, 140.39, 168.57, 169.07 **(**C=O**)**. MS (ES m/z): 537.00 (M^++3). Elemental analysis for $C_{23}H_{15}BrN_6O_3S$ Calculated/Found (%): C: 51.60/51.53, H: 2.82/2.79, N: 15.70/15.71, S: 5.99/6.03.

6-Bromo-3-{4-[5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl] phenyl}-2-methylquinazolin-4(3*H*)-one (4): Brown solid. Yield 87%; m.p. 252-254 °C; MW: 496.42 g/mol; FT-IR $u_{max.}$ (cm^{-1}): 3358 (N-H), 1672 (C=O), 1089 (Ar-Br). ^{1}H-NMR (DMSO-d_6/TMS) d (ppm): 1.22-2.11 (11H, m, cyclohegzyl – CH_2 – and – CH-), 2.16 (3H, s, Ar-CH_3), 7.56 (2H, d, *J:* 8.00 Hz, Ar-H), 7.64 (1H, d, *J:* 9.20 Hz, Ar-H), 7.66 (0.37H, s, – NH-), 7.93 (2H, d, *J:* 8.40 Hz, Ar-H), 7.99-8.01 (1H, dd, *J:* 8.60 Hz, *J:* 2.20 Hz, Ar-H), 8.18 (1H, d, *J:* 2.40 Hz, Ar-H). ^{13}C-NMR (DMSO-d_6/TMS) d (ppm): 24.59, 24.77, 25.70, 32.55, 54.25, 119.21, 122.60, 127.84, 128.81, 129.63, 129.74, 132.05, 137.96, 138.79, 146.82, 154.89, 155.53, 160.76, 168.45 **(**C=O**)**. MS (ES m/z): 497.89 (M^++2). Elemental analysis for $C_{23}H_{22}BrN_5OS$ Calculated/Found (%): C: 55.65/55.29, H: 4.47/4.47, N: 14.11/13.77, S: 6.46/6.51.

2.2. Biofilm Assay

Anti-biofilm capacity of new substituted-4(3*H*)-quinazolinone derivatives was investigated using Crystal Violet (CV) staining, as described by Ulusoy et al (37). An overnight culture of *Pseudomonas aeruginosa* PAO1 was prepared and diluted to an optical density (OD600) of 0.05. One milliliter of the diluted culture was transferred to polystyrene tubes and incubated at 37 °C in the presence of new substituted-4(3*H*)-quinazolinone derivatives. After 24 hours, the nonadherent cells were removed by washing the tubes with distilled water. The remaining biofilms were stained with a 0.4% CV solution. The CV bound to the biofilms was then solubilized using 95% ethanol, and the absorbance was measured at 595 nm using a microplate reader. Biofilm inhibition was calculated using the formula:

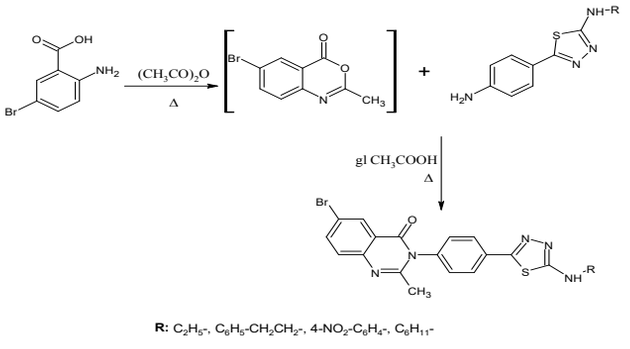
%BI=(ODC−ODFODC)×100\%\text{BI}=\left(\frac{\text{ODC}} – \text{ODF}}{\text{ODC}} \right) \times 100%BI=(ODCODC−ODF)×100

Where **%BI** is the percentage of biofilm inhibition. **ODC** is the absorbance value at 595 nm of the control sample (without nanofibers). **ODF** is the absorbance value at 595 nm of the sample with the substituted-4(3*H*)-quinazolinone derivatives.

3. RESULTS

3.1. Chemistry

Within the context of this study, four novel 4(3*H*)-quinazolinone compounds **(1-4)** were synthesized via a two step reaction starting from 5-bromoanthranilic acid. Firstly, anthranilic acid was reacted with acetic anhydride to obtain the intermediate product. Secondly, amine compounds were added to the reaction in acetic acid media and refluxed. The synthesis route of the final compounds is given in **Scheme 1**. The amine derivatives used in this reaction were achived by a four step reaction starting from benzocaine. The intimate description of the reaction methods were given in our previous literature (35,36).



Scheme 1. *Synthetic route of compounds* ***1-4****.*

The final compounds' purity was checked by TLC and their melting points were calculated. Their yield was between 52-95%. The physicochemical properties belonging to compounds **(1-4)** were given in Table 1.

Table 1. *The physicochemical properties of compounds* ***(1-4)****.*

Compound	R:	Molecular formula	M.A (g/mol)	M.p. (°C)	Yield (%)	Colour
1	C_2H_5-	$C_{19}H_{16}BrN_5OS$	442.33	261-262	95	Light yellow
2	C_6H_5-CH_2CH_2-	$C_{25}H_{20}BrN_5OS$	518.43	218	94	Light brown
3	4-NO_2-C_6H_4-	$C_{23}H_{15}BrN_6O_3S$	535.37	218-220	52	Yellow
4	C_6H_{11}-	$C_{23}H_{22}BrN_5OS$	496.42	252-254	82	Brown

3.2. Biofilm Assay

Antibiotics are able to target free floating planktonic cells but they could not penetrate the biofim matrix. The ability to form biofilm makes bacteria resistant to several antibiotics. To enhance the anti-biofilm capacity could give way to get over bacterial resistance. *P. aeruginosa* is a well known biofilm former (2). From this point of view, the newly synthesized compounds' activity was tested on biofilm formation of *P.* aeruginosa PAO1.The results were presented in Table 2.

Table 2. *The biofilm formation inhibition (%) values of compounds* ***(1-4)****.*

Compound	Biofilm Formation Inhibition (%)
6-Bromo-3-{4-[5-(ethylamino)-1,3,4-thiadiazol-2-yl] phenyl}-2-methylquinazolin-4(3*H*)-one (1)	26.0
6-Bromo-3-{4-[5-(phenethylamino)-1,3,4-thiadiazol-2-yl]phenyl}-2-methylquinazolin-4(3*H*)-one (2)	27.2
6-Bromo-3-{4-[5-(4-nitrophenylamino)-1,3,4-thiadiazol-2-yl]phenyl}-2-methylquinazolin-4(3*H*)-one (3)	30.0
6-Bromo-3-{4-[5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl]phenyl}-2-methylquinazolin-4(3*H*)-one (4)	27.5

4. DISCUSSION

The newly synthesized (1-4) compounds' structures were identified by a variety of spectroscopic methods (FT-IR, ^{1}H-NMR, ^{13}C-NMR and MS). According to the IR absorption spectra, amide C=O stretching bands, which were distinctive for 4(3*H*)-quinazolinone structure, were confirmed at 1711-1670 cm^{-1}. Also, N-H stretching and C-Br stretching bands were detected at 3377-3173 cm^{-1} and 1096-1089 cm^{-1}, respectively. In regard to the ^{1}H-NMR spectra, 4(3*H*)-quinazolinone C_5-H, C_7-H and C_8-H protons became prominent with their *J* value calculations. C_5-H protons were recorded at 8.17-8.19 ppm as dublets with meta interaction. C_7-H protons were detected between 7.97-8.02 ppm as double doublets. C_8-H protons were determined at 7.64 ppm as doublets, except compound 3. Also, at ^{13}C-NMR spectra, the amide C=O were retained at 168.45-169.23 ppm. In addition, with other spectroscopic data, the elemental analysis and MS spectral analysis results confirmed the (1-4) compounds' structure and they were in accordance with the literature (34,38).

In reference to biofilm assay results, all of the compounds showed remarkale antibiofilm activity with 26-30% biofilm inhibition values. Among them

6-bromo-3-{4-[5-(4-nitrophenylamino)-1,3,4-thiadiazol-2-yl]phenyl}-2-methylquinazolin-4(3*H*)-one **(3)** was the most effective one and 6-bromo-3-{4-[5-(ethylamino)-1,3,4-thiadiazol-2-yl]phenyl}-2-methylquinazolin-4(3*H*)-one **(1)** was the least effective one with biofilm formation inhibition values 30.0 and 26.0, respectively.

5. CONCLUSION

Four novel 4(3*H*)-quinazolinone compounds were synthesized and their structures were elucidated by elemental analysis and various spectroscopic methods (IR, ^{1}H-NMR, ^{13}C-NMR and MS). According to the anti-biofilm assay results, 4-nitrophenyl substituted compound 3 was the most effective one with 30.0% inhibition value and it could be evaluated as a lead compound for further studies.

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study didn't need an Ethics Committee Approval.*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: ST, SK

Design of the study: ST, SU, SK, GBT

Acquisition of data for the study: ST, SU, SK, GBT

Analysis of data for the study: ST, SU, SK, GBT

Interpretation of data for the study: ST, SU, SK, GBT

Drafting the manuscript: ST, SU, SK, GBT

Revising it critically for important intellectual content: ST, SU, SK, GBT

Final approval of the version to be published: ST, SU, SK, GBT.

REFERENCES

[1] Gupta P, Gupta RK, Harjai K. Multiple virulence factors regulated by quorum sensing may help in establishment and colonisation of urinary tract by Pseudomonas aeruginosa during experimental urinary tract infection. Indian J Med Microbiol. 2013; 31(1): 29-33. https://doi.org/10.4103/0255-0857.108715

[2] Brindhadevi K, LewisOscar F, Mylonakis E, Shanmugam S, Verma TN, Pugazhendhi A. Biofilm and Quorum sensing mediated pathogenicity in Pseudomonas aeruginosa. Process Biochem. 2020; 96: 49-57. https://doi.org/10.1016/j.procbio.2020.06.001

[3] Rodriguez-Urretavizcaya B, Vilaplana L, Marco MP. Strategies for Quorum Sensing inhibition as a tool for controlling Pseudomonas aeruginosa infections. Int J Antimicrob Agents. 2024 August 29. [Epub ahead of print]. https://doi.org/10.1016/j.ijantimicag.2024.107323

[4] Zalcberg J. Overview of the tolerability of 'Tomudex' (raltitrexed) collective clinical experience in advanced colorectal cancer. Anti-Cancer Drugs. 1997; 8(2): 17-22. https://doi.org/10.1097/00001813-199708002-00004

[5] Cutsem EV, Cunningham D, Maroun, J, Cervantes, A, Glimelius, B. Raltitrexed: current clinical status and future directions. Ann Oncol. 2002; 13: 513-522. https://doi.org/10.1093/annonc/mdf054

[6] Fozard JR. Mechanism of the hypotensive effect of Ketanserin. J Cardiovasc Pharmacol. 1982; 4(5): 829-838. https://doi.org/10.1097/00005344-198209000-00020

[7] Irannejad H, Emami S, Mirzaei H, Hashemi SM. Data on molecular docking of tautomers and enantiomers of ATTAF-1 and ATTAF-2 selectivity to the human/fungal lanosterol-14 α-demethylase. Data Brief. 2020; 31: 105942. https://doi.org/10.1016/j.dib.2020.105942

[8] Cao X, Xu Y, Cao Y, Wang R, Zhou R, Chu W, Yang Y. Design, synthesis, and structure activity relationship studies of novel thienopyrrolidone derivatives with strong antifungal activity against Aspergillus fumigates. Eur J Med Chem. 2015; 102: 471-476. https://doi.org/10.1016/j.ejmech.2015.08.023

[9] Ding Z, Nib T, Xie F, Hao Y, Yu S, Chai X, Jin Y, Wang T, Jian Y, Zhang D. Design, synthesis, and structure-activity relationship studies of novel triazole agents with strong antifungal activity against Aspergillus fumigatus. Bioorg Med Chem Lett. 2020; 30: 126951. https://doi.org/10.1016/j.bmcl.2020.126951

[10] Baranauskiene L, Skiudaite L, Michailoviene V, Petrauskas V, Matulis D. Thiazide and other Cl-benzenesulfonamide bearing clinical drug affinities for human carbonic anhydrases. PLoS One 2021; 16(6): e0253608. https://doi.org/10.1371/journal.pone.0253608

[11] Sen D, Banerjee A, Ghosh AK, Chatterjee TK. Synthesis and antimalarial evaluation of some 4-quinazolinone derivatives based on febrifugine. J Adv Pharm Tech Res. 2010; 1(4): 401-405. https://doi.org/10.4103/0110-5558.76439

[12] Back H, Pradhan S, Yoon Y, Kang W, Chae J, Han N, Miki N, Kwon K, Kim S, Yun H. Population pharmacokinetic modeling and simulation of Afloqualone to predict steady-state exposure levels. Int J Pharmacol. 2018; 14(2): 276-284. https://doi.org/10.3923/ijp.2018.276.284

[13] Hussain MA, Chiu AT, Price WA, Timmermans PB, Shefter E. Anthihypertensive activity of 2-[(2-hydroxyphenyl)amino]-4(3H)-quinazolinone. Pharm Res. 1988; 5(4): 242-244. https://doi.org/10.1023/a:1015949931218

[14] Rathod B, Joshi S, Regu S, Manikanta KVNS, Kumar H, Dubey S, Chowdhury A, Shaikh RP, Das A, Patel S, Satvase R, Chatterjee DR, Jain A, Garg R, Shard A. Design and synthesis of novel quinazolinone-based pyruvate kinase M2 activators as selective inhibitors of oral cancer cells. J Mol Struct. 2024; 1304: 134595. https://doi.org/10.1016/j.molstruc.2024.137595

[15] El-Karim SSA, Syam YM, El Kerdawy AM, Abdel-Mohsen HT. Rational design and synthesis of novel quinazolinone N-acetohydrazides as type II multi-kinase inhibitors and potential anticancer agents. Bioorg Chem. 2024; 142: 106920. https://doi.org/10.1016/j.bioorg.2023.106920

[16] Cheke RS, Shinde SD, Ambhore JP, Chaudhari SR, Bari SB. Quinazoline: An update on current status against convulsions. J Mol Struct. 2022; 1248: 131384. https://doi.org/10.1016/j.molstruc.2021.131384

[17] Mravljak J, Slavec L,Hrast M, Sova M. Synthesis and evaluation of antioxidant properties of 2-substituted quinazolin-4(3H)-ones. Molecules 2021; 26: 6585. https://doi.org/10.3390/molecules26216585

[18] Abbas SE, Awadallah FM, Ibrahin NA, Said EG, Kamel GM. New quinazolinone pyrimidine hybrids: Synthesis, anti-inflammatory, and ulcerogenicity studies. Eur J Med Chem. 2012; 53: 141-149. https://doi.org/10.1016/j.ejmech.2012.03.050

[19] Mhetre UV, Haval NB, Bondle GM, Rathod SS, Choudhari PB, Kumari J, Sriram D, Haval KP. Design, synthesis and molecular docking study of novel triazole-quinazolinone hybrids as antimalarial and antitubercular agents. Bioorg Med Chem Lett. 2024; 108: 129800.
https://doi.org/10.1016/j.bmcl.2024.129800

[20] Hemalatha K, Madhumitha G. Study of binding interaction between anthelmintic 2,3-dihydroquinazolin-4-ones with bovine serum albumin by spectroscopic methods. J Lumin. 2016; 178: 163-171. https://doi.org/10.1016/j.jlumin.2016.05.041

[21] Alamri MA, Afzal O, Akhtar MJ, Karim S, Husain M, Alossaimi MA, Riadi Y. Synthesis, in silico and in vitro studies of novel quinazolinone derivatives as potential SARS-CoV-2 3CLpro inhibitors. Arab J Chem. 2024; 17: 105384.
https://doi.org/10.1016/j.arabjc.2023.105384

[22] Deng Y, Chen M, Yi J, Zheng Y. Design, synthesis, and anti–tobacco mosaic virus activity evaluation of quinazolinone derivatives containing purine moieties. Phytochem Lett. 2024; 59: 10-14. https://doi.org/10.1016/j.phytol.2023.11.003

[23] Qhobosheane MA, Legoabe LJ, Petzer A, Petzer JP. The monoamine oxidase inhibition properties of C6-mono- and N3/C6-disubstituted derivatives of 4(3H)-quinazolinone. Bioorg Chem. 2019; 85: 60-65.
https://doi.org/10.1016/j.bioorg.2018.12.030

[24] Khalifa MM, Sakr HM, Ibrahim A, Mansour AM, Ayyad RR. Design and synthesis of new benzylidene-quinazolinone hybrids as potential anti-diabetic agents: In vitro α-glucosidase inhibition, and docking studies. J Mol Struct. 2022; 1250: 131768. https://doi.org/10.1016/j.molstruc.2021.131768

[25] Tokalı FS, Sağlamtaş R, Öztekin A, Yırtıcı Ü, Çomaklı V. New diacetic acids containing quinazolin-4(3H)-one: Synthesis, characterization, anticholinergic properties, DFT analysis and molecular docking studies. Chemistry Select 2023; 8(e202205039): 1-9. https://doi.org/10.1002/slct.202205039

[26] Moftah HK, Mousa MHA, Elrazaz EZ, Kamel AS, Lasheen DS, Georgey HH. Novel quinazolinone Derivatives: Design, synthesis and in vivo evaluation as potential agents targeting Alzheimer disease. Bioorg Chem. 2024; 143: 107-065.
https://doi.org/10.1016/j.bioorg.2023.107065

[27] Bouley R, Ding, D, Peng Z, Bastian, M, Lastochkin E, Song W, Suckow MA, Schroeder VA, Wolter WR, Mobashery S, Chang M. Structure−activity relationship for the 4(3H)-quinazolinone antibacterials. J Med Chem. 2016; 59: 5011-5021.
https://doi.org/10.1021/acs.jmedchem.6b00372

[28] Gatadi S, Lakshmi TV, Nanduri S. 4(3H)-Quinazolinone derivatives: Promising antibacterial drug leads. Eur J Med Chem. 2019; 170: 157-172. https://doi.org/10.1016/j.ejmech.2019.03.018

[29] Liu T, Peng F, Cao X, Liu F, Wang Q, Liu L, Xue W. Design, synthesis, antibacterial activity, antiviral Activity, and mechanism of myricetin derivatives containing a quinazolinone moiety. ACS Omega 2021; 6: 30826-30833.
https://doi.org/10.1021/acsomega.1c05256

[30] Rasapalli S, Murphy ZF, Sammeta VR, Golen JA, Weig AW, Melander RJ, Melander C, Macha P, Vasudev MC. Synthesis and biofilm inhibition studies of 2-(2-amino-6-arylpyrimidin-4-yl)quinazolin-4(3H)-ones. Bioorg Med Chem Lett. 2020; 30: 127550. https://doi.org/10.1016/j.bmcl.2020.127550

[31] Rakesh KP, Kumara HK, Ullas BC, Shivakumara J, Gowda DC. Amino acids conjugated quinazolinone-Schiff's bases as potential antimicrobial agents: Synthesis, SAR and molecular docking studies. Bioorg Chem. 2019; 90: 103093.
https://doi.org/10.1016/j.bioorg.2019.103093

[32] Türk S, Karakuş S, Maryam A, Oruç-Emre EE. Synthesis, characterization, antituberculosis activity and computational studies on novel Schiff bases of 1,3,4-thiadiazole derivatives. J Res Pharm. 2020; 24(6): 793-800.
https://doi.org/10.35333/jrp.2020.232

[33] Kadi AA, El-Brollosy NR, Al-Deeb OA, Habib EE, Ibrahim TM, El-Emam AA. Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. Eur J Med Chem. 2007; 42: 235-242.
https://doi.org/10.1016/j.ejmech.2006.10.003

[34] Türk S, Karakuş S, Ece A, Ulusoy S, Boşgelmez-Tınaz G. Synthesis, structure elucidation and biological activities of some novel 4(3H)-quinazolinones as anti-biofilm agents. Lett Drug Des Discov. 2019; 16: 313-321.
https://doi.org/10.2174/1570180815666180621101123

[35] Türk S, Turan K, Ulusoy S, Karakuş S, Boşgelmez-Tınaz G. Synthesis, characterization and biological activity studies on amide derivatives. Istanbul J Pharm. 2018; 48(3): 76-81. https://doi.org/10.26650/IstanbulJPharm.2018.18007

[36] Karakus S, Kocyigit-Kaymakcioglu B, Toklu HZ, Aricioglu F, Rollas S. Synthesis and anticonvulsant activity of new N-(alkyl/substitutedaryl)-N'-4-(5-cyclohexylamino)-1,3,4-thiadiazole-2-yl)phenythioureas. Arch Pharm. 2009; 342(1): 48-53. https://doi.org/10.1002/ardp.200800118

[37] Ulusoy S, Akalın RB, Çevikbaş H, Berisha A, Oral A, Boşgelmez-Tinaz G. Zeolite 4A as a jammer of bacterial communication in Chromobacterium violaceum and Pseudomonas aeruginosa. Future Microbiol. 2022; 17(11), 861-871.
https://doi.org/10.2217/fmb-2021-0174

[38] Uraz M, Karakuş S, Mohsen UA, Kaplancıklı ZA, Rollas S. The synthesis and evaluation of anti-acetylcholinesterase activity of some 4(3H)-quinazolinone derivatives bearing substituted 1,3,4-thiadiazole. Marmara Pharm J. 2017; 21: 96-101.
https://doi.org/10.12991/marupj.259886

How to cite this article: Türk S, Ulusoy S, Karakuş S, Boşgelmez Tınmaz G. Synthesis and Anti-Biofilm Activity Studies on Novel Quinazolinone-Thiadiazole Hybrids. Clin Exp Health Sci 2025; 15: 170-174. https://doi.org/10.33808/clinexphealthsci.1548035

Clinical and Experimental Health Sciences

Modulation of MMP9 and AKT by Escin in Retinal Pigment Epithelial Cells: Exploring Novel Therapeutic Approaches for Proliferative Vitreoretinopathy

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Received: September 14, 2024 **Accepted:** January 20, 2025

ABSTRACT

Objective: The aim of this study was to investigate the anti-inflammatory and antioxidant effects of Escin molecule obtained from horse chestnut seed extract on retinal pigment epithelial cell lines (ARPE-19).

Methods: In this research, the ARPE-19 cell line, which is a commercially available retinal pigment epithelial cell line derived from the normal eyes of a 19-year-old male, was utilized. Escin was administered to the cells in varying concentrations of 100, 50, 10, 5, and 1 micromolar throughout a 48-hour timeframe. The IC50 concentration was subsequently determined through MTT cell viability assays. To determine cell migration, a wound healing assay was executed. To quantify MMP9 and AKT protein levels, analysis was conducted using Western blot. Additionally, the mRNA expression levels of *EGF, EGFR, PDGF-β, PDGFβ-R*, and *HIF1A* were analyzed using RT-PCR.

Results: Escin inhibited cell migration in RPE cells. Western blot analysis showed that escin decreased the levels of AKT and MMP9 proteins. Furthermore, it was found that the mRNA expression levels of *PDGFß, PDGFβ-R,* and *HIF1A* were suppressed following escin administration.

Conclusion: Escin has the potential to slow disease progression by suppressing cell migration in retinal pigment epithelial cells. With its anti-angiogenic properties, escin shows promise for developing new therapeutic approaches for the treatment of retinal diseases.

Keywords: Escin, cell migration, retinal pigment epithelial cell, MMP9, AKT

1. INTRODUCTION

The retinal pigment epithelium (RPE) is integral to the retina, carrying out important functions necessary for vision. This monolayer consists of regular polygonal cells situated in the retina's outermost layer. On its outer side, the RPE is linked to Bruch's membrane and the choroid, while its internal face interacts pertaining to the outer segments of photoreceptor cells. The RPE handles a diverse array of functions that are crucial for preserving visual health. These include light absorption, formation of the outer blood-retina barrier, continuity of the visual cycle, regulation of ion and fluid flow, as well as providing protection against oxidative damage, immunomodulation, and phagocytosis of the outer segments of photoreceptors (1).

Retinal diseases can arise from various causes and often have serious clinical consequences. The most common include retinal detachment (2), macular degeneration (3), diabetic retinopathy (4) and retinal vein occlusions (5). These conditions can lead to vision loss and even blindness. Although treatment options vary depending on the patient's condition and severity of the disease, these clinical conditions can be effectively managed with appropriate treatment and care.

Proliferative vitreoretinopathy (PVR) is one of the serious consequences of retinal detachment, characterized by abnormal proliferation, migration, and extracellular matrix (ECM) production of retinal cells. The surgical success rate in PVR cases is very low (6,7). Most other diseases that cause permanent visual damage, such as PVR, are characterized by excessive proliferation. All of these conditions can be referred to as proliferative retinopathy (PR).

Escin is a triterpenoid saponin obtained from the grains of the buckeye tree (*Aesculus hippocastanum*). It is acknowledged in modern medicine for its anti-inflammatory, antioxidant, and vascular-protective properties, which make it useful in treating a number of conditions, such as wound healing and venous insufficiency (8). Research indicates that escin as a favorable impact on the wound healing workflow and enhances the quality of healing (9). Specifically, it has been

https://doi.org/10.33808/clinexphealthsci.1550061

noted that escin facilitates fibroblast proliferation and collagen synthesis, thus speeding up wound healing (10). Moreover, escin's anti-inflammatory properties aid the healing process by mitigating inflammation in affected wounds (11). In light of this, further investigation is essential to gain a deeper understanding of escin's effects on wound healing and to assess its clinical effectiveness. These potential therapeutic benefits of escin may mark a significant advancement in developing new treatment strategies, particularly for wound healing and chronic wounds.

Matrix metalloproteinases (MMPs) are enzymes that are essential for tissue remodeling and wound healing. Among them, matrix metalloproteinase-9 (MMP-9) is particularly important for cell migration, angiogenesis, and the reorganization of the extracellular matrix (ECM), especially during the wound healing process (12). In the inflammatory phase of wound healing, MMP-9 aids the migration of inflammatory cells to the injury site and assists in reorganizing wound tissue by breaking down ECM components (13). Additionally, MMP-9's effects on angiogenesis contribute for the growth of novel blood vessels, which enhances the distribution of oxygen and nutrients to the wound area, thus speeding up the healing process (14). On the other hand, an overexpression of MMP-9 can result in tissue damage and the formation of chronic wounds, emphasizing the necessity for careful regulation of its levels (15). These dual roles of MMP-9 underline its potential as both a therapeutic target and a biomarker in the wound healing process.

AKT (protein kinase B) is a pivotal element of a signaling pathway that significantly influences cellular growth, proliferation, and survival. The stimulation of the AKT pathway can result in the enhanced expression of MMP-9, initiating a variety of cellular processes. Once activated, AKT alters gene expression in the nucleus by phosphorylating transcription factors that govern intracellular signaling pathways (16). Specifically, transcription factors like NF-κB and AP-1 bind to the regulatory domain of the MMP-9 gene, leading to increased levels of MMP-9 mRNA (17). This regulatory influence of AKT on MMP-9 is essential for processes such as cell migration, invasion, and tissue remodeling. Notably, in cancer cells and during wound healing, the AKT-mediated upregulation of MMP9 aids the healing process by promoting the breakdown of the ECM and enhancing cellular migration (18). Therefore, gaining a deeper understanding of the AKT-MMP-9 interaction may be crucial for developing novel therapeutic strategies for wound healing.

This study focused on examining how escin influences cell migration in RPE cells, specifically regarding the roles of MMP-9 and AKT.

2. METHODS

Escin was obtained from Sigma-Aldrich (Cat. No: 6805-41-0). The ARPE-19 cell line was sourced from the ATCC (CRL-2302, Manassas, VA, USA). The primer antibodies for MMP-9 (Cat No: 10176-2-AP), AKT (Cat. No: 10375-2-AP), and beta actin (Cat. No: 20536-1-AP) were purchased from Proteintech (IL, USA). All PCR primers were synthesized by Bioligo Biotechnology (Ankara, Turkey).

2.1. Cell Culture

Complete medium was prepared by adding 10 ml of fetal bovine serum, 1 ml of L-Glutamine, and 1 ml of penicillin-streptomycin to 100 ml of DMEM. Cells were then incubated in this medium at 37°C with 5% CO2. All experiments were conducted using commercially available ARPE-19 cell lines to represent RPE cells.

2.2. Determination of IC50 Doses Using the MTT Cell Viability Assay

Cells were seeded into 96-well plates at a density of 10^4 cells per well and exposed to different concentrations of escin (100, 50, 10, 5, and 1 micromolar) for 48 hours. Following the incubation period, 10 µl of MTT solution (5 mg/ml, SERVA, Heidelberg, Germany) was added to each well and the plates were incubated for another 4 hours at 37°C in a 5% CO2 environment. Upon completion of the incubation, 100 µl of dimethyl sulfoxide (DMSO) was introduced to each well, and the absorbance was assessed using a multiplate reader (Epoch, Biotek, USA) at a wavelength of 572 nm. IC50 values were determined using GraphPad Prism version 8.0.1 (GraphPad Software, Inc., CA, USA). All analyses were conducted in triplicate.

2.3. Protein assessment from cell lysates

Following two washes with PBS, they were lysed with RIPA buffer. The lysate obtained was centrifuged at 16,000 g for 15 minutes at 4°C and the supernatant was transferred to a clean Eppendorf tube. After protein isolation, quantification was performed with the BCA assay (TaKaRa, Shiga, Japan).

2.4. Western Bloting

Thirty micrograms of the isolated protein were combined with 5X SDS PAGE Sample Loading Buffer (ABP Biosciences, Cat. No: P013), 10X Sample Reducing Agent (Novex Bolt, Ref: B0009), and water to achieve a final volume of 20 µl. This mixture was heated at 95°C for 10 minutes using a BIORAD T100 Thermal Cycler and then rapidly cooled to +4°C. Separation of denatured proteins was achieved through SDS-PAGE on a 4-12% Bis-Tris gradient gel (Invitrogen NuPAGE, Cat. No: NP0323BOX) and transferred to a nitrocellulose membrane using the iBLOT2 Gel Transfer System (Invitrogen, Ref. No: IB23001). The membrane was treated with a 5% BCA-PBST solution at room temperature for 1 hour. Primary antibodies utilized included Beta Actin (Ptglab, Cat. No: 20536-1-AP), MMP9 (Ptglab, Cat No: 10176-2-AP), and AKT (Ptglab, Cat. No: 10375-2-AP), while IgG (Ptglab, Cat. No: SA000001-2) served as the secondary antibody. For chemiluminescence imaging, the membrane was treated with ECL buffer and

photographed using a Syngene chemiluminescence imager, with bands quantified using the GeneTools software.

2.5. Isolation of Total RNA, cDNA Synthesis, and Quantitative PCR

After 48 hours of incubation, cells were harvested and rinsed with cold PBS. Total RNA was purified using the RNA Purification Kit (Thermo Scientific, Catalog No: K0731), and its amount and quality were assessed with the Epoch Take3 plate system (Agilent, USA). Following the manufacturer's instructions (Biorad Catalog No: BR1708891), cDNA was synthesized from 1 µg of RNA using reverse transcriptase. Subsequently, Each 20 µl PCR reaction mixture contained 1 µl of cDNA, 10 µl of 2X SYBR Green PCR Master Mix (diluted to 1X as per the manufacturer's instructions), and specific primers at a final concentration of approximately 750 nM per reaction. All procedures were performed under cold chain and sterile conditions Target gene expression was normalized against the housekeeping gene GAPDH. The gene expression values were calculated using the RQ = $2^{-\Delta\Delta Ct}$ formula, based on the ΔΔCt method, with analysis performed using the REST2009 program. Table 1 provides the details of the primer sequences used in the PCR reactions and their respective conditions. Each analysis was conducted in quadruplicate.

Table 1. *Oligonucleotide Primer Sequences and PCR Programs*

Genes	Primer sequences (5' → 3')	RT-PCR Programs	Cycle
GAPDH	F-5'GATTTGGTCGTATTGGGCGC 3' R-5'AGTGATGGCATGGACTGTGG 3'	95˚C-30s/59˚C-1m/72˚C-30s	35
EGF	F-5'CTGAATGTCCCCTGTCCCAC-3' R-5 CTCGGTACTGACATCGCTCC-3'	95˚C-30s/59˚C-1m/72˚C-30s	35
EGFR	F-5'CGCAAAGTGTGTAACGGAATAGG-3' R-5'GGCTGACGACTGCAAGAGAA-3'	95˚C-30s/58˚C-1m/72˚C-30s	35
PDGF-ß	F-5'CTCGTCCGTCTGTCTCGATG-3' R-5' CACACCCACCAAGAGGAGTC-3'	95˚C-30s/59˚C-1m/72˚C-30s	35
PDGFß-R	F – 5'CACCAACGTGGCTTTTCTGG-3' R – 5' GGTGCGGTTGTCTTTGAACC-3'	95˚C-30s/57˚C-1m/72˚C-30s	35
HIF1A	F – 5'TGCTGGGGCAATCAATGGAT-3' R – 5'CTACCACGTACTGCTGGCAA-3'	95˚C-30s/60˚C-1m/72˚C-30s	35

2.6. Wound Healing Assay

6-well plates were used for the assay, and cells were seeded at a density of 300,000 cells per well and incubated overnight. After creating scratches in the monolayer using a sterile 200 µl pipette tip, the cells were rinsed twice with PBS. Following washing, escin at the previously determined IC50 dose from the MTT assay was applied to the wells for 48 hours. Control wells contained 0.5% DMSO in the culture medium. The culture medium was standardized to 5% FBS for all groups. Wound images were taken immediately after scratching and after 48 hours, with three measurements taken at different points across the wound area for each sample. Wound closure rates were analyzed using the ImageJ program. Each experiment was repeated in triplicate (n = 3) to ensure statistical significance.

2.7. Statistical Analysis

Data analysis for the study was performed using GraphPad Prism version 8.0.1 (GraphPad Software, Inc., CA, USA). To assess whether the data followed a normal curve, the Shapiro-Wilk normality test was applied. After confirming normal distribution, a two-tailed t-test, which is a parametric test, was used to compare the two groups. A p-value of <0.05 was considered statistically significant.

3. RESULTS

3.1. IC50 doses of bioactive compounds

The IC50 value of escin for a 48-hour treatment was determined to be 18.99 µM (Figure 1).

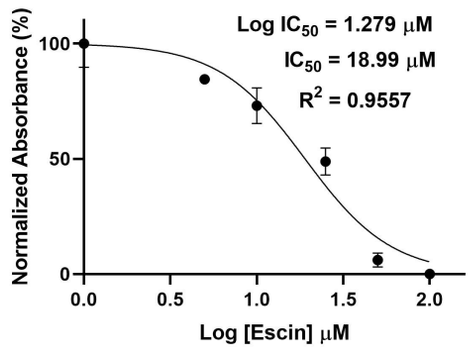

Figure 1. *IC50 dose of Escin*

3.2. The IC50 dose of escin slowed down cell migration

According to the results of the wound healing model, wound closure was observed at 71.16 ± 5.511% in the control group, while it was 51.81 ± 4.374% in the escin group (Table 2, Figure 2). Wound healing was notably slower in the group treated with escin compared to the control group, with a p-value of .0015.

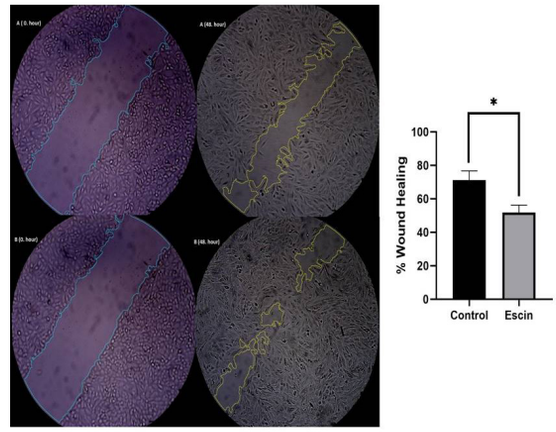

Figure 2. *The effect of Escin at IC50 dose (18.99 µM) on wound healing. A: Escin group, B: Control group, 0th hour on the left, 48th hour on the right in all groups. Wound healing measurements were made using ImageJ software.*

Table 2. *Comparison of % wound healing assay, western blot findings and RT-PCR data between groups.*

% Wound Healing Assay			
	Control	Escin	*p* value
% Wound Healing	71.16±5.511[a]	51.81±4.374[b]	.0015
Normalized Band Intensity			
MMP9	1.095 ± 0.1267[a]	0.4556 ± 0.01944[b]	.0010
AKT	1.001 ± 0.02690[a]	0.4878 ± 0.04081[b]	<.0001
mRNA Expression Levels			
EGF	1[a]	0.917[a]	.604
EGFR	1[a]	0.959[a]	.824
PDGFβ	1[a]	0.710[b]	.044
PDGFβ-R	1[a]	0.645[b]	.025
HIF1A	1[a]	0.684[b]	.036

There is a statistical difference between the values represented by different superscript the rows. (p < .05). All expression levels were compared to the control group. The expression level data represent fold increases or decreases. P < .05 was considered statistically significant. Different superscripts between groups indicate that there is a statistical difference between these groups. MMP9: Matrix metalloproteinase 9, AKT: Protein kinase B, PDGF-β: Platelet Derived Growth Factor Beta, PDGFβ-R: Platelet Derived Growth Factor Beta Receptor, HIF1A: Hypoxia-inducible factor-1α

3.3. Escin decreased MMP9 levels via AKT

As a result of the Western blot analysis, AKT protein levels were suppressed to 0.4878 ± 0.04081 fold ($p < .0001$), while MMP9 protein levels were suppressed to 0.4556 ± 0.01944 fold ($p = .001$) in the escin-treated group compared to the control group (Table 2, Figure 3). This result suggests that escin may impair migration pathways critical for wound closure, potentially through its suppressive effects on AKT signaling and MMP9 protein levels. These findings align with previous studies indicating that MMP9 suppression is associated with reduced cellular migration in cancer models.

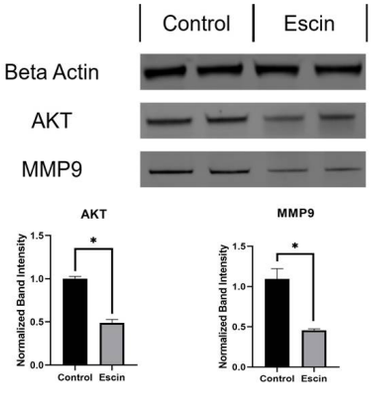

Figure 3. *Western blot bands and graphs. MMP9: Matrix metalloproteinase 9, AKT: Protein kinase B, *p≤ .0001*

3.4. Escin had a suppressive effect on the mRNA expression levels of PDGFβ, PDGFβ-R and HIF1A.

In the escin-treated group, the mRNA expression levels of *PDGFβ*, *PDGFβ-R*, and *HIF1A* were significantly suppressed compared to the control group ($p = .044$, $p = .025$, and $p = .036$, respectively)(Figure 4). These results suggest that escin exerts a regulatory effect on genes associated with angiogenesis and cell proliferation. The observed suppression of *HIF1A*, a key transcription factor activated under hypoxia, supports the notion that escin may interfere with hypoxia-induced signaling pathways, thereby limiting the cellular responses to low oxygen conditions.

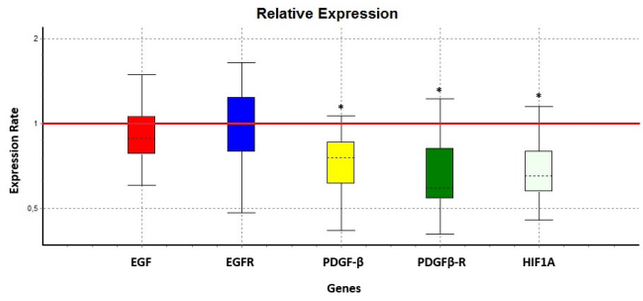

Figure 4. *Relative mRNA expression levels of PDGF-β, PDGFβ-R and HIF1A. Values are expressed as the mean ± SD. All groups were compared with the control group, and the results were given as fold increase/ decrease. The REST 2009 software (Qiagen) was used for statistical analysis and graphing. The red line parallel to the x-axis shows the position of the control group. p < 0.05 was considered statistically significant. EGF: Epidermal Growth Factor, EGFR: Epidermal Growth Factor Receptor, PDGF-β: Platelet Derived Growth Factor Beta, PDGFβ-R: Platelet Derived Growth Factor Beta Receptor, HIF1A: Hypoxia-inducible factor-1α*

Interestingly, no significant changes were detected in the mRNA expression levels of *EGF* and *EGFR* ($p = .604$ and $p = .824$, respectively), indicating that escin's effects might be more specific to the *PDGFβ* signaling axis rather than broadly targeting all growth factor pathways. This specificity may reflect a targeted mechanism of action that distinguishes escin from other agents with broader, less selective effects. Similarly, the selective downregulation of *PDGFβ* signaling has been implicated in reduced cell migration and proliferation.

Taken together, these results highlight escin's potential as a modulator of angiogenic and hypoxia-related signaling pathways, making it a promising candidate for therapeutic applications targeting tumor progression or pathological angiogenesis.

4. DISCUSSION

Oxidative stress has been shown to contribute to various acquired and hereditary diseases in the retinal pigment epithelial (RPE) layer. Factors such as aging, exposure to sunlight, and inflammation significantly increase the risk of conditions like macular degeneration, proliferative vitreoretinopathy (PVR), and Stargardt disease (19). A critical aspect of PVR is the migration and proliferation of RPE cells, which transforms into fibroblast-like cells that contribute to the formation of epiretinal membranes. Therefore,

suppressing cell migration represents a promising therapeutic strategy to prevent the progression of PVR (20,21).

Recent studies have focused on the role of various bioactive agents and molecular targets in modulating RPE cell behavior, particularly in suppressing migration. Biological molecules and antibodies that inhibit cell migration have shown potential for use in treating PR, with promising results in preventing recurrence following surgical treatments (22,23). However, further research is essential to establish the safety and efficacy of these therapeutic approaches in clinical settings.

A growing body of work has explored the use of bioactive compounds to target key biochemical pathways in RPE cells. For example, Wang et al. (24) reported that escin exhibited both cytoprotective and anti-protective effects against oxidative stress in RPE cells, specifically activating AKT-Nrf2 signaling to shield RPE cells from oxidative damage. In addition, escin has been shown to mitigate ischemic damage in the brain by enhancing antioxidant enzyme activities, including superoxide dismutase and glutathione peroxidase.

Furthermore, studies such as those by Hollborn et al. (25) have analyzed the impact of matrix metalloproteinases (MMPs) like MMP-2 and MMP-9, along with vascular endothelial growth factor (VEGF), in RPE cell proliferation and migration. Their findings indicate that hypoxic conditions elevate the expression of MMP-2 and MMP-9, with MMP-9 subsequently enhancing VEGF expression in RPE cells. In a similar vein, Sen et al. (26) examined the protective effects of rosmarinic acid (RA) and thymoquinone (TQ) against retinal damage, noting that overdose of these compounds hindered the wound healing process, emphasizing the need for optimal dosing.

In this study, the effects of escin on cell migration in RPE cells were investigated, leading to significant findings. The results indicate that escin suppresses the wound healing process by decreasing cell migration. This finding is consistent with previous studies and supports escin's properties in suppressing cell migration (27).

Escin treatment was shown to suppress the mRNA expression levels of key signaling molecules, including PDGF-β, PDGFβ-R, and HIF1A in RPE cells, which are critical for regulating cell migration. PDGF-β is involved in cell proliferation, migration, and angiogenesis, while PDGFβ-R plays an essential role in the signaling pathway, enhancing cell motility (28). Our findings suggest that escin's suppression of these molecules may contribute to the inhibition of RPE cell migration.

We also assessed the expression of epidermal growth factor (EGF) and its receptor EGFR in RPE cells. Interestingly, escin treatment did not significantly alter the expression levels of EGF or EGFR compared to controls, with relative expression changes of 0.917 ($p = 0.604$) for EGF and 0.959 ($p = 0.824$) for EGFR. These results imply that escin may not directly influence the EGF/EGFR signaling axis in RPE cells, which distinguishes it from other agents that act through this pathway.

The role of EGF and EGFR in angiogenesis and pathological cell behaviors has been well established. Studies by Keller and Schmidt (29) demonstrated that EGFR and its variant EGFRvIII significantly contribute to tumor progression, promoting angiogenesis and cell invasion, particularly in glioblastoma. In retinal diseases such as diabetic retinopathy and age-related macular degeneration, EGFR activation also promotes pathological neovascularization, as demonstrated by Deng et al. (30). However, our study suggests that escin's mechanism of action does not primarily involve this pathway, highlighting its potential to avoid EGFR-mediated toxicity in normal tissues.

The lack of significant changes in EGF and EGFR expression suggests that escin may exert its effects through alternative pathways, such as PDGFβ, PDGFβ-R, and HIF1A. This specificity of action could be advantageous in contexts where broad EGFR inhibition is undesirable. Furthermore, escin's ability to suppress cell migration by targeting these signaling pathways may be particularly beneficial in treating retinal diseases, including PVR.

Additionally, escin's suppression of PDGF-β, PDGFβ-R, and HIF1A expression may contribute to the inhibition of cell migration under both normoxic and hypoxic conditions. This could be valuable in preventing pathological migration and proliferation of RPE cells, a hallmark of PVR. MMP9, a crucial player in wound healing and extracellular matrix remodeling, was also significantly downregulated in response to escin, consistent with its known regulation by the AKT pathway (16,17). The reduction in MMP9 expression may explain the observed inhibition of wound healing and cell migration, reinforcing escin's role in modulating these processes.

While the findings of this study suggest that escin may be a promising therapeutic agent for treating proliferative retinal diseases, further in vivo studies are necessary to confirm the clinical applicability of these results. In addition, this study focused on a limited number of cell signaling pathways and proteins, and future investigations should explore a broader range of molecular pathways and cell types to better understand the full spectrum of escin's effects on RPE cell migration.

Our study was conducted under in vitro conditions, and the effects of escin on RPE cells in in vivo models remain uncertain. Further research involving in vivo models is needed to validate the potential of escin as a therapeutic agent. Additionally, this study examined only a limited subset of molecular pathways, and a more comprehensive analysis of other signaling pathways is required to fully characterize escin's effects.

5. CONCLUSION

In this study, we investigated the effects of escin on retinal pigment epithelial (RPE) cells, focusing on its potential to modulate key signaling pathways involved in cell migration and angiogenesis. Our findings demonstrated that escin significantly suppresses the wound healing process by

reducing cell migration, likely through the downregulation of PDGFβ, PDGFβ-R, and HIF1A mRNA expression levels. Additionally, escin was shown to decrease MMP9 and AKT protein levels, suggesting a potential mechanism for its inhibitory effects on cell migration. These results align with existing literature and support the hypothesis that escin may impair cellular pathways critical for RPE cell migration, particularly in pathological conditions like proliferative vitreoretinopathy (PVR).

Despite these promising results, the study acknowledges certain limitations, including its in vitro nature and the focus on a limited set of molecular pathways. Further in vivo research is essential to confirm the therapeutic potential of escin in retinal diseases and to explore its broader impacts on other signaling pathways. Overall, escin shows promise as a potential therapeutic agent for managing proliferative retinal conditions, but comprehensive studies are required to validate its efficacy and safety in clinical settings.

Acknowledgements: *We would like to thank Marmara University for providing access to academic databases.*

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *Since this is a cell culture study, ethical approval is not required according to relevant guidelines.*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: SŞ and MK
Design of the study: SŞ, MK, SS and OT
Acquisition of data for the study: SŞ
Analysis of data for the study: SŞ, MK and SS
Interpretation of data for the study: MK, SŞ and OT
Drafting the manuscript: SŞ, MK, SS and OT
Revising it critically for important intellectual content: SŞ, MK, SS and OT
Final approval of the version to be published: SŞ, MK, SS and OT

REFERENCES

[1] Strauss O. Theretinal pigment epithelium in visualfunction. *PhysiolRev.* 2005;85(3):845-881. https://doi.org/10.1152/physrev.00021.2004

[2] Wilkinson C. P. Retinal detachment. *Pathologic Myopia*. 2021;347-356. https://doi.org/10.1007/978-3-030-74334-5_23

[3] Fleckenstein M, Keenan TD, Guymer RH, Chakravarthy U, Schimitz-Valckenberg S, Klaver CC, Wong WT, Chew EY. Age-related macular degeneration. *Nature reviews Disease primers*. 2021;7(1):31. https://doi.org/10.1038/s41572-021-00265-2

[4] Amjad M, Gupta H, Anamika, Kumar R. (2024). DiabeticRetinopathy: CurrentUnderstanding, MechanismsandTreatmentStrategies. *JournalforResearch in AppliedSciencesandBiotechnology*. 2024;3(2):252-260. https://doi.org/10.55544/jrasb.3.2.42

[5] Lendzioszek M, Bryl A, Poppe E, Zorena K, Mrugacz M. Retinal Vein Occlusion–Background Knowledge and Foreground Knowledge Prospects—A Review. *Journal of ClinicalMedicine*. 2024;13(13):3950. https://doi.org/10.3390/jcm13133950

[6] Idrees S, Sridhar J, Kuriyan AE. Proliferative Vitreoretinopathy: A Review. *IntOphthalmolClin*. 2019;59(1):221-240. https://doi.org/10.1097/IIO.0000000000000258

[7] Kwon OW, Song JH, RohMI. Retinal detachment and proliferative vitreoretinopathy. *Retinal Pharmaco therapeutics*. 2016;55:154-162. https://doi.org/10.1159/000438972

[8] Rogers SL, McIntosh RL, Cheung N, Lim L, Wang JJ, Mitchell P, Kowalski JW, Nguyen H, Wong TY, . The prevalence of retinal vein occlusion: pooled data from population studies from the United States, Europe, Asia, and Australia. *Ophthalmology*. 2010;117(2):313-319.e1 https://doi.org/10.1016/j.ophtha.2009.07.017

[9] Yue S, Wang T, Yang Y, Fan Y, Zhou L, Li M, Fu F. Lipopolysaccharide/D-galactosamine-induced acute liver injury could be attenuated by dopamine receptor agonist rotigotine via regulating NF-κB signaling pathway. Int Immunopharmacol. 2021;96:107798. https://doi.org/10.1016/j.intimp.2021.107798.

[10] Zhang L, Chen X, Wu L, Li Y, Wang L, Zhao X, Zhao T, Zhang L, Yan Z, Wei G. Ameliorative effects of escin on neuropathic pain induced by chronic constriction injury of sciatic nerve. J Ethnopharmacol. 2021 Mar 1;267:113503. https://doi.org/10.1016/j.jep.2020.113503.

[11] Yang Y, Wang L, Yuan M, Yu Q, Fu F. Anti-Inflammatory and Gastroprotective Effects of Escin. Natural Product Communications. 2020;15(12). https://doi.org/10.1177/1934578X20982111

[12] Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: Regulators of the tumor microenvironment. *Cell*. 2010; 141(1), 52-67. https://doi.org/10.1016/j.cell.2010.03.015

[13] Caley MP, Martins VL, O'Toole EA. Metalloproteinases and wound healing. *Advances in Wound Care.* 2015*;4*(4):225-234. https://doi.org/10.1089/wound.2014.0581

[14] Rundhaug JE. Matrix metalloproteinases and angiogenesis. *Journal of Cellular and Molecular Medicine.* 2005;9(2):267-285. https://doi.org/10.1111/j.1582-4934.2005.tb00355.x

[15] Armstrong DG, Jude EB. (2002). The role of matrixmetallo proteinases in wound healing. *Journal of the American Podiatric Medical Association.* 2002;92(1):12-18. https://doi.org/10.7547/87507315-92-1-12

[16] Fang J, Shing Y, Wiederschain D. Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. *Proceedings of the National Academy of Sciences.* 2000;97(8), 3884-3889. https://doi.org/10.1073/pnas.97.8.3884

[17] Han YP, Tuan TL, Wu H, Hughes M, Garner WL. TNF-α stimulates activation of pro-MMP2 in human skin through NF-(κ)B mediated induction of MT1-MMP. *Journal of Cell Science.* 2001;114(1), 131-139. https://doi.org/10.1242/jcs.114.1.131

[18] Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. *Molecular CancerResearch.* 2011;9(5), 259-270. https://doi.org/10.1158/1541-7786.MCR-05-0261

[19] Yang S, Zhou J, Li D. Functions and Diseases of the Retinal Pigment Epithelium. *Front Pharmacol*. 2021;12:727870. https://doi.org/10.3389/fphar.2021.727870

[20] Chaudhary R, Scott RA, Wallace G, Berry M, Logan A, Blanch RJ. Inflammatory and fibrogenic factors in proliferative vitreoretinopathy development. *Translational vision science&technology*. 2020;9(3):23-23. https://doi.org/10.1167/tvst.9.3.23

[21] Kaczmarek R, Misiuk-Hojło M. Patomechanisms in proliferative vitreoretinopathy. *Klinika Oczna*. 2011;113(1-3):64-67. PMID: 21853955.

[22] Du YH, Hirooka K, Miyamoto O, Bao YQ, Zhang B, An JB, Ma JX. . Retinoic acid suppresses the adhesion and migration of human retinal pigment epithelial cells. *ExperimentalEyeResearch*. 2013;109:22-30. https://doi.org/10.1016/j.exer.2013.01.006

[23] Umazume K, Liu L, Scott PA, Fernandez de Castro JP, McDonald K, Kaplan HJ, Tamiya S. Inhibition of PVR with a tyrosine kinase inhibitor, dasatinib, in theswine. *Investigative ophthalmology&visual science*. 2013;54(2):1150-1159. https://doi.org/10.1167/iovs.12-10418

[24] Wang K, Jiang Y, Wang W, Ma J, Chen M. Escin activates AKT-Nrf2 signaling to protect retinal pigment epithelium cells from oxidative stress. *Biochem BiophysResCommun*. 2015;468:541-547. https://doi.org/10.1016/j.bbrc.2015.10.117

[25] Hollborn M, Stathopoulos C, Steffen A, Wiedemann P, Kohen L, Bringmann A. Positive feedback regulation between MMP-9 and VEGF in human RPE cells. *InvestOphthalmolVisSci*. 2007;48:4360-4367. https://doi.org/10.1167/iovs.06-1234

[26] Sen S, Kasikci M. Low-dose rosmarinic acid and thymoquinone accelerate wound healing in retinal pigment epithelialcells. *IntOphthalmol*. 2023;43:3811-3821. https://doi.org/10.1007/s10792-023-02799-8

[27] Wang XH, Xu B, LiuJT, Cui JR. Effect of β-escin sodium on proliferation, migration and apoptosis of endothelial cells. *Vascular pharmacology*. 2008;49(4-6):158-165. https://doi.org/10.1016/j.vph.2008.07.005

[28] Zou X, Tang XY, Qu ZY, Sun ZW, Ji CF, Li, YJ, Guo SD. Targeting the PDGF/PDGFR signaling pathway for cancer therapy: A review. *International Journal of Biological Macromolecules*. 2022;202:539-557. https://doi.org/10.1016/j.ijbiomac.2022.01.113

[29] Keller S, Schmidt MH. EGFR and EGFRvIII promote angiogenesis and cell invasion in glioblastoma: combination therapies for an effective treatment. *International journal of molecular sciences*, 2017; 18(6), 1295. https://doi.org/10.3390/ijms18061295

[30] Deng W, Huang K, Cui L, Niu Z, Ke D, Jiang L, Tang N, Zhong H, Lan Q, Xu F, Tang F. SN promote retinal pathological neovascularization through activation of EGFR, IR and IGF-1R. *ExpEyeRes.* 2024;15(250):110158. https://doi.org/10.1016/j.exer.2024.110158

[31] Mudhar HS. A brief review of the histopathology of proliferative vitreoretinopathy (PVR). *Eye*. 2020;*34*(2):246-250. https://doi.org/10.1038/s41433-019-0724-4

[32] Wu Q, You L, Nepovimova E, Heger Z, Wu W, Kuca K, Adam V. Hypoxia-inducible factors: master regulators of hypoxic tumor immune escape. *Journal of hematology&oncology*. 2022;15(1):77. https://doi.org/10.1186/s13045-022-01292-6

How to cite this article: Şen S, Kaşıkcı M, Sul S, Tekin O. Modulation of MMP9 and AKT by Escin in Retinal Pigment Epithelial Cells: Exploring Novel Therapeutic Approaches for Proliferative Vitreoretinopathy. Clin Exp Health Sci 2025; 15: 175-181. DOI: 10.33808/clinexphealthsci.1550061

Clinical and Experimental Health Sciences

Impact of the COVID-19 Pandemic on the Emotional State of School Children

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Received: September 17, 2024 **Accepted:** January 30, 2025

ABSTRACT

Objective: This study was conducted to determine school children's (8–10 years of age) feelings during the COVID-19 pandemic.

Methods: This study was descriptive. The study was conducted online between March 1 and June 17, 2022, with 49 children and their parents living in Türkiye who met the inclusion criteria and agreed to participate.

Results: Our study found that the anxiety scores of children who were diagnosed themselves or had someone in their families diagnosed were higher than those who were not diagnosed. According to the drawing technique, the rate of insecurity and anger was higher in children with a family member diagnosed with COVID-19 compared to those without a diagnosis. According to the results of the correlation, statistically significant relationships were found between anxiety scores and shyness (p=.031), between impulsivity and insecurity (p=.000), between impulsivity and anger (p=.029), and between insecurity and anger (p=.029).

Conclusion: Nurses should be aware of the social and emotional impact of pandemics on children and be able to use the drawing method, a therapeutic communication technique, to determine children's perceptions of the pandemic process and how they are affected by it.

Keywords: Child, COVID-19, drawing, anxiety, nurse

1. INTRODUCTION

The COVID-19 pandemic emerged in December 2019 as a global health issue affecting the whole world (1). The pandemic can cause physical and psychological health problems and negative social and economic impacts (2,3). Measures such as hand hygiene, protective masks, and social isolation have been introduced to prevent the rapid spread of the virus and control the disease (4). Children are one of the groups most affected and vulnerable to the problems caused by the pandemic (5,6). Children may be susceptible to stressors due to their developmental characteristics. Children diagnosed with COVID-19 may experience various difficulties in treatment, care, and quarantine. All over the world, health systems have been established to assist children during epidemics and quarantine periods. Some of these are international organizations such as UNICEF and WHO, while others are national ministries, municipalities, universities, and associations (7, 8, 9).

Pandemics may disrupt children's social relationships, adversely affect their school life, result in significant changes in their daily lives, and lead them to experience emotions such as fear, anxiety, anger, insecurity, reduced attention span, loneliness, and distress (2, 4, 7, 10-13). One of the most common negative emotions experienced by children is anxiety. Several factors cause anxiety. These factors include physical, psychological, social, and emotional situations (14-17).

Children who experience intense social isolation, stress, fear, and anxiety during the pandemic may be negatively impacted developmentally in their current and future life stages (12, 18). Voltmer and Salisch, 2024 revealed in their study that the COVID-19 pandemic increased the future anxiety of primary school 3rd and 4th-grade students (19). Many national and international health authorities have stated that children experiencing the pandemic need support and assistance to identify their feelings, manage their current emotions, and look to the future with confidence (20, 21). Although the pandemic does not continue with as much impact as it did

https://doi.org/10.33808/clinexphealthsci.1542187

in the early days, the pandemic and related long-term effects are seen in school children. These effects can be found even 10–20 years after a disastrous event (22). The primary goals in alleviating the traumatic impact of the pandemic are to ensure that children regain their sense of trust, reduce the impact of the pandemic, prevent the adverse effects that may be caused by it, improve/enhance their level of functioning, and enable children to express themselves (11, 13). The role of the pediatric nurse as a researcher, caregiver, and educator in this process is crucial.

Children may not be able or willing to verbally express their feelings and thoughts about themselves, their family, and their social environment. Therapeutic communication techniques help children express their feelings and thoughts (23, 24). Drawing is one of the most effective techniques in this context. Drawing is a communication tool with a stronger and simpler expression than the words and expressions the child has learned (25, 26). Drawing allows children to reveal and express their inner world and how they perceive the external world, feelings, thoughts, fears, anxieties, violence, and frustrations (27). No study on the effect of the COVID-19 pandemic on the emotional state of school children has been reported in the literature. This study aimed to determine the emotions of school children (8–10 years of age) during COVID-19.

A comparison of children between the ages of 8 and 10 years who were either diagnosed themselves or had a family member diagnosed with COVID-19 and those who were not will be used to answer the following questions:

- Is there a difference between the two groups regarding the emotional indicators obtained from the Draw-a-Person test drawings?
- Is there a difference between them in terms of the mean scores on the Trait Anxiety Inventory for Children?

2. METHODS

This descriptive study was carried out on Turkish children between the ages of 8 and 10 years old after obtaining their parents' permission.

2.1. Sample and Population Selection

The study was conducted online with 49 children and their parents living in Türkiye who agreed to participate. Inclusion criteria for the study were that the child was between the ages of 8 and 10, the child or parent had an Android phone/tablet/PC and internet access, and the child and parent agreed to participate. Exclusion criteria were that the child had an illness that prevented communication, had a medical diagnosis of a mental disorder, and had experienced a significant life event other than the pandemic (e.g., moving to a new house in the past three months or divorce of parents) and any condition that prevented the child from drawing. Convenience sampling, one of the purposive sampling methods, was used to select the sample. The rationale for using this method was to reach participants who were accessible online due to the pandemic.

2.2. Data Collection Tools

The data was collected using the Descriptive Characteristics Form, State-Trait Anxiety Inventory for Children (STAI-CH), and Draw-a-Person test.

The Descriptive Characteristics Form consists of 14 closed-ended questions about the age, education, and employment status of the parents, the age and sex of the child, and the COVID-19 diagnosis status of the child and family members. According to the literature, this form was developed by the researchers (13, 28, 29).

The State-Trait Anxiety Inventory for Children (STAI-CH) instrument was developed by Spielberger (1973) for measuring state and trait anxiety in children between the ages of nine and 12 years. The inventory consists of two parts: the State Anxiety Inventory and the Trait Anxiety Inventory. Each inventory consists of 20 items, making 40 items in total. The Trait Anxiety Inventory is designed to determine how a child feels in general. The inventory uses a 3-point Likert-type scale, and items are scored using the following options: almost never = 1, sometimes = 2, and almost always = 3. The lowest score of 1 indicates the absence of the included emotions. The highest score obtained from each item is 3, which indicates that the presence of emotions is very high. The total score obtained from the Trait Anxiety Inventory varies between 20 and 60 (30).

The Draw-a-Person Test was developed by Koppitz (31). The test is used in the developmental assessment of drawings by children between the ages of 5 and 12 years, and it helps determine the presence of emotional indicators. Koppitz studied children's human figure drawings to assess the presence of emotional indicators. The 28 emotional indicator items included in the test have clinical validity, and they are grouped into the five following categories: impulsivity, insecurity/inadequacy, anxiety, shyness, and anger. Raters score each item as 'present' or 'absent' based on a child's drawing (31, 32).

2.3. Data Collection and Procedure

The research began after the necessary permissions were obtained from the Ethics Committee, along with written consent from the parents of the children and verbal consent from the children. The research was conducted between March 1 and June 17, 2022. Data collection forms were completed by the children and their parents using Google Forms. Parents who agreed to participate in the study were told they could help the children fill out the Descriptive Data Form and the State-Trait Anxiety Inventory for Children. After filling out the forms, children were asked to draw pictures for the Draw a Person test. Parents were asked to provide

A4 paper, eraser, pencil, and other necessary materials for the drawing test. It was emphasized that parents should not interfere with children's drawings, should not direct them, and that drawing should only be done by children. The child was asked to determine the pencil, colors, and figures that the child would use while drawing. Parents took photos of their children's drawings and sent them to the researchers online.

2.4. Ethical Considerations

The research was started after obtaining permission from the Ethics Committee of the Gazi University (E-77082166-604.01.02-279409), the parents' written consent, and the children's verbal consent. The parental consent form was created using Google Forms and sent to parents online. Children of parents who signed the informed consent form verbally agreed to participate in the study and whose forms were properly completed were included in the study. Their personal information will be kept confidential to protect the children's privacy.

2.5. Statistical Analysis

The study data was evaluated using the SPSS 22.0 (IBM Corp. in Armonk, New York, USA) packaged software, and the values were expressed as numbers and percentages. The Mann–Whitney U test was used to assess whether there was a difference between the anxiety states of children with and without a COVID-19 diagnosis. The children's drawings were scored according to Koppitz's scoring criteria, and the children's emotional indicators were determined. The consistency between two experts in the field of painting who evaluated the children's drawings was determined by the Kappa test, and a significant level of consistency (K=0.784, p=0.00) was found among the raters who analyzed the drawings. The chi-squared test was used for two variables to determine whether the relationship between the emotional indicators determined by the drawings of children with and without a COVID-19 diagnosis was significant. The correlation test was used to evaluate the relationship between the children's anxiety scores and the results of the drawing analysis.

3. RESULTS

The mean age of the children in our study was 8.47 ± 0.67 years, while the mean ages of parents were 36 ± 6.09 years for mothers and 40.85±5.37 years for fathers. Regarding educational status, 38.80% of the mothers were primary school graduates, while 30.60% were high school graduates; furthermore, 42.80% of the fathers were college or university graduates. The unemployment rate was 75.50% for mothers, whereas it was 85.70% for fathers. At least one family member had been diagnosed with COVID-19 for 59.20% of the children. The rate of children diagnosed with COVID-19 was 16.30% (Table 1).

Table 1. *Sociodemographic characteristics of the child and family*

Characteristics	M±SD	Min-Max
Child's age (years)	8.47±0.67	7-10
Mother's age	36±6.09	24-48
Father's age	40.85±5.37	28-54
	n	%
The educational level of the mother		
Primary School	19	38.80
High School	15	30.60
University	15	30.60
Educational level of father		
Primary School	14	28.60
High School	14	28.60
University	21	42.80
Employment status of the mother		
Employed	12	24.50
Unemployed	37	75.50
Employment status of the father		
Employed	42	85.70
Unemployed	7	14.30
COVID-19 Diagnosis Status in the Family		
Diagnosed	29	59.20
Not Diagnosed	20	40.80
The child's diagnosis of COVID-19 in the last six months		
Diagnosed	8	16.30
Not Diagnosed	41	83.70

The mean anxiety score of children with at least one family member diagnosed with COVID-19 was 34.20 ± 6.24. The mean anxiety score of children diagnosed with COVID-19 in the last six months was 36.40 ± 4.97. There was no significant relationship between the mean anxiety scores of children with family members who had been diagnosed with COVID-19 versus those without or those who had been diagnosed with COVID-19 versus those who had not. Children diagnosed with COVID-19 or who had a family member diagnosed with COVID-19 had higher anxiety scores than those who did not have a family member diagnosed with COVID-19. However, the difference was not statistically significant (Table 2).

Table 2. *The effect of the presence or absence of a COVID-19 diagnosis in the children themselves and in one of their family members on the anxiety score.*

Characteristics	M±SD	Min-Max	U	p	z
Children diagnosed with COVID-19	36.40±4.97	29-44	158.500	.364	-0.907
Children not diagnosed with COVID-19	34.79±6.96	23-50			
Family member diagnosed with COVID-19	34.20±6.24	23-47	240.000	.308	-1.019
Family member not diagnosed with COVID-19	36.45±7.01	26-50			
Total	35.12±6.59	23-50			

Indications of impulsivity and shyness were found in the drawings of 50% of the children diagnosed with COVID-19,

while indications of insecurity were found in 70% of the drawings by these children. In contrast, symptoms of impulsivity, insecurity, and shyness were observed in about half of the children whose family members were diagnosed with COVID-19. Anger, anxiety, shyness, insecurity, and impulsivity combined are moderately related to anxiety scores.

According to the correlation analysis results, there was a very weak positive correlation between the anxiety score and the anxiety state in the drawing analysis (r= 0.096). On the contrary, there was a very weak negative correlation between impulsivity (r= – 0.061), insecurity (r= – 0.173), and anger (r= – 0.068), as well as a weak negative relationship with shyness (r= – 0.274). There were statistically significant differences between anxiety and shyness (p=.031), impulsivity and insecurity (p=.000), impulsivity and anger (p=.029), and insecurity and anger (p=.029) (Table 3).

Table 3. *The relationship between children's anxiety score and picture analysis results (r)*

	Anxiety score	Impulsivity	Insecurity	Anxiety	Shyness	Anger
Anxiety score		-0.061	-0.173	0.096	-0.274	-0.068
Anxiety score	.	0.341	0.122	0.260	0.031	0.325
Impulsivity	0.341	.	0.000	0.425	0.071	0.029
Insecurity/ inadequacy	0.122	0.000	.	0.084	0.388	0.029
Anxiety	0.260	0.425	0.084	.	0.388	0.178
Shyness/ timidity	0.031	0.071	0.388	0.388	.	0.125
Anger/ aggressiveness	0.325	0.029	0.029	0.178	0.125	.

The multiple correlation coefficient is R=362.

Table 4. *The results of picture analysis of children's family members diagnosed with COVID-19 versus those who had not in*

		Diagnosed		Not diagnosed			
Emotional Indicators Categories		Count	Percent[a]	Count	Percent[b]	X^2	p
Impulsivity	Yes	11	40.74	8	40.00	0.003	.959
	No	16	59.26	12	60.00		
Insecurity/ inadequacy	Yes	12	44.44	7	35.00	0.426	.514
	No	15	55.56	13	65.00		
Anxiety	Yes	9	33.33	10	50.00	1.325	.250
	No	18	66.67	10	50.00		
Shyness/ timidity	Yes	13	48.14	13	65.00	1.320	.251
	No	14	51.86	7	35.00		
Anger/ aggressiveness	Yes	4	14.82	1	05.00	*	*
	No	23	85.18	19	95.00		

[a]*n=27*
[b]*n=20*
**Fisher Exact Sig. (2-tailed)=0.377*

The rates of mistrust and anger were higher in children whose family members were diagnosed with COVID-19 than in those who were not. Feelings of anxiety and shyness were higher in those whose family members had not been diagnosed with COVID-19. The feeling of impulsivity was similar in both groups. However, the difference in emotions between children with and without a family member diagnosed with COVID-19 was not statistically significant (Table 4). Feelings of insecurity and anger were significantly higher in children diagnosed with COVID-19 than they were in those without a diagnosis, but the result was not statistically significant (Table 5).

Tablo 5. *The results of picture analysis of children diagnosed with COVID-19 versus those who had not.*

		Diagnosed		Not diagnosed		
Emotional Indicators Categories		Count	Percent[a]	Count	Percent[b]	p*
Impulsivity	Yes	5	50.00	14	37.83	.496
	No	5	50.00	23	62.17	
Insecurity/ inadequacy	Yes	7	70.00	12	32.43	.066
	No	3	30.00	25	67.57	
Anxiety	Yes	3	30.00	16	43.24	.718
	No	7	70.00	21	56.76	
Shyness, timidity	Yes	5	50.00	21	56.76	.734
	No	5	50.00	16	43.24	
Anger/ aggressiveness	Yes	2	20.00	3	08.11	.285
	No	8	80.00	34	91.89	

[a]*n=10*
[b]*n=37*
** Fisher Exact Sig*

4. DISCUSSION

The COVID-19 pandemic has been a time of many negative experiences, both physical and mental. Children were especially away from their families, school, activities, and friends due to the quarantine process. Investigating the psychological effects of this will be useful in terms of taking the necessary precautions in similar situations in the future. In our study, we aimed to evaluate the anxiety of children during the COVID-19 pandemic using both quantitative method and drawing methods. Drawing is one of several therapeutic techniques that can help children express themselves, and it is a highly effective tool for communicating with children, especially in situations where communication is interrupted and insufficient, such as during a pandemic (33, 34). There are many studies in the literature to evaluate childhood anxiety during the COVID-19 pandemic (35-37). In the literature review, no study was found in which the quantitative and drawing methods were used together in children during the Covid 19 pandemic.

In our study, children diagnosed with COVID-19 and those not diagnosed with COVID-19 had similar anxiety scores. A study by Zhang et al. (38) found that similar to our study, the anxiety scores of children during the COVID-19 pandemic were not high. Studies in the literature at the beginning of the quarantine period (especially the first six weeks) reported that anxiety symptoms were higher in children (39-41). Still, studies conducted after this first period indicated that children's anxiety symptoms were at normal levels (13,

38, 42, 43). A systematic review and meta-analysis conducted by Miao et al. determined that the anxiety level, which was high in the early days of quarantine, increased again towards the second quarantine (44). The fact that the children in our study, whether diagnosed with COVID-19 or not, had similar levels of anxiety might be due to the study data being collected after the first six months of the pandemic. This can be explained by the fact that children's knowledge of and experience with quarantine and isolation measures, mask use, and social distancing measures increased over time. In addition, some studies have reported that academic success, academic process anxiety, and social isolation, which have been essential sources of anxiety during the pandemic, are experienced more by adolescents than by children (36, 45). Adolescents have higher anxiety scores than children, and children experience less anxiety because they are less affected by difficulties during these periods (46, 47, 48). It is thought that the children 8 and 10 in our study did not have high anxiety scores because they were at the beginning of their academic lives.

In our study, the anxiety levels of children whose family members were diagnosed with COVID-19 were very close to those of children who were diagnosed themselves. This shows that children are affected by their parents' anxiety (e.g., job loss, loss of loved ones, and deterioration of financial and psychological conditions) (14, 33). It has been observed that children who have lost a family member due to COVID-19 experience anger and express this loss in their drawings (Figure 1). The analysis of the children's drawings shows that children who were not themselves diagnosed and whose family members were not diagnosed with COVID-19 expressed more anxiety in their drawings (Figure 2). This result led us to believe that previously undiagnosed children and their families were worried about being diagnosed with COVID-19 and infecting their families with the disease. Our results are similar to those observed in the literature (11, 33, 38, 49-53).

Figure 1. *Boy, 8 years, His grandfather died due to covid-19. He drew a heart between his grandfather's house and his grave. He drew the coronavirus next to his grandfather's grave.*

Figure 2. *Girl, 8 years, She draws dark clouds and rain. Signs of intense anxiety appear.*

There was a positive relationship between anxiety and anxiety scores reflected in the children's drawings. In particular, anger and insecurity were seen more often in the drawings of children who had been diagnosed with COVID-19 or who had a family member diagnosed with COVID-19 (Figure 3). At the same time, anxiety was more common in children who had not been diagnosed (Figure 4). It has been interpreted that the reason for the high level of anxiety in undiagnosed children is that they have a fear of the unknown and that those who have been diagnosed may have less anxiety because they have been through the process. Studies in the literature show that knowledge about the pandemic and the disease reduces anxiety (54, 55). Our study supports these results.

Figure 3. *Girl, 8 years. The absence of hands indicates insecurity, asymmetry of arms and legs indicates impulsivity, exaggerated teeth indicate anger, and legs together indicate anxiety.*

Figure 4. *Gril, 8 years. The absence of feet is a sign of insecurity, the absence of a nose is a sign of shyness. In addition, the child is seen to have a sad facial expression.*

Our study has shown that it is easier for children to express the psychological distress and emotions caused by the pandemic when they transfer their pandemic experiences to paper through drawing. This technique is helpful in communicating with children and enabling them to express themselves. Idoiaga et al. (56) used the drawing in their study to examine how children aged 6-12 years understand the COVID-19 pandemic in their daily thoughts. The drawings evaluated in the study were examined under four themes. These were related to pandemic symbols (32.34%), emotions (30.55%), actions taken (27.18%), and socialization (9.90%). In our study, children also drew symbols, emotions, and events related to the pandemic (56). Also, in two studies conducted during the COVID-19 pandemic, children were asked to express their pandemic experiences through drawings. These studies found that the drawing reducing effectively facilitated the expression of children's experiences and reduced emotional distress (33, 51). Drawing can be an effective method for expressing and regulating emotions. Drawing is an effective and reliable tool to help children cope with a crisis, increase emotional expression and awareness, and express themselves (57). The use of drawings and visual models can assist in understanding and communicating appropriately with children who may not be able to fully express their emotions verbally, especially in situations where the disease process is severe, clinical symptoms are prolonged, and deaths occur, such as in pandemics.

4.1. Strength and Limitations

The strongest aspect of our study is its originality due to the absence of a similar study on this topic in the literature. Our results allow us to better understand the emotional states of children and the role of families in this process, with its content and the parameters it measures. In addition, it will be a guide for programs to be developed in this sense.

Although our study was conducted with children from many geographical regions, due to the pandemic's constraints, observing the children during their drawing activities or talking to them about their emphases and explanations was impossible. In addition, the study cannot be generalized to any region or area because the children reached in our study cannot represent any universe. One limitation of this study was that the researchers stayed away from the children and parents during the drawings.

5. CONCLUSION

Our findings showed that symptoms of impulsivity and shyness were determined in the drawings of half of the children diagnosed with COVID-19. Symptoms of impulsivity, insecurity, and shyness were observed in half of the children whose parents were diagnosed with COVID-19. The relationship between the draw-a-man test scores and the children's anxiety scale scores was analyzed insecurity analyzed, and according to this, anger, fear, shyness, insecurity analyzed, and according to this, anger, fear, shyness, insecurity, and impulsivity together are moderately related to the anxiety score. There were statistically significant differences between "anxiety and shyness", "impulsivity and insecurity", "impulsivity and anger", and "insecurity and anger". Nurses should be aware of the social and emotional impact of pandemics on children and be able to use drawing, a therapeutic communication technique, to determine children's perceptions of the pandemic process and how they are affected by it.

Acknowledgements: *We would like to thank the children and their parents who participated in the study. Also the authors would like to thank Gazi University Academic Writing Application and Research Center for proofreading the article*
Funding: *The authors received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Gazi University,*
Noninvasive Clinic Ethics Committee (Approval date: January 25, 2022; Number: E-77082166-604.01.02-279409)
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: RC, BE, GC, NA, EK
Design of the study: RC, BE, GC, NA, EK
Acquisition of data for the study: RC, BE, GC
Analysis of data for the study: RC, BE, GC
Interpretation of data for the study: RC, BE, GC, NA, EK
Drafting the manuscript: RC
Revising it critically for important intellectual content: NA, EK
Final approval of the version to be published: RC, BE, GC, NA, EK

REFERENCES

[1] Umakanthan S, Chauhan A, Gupta, MM, Sahu PK, Bukelo MM, Chattu VK. COVID-19 pandemic containment in the Caribbean Region: A review of case-management and public health strategies. AIMS Public Health. 2021;8(4):665. https://doi.org/10.3934/publichealth.2021053

[2] Kaur R, Boobna T, Kallingal P Effect of Covid-19 lock down on indian children with autism. Research in Developmental Disabilities. 2022;125:104230.
https://doi.org/10.1016/j.ridd.2022.104230

[3] Serlachius A, Badawy SM, Thabrew H. Psychosocial challenges and opportunities for youth with chronic health conditions during the COVID-19 pandemic. JMIR Pediatrics and Parenting. 2020;3(2):e23057.
https://doi.org/10.2196/23057

[4] Richter SA, Ferraz-Rodrigues C, Schilling LB, Camargo NF, Nunes ML. Effects of the COVID-19 pandemic on sleep quality in children and adolescents: A systematic review and meta-analysis. Journal of sleep research. 2023;32(1):e13720. https://doi.org/10.1111/jsr.13720

[5] Buheji M, Hassani A, Ebrahim A, da Costa Cunha K, Jahrami H, Baloshi M, Hubail S. Children and coping during COVID-19: A scoping review of bio-psycho-social factors. International Journal of Applied Psychology. 2020;10(1):8-15.
https://doi.org/10.5923/j.ijap.20201001.02

[6] Christakis DA School reopening—the pandemic issue that is not getting its due. JAMA pediatrics. 2020;174(10):928-928. DOI:10.1001/jamapediatrics.2020.2068

[7] Anand P, Patil RS, Puri P, Patil S. The psychosocial effects of lockdown due to the COVID-19 Pandemic on children in 2021. Cureus, 2024;16(2): e53614.
https://doi.org/10.7759/cureus.53614

[8] UNICEF. Psychosocial Support for Children during COVID-19. A Manual for Parents and Caregivers. 2024. https://www.unicef.org/india/media/3401/file/PSS-COVID19-Manual-ChildLine.pdf

[9] TC Sağlık Bakanlığı. Covid Bilgilendirme Platformu. 2024. https://covid19.saglik.gov.tr/TR-66158/81-il-psikososyal-destek-hat-bilgileri.html

[10] Becker SP, Gregory AM. Editorial Perspective: Perils and promise for child and adolescent sleep and associated psychopathology during the COVID-19 pandemic. Journal of Child Psychology and Psychiatry. 2020;61(7):757-759.
https://doi.org/10.1111/jcpp.13278

[11] Jefsen OH, Rohde C, Nørremark B, Østergaard SD. Editorial Perspective: COVID-19 pandemic-related psychopathology in children and adolescents with mental illness. Journal of Child Psychology and Psychiatry. 2020;62:798–800.
https://doi.org/10.1111/jcpp.13292

[12] Phelps C, Sperry LL. Children and the COVID-19 pandemic. Psychological Trauma: Theory, Research, Practice, and Policy. 2020. https://doi.org/10.1037/tra0000861

[13] Ravens-Sieberer U, Kaman A, Erhart M, Devine J, Schlack R, Otto C. Impact of the COVID-19 pandemic on quality of life and mental health in children and adolescents in Germany. Eur Child Adolesc Psychiatry. 2021;31(6):879-889.
https://doi.org/10.1007/s00787-021-01726-5

[14] Courtney D, Watson P, Battaglia M, Mulsant BH, Szatmari P. COVID-19 impacts on child and youth anxiety and depression: challenges and opportunities. The Canadian Journal of Psychiatry. 2020;65(10):688-691.
https://doi.org/10.1177/0706743720935646

[15] Liu JJ, Bao Y, Huang X, Shi J, Lu L Mental health considerations for children quarantined because of COVID-19. The Lancet Child & Adolescent Health. 2020;4(5):347-349.
https://doi.org/10.1016/S2352-4642(20)30096-1

[16] Racine N, McArthur BA, Cooke JE, Eirich R, Zhu J, Madigan S. Global prevalence of depressive and anxiety symptoms in children and adolescents during COVID-19: a meta-analysis. JAMA Pediatrics. 2021;175(11):1142-1150.
https://doi.org/10.1001/jamapediatrics.2021.2482

[17] Şahbudak B, Emiroğlu Nİ. COVID-19 pandemic and mood disorders in children and adolescents. Turk J Child Adolesc Ment Health. 2020;27(2):59-63. *(Turkish)*

[18] Wang G, Zhang Y, Zhao J, Zhang J, Jiang F. Mitigate the effects of home confinement on children during the COVID-19 outbreak. The Lancet. 2020;395(10228):945-947.
https://doi.org/10.1016/S0140-6736(20)30547-X

[19] Voltmer K, von Salisch M. Longitudinal prediction of primary school children's COVID-related future anxiety in the second year of the pandemic in Germany. Plos one. 2024;19(5):e0302065.
https://doi.org/10.1371/journal.pone.0302065

[20] The American Academy of Pediatrics COVID-19 guidance for safe schools and promotion of ın-person learning. The American Academy of Pediatrics. 2023. https://www.aap.org/en/pages/2019-novel-coronavirus-covid-19-infections/clinical-guidance/covid-19-planning-considerations-return-to-in-person-education-in-schools

[21] World Health Organization. Considering the impact of COVID-19 on children. World Health Organization. 2023. https://www.who.int/europe/activities/considering-the-impact-of-covid-19-on-children

[22] Wolf K, Schmitz J. Scoping review: longitudinal effects of the COVID-19 pandemic on child and adolescent mental health. Eur Child Adolesc Psychiatry. 2023;33(5):1257–1312.
https://doi.org/10.1007/s00787-023-02206-8

[23] Lyon P. Using drawing in visual research: materializing the invisible. In The SAGE Handbook of Visual Research Methods (Second ed., pp. 297-308). SAGE Publications, Inc., 2020. https://doi.org/10.4135/9781526417015

[24] Volans A, Brown E. Children expressing themselves. Oxford Textbook of Palliative Care for Children, 2021;95.

[25] Brechet C, D'Audigier L, Audras-Torrent L. The use of drawing as an emotion regulation technique with children. Psychology of Aesthetics, Creativity, and the Arts. 2022;16(2):221–232. https://doi.org/10.1037/aca0000314

[26] Mondragon IN, Munitis EA, Sancho BN, Etxebarria ON. Drawing the COVID-19 pandemic: how do children incorporate the health crisis and its consequences into their everyday thinking? Psychology & Health. 2022.
https://doi.org/10.1080/08870446.2022.2066103

[27] Farley L, Mishra Tarc A. Drawing trauma: The therapeutic potential of witnessing the child's visual testimony of war. Journal of the American Psychoanalytic Association. 2014;62(5):835-854. https://doi.org/10.1177/0003065114554419

[28] Schmidt SJ, Barblan LP, Lory I, Landolt MA. Age-related effects of the COVID-19 pandemic on mental health of children and adolescents. Eur J Psychotraumatol. 2021;12(1):1901407. https://doi.org/10.1080/20008198.2021.1901407

[29] Zengin M, Yayan EH, Vicnelioğlu E. The effects of the COVID-19 pandemic on children's lifestyles and anxiety levels. Journal of Child and Adolescent Psychiatric Nursing. 2021;34(3):236-242. https://doi.org/10.1111/jcap.12316

[30] Ozusta HS. Çocuklar için durumluk-sürekli kaygı envanteri uyarlama, geçerlik ve güvenirlik çalışması [Adaptation, validity and reliability study of state-trait anxiety inventory for

children]. Turkish Journal of Psychology. 1995;10(34):32-44. *(Turkish)*

[31] Koppitz EM. Psychological evaluation of children's human figure drawings. NewYork: Grune&Stratton. 1968.

[32] Koppitz, EM. Psychological evaluation of human figure drawings by middle school pupils. London: Grune&Stratton. 1984.

[33] Abdulah DM, Abdulla BMO, Liamputtong P. Psychological response of children to home confinement during COVID-19: A qualitative arts-based research. International Journal of Social Psychiatry. 2021;67(6):761-769.
https://doi.org/10.1177/0020764020972439

[34] Moitra M, Rahman M, Collins PY et al. Mental health consequences for healthcare workers during the COVID-19 pandemic: A scoping review to draw lessons for LMICs. Frontiers in psychiatry. 2021;12:602-614.
https://doi.org/10.3389/fpsyt.2021.602614

[35] Cornaggia A, Bianco F, Gilli G, Marchetti A, Massaro D, Castelli I. Children's representations of the COVID-19 lockdown and pandemic through drawings. Frontiers in Psychology. 2022;13:960893.
https://doi.org/10.3389/fpsyg.2022.960893

[36] Idoiaga Mondragon N, Eiguren Munitis A, Berasategi Sancho N, Ozamiz Etxebarria N. Drawing the COVID19 pandemic: how do children incorporate the health crisis and its consequences into their everyday thinking? Psychology & Health. 2022a;19:1-20. https://doi.org/10.1080/08870446.2022.2066103

[37] Le Vu MN, Do AL, Boyer L, et al. A review of the effectiveness, feasibility, and acceptability of art therapy for children and adolescents during the COVID-19 pandemic. International Journal of Environmental Research And Public Health. 2022;19(18):11612.
https://doi.org/10.3390/ijerph191811612

[38] Zhang L, Zhang D, Fang J, Wan Y, Tao F, Sun Y. Assessment of mental health of Chinese primary school students before and after school closing and opening during the COVID-19 pandemic. JAMA Network Open 3. 2020:e2021482.
https://doi.org/10.1001/jamanetworkopen.2020.21482

[39] Qi M, Zhou SJ, Guo, ZC, Zhang LG, Min HJ, Li XM, Chen JX. The effect of social support on mental health in chinese adolescents during the outbreak of COVID-19. The Journal of Adolescent Health. 2020a;67:514–518.
https://doi.org/10.1016/j.jadohealth.2020.07.001

[40] Yeasmin S, Banik R, Hossain S, Hossain MN, Mahumud R, Salma N, Hossain MM. Impact of COVID-19 pandemic on the mental health of children in Bangladesh: A cross-sectional study. Children and Youth Services Review. 2020;117:105277. https://doi.org/10.1016/j.childyouth.2020.105277

[41] Zhou SJ, Wang LL, Yang R, Yang XJ, Zhang LG, Guo ZC, et al. Sleep problems among Chinese adolescents and young adults during the coronavirus-2019 pandemic. Sleep Medicine. 2020a;74:39–47. https://doi.org/10.1016/j.sleep.2020.06.001

[42] Cao Y, Huang L, Si T, Wang NQ, Qu M, Zhang XY. The role of only-child status in the psychological impact of COVID-19 on mental health of Chinese adolescents. Journal of Affective Disorders. 2021;282:316–321.
https://doi.org/10.1016/j.jad.2020.12.113

[43] Tang S, Xiang M, Cheung T, Xiang YT. Mental health and its correlates among children and adolescents during COVID-19 school closure: The importance of parentchild discussion. Journal of Affective Disorders. 2021;279:353–360.
https://doi.org/10.1016/j.jad.2020.10.016

[44] Miao, R., Liu, C., Zhang, J., & Jin, H. Impact of the COVID-19 pandemic on the mental health of children and adolescents: a systematic review and meta-analysis of longitudinal studies. Journal of Affective Disorders. 2023;340:914-922.
https://doi.org/10.1016/j.jad.2023.08.070

[45] Carter C, Barley R, Omar A. 'I wish that COVID would disappear, and we'd all be together': Maintaining Children's friendships during the Covid-19 pandemic. Children & Society. 2023. https://doi.org/10.1111/chso.12693

[46] Idoiaga Mondragon N, Eiguren Munitis A, Berasategi Sancho N, Picaza Gorrotxategi M, Dosil Santamaria M. How are children coping with COVID-19 health crisis? Analysing their representations of lockdown through drawings. Childhood. 2022b;29(4):545-560.
https://doi.org/10.1177/09075682221101199

[47] Ellis WE, Dumas TM, Forbes LM. Physically isolated but socially connected: Psychological adjustment and stress among adolescents during the initial COVID-19 crisis. Canadian Journal of Behavioural Science/Revue Canadienne Des Sciences Du Comportement. 2020;52:177–187.
https://doi.org/10.1037/cbs0000215

[48] O'Sullivan K, Clark S, McGrane A et al. A qualitative study of child and adolescent mental health during the COVID-19 pandemic in Ireland. International Journal of Environmental Research and Public Health. 2021;18(3):1062.
https://doi.org/10.3390/ijerph18031062

[49] Abawi O, Welling MS, van den Eynde E et al. COVID-19 related anxiety in children and adolescents with severe obesity: A mixed-methods study. Clinical Obesity. 2020;10:e12412. https://doi.org/10.1111/cob.12412

[50] Evans S, Mikocka-Walus A, Klas A, Olive L, Sciberras E, Karantzas G, Westrupp EM. From "It has stopped our lives" to "spending more time together has strengthened bonds": The varied experiences of Australian families during COVID-19. Frontiers in Psychology. 2020;11:588-667.
https://doi.org/10.3389/fpsyg.2020.588667

[51] Jones K, Hughes B. Children's Experiences of Death Anxiety and Responses to the Covid-19 Pandemic. Illness, Crisis & Loss. 2022;31(3):1-18.
https://doi.org/10.1177/10541373221100899

[52] Jiao WY, Wang LN, Liu J, Fang SF, Jiao FY, Pettoello-Mantovani M, Somekh E. Behavioral and emotional disorders in children during the COVID-19 epidemic. The Journal of Pediatrics. 2020;221:264–266.e1.
https://doi.org/10.1016/j.jpeds.2020.03.013

[53] Xue Q, Xie X, Liu Q et al. Knowledge, attitudes, and practices towards COVID- 19 among primary school students in Hubei Province, China. Children and Youth Services Review. 2021;120:105735.
https://doi.org/10.1016/j.childyouth.2020.105735

[54] Qi H, Liu R, Chen X, Yuan XF, Li YQ, Huang HH, et al. Prevalence of anxiety and associated factors for Chinese adolescents during the COVID-19 outbreak. Psychiatry and Clinical Neurosciences. 2020b;74:555–557.
https://doi.org/10.1111/pcn.13102

[55] Zhou SJ, Zhang LG, Wang LL, Guo ZC, Wang JQ, Chen JC, et al. Prevalence and sociodemographic correlates of psychological

health problems in Chinese adolescents during the outbreak of COVID-19. European Child and Adolescent Psychiatry. 2020b;29:749–758.
https://doi.org/10.1007/s00787-020-01541-4

[56] Idoiaga MN, Eiguren MA, Berasategi SN, Ozamiz EN. Drawing the COVID-19 pandemic: How do children incorporate the health crisis and its consequences into their everyday thinking?. Psychology & Health. 2024;39(3):379-398.
https://doi.org/10.1080/08870446.2022.2066103

[57] Drake JE. How children can use drawing to regulate their emotions. Theory Into Practice. 2023;62(2):181-192.
https://doi.org/10.1080/00405841.2023.2202132

How to cite this article: Çelik R, Erdoğan B, Çakır G, Altay N, Kılıçarslan E. Impact of the COVID-19 Pandemic on the Emotional State of School Children. Clin Exp Health Sci 2025; 15: 182-190. DOI: 10.33808/clinexphealthsci.1542187

Clinical and Experimental Health Sciences

The Effects of Mental Health First Aid Training on Positive Mental Health and Mental Health Literacy on Office Workers: A Randomized Controlled Trial

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Received: September 21, 2024 **Accepted:** March 6, 2025

ABSTRACT

Objective: Mental health problems are among the most common health problems today.

This study was a randomized controlled trial in order to determine the effect of nurse-led mental health first aid training (MHFA) on mental health and mental health literacy (MHL) of office workers in Turkey.

Methods: The study was performed with 88 employees in the intervention group and 90 employees in the control group, who were randomly allocated. The data were collected with positive mental health (PMH) scale and MHL scale via online forms. Intervention group received 8-hour MHFA course. The primary outcomes included autonomy, personal satisfaction, problem solving and self-actualization, self-control, prosocial attitude and interpersonal relationship skills of PMH sub-dimensions. The secondary outcome was mental health literacy. Post-test was administered one week following the completion of the MHFA training. Data were analyzed using chi-square, independent sample t test and repeated-measures ANOVA.

Results: Following the MHFA training, post-test scores of the PMH scale personal satisfaction, self-control, autonomy, problem solving and self-actualization, prosocial attitude and interpersonal relationship skills were found to increase in favor of the intervention group ($p<.05$). It was determined that MHL scores of the intervention group increased ($p<.001$).

Conclusion: Mental health first aid training was determined to be an effective program and can be used in workplaces.

Keyword: Mental health first aid, mental health literacy, positive mental health

1. INTRODUCTION

Mental disorder is a serious public health issue that is common enough to be diagnosed in 1 of 3 adults. However, many people with mental disorder remain undiagnosed and untreated (1). According to the data of the World Health Organization (2), 76-85 percent of people with mental illness do not receive treatment in low – and middle-income countries. By contrast, in high-income countries, 35-50 percent of these people do not receive treatment. Identifying individuals at an early stage of the disease or when they are at risk and referring them for appropriate intervention can reduce the burden of disease accordingly (1).

The World Health Organization (2), draws attention to the fact that the care and treatment of people with the disease is hindered due to reasons such as the perception of mental illnesses as a shameful situation and the anxiety of being exposed to discrimination and negligence. In days of yore, employees might conceal their status of mental health problem (MHP) or were stigmatized by their managers and co-workers in the workplace. The increase in the rate of mental illness in the society over the years has made it imperative now to address the problem in the workplace. Although the main purpose of occupational health nurses is to protect the health and well-being of employees, results such as absenteeism due to mental illnesses, high costs of health care and disability affect services, decreased productivity and feeling of unsafe. Presenteeism – attending work even though the worker is sick – produces indirect costs. The case of an employee struggling with a mental health (MH) issue can effect other workers or team members at the same workplace. Occupational safety concerns and social interaction issues may arise, workload could increase for the other co-workers. To seek support is the most important step for employees to be able to cope with mental disorders as

https://doi.org/10.33808/clinexphealthsci.1553470

these are generally treatable. A study found that 60% of adults with mental ill-health were not treated in the previous year as they were not able to afford it, had no time to allocate or their lack of knowledge about available support. Additionally, many people showed doubts about confidentiality and fear of getting fired. The occupational health nurse plays a key role in encouraging employees to seek help and signposting them the most feasible support tools and also ensuring strict confidentiality, helps employees to keep their work (3,4).

One way of increasing the number of people with mental illness to seek help is to educate the public on recognizing significant symptoms, strengthen first aid skills for people affected by MHP, and recommend that they seek professional help accordingly. Mental Health First Aid (MHFA) training was created in 2001 by Betty Kitchener, a nurse specializing in health education in Australia, and Anthony Jorm, professor of mental health literacy, in order to address this problem. The MHFA is designed for all levels of health literacy and emphasizes the use of appropriate terminology. The training is a program that teaches participants how to identify, understand and respond to the symptoms of mental illnesses and substance use disorders (5, 6). Mental Health First Aid Training is currently applied in 21 countries around the world (7, 8).

Many studies have demonstrated that MHFA is effective in eliminating the lack of understanding of MHP and effective treatments in the society, and reducing the effect of stigma that prevents people from getting help at an earlier stage, delays their recovery and significantly reduces their quality of life (6, 9-11). In addition, in the meta-analysis studies, it was found that the education program improved MH knowledge, reduced stigmatizing attitudes and increased the attitude to seek help (8, 10, 11).

No study could be found involving the application of MHFA in Turkey. A limited number of studies conducted have included psychological first aid (12, 13). Unlike MHFA, psychological first aid is a kind of early psychosocial intervention approach that is carried out during or after a disaster, accident, terrorist attack or any event that causes negative effects at the individual/social level (14). Mental Health First Aid, on the other hand, is not an early psychosocial intervention approach, but a program that aims to increase the help-seeking behavior of individuals.

This study was planned to determine the effect of MHFA training delivered by occupational health nurse on mental health and mental health literacy of office workers in Turkey. This particular study, in which MHFA training was implemented for the first time in Turkey, will provide an example of a program on MH improvement in the workplace.

As a result, the following hypotheses are proposed in this study:

H1: Positive mental health (PMH) scale score will be higher in the intervention group than in the control group one week after MHFA training.

H2: The knowledge, belief and resource factors score of the mental health literacy (MHL) scale for the employees in the intervention group will be higher than the control group one week after MHFA training.

H3: Employees in the intervention group will have a higher PMH scale post-test score compared to the pre-test.

H4: The post-test scores of the employees in the intervention group for the knowledge, belief and resource factors of the MHL scale will increase compared to the pre-test.

2. METHODS

2.1. Study design

This randomized controlled trial was conducted at a fast-moving consumer goods company with office employees. The primary outcomes of the study included autonomy, prosocial attitude and interpersonal relationship skills that PMH method sub-dimensions. The secondary outcome was MHL.

2.2. Ethical Approval

Ethical approval for this study was received by the Marmara University Health Sciences Faculty, Ethics Committee (Reference: 19.12.2019-230). Institutional consent was obtained in order to conduct this study in the workplace and to reach out to the employees. Participants signed a written informed consent including that their data could be used in research.

2.3. Settings and Participants

The MHFA training invitation and details were sent to the all employees at the company (N= 920) via e-mail. 178 of employees accepted to participate in the program. For the sample size; when power analysis was performed according to the pre-test scores the following were found: Alpha= 0.01, Beta=0.2, Group 1 PMH Scale score= 2.35±0.66, Group 2 PMH Scale score= 2.75, and when power was calculated as 0.80, the sample was determined to be at least 128 (Group 1=64 Group 2=64) according to the pre-test scores. We recruited all participants who consented to participate in the MHFA program and listed the participants. Then, we randomly divided into Group 1 (n=88) and Group 2 (n=90) in the statistical program with the help of a statistician as approximately 50% of the participants (Figure 1).

Concealment of participants and researchers was not possible due to the context of the intervention. However, since group data were coded as group 1 and group 2, masking was performed for statistical analysis.

Those who accepted to participate in the MHFA training were applied pre-test. The MHFA training was conducted one week later the pre-test. The post-test was administered one week following the completion of the training.

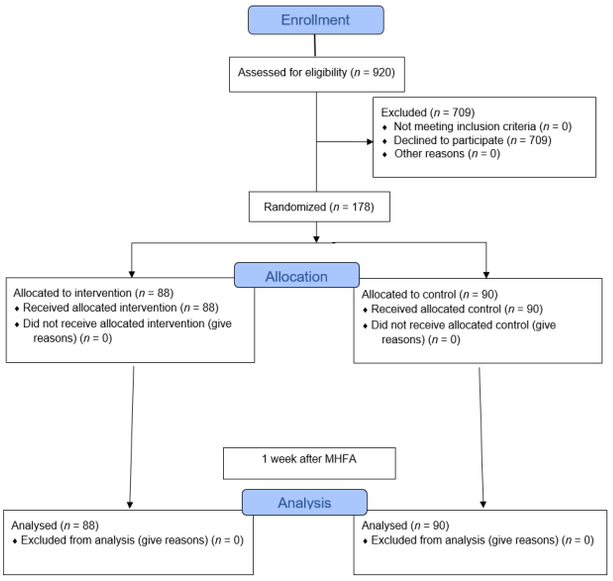

Figure 1. *Consort Diagram*

2.4. Assessment and scales

The data were collected with the Employee Assessment Survey, PMH Scale and MHL Scale via online self-report forms. We measured problem solving and self-actualization, autonomy, interpersonal relationship skills, self-control, prosocial attitude and personal satisfaction that are the primary outcomes of the study, with the PMH scale sub-dimension scores. The secondary outcome was assessed with MHL scale.

The Employee Assessment Survey includes four questions on the attendee's age, gender, presence of mental illness and having a relative with mental illness. These questions were excluded in the post intervention.

Positive Mental Health scale was developed to determine the conceptual model of PMH and to assess PMH by Lluch (15), and its Turkish adaptation was performed by Teke and Arabacı (16). The Cronbach's alpha coefficient of the PMH-Scale is 0.93, which consists of 39 items and 6 factors. In this study, it was found to be 0.87. The high score obtained from the scale indicates that mental health is positive.

Mental Health Literacy (MHL) scale was developed by Jung et al (17) and adapted to Turkish by Göktaş et al (18). The scale consists of three factors, which are knowledge, belief and resource oriented, and 22 items. The score to be taken from the scale varies between 0-22 and is evaluated to be positive as the score increases. The Cronbach's alpha coefficient of the MHL-Scale was found to be 0.75 in our study, while the original coefficient was 0.71.

2.5. Intervention

The MHFA program was translated and modified to suit the Turkish context. The MHFA program adopts the ALGEE approach, which is a 5-step action plan to recognize and manage MHP. ALGEE; consists of steps such as Approach the person, Assess the risk, Listen non-judgmentally, Give support and information, Encourage the person to get appropriate professional help and Encourage other support (5). The first part of the training includes information about the definition of mental health, its epidemiology, its effects, and prevention, early intervention, treatment and assistance. Common cultural and social discourses such as "people with mental illness are dangerous" and "people with mental illness should be segregated" are discussed, as well as the impact of stigma and discrimination on the help-seeking behavior of people with mental health problems. In the second part of training, common mental health problems such as depression, anxiety, stress and substance abuse are discussed. For each disease, information is given about risk factors, appropriate interventions, the importance of early intervention, disease-related crises and helpful resources. The ALGEE action plan is applied to each mental health problem, specifying the actions that need to be taken. The third part includes first aid for mental health crises such as suicidal ideation and behavior, self-harm, acute effects of drug and alcohol abuse and aggressive behavior (39).

The training was comprising of knowledge presentations, quiz, case video and discussions. Group 1 participants received MHFA training. First author who is an occupational health nurse with MHFA certified, delivered 2-day online courses consisting of 8-hours were delivered. Control group received no intervention during the data collection period.

2.6. Data Analysis

To compare of presence of mental illness, demographic and having a relative with mental illness characteristics in both groups, we used chi-square (χ^2) test and independent sample t test.

Mann Whitney U test was applied to compare the positive mental health scale and mental health literacy scale and subscale scores of the intervention and control groups, and the Wilcoxon signed-rank test was used to compare the pre-test and post-test results. The significance level was evaluated at $p<0.05$ level.

3. RESULTS

3.1. Sample characteristics

Figure 1 shows the flow of participants from recruitment through randomization and data collection. The study was performed with 88 office employees in the intervention group and 90 office employees in the control group who were randomly allocated, consented to participate and working in a fast-moving consumer goods company (N= 920)

(Figure 1). The majority of participants in both groups were female (60.2%, 56.7%). There was no dropout from the study in both groups. Post-test data were obtained one week after the MHFA training, and we did reminders for those did not fill out the questionnaires via e-mail several times and full participation was ensured.

3.2. Findings on descriptive characteristics

There was no difference between the intervention and control groups in terms of mean age, gender and any health problem (p>.05) (Table 1).

Table 1. *Demographic characteristics of the participants in the two groups*

Characteristic	Intervention (n=88)	Control (n=90)	Statistics
	Mean±SD	**Mean±SD**	**t. p**
Age (Mean +SD)	35.1±5.6	34.0±5.8	1.25; 0.21
	n (%)	**n (%)**	**χ^2. p**
Gender			
Female (n=104)	53 (60.2)	51 (56.7)	0.23; .65
Male (n=74)	35 (39.8)	39 (43.3)	
Presence of mental illness			
Yes	6 (6.8)	11 (12.2)	1.50; .31
No	82 (93.2)	79 (87.8)	
t= Independent sample t test; χ^2= Chi-square test			

When the distribution of the descriptive characteristics of the participants in the study was examined, the mean age of the intervention group was 35.1+5.6, and the mean age of the control group was 34+5.8. When examined in terms of gender, 60.2% of the intervention group and 56.7% of the control group were female. 93.2% of the intervention group and 87.8% of the control group did not have any health problems. (Table 1).

3.3. Findings on positive mental health and mental health literacy in intervention and control groups

There was no difference between the pre-test scores of positive mental health scale in the intervention and control groups except the Factor 5: Problem solving and self-actualization (p>.05) (Table 2).

The score of the intervention group was found to be significantly higher than the control group in terms of all sub-factors and total PMH score except Factor 4: Autonomy, when the positive mental health scale post-test scores of the groups were compared (p<.05). It was determined that the post-test score of Factor 4: Autonomy in the intervention group was higher than the pre-test (p<.05).

When the PMH scale pre-test and post-test scores of the intervention group were compared, total PMH and all factor post-test scores except Factor 2: Prosocial attitude were found to be higher than the pre-test (p<.05) (Table 2).

Table 2. *Comparison of scores based on Positive Mental Health Scale in intervention and control groups*

Positive Mental Health Scale Factors		Intervention		Control		Statistic	
		Mean	SD	Mean	SD	U^z	p
Factor 1: Personal Satisfaction	Pretest	27.9	3.8	28.1	3.1	-0.3	.779
	Posttest	29.5	2.0	28.2	3.6	-2.2	**.03**
Statistic	W^z. p	-3.857	**<.001**	-.204	0.838		
Factor 2: Prosocial Attitude	Pretest	18.2	1.7	17.8	1.6	-1.6	.103
	Posttest	18.3	1.7	17.6	2.1	-2.4	**.02**
Statistic	W^z.p	-.771	.440	-.368	0.713		
Factor 3: Self Control	Pretest	15.8	2.5	15.8	1.9	-0.7	.456
	Posttest	17.0	1.9	15.9	2.4	-2.2	**.03**
Statistic	W^z.p	-4.055	**<.001**	-.072	0.943		
Factor 4: Autonomy	Pretest	16.5	2.7	16.9	2.3	-0.9	.371
	Posttest	17.6	1.5	16.8	2.3	-1.9	.06
Statistic	W^z.p	-3.398	**.001**	-.598	0.550		
Factor 5: Problem Solving and Self-Actualization	Pretest	31.1	3.7	29.9	3.0	-3.0	**.003**
	Posttest	32.3	2.9	30.3	4.1	-3.3	**>.01**
Statistic	W^z.p	-2.278	**.023**	-1.055	0.292		
Factor 6: Interpersonal Relationship Skills	Pretest	23.5	2.8	23.2	2.6	-1.2	.234
	Posttest	24.6	2.5	23.3	3.3	-2.7	**.01**
Statistic	W^z.p	-3.354	**.001**	-.377	0.706		
Total Positive Mental Health Scale	Pretest	133.0	12.4	131.7	7.8	-1.6	.112
	Posttest	139.4	9.9	132.1	13.3	-3.9	**<.001**
Statistic	W^z.p	-4.697	**<.001**	-.667	0.505		

Uz = Mann Whitney U; Z = Wilcoxon Signed Ranks Test

Table 3. *Comparison of scores based on mental health literacy scale in intervention and control groups*

Mental Health Literacy Scale Factors		Intervention		Control		Statistic	
		Mean	SD	Mean	SD	U^z	p
Factor 1: Knowledge-Oriented MHL	Pretest	8.2	2.1	7.5	2.0	-2.7	**.007**
	Posttest	9.3	1.3	7.5	2.2	-6.7	**<.001**
Statistic	W^z.p	-4.280	**<.001**	-.395	0.693		
Mean Difference		1.1	2.4	-0.1	2.9	-2.6	**.01**
Factor 2: Belief-Oriented MHL	Pretest	4.4	2.0	1.9	1.6	-7.5	**<.001**
	Posttest	5.5	1.8	2.2	1.6	-9.3	**<.001**
Statistic	W^z.p	-4.028	**<.001**	-1.367	0.172		
Mean Difference		1.1	2.3	0.3	2.3	-1.9	.06
Factor 3: Resource-Oriented MHL	Pretest	2.0	1.3	2.4	1.2	-2.2	**.03**
	Posttest	3.9	0.4	2.6	1.2	-7.7	**<.001**
Statistic	W^z.p	-7.384	**<.001**	-1.104	0.270		
Mean Difference		1.9	1.3	0.2	1.7	-6.2	**<.001**
MHL Total	Pretest	14.5	3.5	11.8	3.5	-5.0	**<.001**
	Posttest	18.6	1.9	12.3	4.0	-10.0	**<.001**
Statistic	W^z.p	-7.191	**<.001**	-.815	0.415		
Mean Difference		4.1	3.9	0.4	5.3	-4.7	**<.001**

Uz:Mann Whitney U; WZ: Wilcoxon Signed Ranks Test

A significant difference was found between the pre-test scores of mental health literacy scale in the intervention and control groups. Therefore, posttest-pretest differences

of the groups were analyzed. It was determined that the mean difference of knowledge sub-dimension, resource sub-dimension and total MHL in the intervention group was significantly higher than the control group, when evaluated according to their mean differences (p>.01) (Table 3).

In addition, the post-test scores of knowledge, belief and resource sub-dimensions and total MHL scores in the intervention group were found to be statistically significantly higher than the pre-test (p<.01). There was no difference between all sub-dimensions and total MHL pre-test and post-test scores in the control group (p>.05) (Table 3).

4. DISCUSSION

In this study, which was conducted to determine the effect of an occupational health nurse-led MHFA, on the behaviors, attitudes and knowledge of employees about MH, it was determined that PMH and MHL developed positively. Interpersonal relationship skills, problem solving and self-actualization, autonomy, personal satisfaction, self-control and prosocial attitude, which was our primary outcomes, were found to be improved significantly compared to the control group after MHFA, which includes health training of the employees in the intervention group on communicating/contacting, speaking, helping, and directing to get professional help with a person with a MHP. At the same time, it was determined that the knowledge, belief and resource-oriented MHL scores that are secondary outcome of the intervention group increased.

Mental Health First Aid training helps to create a work culture with transparent and holistic management policies, where employees can freely express their MHP (9). In this study, the implementation of the program in the workplace and its positive results showed that MHFA can be used in workplaces.

Although studies in the workplace are limited, in a study involving employees in various business lines such as public, private sector and non-governmental organizations, it was found that employees' confidence in contacting, speaking and helping people with MHP improved (7). A study with student affairs staff of a university demonstrated the benefits of MHFA in terms of increasing confidence and knowledge, recognizing and interacting with people with MHP (19). Other workplace studies have shown that MHFA is quite acceptable in a workplace setting and that the employee group may benefit from this training (20, 21).

In our study, it was found that the PMH sub-dimension scores increased in favor of the intervention group. These results confirm the hypotheses 'H1: The PMH scale score will be higher in the intervention group than in the control group' and 'H3: Employees in the intervention group will have a higher PMH scale post-test score.'

On the other hand, we could not find a study in the literature in which the measurement tools used in our study were used to evaluate the MHFA results. However, there are studies evaluating the effects of different measurement tools that evaluate the knowledge, attitudes and behaviors of the participants on the MHFA results. The results of these studies show positive changes in the knowledge, attitudes and behaviors of the participants, similar to our results (10,19,20,23-25).

In some studies in which MHFA training is conducted, it is seen that different outcome criteria such as trust, intention, and stigma are addressed (7,26-28). In a study with nursing students, it was found that self-confidence in helping others increased in the intervention group after MHFA (29). In another trial conducted with medical students, it was found that confidence in helping a person with a MHP increased in the intervention group (30). In addition, significant positive changes were observed from baseline to 6 months post-training in terms of self-confidence and behavioral intention to perform MHFA actions (1,7,20, 31). Although trust and intention were not evaluated in our study, positive changes in personal satisfaction and interpersonal relationship skills can be evaluated in parallel with trust and intention.

In meta-analysis studies, it was found that MH knowledge improved, stigmatizing attitudes decreased and helping behaviors increased (8,10,11). Similarly, it was determined that MHL increased in our study, and the hypothesis 'H4: The post-test scores of the employees in the intervention group for the knowledge, belief and resource sub-dimensions of the MHL scale will increase' was confirmed. It is important to improve the level of MHL in the society so that individuals can recognize MH disorders and manage their own MH more effectively (32). Studies have shown that information obtained about MH and diseases creates a better awareness for help and treatment, and increases the behavior of properly using appropriate treatment resources in individuals, and that reducing stigma against mental illness at individual, social and institutional levels is beneficial in early diagnosis of mental disorders, may improve MH results, and has increased the effective use of health services (17,33,34).

In the literature, positive results were obtained in studies evaluating the effect of MHFA on MHL. In a study conducted with university students, significant findings were obtained showing that the program significantly improved the participants' MH knowledge and literacy (35). In another study conducted with public sector employees, it was determined that participants showed significant improvement in their MH knowledge and helping behaviors (20). In another study conducted with a group of adults and young individuals, it was emphasized that MHFA contributed positively to an increased MHL (1). Additionally, in some studies, participants stated that MHFA improved their knowledge of recognizing the signs and symptoms of mental illnesses and how to approach individuals (20,36). Similar to the results of the studies in the literature, this study confirmed that MHFA positively increased the MHL of the participants, and it was determined that the knowledge, belief and resource-oriented MHL level of the intervention group increased compared to the control group. These results confirm the hypothesis 'H2:

The knowledge, belief and resource sub-dimensions scores of the MHL scale for the employees in the intervention group will be higher than the control group.'

At the same time, studies have shown that MHFA reduces participants' stigmatizing thoughts about MHP of people who are important to them (1,8). Although stigma was not evaluated in our study, it is stated in the literature that stigma is associated with MHL (37-39). Cognitive stigma is related to people's attitudes and is based on their perception of problems in social relationships. In future studies, the effect of MHFA on stigma can be evaluated.

5. CONCLUSION

Mental health first aid training was determined to be an effective program and can be used in workplaces. Furthermore, MHFA can be beneficial in terms of improving MH and increasing awareness in studies conducted in different community groups and working population.

The strength of the study is that it was conducted in a randomized controlled design. The randomized controlled design is the gold standard for determining differences between groups in examining the effect of the MHFA intervention. In addition, the sample size and high participation rate in this study are other strengths of the study.

This study has several limitations. First, a double-blind design was not possible due to the feature of intervention. However, we coded group data thus outcome assessors were masked. Second limitation of this study is that the long-term results of MHFA were not evaluated. It is recommended to evaluate the long-term effects of the program by including a follow-up period in further studies. In addition, in order to evaluate the effect of MHFA on individuals with MHP, after the interviews made by mental health first aiders, the individuals who benefit from it can be evaluated in terms of sharing their problems and seeking professional help.

Acknowledgement: *We would like to thank the occupational health nurse and employees who support our study on data collection.*

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by Ethics Committee of Marmara University Health Sciences Faculty, Ethics Committee (Approval Date: 19.12.2019, Number: 230)*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: IY
Design of the study: IY, AE
Acquisition of data for the study: IY
Analysis of data for the study: IY, AE
Interpretation of data for the study: IY, AE
Drafting the manuscript: IY
Revising it critically for important intellectual content: IY
Final approval of the version to be published: IY, AE

REFERENCES

[1] Banh MK, Chaikind J, Robertson HA, Troxel M, Achille J, Egan C, Anthony BJ. Evaluation of mental health first aid USA using the mental health beliefs and literacy scale. Am J Health Promot. 2019;33(2):237-247. https://doi.org/10.1177/0890117118784234

[2] World Health Organisation. Fact sheets on mental disorders 2018. Accessed 4 January 2022. Mental disorders (who.int)

[3] Cadorette M, Agnew J. Mental health in the workplace. Workplace Health Saf. 2017; 65(9):448-448. https://doi.org/10.1177/2165079917716188

[4] Kinman G. Sickness presenteeism at work: prevalence, costs and management. Br Med Bull. 2019;129(1):69-78. doi: 10.1093/bmb/ldy043. PMID: 30649219.

[5] Kolmetz MJ. Mental health first aid training: Removing the stigma and empowering recovery. J Am Acad Physician Assist. 2019;32(2):1-2. https://doi.org/10.1097/01.JAA.0000552721.83241.d9

[6] Dollar KJ, Ruisinger JF, Graham EE, Prohaska ES, Melton BL. Public awareness of mental health first aid and perception of community pharmacists as mental health first aid providers. J Am Pharm Assoc. 2020;60(5):S93-S97. https://doi.org/10.1016/j.japh.2020.01.017

[7] Jensen KB, Morthorst BR, Vendsborg PB, Hjorthøj C, Nordentoft M. Effectiveness of mental mealth first aid training in Denmark: A randomized trial in waitlist design. Soc Psychiatry Psychiatr Epidemiol. 2016;51(4):597-606. https://doi.org/10.1007/s00127-016-1176-9

[8] Hadlaczky G, Hökby S, Mkrtchian A, Carli V, Wasserman D. Mental Health First Aid is an effective public health intervention for improving knowledge, attitudes, and behaviour: A meta-analysis. Int Rev Psychiatry. 2014;26(4):467-475. https://doi.org/10.3109/09540261.2014.924910

[9] Kroll H. Mental health first aid: Addressing mental health as a public health priority. Perspect Public Health. 2015; 135(1):12. https://doi.org/10.1177/1757913914562120

[10] Wong EC, Collins RL, Cerully JL. Reviewing the evidence base for mental health first aid: Is there support for its use with key target populations in California? Rand Health Q. 2015; 5(1):19. https://doi.org/10.7249/RR972

[11] Morgan AJ, Ross A, Reavley NJ. Systematic review and meta-analysis of mental health first aid training: effects on knowledge, stigma, and helping behaviour. Plos One 2018; 13(5):e0197102. https://doi.org/10.1371/journal.pone.0197102

[12] Tetik S, Mutlu A, Ünlübilgin E. Cinsel saldırı olgularında psikolojik ilk yardım [psychological first aid in sexual assault cases]. Psikiyatr Güncel Yaklaşımlar2021;13(4):751-762. https://doi.org/10.18863/pgy.880465 (Turkish)

[13] Gerdan S, Kırıkkaya EB, Özdemir A. Kocaeli mahalle halkı afetlere hazırlık eğitimi projesi [Disaster training project of Kocaeli neighborhood]. Published 4 June 2018. Accessed 6 October 2021. https://www.ishad.info/PastConferences/ISHAD2018/ISHAD2018/papers/A1.9-ISHAD2018ID97.pdf (Turkish)

[14] Brymer M, Layne C, Jacobs A, Pynoos R, Ruzek J, Steinberg A, Watson P. Psychological first aid field operations guide. National Child Traumatic Stress Network. Published 2006. Accessed 6 October 2021. https://www.nctsn.org/resources/psychological-first-aid-pfa-field-operations-guide-2nd-edition

[15] Lluch Canut M. Construcción de una escala para evaluar la salud mental positiva. Universitat de Barcelona. Published 25 January 2000. Accessed 6 October 2021. http://hdl.handle.net/2445/42359

[16] Teke C, Baysan Arabacı L. The validity and reliability of Positive Mental Health Scale. Anat J Psychiatry. 2018; 19:21-28. https://doi.org/10.5455/apd.284116

[17] Jung H, von Sternberg K, Davis K. Expanding a measure of mental health literacy: development and validation of a multicomponent mental health literacy measure. Psychiatr Res. 2016; 243:278-286. https://doi.org/10.1016/j.psychres.2016.06.034

[18] Göktaş S, Işıklı B, Metintaş S. Mental health literacy. Turk J Public Health. 2018;3(2),67-75. Accessed 6 October 2021. http://www.estudamdergi.org/index.php/Halk/article/view/83

[19] Massey J, Brooks M, Burrow J. Evaluating the effectiveness of mental health first aid training among student affairs staff at a Canadian university. J Stud Aff Res Pract. 2014; 51(3):323-336. https://doi.org/10.1515/jsarp-2014-0032

[20] Svensson B, Hansson L. Effectiveness of mental health first aid training in Sweden. A randomized controlled trial with a six-month and two-year follow-up. J Plos One 2014; 9(6):e100911. https://doi.org/10.1371/journal.pone.0100911

[21] Kitchener BA, Jorm AF. Mental health first aid training in a workplace setting: A randomized controlled trial. BMC Psychiatry 2004;4(1):1-8. https://doi.org/10.1186/1471-244X-4-23

[22] McLeod S. Maslow's hierarchy of needs. Simply psychology. 2007;1(1-18). Accessed 2 January 2022. https://www.simplypsychology.org/maslow.html

[23] Morawska A, Fletcher R, Pope S, Heathwood E, Anderson E, McAuliffe C. Evaluation of mental health first aid training in a diverse community setting. Int J Ment Health Nurs. 2013;22(1):85-92. https://doi.org/10.1111/j.1447-0349.2012.00844.x

[24] Mohatt NV, Boeckmann R, Winkel N, Mohatt DF, Shore J. Military mental health first aid: development and preliminary efficacy of a community training for improving knowledge, attitudes, and helping behaviors. Mil Med. 2017;182(1-2):e1576-e1583. https://doi.org/10.7205/MILMED-D-16-00033

[25] Rose T, Leitch J, Collins KS, Frey JJ, Osteen PJ. Effectiveness of youth mental health first aid USA for social work students. J Res Soc Work Pract. 2019;29(3):291-302. https://doi.org/10.1177%2F1049731517729039

[26] Edgar S, Connaughton J. Using mental health first aid training to improve the mental health literacy of physiotherapy students. Physiother Can. 2021;73(2):188-193. https://doi.org/10.3138/ptc-2019-0036

[27] Lipson SK, Speer N, Brunwasser S, Hahn E, Eisenberg D. Gatekeeper training and access to mental health care at universities and colleges. J Adolesc Health. 2014;55(5):612-619. https://doi.org/10.1016/j.jadohealth.2014.05.009

[28] O'Reilly CL, Bell JS, Kelly PJ, Chen TF. Impact of mental health first aid training on pharmacy students' knowledge, attitudes and self-reported behaviour: A controlled trial. Aust N Z J Psychiatry 2011;45(7):549-557. https://doi.org/10.1371/journal.pone.0197102

[29] Burns S, Crawford G, Hallett J, Hunt K, Chih HJ, Tilley PJ. What's wrong with John? A randomized controlled trial of mental health first aid (MHFA) training with nursing students. BMC psychiatry. 2017;17(1):1-12. https://doi.org/10.1186/s12888-017-1278-2

[30] Davies EB, Beever E, Glazebrook C. A pilot randomized controlled study of the mental health first aid eLearning course with UK medical students. BMC Med Educ. 2018;18(1):1-12. https://doi.org/10.1186/s12909-018-1154-x

[31] Jorm, AF, Blewitt KA, Griffiths KM, Kitchener BA, Parslow RA. Mental health first aid responses of the public: results from an Australian national survey. BMC Psychiatry 2005;5:1-9.

[32] Kutcher S, Wei Y, Coniglio C. Mental health literacy: past, present, and future. Can J Psychiatry. 2016;61(3):154-158. https://doi.org/10.1177/0706743715616609

[33] Rüsch N, Evans-Lacko SE, Henderson C, Flach C, Thornicroft G. Knowledge and attitudes as predictors of intentions to seek help for and disclose a mental illness. Psychiatr Serv. 2011;62(6):675-678.

[34] Bonabi H, Müller M, Ajdacic-Gross V, Eisele J, Rodgers S, Seifritz E, Rüsch N. Mental health literacy, attitudes to help seeking, and perceived need as predictors of mental health service use: a longitudinal study. J Nerv Ment Dis. 2016;204(4):321-324. https://doi.org/10.1097/NMD.0000000000000488

[35] Morrissey H, Moss S, Alexi N, Ball P. Do mental health first aid™ courses enhance knowledge? J Ment Health Train Educ Pract. 2017;12(2):69-76.

[36] Minas H, Colucci E, Jorm AF. Evaluation of mental health first aid training with members of the Vietnamese community in Melbourne, Australia. Int J Ment Health Syst. 2009;3(1):1-10. https://doi.org/10.1186/1752-4458-3-19

[37] Crowe A, Mullen PR, Littlewood K. Self-stigma, mental health literacy, and health outcomes in integrated care. J Couns Dev. 2018;96(3):267-277.

[38] Kim HC. Mediating effect of stigma on the relationship between mental health literacy and help-seeking attitudes among university students in South Korea. Int J Ment Health. 2021;1-16. https://doi.org/10.1080/00207411.2021.1965397

[39] Wong DF, Lau Y, Kwok S, Wong P, Tori C. Evaluating the effectiveness of mental health first aid program for Chinese people in Hong Kong. Res Soc Work Pract. 2017;27(1):59-67. https://doi.org/10.1177%2F1049731515585149

How to cite this article: Yegen İ, Ergün A. The Effects of Mental Health First Aid Training on Positive Mental Health and Mental Health Literacy on Office Workers: A Randomized Controlled Trial. Clin Exp Health Sci 2025; 15: 191-197. DOI: 10.33808/clinexphealthsci.1553470

Clinical and Experimental Health Sciences

Unveiling the Expression of UNC13C in Healthy Brain and Glioblastoma Cells

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Received: 25.10.2024 **Accepted:** 25.11.2024

ABSTRACT

Objective: Glioblastoma (GBM) is the most aggressive type of brain tumor, accounting approximately half of malignant central nervous system tumors. Median overall survival remains below 15 months post-diagnosis. Current treatments include surgical resection, radiotherapy, and chemotherapy, primarily with temozolomide, yet the median overall survival remains below 15 months post-diagnosis. Understanding the molecular mechanisms of GBM is essential for developing novel therapeutic approaches. Among the implicated genes, the UNC13 protein family, particularly UNC13C, is of interest. While UNC13A and UNC13B have been linked to various neurological disorders, UNC13C has been less studied despite its involvement in neurotransmitter release and potential tumor-suppressive effects in other cancers. Our previous work indicated low expression levels of UNC13C in glioblastoma cell lines compared to healthy brain tissue, suggesting a role in GBM pathogenesis. In this study, we aimed to comprehensively evaluate UNC13C expression using web based bioinformatics tools and experimental approaches.

Methods: We analyzed UNC13C expression across various tissues via Correlation Analyzer, confirming in glioblastoma tissues compared to healthy brain samples using the GEPIA and UALCAN databases. Additionally, we assessed UNC13C levels in glioblastoma cell lines (LN-18, A-172, U-87), human microglia (HMC3), and healthy astrocytes through quantitative real-time polymerase chain reaction (qRT-PCR).

Results: Our findings reveal that UNC13C expression is notably reduced in glioblastoma cells, with the highest expression observed in healthy astrocytes, albeit at low levels. These results underscore the importance of UNC13C in GBM and highlight the need for further investigation into its role in tumor development and progression.

Conclusion: This study provides the first report of UNC13C expression detailed in human cell lines both normal and glioblastoma, emphasizing its significance from a developmental perspective.

Keywords: UNC13C, gene expression, glioblastoma, brain cells

1. INTRODUCTION

Glioblastoma (GBM) is the most aggressive type of brain tumor, accounting for 14.5% of all and 48.6% of malignant central nervous system tumors (1). The incidence of GBM ranges from 3.19 to 4.17 (cases per 100,000 people per year, and it is 1.58 times more common in men than in women (2). The current standard treatment for glioblastoma includes surgical resection of the tumor, followed by radiotherapy and chemotherapy (3). Since its FDA approval in 2005, temozolomide, an alkylating agent, has been the primary chemotherapeutic used in GBM treatment (4) Despite these multimodal treatment strategies, the median overall survival remains less than 15 months post-diagnosis. Therefore, identifying the pathophysiology of GBM is crucial for developing novel therapeutic approaches.

Understanding the molecular mechanisms underlying the central nervous system is crucial for elucidating the pathogenesis of neurological diseases. In the quest to identify the pathogenesis of glioblastoma (GBM), numerous studies are ongoing, leading to the discovery of various implicated genes. Among these, the UNC13 (uncoordinated-13) protein family, comprising the evolutionarily conserved members UNC13A, UNC13B, and UNC13C, UNC13D plays a pivotal role. These proteins are essential regulators of synaptic vesicle priming and are also involved in modulating immune responses (5,6).

UNC13A plays a crucial role in neurotransmitter release at nerve terminals and has been implicated in neurodegenerative diseases (7,8). UNC13B is involved in priming and fusing synaptic vesicles, with polymorphisms in this gene being

https://doi.org/10.33808/clinexphealthsci.1573250

linked to diabetic kidney disease, ALS, and epilepsy. UNC13D regulates immune cell function, and mutations in this gene are associated with familial hemophagocytic lymphohistiocytosis (FHL) (9,10). In contrast, UNC13C is less studied than its counterparts, though it is known to be involved in neurotransmitter release. The tumor-suppressive effect of UNC13C has been demonstrated in oral squamous cell carcinoma and hepatocellular carcinoma (11–13). Notably, UNC13C was previously reported by our group to have low expression levels in glioblastoma (GBM) compared to healthy brain tissue samples. This differential expression indicates a possible role for UNC13C in GBM pathogenesis, highlighting the need for further research into its involvement in tumor development and progression (14).

In this study, we will comprehensively evaluate the expression profile of the UNC13C gene using web-based bioinformatics tools. Additionally, we will put forward the expression levels of UNC13C in glioblastoma cancer cell lines, neural stem cells and healthy brain cell lines.

2. METHODS

2.1. Expression Profiling via web based tools

We examined UNC13C expression across multiple tissue types, including both cancerous and healthy tissues, using the Correlation AnalyzeR web tool (https://gccri.bishop-lab.uthscsa.edu/shiny/correlation-analyzer/) (15). To validate its differential expression in glioblastoma tumors and healthy tissues, we utilized the GEPIA database (http://gepia.cancer-pku.cn/), which is commonly used for analyzing data from The Cancer Genome Atlas (TCGA). Additionally, we employed the UALCAN database (http://ualcan.path.uab.edu/index.html) to confirm UNC13C expression levels in relation to different clinical characteristics.

2.2. Cell Culture

To assess the expression of the UNC13C gene, in addition to LN-18, A-172, and U-87 glioblastoma cell lines, healthy immortalized astrocytes, human microglia cells (HMC3), and neural stem cells were utilized. All cell lines were cultured in complete DMEM medium (DMEM, Gibco, New York, USA) supplemented with 10% fetal bovine serum (Gibco, New York, USA) and 1% Penicillin-Streptomycin (Gibco, New York, USA). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2. Regular checks for mycoplasma contamination were performed. Two different passages of each cell line were used as biological replicates. Cells were trypsinized upon reaching 80% confluence, and the pellets were stored at – 80°C for further analysis.

2.3. RNA Isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was isolated from the cell pellets using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The isolated RNA was then reverse-transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The quantitative PCR reactions were performed using Universal Master Mix, TaqMan probe specific for UNC13C and the synthesized cDNA. The reactions were run on the StepOnePlus Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) to accurately quantify the gene expression levels. GAPDH was used as the reference gene as a control of cDNA. The experiment was performed in triplicate, and the mean CT values with error bars were presented.

2.4. Statistical Analysis

The box plot illustrates the differential expression of UNC13C between cancerous and normal tissue samples across various tissue types. Statistical significance was assessed using the Wilcoxon rank sum test (15), The significance of differences performed by UALCAN in expression levels between normal tissues and primary tumors was evaluated using Welch's t-test (16). GEPIA used Differential gene expression analysis by conducting one-way ANOVA, as the independent variable to assess variations in expression levels (17). (significance levels in figures denoted as follows: *p < .05, **p < .01, ***p < .001, ****p < .0001).

3. RESULTS

UNC13C expression was analyzed across multiple tissue types, including cancerous tissues by Correlation Analyzer. Elevated levels of UNC13C mRNA were identified in the pancreas, intestine, immune cells, respiratory tissues, muscles, mammary glands, kidney, stomach, bone, skin, adipose tissue, and brain compared to cancerous samples (Figure 1).

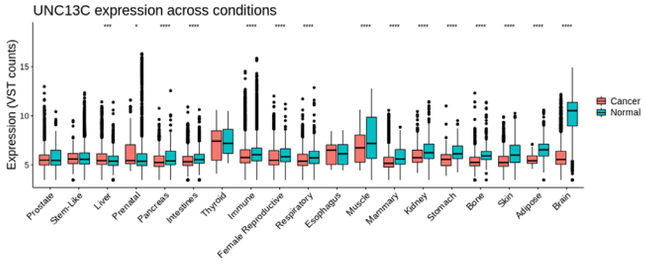

Figure 1. *UNC13C expression across multiple tissue types *: p < .05, **: p < .01, ***: p < .001, ****: p < .0001*

Since our focus was on UNC13C expression in glioblastoma and brain tissue, we specifically investigated its expression in glioblastoma tissues and healthy brain samples using GEPIA analysis, based on data from the TCGA database.A significant decrease in UNC13C expression was observed in glioblastoma tissues, as demonstrated by the boxplot analysis (Figure 2a). This finding was further validated through UALCAN analysis,

which confirmed the reduction of UNC13C expression at both the gene and protein levels (Figure 2b, c).

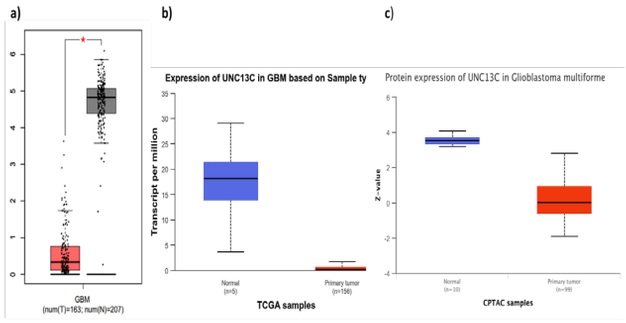

Figure 2. *UNC13C Expression in Glioblastoma Tissues. a) Boxplot from GEPIA analysis showing decreased UNC13C mRNA levels in glioblastoma tissues compared to healthy brain tissues ($p < .05$). b) Boxplot from UALCAN analysis confirming reduced UNC13C mRNA levels in glioblastoma tissues. c) Protein expression analysis from UALCAN further demonstrates reduced UNC13C protein levels in glioblastoma tissues compared to healthy brain tissue.*

We further assessed the expression of the UNC13C mRNA in glioblastoma cell lines U87, A172, and LN18, alongside healthy brain cells such as immortalized astrocytes and Human Microglia Cells (HMC3). Notably, late Ct values were observed in U87 and LN18 cells, and no expression was detected in the A172 cell line. Interestingly, the Ct values for HMC3 cells were very close to those of U87, indicating reduced UNC13C gene expression in both glioblastoma cells and microglia. In healthy astrocytes, UNC13C mRNA was detectable, although the Ct values were over 30, suggesting only basal expression levels. GAPDH was used as a control gene to validate the quality of cDNA from the cell lines (Figure 3b).

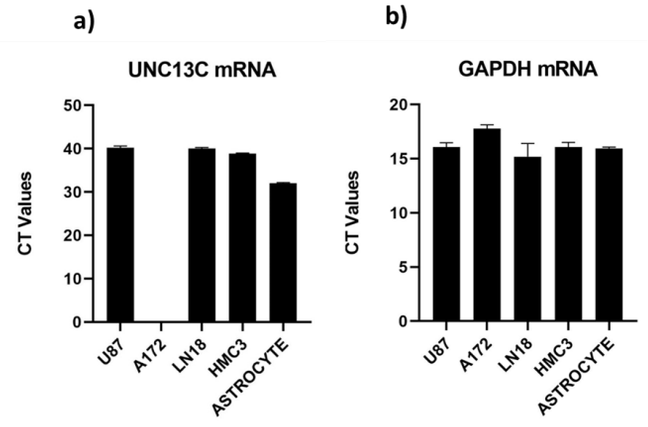

Figure 3. *UNC13C gene expression in cell lines a) The mean CT values of UNC13C in cell lines b) The mean CT values of control GAPDH gene in cell lines*

4. DISCUSSION

UNC13C, the mammalian homolog of Munc13 in mice, is evolutionarily conserved and orthologous to the human UNC13A variant. Mice deficient in UNC13C exhibit complete paralysis and die before birth, highlighting the gene's critical role in early development. Munc13-3 deletion mutants specifically demonstrate increased paired-pulse facilitation at parallel fiber–Purkinje cell synapses, which impacts synaptic plasticity. While these mutants display normal spontaneous motor activity, they show significant impairment in learning complex motor tasks, underscoring the importance of UNC13C in motor coordination and learning (18). Meunier et al. demonstrated that UNC13C plays a critical role in positional or molecular superpriming, which enhances the activation of calcium (Ca^{2+}) release (19) . This process is essential for efficient synaptic transmission, as the elevated Ca^{2+} levels facilitate the release of neurotransmitters, thereby contributing to proper synaptic function and signaling. Recently, a transcriptomic study on mouse astrocytes revealed that UNC13C exhibits high expression levels, highlighting its potential significance in astrocyte function (20).

In our previous study, we demonstrated a decreased level of UNC13C gene expression in the A172 and U-87 human glioblastoma cell lines (14). To gain a more comprehensive understanding, we expanded our analysis to evaluate the gene's expression across various tissue types using transcriptomic data. The highest expression of UNC13C was observed in healthy brain samples compared to tumor tissues. This finding was further validated by UALCAN and GEPIA analyses, both of which showed a significant decrease in UNC13C expression in glioblastoma samples at both the mRNA and protein levels.

To assess the mRNA levels of UNC13C in glioblastoma, we conducted RT-PCR, analyzing its expression in A172, U87, and LN18 glioblastoma cell lines, as well as HMC3 microglia cells and immortalized astrocytes. Consistent with the transcriptomic data, UNC13C expression was undetectable in A172 cells, while very late CT values indicated low expression in U-87 and LN18 cell lines. Wang et al. previously demonstrated that synaptic plasticity in the hippocampus is compromised in C6 glioma-bearing rats, providing support for our findings (21).

Among all the cell lines tested, human astrocytes showed the highest expression, although the CT values did not suggest early expression. While this aligns with transcriptomic data, we had anticipated higher levels of expression. The discrepancy may be due to the differences between cell lines and tissue samples. It could also be influenced by several other factors. These may include variations in the microenvironment, cellular differentiation states, or the influence of external factors such as culture conditions and passage number. Interestingly, basal levels of UNC13C expression were detected in HMC3 cells, a cell line established from 8-10 week-old embryos (22). We hypothesize that the basal UNC13C expression observed in HMC3 cells may reflect a developmental stage, with expression potentially increasing as astrocytes mature in adulthood. While

our findings are significant, the use of a healthy cell line rather than actual brain tissue presents a limitation. Future research that incorporates a broader range of cell lines and samples would yield more comprehensive insights into the role of UNC13C.

5. CONCLUSION

In conclusion, this study provides the first report of UNC13C expression in LN18, HMC3, and healthy astrocytes. Our findings underscore the importance of UNC13C from a developmental perspective.

Acknowledgements: *We would like to thank Marmara University for providing access to academic databases.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: DS
Design of the study: DS
Acquisition of data for the study:
Analysis of data for the study: DS
Interpretation of data for the study: DS
Drafting the manuscript: DS, ABD
Revising it critically for important intellectual content: DS, ABD, ÖFB
Final approval of the version to be published: DS, ABD, ÖFB,

REFERENCES

[1] Batash R, Asna N, Schaffer P, Francis N, Schaffer M. Glioblastoma Multiforme, Diagnosis and Treatment; Recent Literature Review. Curr Med Chem. 2017;24(27):3002-3009. https://doi.org/10.2174/0929867324666170516123206

[2] Fabbro-Peray P, Zouaoui S, Darlix A, Fabbro M, Pallud J, Rigau V, Mathieu-Daude H, Bessaoud F, Bauchet F, Riondel A, Sorbets E, Charissoux M, Amelot A, Mandonnet E, Figarella-Branger D, Duffau H, Tretarre B, Taillandier L, Bauchet L. Association of patterns of care, prognostic factors, and use of radiotherapy-temozolomide therapy with survival in patients with newly diagnosed glioblastoma: A French national population-based study. J Neurooncol. 2019 Mar;142(1):91-101. https://doi.org/10.1007/s11060-018-03065-z

[3] Rajaratnam V, Islam MM, Yang M, Slaby R, Ramirez HM, Mirza SP. Glioblastoma: Pathogenesis and Current Status of Chemotherapy and Other Novel Treatments. Cancers (Basel). 2020 Apr 10;12(4):937. https://doi.org/10.3390/cancers12040937

[4] Wesolowski JR, Rajdev P, Mukherji SK. Temozolomide (Temodar). AJNR Am J Neuroradiol. 2010 Sep;31(8):1383-4. https://doi.org/10.3174/ajnr.A2170

[5] Ansari U, Chen V, Sedighi R, Syed B, Muttalib Z, Ansari K, Ansari F, Nadora D, Razick D, Lui F. Role of the UNC13 family in human diseases: A literature review. AIMS Neurosci. 2023 Dec 6;10(4):388-400. https://doi.org/10.3934/Neuroscience.2023029

[6] Dittman JS. Unc13: a multifunctional synaptic marvel. Curr Opin Neurobiol. 2019 Aug;57:17-25. https://doi.org/10.1016/j.conb.2018.12.011

[7] Varoqueaux F, Sons MS, Plomp JJ, Brose N. Aberrant morphology and residual transmitter release at the Munc13-deficient mouse neuromuscular synapse. Mol Cell Biol. 2005 Jul;25(14):5973-84. https://doi.org/10.1128/MCB.25.14.5973-5984.2005

[8] Willemse SW, Harley P, van Eijk RPA, Demaegd KC, Zelina P, Pasterkamp RJ, van Damme P, Ingre C, van Rheenen W, Veldink JH, Kiernan MC, Al-Chalabi A, van den Berg LH, Fratta P, van Es MA. UNC13A in amyotrophic lateral sclerosis: from genetic association to therapeutic target. J Neurol Neurosurg Psychiatry. 2023 Aug;94(8):649-656. https://doi.org/10.1136/jnnp-2022-330504

[9] Aricò M, Boggio E, Cetica V, Melensi M, Orilieri E, Clemente N, Cappellano G, Buttini S, Soluri MF, Comi C, Dufour C, Pende D, Dianzani I, Ellis SR, Pagliano S, Marcenaro S, Ramenghi U, Chiocchetti A, Dianzani U. Variations of the UNC13D gene in patients with autoimmune lymphoproliferative syndrome. PLoS One. 2013 Jul 1;8(7):e68045. https://doi.org/10.1371/journal.pone.0068045

[10] Zhang K, Jordan MB, Marsh RA, Johnson JA, Kissell D, Meller J, Villanueva J, Risma KA, Wei Q, Klein PS, Filipovich AH. Hypomorphic mutations in PRF1, MUNC13-4, and STXBP2 are associated with adult-onset familial HLH. Blood. 2011 Nov 24;118(22):5794-8. https://doi.org/10.1182/blood-2011-07-370148

[11] Ariel P, Ryan TA. New insights into molecular players involved in neurotransmitter release. Physiology (Bethesda). 2012 Feb;27(1):15-24. https://doi.org/10.1152/physiol.00035.2011

[12] Dong W, Zhao L, Zhang S, Zhang S, Si H. Circ-KIAA0907 inhibits the progression of oral squamous cell carcinoma by regulating the miR-96-5p/UNC13C axis. World J Surg Oncol. 2021 Mar 14;19(1):75. https://doi.org/10.1186/s12957-021-02184-8

[13] Kumar VB, Lee CH, Su TC, Lin CC, Mohammedsaleh ZM, Yeh CM, Kiefer R, Lin SH. Prognostic and Clinical Implications of UNC13C expression in Hepatocellular Carcinoma Patients. Int J Med Sci. 2023 Aug 6;20(9):1235-1239. https://doi.org/10.7150/ijms.80488

[14] Gulluoglu S, Tuysuz EC, Sahin M, Kuskucu A, Kaan Yaltirik C, Ture U, Kucukkaraduman B, Akbar MW, Gure AO, Bayrak OF, Dalan AB. Simultaneous miRNA and mRNA transcriptome profiling of glioblastoma samples reveals a novel set of OncomiR candidates and their target genes. Brain Res. 2018 Dec 1;1700:199-210. https://doi.org/10.1016/j.brainres.2018.08.035

[15] Miller HE, Bishop AJR. Correlation AnalyzeR: functional predictions from gene co-expression correlations. BMC Bioinformatics. 2021 Apr 20;22(1):206. https://doi.org/10.1186/s12859-021-04130-7

[16] Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon AR, Athar M, Netto GJ, Qin ZS, Kumar S, Manne U, Creighton CJ, Varambally S. UALCAN: An update to the integrated cancer data analysis platform. Neoplasia. 2022 Mar;25:18-27. https://doi.org/10.1016/j.neo.2022.01.001

[17] Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017 Jul 3;45(W1):W98-W102. https://doi.org/10.1093/nar/gkx247

[18] Augustin I, Korte S, Rickmann M, Kretzschmar HA, Südhof TC, Herms JW, Brose N. The cerebellum-specific Munc13 isoform Munc13-3 regulates cerebellar synaptic transmission and motor learning in mice. J Neurosci. 2001 Jan 1;21(1):10-7. https://doi.org/10.1523/JNEUROSCI.21-01-00010.2001

[19] Meunier FA, Hu Z. Functional Roles of UNC-13/Munc13 and UNC-18/Munc18 in Neurotransmission. Adv Neurobiol. 2023;33:203-231. https://doi.org/10.1007/978-3-031-34229-5_8

[20] Batiuk MY, Martirosyan A, Wahis J, de Vin F, Marneffe C, Kusserow C, Koeppen J, Viana JF, Oliveira JF, Voet T, Ponting CP, Belgard TG, Holt MG. Identification of region-specific astrocyte subtypes at single cell resolution. Nat Commun. 2020 Mar 5;11(1):1220. https://doi.org/10.1038/s41467-019-14198-8

[21] Wang YY, Liu SC, Yang Z, Zhang T. Impaired hippocampal synaptic plasticity in C6 glioma-bearing rats. J Neurooncol. 2011 Jul;103(3):469-77. https://doi.org/10.1007/s11060-010-0447-7

[22] Janabi N, Peudenier S, Héron B, Ng KH, Tardieu M. Establishment of human microglial cell lines after transfection of primary cultures of embryonic microglial cells with the SV40 large T antigen. Neurosci Lett. 1995 Aug 4;195(2):105-8. https://doi.org/10.1016/0304-3940(94)11792-h

How to cite this article: Seven D, Dalan AB, Bayrak ÖF. Unveiling the Expression of UNC13C in Healthy Brain and Glioblastoma Cells. Clin Exp Health Sci 2025; 15: 198-202. DOI: 10.33808/clinexphealthsci.1573250

Clinical and Experimental Health Sciences

Investigating the Effect of School Climate on Adolescents' Psychological Resilience and Healthy Lifestyle Beliefs

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Received: November 18, 2024 **Accepted:** February 5, 2025

ABSTRACT

Objective: Adolescents are prone to many unhealthy behaviors and psychological problems such as anxiety. It is fair to say that the psychological resilience and healthy life behaviors of students who spend most of this transition period in school are largely influenced by the school climate.

Methods: This descriptive, correlational study was conducted to examine the effect of school climate on adolescents' psychological resilience and healthy lifestyle beliefs. A total of 767 students were included in the study. To ensure a homogeneous distribution of students, a stratified random sample was drawn by school and the data were collected by the researchers in the classroom after informing the students about the study. The research data were collected using the 'Adolescent Data Collection Form', 'Delaware School Climate Survey', 'Child and Youth Resilience Measure', and 'Healthy Lifestyle Belief Scale for Adolescents'.

Results: In this study, which aimed to investigate the effects of school climate on psychological resilience and healthy lifestyle beliefs of adolescents, it was found that there was a moderate positive relationship between school climate and students' psychological resilience and healthy lifestyle beliefs. A number of factors have been identified as contributors to school climate, including gender, feeling healthy, and the resilience of children and youth.

Conclusions: The results of this study show that school climate is important for adolescents' psychological resilience and healthy life beliefs.

Keywords: school climate; adolescents; psychological resilience; healthy lifestyle

1. INTRODUCTION

A significant part of adolescence, during which relationships with peers become more important and social interactions more complex, is spent in the school environment. Schools are not only institutions where students learn courses for their academic development but also environments where they acquire social skills and autonomy and learn many healthy behaviors for life (1,2). For this reason, the physical structure of the school and the surrounding environment in which it is located, as well as the parents, teachers, and administrators in the school, are the cornerstones of the school where students continue their development after the home environment. When the school climate is considered as an ecological framework in which the social and physical characteristics of a school come to the fore, it reflects the patterns of experience and norms, values, group relationships, teaching-learning practices and organizational structures in people's school life (1,3,4). School climate, understood as the quality and character of school life, has a direct impact on students' cognitive, social, and psychological development (5). The five general areas of school climate are order-safety-discipline, learning-teaching, social relationships, school environment/structure and school engagement. Safety includes the physical, social, and emotional well-being of students. Teaching and learning includes the learning environment, including academic achievement, expectations, praise and reinforcement, and social, emotional, and ethical learning. The Relationships dimension includes norms related to respect for differences, relationships with adults and peers, and school engagement. Safety includes the physical, social and emotional well-being of students. The final domain, environmental structure, includes aesthetic issues of the environment, such as current books and materials, the physical condition of the school building, cleanliness, and the efforts of school staff and students to preserve the school building and resources (5–7).

https://doi.org/10.33808/clinexphealthsci.1587455

Current evidence shows that school climate is directly correlated with student behavior and academic performance. It has been shown that in schools with poor social relationships, students' attendance decreases, their disruptive behaviors increase and their academic performance decreases (1,4).

School climate is critical to how young people perceive the quality of their school experience. A consistently positive school climate provides physical and social safety and supports students' cognitive, spiritual and emotional development, behavior and learning outcomes (4). Students' personal development, perceived social support, quality of life, school satisfaction, motivation to learn, and health behaviors and psychological resilience develop under the influence of school climate (8). Given that the development of health perceptions begins in childhood and lifelong behaviors are established during adolescence, the importance of school climate becomes clear. It is well known that adolescence influences the psychological, social and intellectual development of students and is considered one of the determining factors for the next stage of life. Adolescents are prone to many unhealthy behaviors and psychological problems such as anxiety (4). It is reasonable to assume that the psychological resilience and healthy life behaviors of students who spend most of this transition period in school are largely influenced by the school climate (4,9,10). Therefore, it was aimed to investigate how school climate affects adolescents' psychological resilience and healthy lifestyle beliefs.

1.1. Research Questions:

– Do adolescent characteristics adolescents affect school climate, healthy lifestyle beliefs and psychological resilience?

– Is there a relationship between school climate and adolescents' healthy lifestyle beliefs and psychological resilience?

2. METHODS

This descriptive, correlational study was conducted to examine the effect of school climate on adolescents' psychological resilience and healthy lifestyle beliefs.

2.1. Design and sample

The universe of the study consists of public high schools in Fethiye district of Muğla province. In the academic year 2022-2023, 6880 students are studying at high schools. The software program G* Power 3.1.9.7 was used to calculate the sample size (11). When calculating the sample, it was determined that 721 high school students were needed to perform the Multiple Logistic Regression analysis with a confidence interval of 95%, a sampling error of 0.05 and a power of 80% (12). A total of 767 students who were studying at the high schools and whose parents gave their written assent to voluntary participation were included in the study. To ensure a homogeneous distribution of students, a stratified random sample was drawn by school and the data were collected by the researchers in the classroom after informing the students about the study.

2.2. Data collection instruments

The research data were collected using the 'Adolescent Data Collection Form', 'Delaware School Climate Survey', 'Child and Youth Resilience Measure (CYRM-12)', and 'Healthy Lifestyle Belief Scale for Adolescents'.

2.2.1. Adolescent Data Collection Form

This form, prepared based on current literature, consisted of six questions about age, gender, educational class, number of members in the family and the state of feeling healthy (4,9,10,13,14).

2.2.2. Delaware School Climate Survey

The scale was developed by Bear et al. (2011) (15). The validity and reliability of this scale were tested for the Turkish language by Durnalı and Filiz (2019) (3). The scale is a 4-point Likert scale: "strongly disagree (1)", "disagree (2)", "agree (3)", "strongly agree (4)". It consists of four sub-dimensions and 17 items. The sub-dimension "teacher-student relations" consists of six items, the sub-dimension "student-student relations" consists of four items, the sub-dimension "liking of school" consists of four items and the sub-dimension "fairness of school rules" consists of three items (3). The Cronbach's alpha coefficient was found to be 0.84. In our study, the Cronbach's alpha coefficient of the scale was found to be 0.87.

2.2.3. Child and Youth Resilience Measure (CYRM-12)

The scale was developed by Liebenberg et al. (2013) (16). The validity and reliability of this scale were tested for the Turkish language by Arslan (2015) (17). The short form of the scale consists of 12 items. The scale has a five-point Likert scale and is graded between "Completely describes me (5)" and "Does not describe me at all (1)". A high score indicates a high level of resilience (Cronbach's alpha= 0.91). In our study, the Cronbach's alpha coefficient of the scale was found to be 0.82.

2.2.4. Healthy Lifestyle Belief Scale for Adolescents (HLB Scale)

The scale was developed by Kelly et al. (2011) (18). The validity and reliability of this scale were tested for the Turkish language by Akdeniz et al. (2020) (19). The scale emphasizes the beliefs in the various aspects of maintaining a healthy lifestyle. It is a Likert-type, with each item in the scale is scored from 1 = strongly disagree to 5 = strongly agree. The increase in the score indicates the increase in the healthy lifestyle beliefs of the adolescents. Cronbach alfa' katsayısı

is 0.90. In our study, the Cronbach's alpha coefficient of the scale was found to be 0.87.

2.3. Data analysis

The data analysis was conducted using the IBM SPSS 20.0 package program (IBM Corp., Armonk, NY, USA). Means and percentages were used to analyze the descriptive data. Skewness and kurtosis were used to test the normality of the data set, and their values ranged from −1.5 and ±1.5, indicating a normal distribution (20). When comparing the characteristics of the adolescents and the scale values, the t-test was used for bivariate data and the one-way ANOVA test for data with more than two variables. The Bonferroni test was used for the post-hoc analysis of the data. The Pearson correlation test was used to examine the relationship between the scales. Multiple linear regression analysis was used to determine the factors influencing school climate.

2.4. Ethical Consideration

Before the start of the study, we sought approval from the Health Sciences Ethics Committee of Muğla Sıtkı Koçman University (Protocol Number: 220150, Decision Number: 1, Date:15.03.2023) and the directorate of national education, with which the schools in Fethiye (Number: E-70004082-605.01-74385002) are affiliated. Data collection took place between May and June 2023. All procedures involving human participants adhered to the ethical standards set by the institutional and/or national research committee and complied with the 1964 Helsinki Declaration, as well as its later amendments or comparable ethical standards. Oral assent was obtained from the children who agreed to participate in the research, while written informed consent was obtained from their parents.

Table 1. *Scale characteristics*

Scale	Mean	Standart Deviation	Minimum	Maximum	Skewness	Kurtosis
School Climate Survey	43.55	8.64	18.00	68.00	-0.146	-0.187
Healthy Lifestyle Belief Scale	57.95	11.01	16.00	80.00	-0.823	1.274
Child and Youth Resilience Measure	42.22	8.51	12.00	60.00	-0.574	0.404

3. RESULTS

It was found that 52% (n=399) of the adolescents were female, 42% were in the 9th grade (n=322) and 51.4% (n=394) lived in a family of four. 30.2% of the adolescents stated that they felt poor health (Table 2).

Table 2. *Distribution of descriptive characteristics of adolescents*

Variables	n	%
Age		
14	98	12.8
15	321	41.9
16	194	25.3
17	128	16.7
18	26	3.4
Gender		
Female	399	52.0
Male	368	48.0
Educational class		
9. grade	322	42.0
10. grade	249	32.5
11. grade	171	22.3
12. grade	25	3.3
Number of members in the family		
3	87	11.3
4	394	51.4
5 and above	286	37.3
The state of feeling healthy		
Yes	535	69.8
No	232	30.2
Total	**767**	**100**

When the mean scale scores were compared according to the descriptive characteristics of adolescents, it was determined that school climate, healthy lifestyle belief and psychometric properties of resilience scores showed significant differences according to gender and feeling healthy. The mean scores of male students on school climate survey (t:-2.322, p<.05), healthy lifestyle belief scale (t:-4.056, p<.05) and child and youth resilience measure (t:-2.603, p<.05) were significantly higher than female students. The mean scores of the school climate survey (t:10.339, p<.05), healthy lifestyle belief scale (t:11.213, p<.05) and child and youth resilience measure (t:9.811, p<.05) of the students who felt healthy were significantly higher than those who felt unhealthy. Conversely, there is no significant difference between the scale values and the variables age, education class, number of family members (Table 3).

When looking at the total scale scores of the adolescents, a statistically significant and positive correlation was found between the total scores of the School Climate Survey and the total scores of the Healthy Lifestyle Belief Scale (r=0.467, p<.01) and the total scores of the Child and Adolescent Resilience Measure (r=0.629, p<.01). A moderate, positive and statistically significant correlation was found between the total scores of the healthy lifestyle belief scale and the total scores of the measure of resilience in children and adolescents (r=0.677, p<.01) (Table 4).

As a result of the multiple linear regression analysis conducted using the Enter method to determine the variables associated with school climate, it was found that the mean scores of students' positive healthy thoughts 0.167 (95% CI= .102-.233) and psychological resilience 0.033 (95% CI= .029-.038) were influenced by school climate (p<.001). The mean values of age, gender and healthy lifestyle beliefs of adolescents were

not influenced by school climate. This model explains 42% of the sample. Thus, adolescents with higher mean scores on the school climate scale had higher mean scores for healthy thoughts and psychological resilience (Table 5).

Table 3. *Comparison of descriptive characteristics and scale scores of adolescents*

	n	School Climate Survey		Healthy Lifestyle Belief Scale		Child and Youth Resilience Measure	
		X̄	Ss	X̄	Ss	X̄	Ss
Age							
14	98	44.41	10.36	57.87	11.27	43.15	8.97
15	321	43.69	8.91	57.76	11.43	42.16	8.98
16	194	42.19	7.70	56.95	11.14	41.60	7.77
17	128	44.68	7.25	60.00	9.39	42.93	7.95
18	26	43.03	10.33	58.07	10.56	40.76	8.71
		F:2.057 p=.085		F:1.530 p=.191		F:0.968 p=.424	
Gender							
Female	399	42.85	8.35	56.42	11.18	41.46	8.55
Male	368	44.30	8.90	59.62	10.58	43.05	8.40
		t:-2.322 **p=.020***		t:-4.056 **p=.000***		t:-2.603 **p=.090***	
Educational class							
9. grade	322	44.05	9.54	57.86	11.05	42.67	8.71
10. grade	249	42.62	7.94	57.40	11.34	41.90	8.36
11. grade	171	43.46	7.67	59.00	10.17	41.90	8.16
12. grade	25	46.84	8.72	57.52	12.67	41.92	9.88
		F:2.546 p=.055		F:0.736 p=.531		F:0.510 p=.675	
Number of members in the family							
3	87	44.48	9.32	59.42	10.24	43.85	9.11
4	394	43.35	8.10	58.17	10.94	42.35	8.22
5 and above	286	43.53	9.15	57.22	11.30	41.56	8.67
		F:0.603 p=.547		F:1.491 p=.226		F: 2.495 p=.083	
The state of feeling healthy							
Yes	535	45.54	8.02	60.68	9.65	44.10	7.84
No	232	38.95	8.28	51.68	11.39	37.90	8.43
		t:10.339 **p=.000***		t:11.213 **p=.000***		t:9.811 **p=.000***	

t: Student T Testi, F: OneWayAnova

Table 4. *The relationship between scale scores of adolescents*

	School Climate Survey	Healthy Lifestyle Belief Scale	Psychometric Properties of Resilience Measure
School Climate Survey	1		
Healthy Lifestyle Belief Scale	0.467**	1	
Child and Youth Resilience Measure	0.629**	0.677**	1

*** p<.01*

Table 5. *Evaluation of factors affecting school climate with linear regression analysis*

Model		Unstandardized Coefficients		Standardized Coefficients	t	P	95,0% Confidence Interval for B		Correlations
		B	SE	Beta			Lower Bound	Upper Bound	
1	Constant	.927	.233		3.97	.000	.470	1.385	
	Age	.001	.014	.002	.07	.945	-.026	.028	-.018
	Gender (male)	.010	.029	.010	.34	.731	-.046	.066	.084
	the state of feeling healthy (yes)	.167	.033	.151	5.01	.000	.102	.233	.350
	CYRM-12 (total)	.033	.002	.556	14.67	.000	.029	.038	.629
	HLB Scale (total)	.001	.002	.032	.82	.411	-.002	.005	.467
F=109.54, p<.001, Durbin-Watson=1.91, R=.65, R^2=.42									
Abbreviations: CI, confidence interval; SE, standard error; β, standardized regression coefficient. *Dependent variable = School Climate.									

4. DISCUSSION

In this study, which aimed to investigate the effects of school climate on psychological resilience and healthy lifestyle beliefs of adolescents, it was found that there was a positive relationship between school climate and students' psychological resilience and healthy lifestyle beliefs. A number of factors have been identified as contributors to school climate, including gender (male), feeling healthy, and the resilience of children and youth.

Although school-level variables such as the average socioeconomic status of the school, school size, or student-teacher ratio determine school climate, student characteristics, the way students perceive, interact with, and feel toward the environment, and variables such as adaptation to the environment, seeking support, and achieving academic goals lead to individual differences in students' perceptions of school climate. Due to its multidimensional nature, perceptions of school climate may vary by student group characteristics such as grade level, gender, and ethnicity. When assessed by gender characteristics, boys generally report lower perceptions of school climate than girls (13,14). In this study, the mean scores of boys on the healthy lifestyle belief scale and child and youth resilience measure were higher than girls, and there was no difference between their perceptions of school climate. One study found that while female students had higher scores for general health than male students, their scores for mental health were significantly lower (14). A study examining school climate and health behavior found that female students were more likely to consume unhealthy foods, exercise less than male students and brush their teeth more often (2). The study by Bhat et al. (2018) reports that female students perceive a more positive school climate. The study found a significant and positive correlation between school climate and students' academic performance, although the perception of school climate varied significantly depending on the type of school. Students in private schools in particular reported a better school climate than students in public schools (21). The results also showed that there were no differences in perceptions of school climate by school type. This could be due to the schools being in the same region and all being public schools.

Teacher support and peer relationships are components of the psychosocial school climate that affect students. Belonging to a positive peer group increases student engagement in school and contributes to healthy behavior (2,7). When students learn in a regular environment where they feel supported, they feel safe and more connected to school. Improving the school climate can be seen as a prerequisite for creating a safe school. Adolescents tend to engage in positive health behaviors when they experience a positive emotional and social climate in a well-organized school. In addition, a good school environment not only promotes student engagement and attendance, but also the adoption of healthy lifestyle habits and psychological well-being among adolescents (4,22). In this study, adolescents who felt healthy had significantly higher scores on the School Climate Survey, the Healthy Lifestyles Scale and the Child and Adolescent Resilience Measure. Nassar et al. (2018) found a correlation between a negative school climate and poor toothbrushing habits. The study found a significant correlation between the sub-dimension of teacher support and physical activity and tooth brushing. In addition, the sub-dimension of peer support was significantly associated with toothbrushing (2). In their study of 47,888 students, Cornell and Huang (2016) found that a positive school climate helps to reduce risky health behaviors in adolescents such as substance abuse, aggression and suicide attempts (22). The school climate is shaped by the behaviors of individuals, including students, teachers, and parents. Consequently, school climate has the potential to influence adolescents' health behaviors in a positive or negative manner. It is noteworthy that the adolescent period, characterized by ongoing development, is a critical juncture for the acquisition of both risky and healthy behaviors, with the school environment playing a pivotal role in shaping these behaviors.

School climate is considered an important indicator of students' emotional and behavioral outcomes. Psychological characteristics of adolescents, including well-being, life satisfaction, ethnic and moral identity, and resilience, are directly related to the school environment. While a positive school environment promotes students' and school staff's social behaviors and supports their learning and psychological development, a negative school climate can hinder growth and development (7,23,24). The results of this study suggest that school climate may have an effect on adolescents' psychological resilience and optimism about their health. It was found that students who reported a more favorable school climate had higher mean scores on psychological resilience and felt positive about their health. A study of secondary school students (5th-8th grade) found that psychological resilience and school climate are significant indicators of life satisfaction (25). In another study, perceived social support and school engagement were found to be important determinants of psychological resilience in adolescents (26). A study of 618 high school students at 15 schools in Australia found statistically significant and positive correlations between students' perceptions of school climate and their self-reported levels of well-being, resilience and moral identity (8). The study's findings align with existing literature on the subject. The significance of the relationship between school climate and health has once again been highlighted.

School climate encompasses the broader environmental characteristics of an academic institution, such as its values, culture, student-teacher relationships, and focus on education. Students' views of school climate have been found to correlate with their psychological well-being (27). Research suggests that school climate is related to the mental and physical health of adolescents. According to László et al. (2019) and Patalay et al. (2020), high school engagement and a sense of safety at school can have a positive impact on students' overall well-being (28,29). This

study found that the school climate has a positive impact on adolescents's healthy lifestyle belief and their psychological resilience. Students who reported a better school climate scored better in both dimensions. A Scottish study of 2,571 students aged 15-16 found that school climate has a positive impact on various aspects of adolescent mental health. Long et al. (2021) reported that adolescents who were bullied by their peers had poorer mental health than those who were supported by their peers and had positive relationships with their teachers (14). In addition, Nie et al. (2020) found that students who experienced a more positive school climate had fewer depressive symptoms than students who experienced a negative school climate (30). In addition to the physical development of adolescents at these ages, their psychological development also continues, with peer and school influences playing a significant role in this process. Consequently, the importance of fostering a positive school climate for the purpose of promoting optimal psychological development in adolescents is underscored.

4.1. Limitations

Due to the preparation time for the university exams, very little data could be collected from grade 12 students. Limitations of the study include the fact that the research was conducted in a single district and that teachers and parents were not included in the determination of school climate. The large sample size is the strength of the study.

6. CONCLUSIONS

Adolescents in secondary school show a high level of involvement in risky health behaviors, such as insufficient physical activity, irregular eating and sleeping habits, consumption of convenience foods and carbonated drinks, and less tooth brushing. On the other hand, the search for identity during this period drives adolescents to many psychological emotions and influences the development of mental health. The results of this study show that school climate is important for adolescents' psychological resilience and healthy life beliefs. In addition, perceptions of school climate were found to vary according to students' gender and health beliefs. These findings suggest that school climate is critical to adolescent development. By examining the relationship between adolescent health and school climate, this study provides a framework for the key characteristics of adolescent health and school climate and sheds light on the goals of school interventions for adolescent development. An authoritative theory of school climate can play an important role in the healthy development of the younger generation by providing a useful conceptual framework for school intervention efforts.

Acknowledgements: *The authors acknowledge the cooperation of the Turkish Ministry of Education and the many principals, teachers, parents, and students who participated in this study.*
Funding: *No funding was received to assist with the preparation of this manuscript.*
Conflicts of interest: *The authors have no competing interests to declare that are relevant to the content of this article.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Muğla Sıtkı Koçman University, Health Sciences Ethics Committee (Approval date: 15.03.2023; Number: 230025/49)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: RK
Design of the study: RK, GM,
Acquisition of data for the study: RK, EA
Analysis of data for the study: RK, GM, ÖA
Interpretation of data for the study: RK, EA, GM, ÖA
Drafting the manuscript: RK, EA
Revising it critically for important intellectual content: GM, ÖA
Final approval of the version to be published: RK, EA, GM, ÖA

REFERENCES

[1] Zullig KJ, Ward RM, Scott Huebner E, Daily SM. Association between Adolescent School Climate and Perceived Quality of Life. Child Indic Res 2018;11(6):1737–1753 https://doi.org/10.1007/s12187-017-9521-4

[2] Nassar O, Shaheen AM, Jarrah SS, Norton ME, Khalaf IA, Mohammad Hamdan K. Jordanian adolescents' health behaviour and school climate. J Res Nurs 2018;23(1):58–73 https://doi.org/10.1177/1744987117741668

[3] Durnalı M, Filiz B. Delaware Okul İklimi Ölçeği Öğrenci Versiyonunun Türk Kültürüne Uyarlanması: Geçerlik ve Güvenirlik Çalışması. Kastamonu Eğitim Derg 2019;27(6):2651–2661 https://doi.org/10.24106/kefdergi.3513

[4] Lester L, Cross D. The Relationship Between School Climate and Mental and Emotional Wellbeing Over the Transition from Primary to Secondary School. Psychol Well Being 2015;5(1):9 https://doi.org/10.1186/s13612-015-0037-8

[5] Grazia V, Molinari L. School climate multidimensionality and measurement: a systematic literature review. Res Pap Educ 2021;36(5):561–587 https://doi.org/10.1080/02671522.2019.1697735

[6] Charlton CT, Moulton S, Sabey C V., West R. A Systematic Review of the Effects of Schoolwide Intervention Programs on Student and Teacher Perceptions of School Climate. J Posit Behav Interv 2021;23(3):185–200 https://doi.org/10.1177/1098300720940168

[7] Hawkins GT, Chung CS, Hertz MF, Antolin N. The School Environment and Physical and Social-Emotional Well-Being: Implications for Students and School Employees. J Sch Health 2023;93(9):799–812 https://doi.org/10.1111/josh.13375

[8] Riekie H, Aldridge JM, Afari E. The role of the school climate in high school students' mental health and identity formation: A South Australian study. Br Educ Res J 2017;43(1):95–123 https://doi.org/10.1002/berj.3254

[9] Colvin S, Egan JE, Coulter RWS. School Climate & Sexual and Gender Minority Adolescent Mental Health. J Youth Adolesc 2019;48(10):1938–1951 https://doi.org/10.1007/s10964-019-01108-w

[10] Aldridge JM, McChesney K. The relationships between school climate and adolescent mental health and wellbeing: A systematic literature review. Int J Educ Res 2018;88(September 2017):121–145 https://doi.org/10.1016/j.ijer.2018.01.012

[11] Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral,

and biomedical sciences. J Mater Environ Sci 2007;39(2):175–191.

[12] Demidenko E. Sample size and optimal design for logistic regression with binary interaction. Stat Med 2008;27(1):36–46 https://doi.org/10.1002/sim.2980

[13] La Salle TP, McCoach DB, Meyers J. Examining Measurement Invariance and Perceptions of School Climate Across Gender and Race and Ethnicity. J Psychoeduc Assess 2021;39(7):800–815 https://doi.org/10.1177/07342829211023717

[14] Long E, Zucca C, Sweeting H. School Climate, Peer Relationships, and Adolescent Mental Health: A Social Ecological Perspective. Youth Soc 2021;53(8):1400–1415 https://doi.org/10.1177/0044118X20970232

[15] Bear GG, Gaskins C, Blank J, Chen FF. Delaware School Climate Survey—Student: Its factor structure, concurrent validity, and reliability. J Sch Psychol 2011;49(2):157–174 https://doi.org/10.1016/j.jsp.2011.01.001

[16] Liebenberg L, Ungar M, LeBlanc JC. The CYRM-12: A Brief Measure of Resilience. Can J Public Heal 2013;104(2):e131–e135 https://doi.org/10.1007/BF03405676

[17] Arslan G. Psychometric Properties of Child and Youth Resilience Measure (CYRM-12): The Study of Reliability and Validity (Çocuk ve Genç Psikolojik Sağlamlık Ölçeği'nin (ÇGPSÖ-12) Psikometrik Özellikleri: Geçerlilik ve Güvenirlik Çalışması). Ege Eğitim Derg 2015;16(1):1–12.

[18] Kelly SA, Melnyk BM, Jacobson DL, O'Haver JA. Correlates Among Healthy Lifestyle Cognitive Beliefs, Healthy Lifestyle Choices, Social Support, and Healthy Behaviors in Adolescents: Implications for Behavioral Change Strategies and Future Research. J Pediatr Heal Care 2011;25(4):216–223 https://doi.org/10.1016/j.pedhc.2010.03.002

[19] Akdeniz Kudubeş A, Bektas M. Orginal Article: Psychometric Properties of the Turkish Version of the Healthy Lifestyle Belief Scale for Adolescents. J Pediatr Nurs 2020;53:e57–e63 https://doi.org/10.1016/j.pedn.2020.02.006

[20] Tabachnick BG, Fidell LS. Using Multivariate Statistics. 6th ed. Scientific Research Publishing: Boston; 2013.

[21] Bhat MS, Mir SA. Perceived school climate and academic achievement of secondary school students in relation to their gender and type of school. Int J Adv Educ Res 2018;3(2):620–628.

[22] Cornell D, Huang F. Authoritative School Climate and High School Student Risk Behavior: A Cross-sectional Multi-level Analysis of Student Self-Reports. J Youth Adolesc 2016;45(11):2246–2259 https://doi.org/10.1007/s10964-016-0424-3

[23] Luo Y, Ma T, Deng Y. School climate and adolescents' prosocial behavior: the mediating role of perceived social support and resilience. Front Psychol 2023;14(July):1–10 https://doi.org/10.3389/fpsyg.2023.1095566

[24] Aldridge JM, Fraser BJ, Fozdar F, Ala'i K, Earnest, J, Afari E. Students' perceptions of school climate as determinants of wellbeing, resilience and identity. Improv Sch 2016;19(1):5–26 https://doi.org/10.1177/1365480215612616

[25] Baş A, Yurdabakan İ. The Predictive Value of Resilience and School Climate in Life Satisfaction among Middle School Students. Mehmet Akif Ersoy Üniversitesi Eğitim Fakültesi Derg 2017;1(41):202 https://doi.org/10.21764/efd.32175

[26] Turgut Ö, Eraslan-Çapan B. The Predıctors of Adolescent Resilience: Perceived Social Support and School Engagement. Mehmet Akif Ersoy Üniversitesi Eğitim Fakültesi Derg 2017;44:162–183 https://doi.org/1021764/maeuefd.309934

[27] La Salle TP, Rocha-Neves J, Jimerson S, Di Sano S, Martinsone B, Albertova SM, Gajdošová E, Baye A, Deltour C, Martinelli V, Raykov M, Hatzichristou C, Palikara O, Szabó É, Arlauskaite Z, Athanasiou D, Brown-Earle O, Casale G, Lampropoulou A, Mikhailova A, Pinskaya M, Zvyagintsev R. A multinational study exploring adolescent perception of school climate and mental health. Sch Psychol 2021;36(3):155–166 https://doi.org/10.1037/spq0000430

[28] Patalay P, O'Neill E, Deighton J, Fink E. School characteristics and children's mental health: A linked survey-administrative data study. Prev Med (Baltim) 2020;141:106292 https://doi.org/10.1016/j.ypmed.2020.106292

[29] László KD, Andersson F, Galanti MR. School climate and mental health among Swedish adolescents: a multilevel longitudinal study. BMC Public Health 2019;19(1):1695 https://doi.org/10.1186/s12889-019-8018-0

[30] Nie Q, Yang C, Teng Z, Furlong MJ, Pan Y, Guo C, Zhang D. Longitudinal association between school climate and depressive symptoms: The mediating role of psychological suzhi. Sch Psychol 2020;35(4):267–276 https://doi.org/10.1037/spq0000374

How to cite this article: Kara R, Üner Asil E, Karayağız Muslu G, Aygün Ö . Investigating the Effect of School Climate on Adolescents' Psychological Resilience and Healthy Lifestyle Beliefs. Clin Exp Health Sci 2025; 15: 203-209. DOI: 10.33808/clinexphealthsci.1587455

Clinical and Experimental Health Sciences

SREBP-1c Deficiency Attenuates Fructose-Induced Lipid Droplet Accumulation

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Received: December 20, 2024 **Accepted:** January 31, 2025

ABSTRACT

Objective: Sterol regulatory element binding protein 1c (SREBP-1c), a transcription factor involved in the biosynthesis of fatty acids, is critical in metabolic dysfunction-associated fatty liver disease (MAFLD) by promoting lipid accumulation and metabolic dysregulation that leads to hepatic pathologies. Fructose, becoming increasingly common in diets, activates SREBP-1c by increasing acetyl-CoA production. Present study aimed to sought the effect of SREBP-1c in fructose induced lipid accumulation.

Methods: A fructose-induced lipid accumulation model was developed in mouse hepatocyte cells (AML12), where SREBP-1c expression was inhibited through siRNA transfection. Following different fructose concentrations, viability was determined by MTT assay, and the protein expression of SREBP-1c protein was determined by western blotting. The number of lipid droplets (LDs) was quantified microscopically, and lipogenic mRNA expressions of FASN, SCD1, GPAM, ACLY, ACSL1 and ACACA were detected by qRT-PCR.

Results: Western blotting and microscopic analysis indicated that 25 mM for 72 hours of fructose increased total LDs, together with SREBP-1c levels, without affecting cell viability. The mRNA expression of SREBP-1c decreased in the presence of siRNA, confirming siRNA efficacy. SREBP-1c silencing reduced the number of fructose-induced total LDs. As lipogenic mRNA expressions, SREBP-1c silencing reduced SCD1 and ACLY, while other genes were unaffected.

Conclusion: Silencing of SREBP-1c in hepatocytes demonstrated its beneficial effect by reducing fructose-induced LD accumulation.

Keywords: Sterol regulatory element binding transcription factor 1c, fructose, lipid accumulation

1. INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) is defined as lipid accumulation in hepatocytes of more than 5% of liver weight without a secondary cause such as alcohol or drugs (1). The progression of the disease varies considerably depending on the extent of damage to hepatocyte cells, the presence of inflammatory processes, and fibrosis development (2). Therefore, mechanisms leading to lipid accumulation in the liver are of crucial importance in steatosis development. Along with an irregular diet and sedentary lifestyle, excessive fructose consumption also promotes the development of MAFLD (3). This is due to the fact that fructose not only serves as a substrate but also induces lipogenesis in hepatic *de novo* lipogenesis (DNL). The glucose transporter 2 (GLUT-2), a transmembrane transporter protein that facilitates the transport of fructose to hepatocytes, is not regulated by insulin or another hormone. Furthermore, the direct incorporation of fructose into the glycolytic pathway, bypassing the regulatory phosphofructokinase step of glycolysis, results in an excess of glycolysis products and increased flux of fructose carbons to lipogenic precursors (3, 4). As a consequence of these effects, there may be a reduction in insulin sensitivity in tissues other than adipose tissue, potentially leading to lipotoxicity in these cells (5).

The main family of transcription factors that enable the activation of DNL are sterol response element binding proteins (SREBPs) (6). One of the three isoforms of the family, SREBP-1c, modulates the expression of genes associated with the biosynthesis of fatty acids, and is tightly controlled by insulin and glucose at both transcriptional and post-translational stages, especially in liver and adipose tissues (7). Studies demonstrated a direct interaction between SREBP-1c and MAFLD (8). In chronic fructose consumption, increased fructose metabolism and acetyl-coA production

https://doi.org/10.33808/clinexphealthsci.1603452

led to SREBP-1c activation, and thus, fructose behaves as a DNL inducer. Therefore, fructose is recognized as the most potent lipogenic carbohydrate inducing hepatic steatosis (9). Similarly, high fructose consumption is observed to be associated with an increase in liver triacylglycerols (TAG) levels. This is achieved by increasing DNL through the induction of expression of proteins, such as fatty acid synthase (FASN) (10) and acetyl-CoA carboxylase (ACC) (10, 11), and transcription factors, including SREBP-1c (11, 12).

Nowadays, the production and easy availability of fructose-containing sugar syrups have revealed that excessive consumption of fructose stimulates SREBP-1c expression and leads to fatty liver diseases through hepatic lipid accumulation (8). However, understanding fructose metabolism and its relationship with SREBP-1c may facilitate the exploration of therapeutic approaches aimed to mitigate lipid accumulation. In this context, we have investigated the influences of fructose-induced lipid metabolism in the connection of SREBP-1c using hepatocyte cells.

2. METHODS

2.1. Cell Culture and Treatments

Mouse hepatocyte cells (AML12, ATCC CRL 2254) were cultured in Dulbeco's Modified Eagle's Medium (DMEM) (Merck KGaA, Darmstadt, Germany) containing 10% FBS (Gibco) (Thermo Fisher Scientific, Massachusetts, USA), 100 U/mL penicillin and 100 mg/mL streptomycin (Gibco) (Thermo Fisher Scientific, Massachusetts, USA) at a constant-temperature incubator (37°C and 5 % CO_2). Intracellular lipid accumulation was established by exposing the cells to fructose (Merck KGaA, Darmstadt, Germany, catalog No. 104007) as indicated in figure legends. All experimental procedures were approved by the Marmara University, School of Medicine Ethics Committee (protocol number 09.2019.188).

2.2. siRNA Mediated Gene Silencing

Cells were transfected with SREBP-1c targeting siRNA, as previously reported by our group (13). Briefly, AML12 cells were seeded at 80% confluency in 6-well plates, and transfection was conducted in accordance with the instructions provided by the manufacturer for the Lipofectamine RNAiMax Reagent (Thermo Fisher Scientific, Massachusetts, USA, catalog No. 13778075). Eighty pmol siRNA (Thermo Fisher Scientific, siRNA ID: 151861) was prepared at the ratio of 1:3 with Lipofectamine RNAiMax Reagent in serum-free culture medium (OptiMEM) (Thermo Fisher Scientific, Massachusetts, USA). Experiments were conducted after incubation for 24 h with siRNA.

2.3. BODIPY Staining

Bodipy staining was used to determine characteristic (size and number) differences in lipid droplets (LDs). AML12 cells were seeded at 25×10^3 density in 48-well plates, and treated with fructose and SREBP-1c targeting siRNA. Following the administrations, cells were rinsed with PBS and subsequently fixed with 4% formaldehyde. After washing with PBS, cells were incubated with BODIPY 493/503 (Thermo Fisher Scientific, Massachusetts, USA, catalog No. D3922) at 0.25 µg/ml for 20 min, then exposed to the nuclear stain DAPI. The images were acquired using a Zeiss LSM700 confocal microscope (Amsterdam, Netherlands). LDs were counted using ImageJ software. Data were obtained from at least 30 cells of each group.

2.4. Gene Expression Analysis

AML12 cells were seeded at 5×10^4 density in 6-well plates, and after the administrations, RNA isolation was performed with Pure Link RNA Mini Kit (Thermo Fischer). High-Capacity cDNA Reverse Transcription kit (Thermo Fischer) was used to synthesize cDNA according to the protocol recommended by the manufacturer. The amplification of the synthesized cDNA samples was performed using Power UP SYBR Green Master Mix kit (Thermo Fischer) and Rotor Gene Q-RT PCR system (Qiagen). After determining the threshold cycle (CT), relative gene expression was calculated as follows: fold change = $2^{-\Delta(\Delta CT)}$, where ΔCT = CT-CT target housekeeping (GAPDH) and Δ(ΔCT) = ΔCT-CT treated control. The primary sequences are presented in Supplementary Table 1.

2.5. Immunoblot Analysis

AML12 cells were seeded at 25×10^3 density in 48-well plates, and after the administrations, lysed with RIPA buffer (Cell Signalling Technology, Massachusetts, USA, catalog No. 9806), which allows for the isolation of proteins. Protein concentration was quantified using BCA assay (Thermo Fisher Scientific, Massachusetts, USA, catalog No. 23225), which was conducted in accordance with the manufacturer's instructions. Thirty µg of protein was subjected to SDS-PAGE, and subsequently, separated proteins were transferred to a nitrocellulose membrane. The membrane was blocked with 5% BSA in TBST. Then, the membrane was incubated with primary antibodies against SREBP1c (Novus Biologicals, catalog No. NB100-2215, 1:500 dil) and GAPDH (Novus Biologicals, catalog No. NB300-221, 1:2000 dil) overnight. After 1 hour of incubation with HRP-conjugated secondary antibody, blots were visualized using a chemiluminescence kit (Thermo Fisher Scientific, Massachusetts, USA, catalog No. 34580). The intensity of the bands was measured using Image J software and normalized to GAPDH.

2.6. Statistical Analysis

The statistical analysis was conducted using the Prism 4 software package (GraphPad). To ascertain the statistical significance of observed differences, a one-way ANOVA was conducted, followed by multiple comparisons using the Student-Newman-Keuls test. A p-value of less than .05 is considered statistically significant.

3. RESULTS

3.1. Twenty-Five mM of Fructose Induces Lipid Accumulation and SREBP-1c Expression in Hepatocytes

Our study was performed in the AML12 mouse hepatocyte cell line, which is generally preferred in studies on the establishment of the fatty liver model (14, 15). Enhanced LD accumulation in hepatocytes is a characteristic feature of hepatic steatosis. Accordingly, the objective of our study was to initially observe the impact of fructose at different concentrations (0.5 mM, 5 mM, 25 mM) for 72 hours. We first checked the effect of fructose on cell viability, and all concentrations had no significant effect as expected (Figure 1A). To determine *in vitro* steatosis establishment, the accumulation of LDs was detected using BODIPY and confocal microscopy. As shown in Figure 1B, fructose at 0.5 and 5 mM showed no significant difference, whereas 25 mM of fructose increased the number of LDs. To further examine the involvement of SREBP-1c in fructose-induced lipid accumulation, we checked its protein expression by western blotting, and observed a significant increase in the 5 mM and 25 mM groups compared to control (Figure 1C). In this context, fructose administration at 25 mM for 72 hours was preferred to induce lipid accumulation for the remainder of the study.

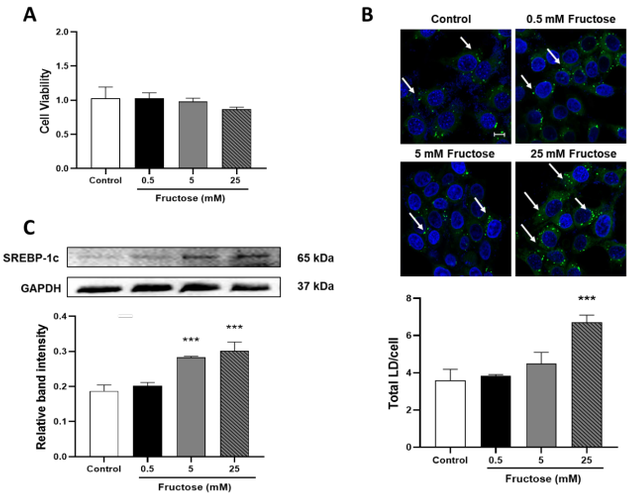

Figure 1. *Twenty-five mM of fructose induces lipid accumulation and SREBP-1c expression in hepatocytes*
*AML12 cells were treated with different concentrations of fructose (0.5 – 5 – 25 mM) for 72 hours. Cell viability was analyzed by MTT assay (A). Lipid droplets are labelled with BODIPY (green) and cell nuclei with DAPI (blue), followed by analysis of the number of LDs per cell. Representative images shows LD-positive areas in hepatocytes (arrowhead). Scale bar = 10 µm (B). SREBP-1c protein expressions in cells were measured by western blotting. Relative ratios were quantified and normalized to GAPDH (C). Data are expressed as mean ± S.D. One-way ANOVA, Student-Newman-Keuls test, (n=3). ***p < .001 vs. control*

3.2. SREBP-1c Mediates Fructose-Induced Lipid Accumulation

To identify the role of SREBP-1c in fructose-induced lipid accumulation, SREBP-1c was silenced by siRNA transfection in accordance with our previous studies (13). As expected, siRNA transfection significantly inhibited SREBP-1c expression at the transcriptional level in both fructose-treated and non-treated conditions (Figure 2A). However, in accordance with Figure 1B, fructose administration in normal conditions increased the number of LDs, which was prevented by siRNA transfection (Figure 2B). Present findings so far reveal the crucial role of SREBP-1c on fructose-induced LD accumulation in hepatocytes.

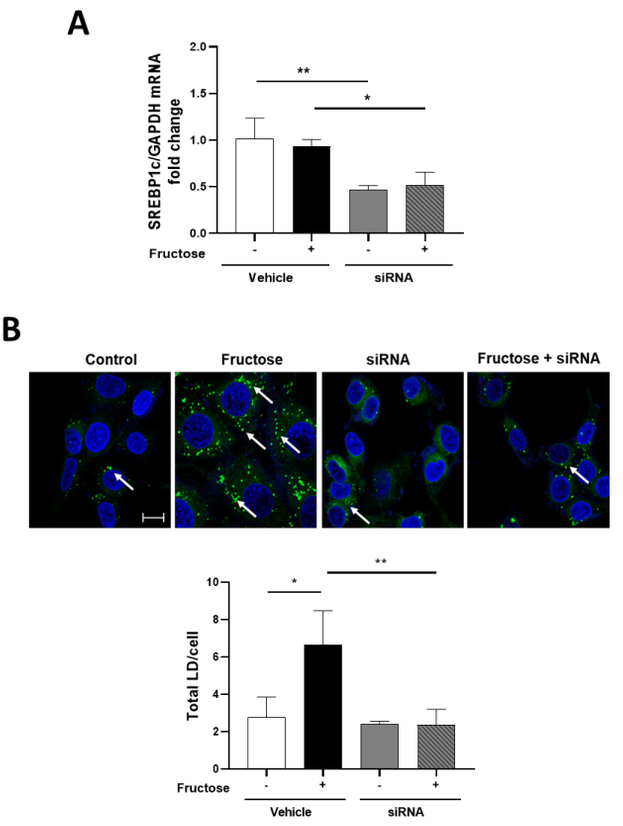

Figure 2. *SREBP-1c mediates fructose-induced lipid accumulation AML12 cells were treated with 25 mM fructose for 72 hours followed by SREBP-1c siRNA transfection. mRNA expression of SREBP-1c was determined by qRT-PCR and normalized to GAPDH (A). Lipid droplets were labelled with BODIPY (green) and cell nuclei with DAPI (blue), followed by analysis of the number LDs per cell. Representative images shows LD-positive areas in hepatocytes (arrowhead). Scale bar = 10 µm (B). Data are expressed as mean ± S.D. One-way ANOVA, Student-Newman-Keuls test, (n=3). *p< .05 and **p < .01*

3.3. SREBP-1c Dependent Lipid Accumulation in Fructose-Induced Hepatocytes is Lipid Metabolism Independent

To further evaluate the alterations in lipid metabolism, we examined the mRNA expression of well-known parameters of lipolysis and lipogenesis, including FASN, SCD-1, GPAM, ACLY, ACSL1, and ACACA. As shown in Figure 3, neither siRNA transfection nor fructose administration had a

significant effect on FASN, GPAM, ACSL1, and ACACA expressions. However, siRNA transfection under normal conditions exhibited a significant decrease in SCD1 and ACLY expressions, while fructose administration in SREBP-1c silenced hepatocytes did not alter any of the parameters (Figure 3).

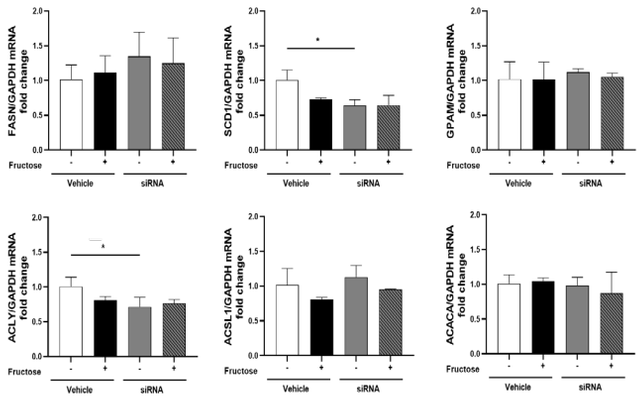

Figure 3. *SREBP-1c dependent lipid accumulation in fructose-induced hepatocytes is lipid metabolism independent*
*AML12 cells were treated with 25 mM fructose for 72 hours followed by SREBP-1c siRNA transfection. mRNA expressions of FASN, SCD1, GPAM, ACLY, ACSL1 and ACACA were determined by qRT-PCR and normalized to GAPDH. Data are expressed as mean ± S.D. One-way ANOVA, Student-Newman-Keuls test, (n=3). *p< .05*

4. DISCUSSION

The most fundamental pathological characteristic of MAFLD, the most prevalent metabolic disorder affecting about 25% of the worldwide population, is the accumulation of lipids in hepatocytes (16). Although MAFLD becomes incurable in its later stages, it is possible to control or reverse hepatosteatosis that develops due to unhealthy diets in the early stages (17). In particular, acute fructose metabolism causes the rapid formation of substrates required for DNL, leading to MAFLD. The consumption of fructose, a highly lipogenic sugar, has recently increased markedly. There is a developing body of findings associating its intake with components of metabolic syndrome, such as insulin resistance, abdominal obesity, dyslipidemia, and hypertension (18). Furthermore, fructose stimulates hepatic DNL more strongly compared to glucose (19-21), starch (22-24) or high-fat diets (25-27). In the short term (4-6 h), only a small percentage of the carbon skeleton of fructose is transformed into lipid in a span of 4-6 h (28). Furthermore, the long-term DNL-promoting impact of fructose was confirmed by labelled acetate infusion studies (19, 20, 29). Thereby, along with the increased level of DNL substrate with chronic fructose exposure, fructose also has an effect on prolipogenic mechanisms (30). Natural fructose from plant sources is relatively beneficial compared to fructose from industrial sources such as sucrose and high-fructose corn syrup. Its slower absorption and components, such as antioxidants and plant fiber, provide metabolic benefits. In contrast, industrial fructose, especially in liquid form, shows rapid absorption and may cause hepatic insulin resistance and MASLD (31).

In this study, we sought to investigate the effect of fructose on lipid metabolism and evaluate potential approaches for steatosis treatment. To establish an *in vitro* steatosis model, we used theAML12 cell line due to its stable phenotypes and replicative capacity and established fructose-induced lipid accumulation. There are various *in vitro* studies involving different concentrations in the fructose-induced steatosis model. For instance, studies in HepG2 cells and primary human hepatocytes have shown enhanced lipid accumulation by 8 mM (32) and 22.2 mM (33) fructose, respectively. Other studies in hepatocytes used 25 mM (34), 88.8 mM (35) and 100 mM (36) fructose for lipid accumulation. These studies have shown that in addition to the lipid accumulation-inducing effect of fructose, it also increases the production of inflammatory cytokines interleukin-1β (IL-1β), IL-6 and tumour necrosis factor-α (TNF-α), and the expression of Ubiquitin carboxyl-terminal hydrolase 2 (USP2), which plays a role in cell cycle and protein degradation (35). Due to the contradictions on dose, we performed an optimization process for the fructose-induced model in the initial part of our study. For this purpose, we aimed to establish our model under optimal conditions by comparing the effect of three different concentrations, 0.5, 5, and 25 mM, on lipid accumulation. Our findings confirmed that 25 mM fructose for 72h increased total LDs. In addition, our study indicated that 5 mM and 25 mM fructose upregulated the SREBP-1c expression without any effect on cell viability. Accordingly, 25 mM fructose was selected as the inducer of lipid accumulation in hepatocytes in the rest of our experiments.

SREBP-1c is a major regulatory factor of DNL-related genes and contributes to MAFLD development (37). The expression of SREBP-1c and its post-translational activation is markedly enhanced by insulin signaling. A diet high in fructose frequently results in insulin resistance and hyperinsulinemia, thereby inducing insulin-mediated SREBP-1c activation and promoting hepatic lipid synthesis (38, 39). In our previous study, silencing SREBP-1c reduced the number of LDs induced by oleic acid (13). Inconsistent, silencing of SREBP-1c caused a reduction in fructose-induced total LDs formation in the present study. These findings indicate that targeting SREBP-1c may serve as a therapeutic strategy against fructose-induced steatosis. Furthermore, various studies have demonstrated the efficacy of SREBP-1c silencing through therapeutic interventions. Ruiz et al. (40) revealed that silencing of SREBP-1 induced the gluconeogenesis genes, while decreasing the glycolysis and glycogen synthesis genes in an animal model of obesity and type 2 diabetes. In another study by Li et al. (41), SREBP-1 knockdown was observed to increase apoptosis and inhibit proliferation, migration and invasion in both MHCC97L and HepG2 cells. These findings suggest that SREBP-1 may contribute to tumor development by enhancing cell growth and metastasis, thus suggesting that it may be a potential therapeutic target for HCC (41).

However, further studies using animal and human samples are crucial to confirm these findings.

The mechanism by which SREBP-1c siRNA reduces fructose-induced LD accumulation might occur through the inhibition of lipogenesis. However, SREBP-1c activation was reported to be associated with increase in enzymes involved in DNL, including FASN (42) SCD1 (43), ATP citrate lyase (ACLY), glycerol-3-phosphate acyltransferase (GPAM) and acyl-CoA synthetase long chain family member 1 (ACSL1) (44). For this reason, the effect of siRNA on mRNA expressions of lipogenic genes was examined in our research. The siRNA only reduced the expression levels of SCD1 and ACLY, while the mRNA expression levels of the other lipogenic genes remained unchanged. However, the reducing effect on SCD1 and ACLY was not on fructose-induced hepatocytes. Therefore, it can be concluded that the therapeutic effect of siRNA on fructose-induced lipid accumulation is independent of lipid metabolism. However, further experiments, such as lipogenic proteins at the translational level, should be conducted to establish that this effect is independent of lipid metabolism.

According to our results, the silencing of SREBP-1c prevented the fructose-induced accumulation of LDs by alternative mechanisms other than lipogenesis suppression. Recent findings indicate that lipophagy, which is essential for maintaining cellular homeostasis, is an effective mechanism for lipid removal in hepatocytes (45). For instance, the inhibition of lipophagy resulted in increased TAG and LDs levels, decreased TAG degradation (46). It was also revealed that autophagy parameters such as Autophagy protein 5 (Atg5), Atg7, LC3II/I, and p62 were inhibited in the liver of mice subjected to a high fructose intake (47). Autophagic dysregulation has also been identified as a factor in the progression of human MAFLD (48, 49). Rather than autophagy, endoplasmic reticulum (ER) stress, which is known to be associated with SREBP in steatosis, might be involved in the therapeutic effect of siRNA(50). In our previous study, the silencing of SREBP-1c resulted in a notable reduction in ER stress (phospho-IRE1, ATF6) and ER stress-triggered apoptosis (JNK, CHOP) parameters in oleic acid-induced steatosis (51). Fructose is also known to increase ER stress in hepatocytes, triggering adverse processes such as inflammation, insulin resistance and apoptosis (17). Finally, siRNA might indirectly reduce fructose-induced LD accumulation by blocking signaling pathways of ER stress or reducing the production of inflammatory cytokines or preventing cellular damage, this should be examined in further studies. The present study provides novel insights into the function of SREBP-1c in fructose-induced lipid accumulation. The silencing of SREBP-1c was found to result in a notable reduction in the accumulation of LDs. Our findings provide promising evidence in developing novel treatment strategies against fructose-induced lipid accumulation and MAFLD.

5. CONCLUSION

It is evident that fructose, a prevalent dietary component nowadays, plays a contributory role in the progression of steatosis. This study investigates the role of SREBP-1c in regulating lipid accumulation induced by fructose. The findings of present study indicate that the silencing of SREBP-1c is an effective method for preventing fructose-induced lipid accumulation. The lack of impact on lipogenesis mRNAs suggests that SREBP-1c siRNA exerts its therapeutic effect through alternative pathways, such as lipophagy, and ER stress. A future examination of the mechanism of action of SREBP-1c siRNA through alternative pathways will provide a different perspective on approaches to reduce the development of MASH.

Acknowledgements: *We would like to thank Marmara University for providing access to academic databases.*
Funding: *This work was supported by the The Scientific and Technological Research Council of Turkey (TUBITAK) [grant number 323S171].*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Marmara University, School of Medicine Ethics Committee (Approval date: 01.02.2019; Number: 09.2019.188)*
Peer-review: *Externally peer-reviewed.*
Author Contributions: (Initials only)
Research idea: SMT, NKO, ES
Design of the study: SMT, ES
Acquisition of data for the study: SMT, II, BC, TDY
Analysis of data for the study: SMT, II, BC, TDY
Interpretation of data for the study: SMT
Drafting the manuscript: SMT, ES
Revising it critically for important intellectual content: SMT, ES, NKO
Final approval of the version to be published: SMT, II, BC, TDY, NKO, ES

REFERENCES

[1] Maurice J, Manousou P. Non-alcoholic fatty liver disease. Clin Med (Lond). 2018;18(3):245-50. https://doi.org/ 10.7861/clinmedicine.18-3-245.

[2] Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, et al. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr Disord. 2022;22(1):63. https://doi.org/10.1186/s12902-022-00980-1

[3] Jegatheesan P, De Bandt JP. Fructose and NAFLD: The Multifaceted Aspects of Fructose Metabolism. Nutrients. 2017;9(3). https://doi.org/10.3390/nu9030230

[4] Dholariya SJ, Orrick JA. Biochemistry, Fructose Metabolism. StatPearls. Treasure Island (FL)2024.

[5] Zhang DM, Jiao RQ, Kong LD. High Dietary Fructose: Direct or Indirect Dangerous Factors Disturbing Tissue and Organ Functions. Nutrients. 2017;9(4). https://doi.org/10.3390/nu9040335

[6] Lee GY, Jang H, Lee JH, Huh JY, Choi S, Chung J, et al. PIASy-mediated sumoylation of SREBP1c regulates hepatic lipid metabolism upon fasting signaling. Mol Cell Biol. 2014;34(6):926-38. https://doi.org/10.1128/MCB.01166-13

[7] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002;109(9):1125-31. https://doi.org/10.1172/JCI15593.

[8] Moslehi A, Hamidi-Zad Z. Role of SREBPs in Liver Diseases: A Mini-review. J Clin Transl Hepatol. 2018;6(3):332-8. https://doi.org/10.14218/JCTH.2017.00061

[9] Herman MA, Samuel VT. The Sweet Path to Metabolic Demise: Fructose and Lipid Synthesis. Trends Endocrinol Metab. 2016;27(10):719-30. https://doi.org/10.1016/j.tem.2016.06.005

[10] Nunes PM, Wright AJ, Veltien A, van Asten JJ, Tack CJ, Jones JG, et al. Dietary lipids do not contribute to the higher hepatic triglyceride levels of fructose- compared to glucose-fed mice. FASEB J. 2014;28(5):1988-97. https://doi.org/10.1096/fj.13-241208

[11] Wang DD, Sievenpiper JL, de Souza RJ, Chiavaroli L, Ha V, Cozma AI, et al. The effects of fructose intake on serum uric acid vary among controlled dietary trials. J Nutr. 2012;142(5):916-23. https://doi.org/10.3945/jn.111.151951

[12] Leibowitz A, Rehman A, Paradis P, Schiffrin EL. Role of T regulatory lymphocytes in the pathogenesis of high-fructose diet-induced metabolic syndrome. Hypertension. 2013;61(6):1316-21. https://doi.org/10.1161/HYPERTENSIONAHA.111.203521

[13] Sozen E, Demirel-Yalciner T, Sari D, Avcilar C, Samanci TF, Ozer NK. Deficiency of SREBP1c modulates autophagy mediated lipid droplet catabolism during oleic acid induced steatosis. Metabol Open. 2021;12:100138. https://doi.org/10.1016/j.metop.2021.100138

[14] Cui W, Chen SL, Hu KQ. Quantification and mechanisms of oleic acid-induced steatosis in HepG2 cells. Am J Transl Res. 2010;2(1):95-104. https://www.ncbi.nlm.nih.gov/pubmed/20182586

[15] Zhu X, Yan H, Xia M, Chang X, Xu X, Wang L, et al. Metformin attenuates triglyceride accumulation in HepG2 cells through decreasing stearyl-coenzyme A desaturase 1 expression. Lipids Health Dis. 2018;17(1):114. https://doi.org/10.1186/s12944-018-0762-0

[16] Cotter TG, Rinella M. Nonalcoholic Fatty Liver Disease 2020: The State of the Disease. Gastroenterology. 2020;158(7):1851-64. https://doi.org/10.1053/j.gastro.2020.01.052

[17] Wang YL, Zhou X, Li DL, Ye JM. Role of the mTOR-autophagy-ER stress pathway in high fructose-induced metabolic-associated fatty liver disease. Acta Pharmacol Sin. 2022;43(1):10-4. https://doi.org/10.1038/s41401-021-00629-0

[18] Aijala M, Malo E, Ukkola O, Bloigu R, Lehenkari P, Autio-Harmainen H, et al. Long-term fructose feeding changes the expression of leptin receptors and autophagy genes in the adipose tissue and liver of male rats: a possible link to elevated triglycerides. Genes Nutr. 2013;8(6):623-35. https://doi.org/10.1007/s12263-013-0357-3

[19] Parks EJ, Skokan LE, Timlin MT, Dingfelder CS. Dietary sugars stimulate fatty acid synthesis in adults. J Nutr. 2008;138(6):1039-46. https://doi.org/10.1093/jn/138.6.1039

[20] Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest. 2009;119(5):1322-34. https://doi.org/10.1172/JCI37385

[21] Lecoultre V, Egli L, Carrel G, Theytaz F, Kreis R, Schneiter P, et al. Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. Obesity (Silver Spring). 2013;21(4):782-5. https://doi.org/10.1002/oby.20377

[22] Laube H, Klor HU, Fussganger R, Pfeiffer EF. The effect of starch, sucrose, glucose and fructose on lipid metabolism in rats. Nutr Metab. 1973;15(4):273-80. https://doi.org/10.1159/000175450

[23] Timlin MT, Parks EJ. Temporal pattern of de novo lipogenesis in the postprandial state in healthy men. Am J Clin Nutr. 2005;81(1):35-42. https://doi.org/10.1093/ajcn/81.1.35

[24] Schwarz JM, Noworolski SM, Wen MJ, Dyachenko A, Prior JL, Weinberg ME, et al. Effect of a High-Fructose Weight-Maintaining Diet on Lipogenesis and Liver Fat. J Clin Endocrinol Metab. 2015;100(6):2434-42. https://doi.org/10.1210/jc.2014-3678

[25] Kennedy AR, Pissios P, Otu H, Roberson R, Xue B, Asakura K, et al. A high-fat, ketogenic diet induces a unique metabolic state in mice. Am J Physiol Endocrinol Metab. 2007;292(6):E1724-39. https://doi.org/10.1152/ajpendo.00717.2006

[26] Samuel VT. Fructose induced lipogenesis: from sugar to fat to insulin resistance. Trends Endocrinol Metab. 2011;22(2):60-5. https://doi.org/10.1016/j.tem.2010.10.003

[27] Softic S, Cohen DE, Kahn CR. Role of Dietary Fructose and Hepatic De Novo Lipogenesis in Fatty Liver Disease. Dig Dis Sci. 2016;61(5):1282-93. https://doi.org/10.1007/s10620-016-4054-0

[28] Chong MF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. Am J Clin Nutr. 2007;85(6):1511-20. https://doi.org/10.1093/ajcn/85.6.1511

[29] Faeh D, Minehira K, Schwarz JM, Periasamy R, Park S, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes. 2005;54(7):1907-13. https://doi.org/10.2337/diabetes.54.7.1907

[30] Ter Horst KW, Serlie MJ. Fructose Consumption, Lipogenesis, and Non-Alcoholic Fatty Liver Disease. Nutrients. 2017;9(9). https://doi.org/10.3390/nu9090981

[31] Malik VS, Hu FB. Fructose and Cardiometabolic Health: What the Evidence From Sugar-Sweetened Beverages Tells Us. J Am Coll Cardiol. 2015;66(14):1615-24. https://doi.org/10.1016/j.jacc.2015.08.025

[32] Huggett ZJ, Smith A, De Vivo N, Gomez D, Jethwa P, Brameld JM, et al. A Comparison of Primary Human Hepatocytes and Hepatoma Cell Lines to Model the Effects of Fatty Acids, Fructose and Glucose on Liver Cell Lipid Accumulation. Nutrients. 2022;15(1). https://doi.org/10.3390/nu15010040

[33] Hoang NA, Richter F, Schubert M, Lorkowski S, Klotz LO, Steinbrenner H. Differential capability of metabolic substrates to promote hepatocellular lipid accumulation. Eur J Nutr. 2019;58(8):3023-34. https://doi.org/10.1007/s00394-018-1847-2

[34] Tsameret S, Chapnik N, Froy O. Differential Effect of Fructose in the Presence or Absence of Fatty Acids on Circadian Metabolism in Hepatocytes. Metabolites. 2023;13(2). https://doi.org/10.3390/metabo13020138

[35] Li C, Li M, Sheng W, Zhou W, Zhang Z, Ji G, et al. High dietary Fructose Drives Metabolic Dysfunction-Associated Steatotic Liver Disease via Activating ubiquitin-specific peptidase 2/11beta-hydroxysteroid dehydrogenase type 1 Pathway in Mice. Int J Biol Sci. 2024;20(9):3480-96. https://doi.org/10.7150/ijbs.97309

[36] Sasi USS, Sindhu G, Raghu KG. Corrigendum to 'Fructose-palmitate based high calorie induce steatosis in HepG2 cells via mitochondrial dysfunction: An in vitro approach' [Toxicology

in Vitro 68 (2020) 104952]. Toxicol In Vitro. 2021;75:105177. https://doi.org/10.1016/j.tiv.2021.105177

[37] Aragno M, Tomasinelli CE, Vercellinatto I, Catalano MG, Collino M, Fantozzi R, et al. SREBP-1c in nonalcoholic fatty liver disease induced by Western-type high-fat diet plus fructose in rats. Free Radic Biol Med. 2009;47(7):1067-74. https://doi.org/10.1016/j.freeradbiomed.2009.07.016

[38] Bezerra RM, Ueno M, Silva MS, Tavares DQ, Carvalho CR, Saad MJ, et al. A high-fructose diet induces insulin resistance but not blood pressure changes in normotensive rats. Braz J Med Biol Res. 2001;34(9):1155-60. https://doi.org/10.1590/s0100-879x2001000900008

[39] Tran LT, Yuen VG, McNeill JH. The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. Mol Cell Biochem. 2009;332(1-2):145-59. https://doi.org/10.1007/s11010-009-0184-4

[40] Ruiz R, Jideonwo V, Ahn M, Surendran S, Tagliabracci VS, Hou Y, et al. Sterol regulatory element-binding protein-1 (SREBP-1) is required to regulate glycogen synthesis and gluconeogenic gene expression in mouse liver. J Biol Chem. 2014;289(9):5510-7. https://doi.org/10.1074/jbc.M113.541110

[41] Li C, Yang W, Zhang J, Zheng X, Yao Y, Tu K, et al. SREBP-1 has a prognostic role and contributes to invasion and metastasis in human hepatocellular carcinoma. Int J Mol Sci. 2014;15(5):7124-38. https://doi.org/10.3390/ijms15057124

[42] Jensen-Urstad AP, Semenkovich CF. Fatty acid synthase and liver triglyceride metabolism: housekeeper or messenger?. Biochimica et biophysica acta. 2012;1821(5):747–53. https://doi.org/10.1016/j.bbalip.2011.09.017

[43] Paton CM, Ntambi JM. Biochemical and physiological function of stearoyl-CoA desaturase. Am J Physiol Endocrinol Metab. 2009;297(1):28-37. https://doi.org/10.1152/ajpendo.90897.2008

[44] Lounis MA, Bergeron KF, Burhans MS, Ntambi JM, Mounier C. Oleate activates SREBP-1 signaling activity in SCD1-deficient hepatocytes. Am J Physiol Endocrinol Metab. 2017;313(6):710-20. https://doi.org/10.1152/ajpendo.00151.2017

[45] Chen CL, Lin YC. Autophagy Dysregulation in Metabolic Associated Fatty Liver Disease: A New Therapeutic Target. Int J Mol Sci. 2022;23(17). https://doi.org/10.3390/ijms231710055

[46] Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, et al. Autophagy regulates lipid metabolism. Nature. 2009;458(7242):1131-5. https://doi.org/10.1038/nature07976

[47] Wang H, Sun RQ, Zeng XY, Zhou X, Li S, Jo E, et al. Restoration of autophagy alleviates hepatic ER stress and impaired insulin signalling transduction in high fructose-fed male mice. Endocrinology. 2015;156(1):169-81. https://doi.org/10.1210/en.2014-1454

[48] Gonzalez-Rodriguez A, Mayoral R, Agra N, Valdecantos MP, Pardo V, Miquilena-Colina ME, et al. Impaired autophagic flux is associated with increased endoplasmic reticulum stress during the development of NAFLD. Cell Death Dis. 2014;5(4):e1179. https://doi.org/10.1038/cddis.2014.162

[49] Fukuo Y, Yamashina S, Sonoue H, Arakawa A, Nakadera E, Aoyama T, et al. Abnormality of autophagic function and cathepsin expression in the liver from patients with non-alcoholic fatty liver disease. Hepatol Res. 2014;44(9):1026-36. https://doi.org/10.1111/hepr.12282

[50] Liu J, Jin X, Yu CH, Chen SH, Li WP, Li YM. Endoplasmic reticulum stress involved in the course of lipogenesis in fatty acids-induced hepatic steatosis. J Gastroenterol Hepatol. 2010;25(3):613-8. https://doi.org/10.1111/j.1440-1746.2009.06086.x

[51] Sozen E, Demırel-yalcıner T, Demır DD, Oznacar B, Ozer NK. SREBP1c silencing reduces endoplasmic reticulum stress and related apoptosis in oleic acid induced lipid accumulation. Marmara Medical Journal. 2021;34(3):241-7.

How to cite this article: Mese Tayfur S, Isot I, Cetinkaya Bengü, Demirel Yalciner T, Kartal Ozer N, Sozen E. SREBP-1c Deficiency Attenuates Fructose-Induced Lipid Droplet Accumulation. Clin Exp Health Sci 2025; 15: 210-216. DOI: 10.33808/clinexphealthsci.1603452

Clinical and Experimental Health Sciences

Cross-sectional Analysis of Health Quality, Treatment Satisfaction and Adherence in Children with Food Allergy

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Received: November 23, 2024 **Accepted:** February 19, 2025

ABSTRACT

Objective: Food allergies (FA) significantly affect the quality of life (QoL) in children and their families. Management involves strict allergen avoidance, which can result in high emotional distress and an increased disease burden.

Objective: To evaluate treatment adherence, satisfaction, and disease-specific health-related quality of life (HRQL) in children aged 0-12 years with FA and their parents.

Methods: A cross-sectional study was conducted with 100 children diagnosed with FA by a physician who required allergen avoidance. Parents completed the Food Allergy Quality of Life Questionnaire Parent Form (FAQLQ-PF), Food Allergy Parent Questionnaire (FAPQ), Treatment Satisfaction Questionnaire (TSQM-9), and Modified Morisky Scale (MMS). Demographic and clinical data were obtained.

Results: The median total score for FAQLQ-PF was 39 (IQR 26-54), indicating moderate impairment in QoL. Emotional impact and social/dietary limitations were notably higher in children older than five years and those with multiple FA. The median FAPQ score was 38 (IQR 29-49), reflecting moderate parental stress and anxiety. Treatment satisfaction (TSQM-9) had a median score of 57 (IQR 41-71), with higher scores for parents of children with multisystem involvement. Treatment adherence (MMS) had a median score of 83 (IQR 67-100), indicating good overall adherence. Significant correlations were observed between higher parental anxiety and lower QoL.

Conclusion: Children with FA and their parents experience significant HRQL impairment, stress, and varying levels of satisfaction with treatment. These results underscore the necessity for comprehensive management strategies that encompass psychosocial support and personalized interventions to enhance outcomes for families dealing with FA.

Keywords: Food Allergies, Health Related Quality of Life, Treatment Adherence, Treatment Satisfaction.

1. INTRODUCTION

Food allergy (FA) is a chronic condition that has become increasingly common worldwide, significantly affecting quality of life (QoL) (1). Recent data suggest a substantial increase in the global prevalence of FA, with estimates reaching up to 10% among children in high-income countries (2, 3). Studies indicate that FA prevalence has been increasing by approximately 1-2% per decade in some areas, driven by environmental and genetic factors (2). This growing prevalence highlights the critical need to better understand the factors influencing the quality of life (QoL), and treatment outcomes in affected populations (4). The condition is most commonly triggered by foods such as milk, eggs, wheat, soy, peanuts, tree nuts, fish, and shellfish (5). In Turkey milk and eggs are the predominant allergens (6). The management of FA typically involves strict allergen avoidance and emergency preparedness. However, the chronic nature of the disease, coupled with difficulties in avoiding allergens and the risk of accidental exposure, places a significant burden on both patients and their families (7).

This burden is reflected in the psychological distress and increased disease burden experienced by patients and parents, which can adversely affect their health-related quality of life (HRQL) (8). Existing studies highlight that stress and anxiety associated with the constant need to avoid allergens and the risk of severe reactions contribute to a significant decline in HRQL (1, 7). Moreover, the QoL of children with FA is reported to be significantly impaired compared to healthy children (9) and those with other chronic conditions such as type 1 diabetes mellitus (10, 11) and juvenile rheumatoid arthritis (12). Despite this, there is a scarcity of studies that evaluate parent-reported compliance, treatment satisfaction, and the HRQL of families managing FA.

The current study aimed to assess treatment adherence, treatment satisfaction, and disease-specific HRQoL in children with FA aged 0-12 years and their parents, as well as the factors affecting these outcomes. By addressing these key areas, we hope to provide valuable insights into the management of FA and its impact on affected families.

https://doi.org/10.33808/clinexphealthsci.1589757

2. METHODS

This current cross-sectional study enrolled 100 patients aged 0-12 years with FA who required allergen food avoidance between January 2022 and June 2022. Clinical and demographic information was obtained from medical records. Additionally, parents were asked to complete questionnaires assessing disease-specific HRQL using the Food Allergy Quality of Life Questionnaire Parent Form (FAQLQ-PF), Food Allergy Parent Questionnaire (FAPQ), and Treatment Satisfaction Scale (TSQM-9) for treatment satisfaction and the Modified Morisky Scale (MMS) for treatment adherence, either in person or via telephone interviews.

Ethics committee approval of the study taken on 04.03.2022 (protocol number 09.2022.389). Each patient's parents provided written informed consent to participate in this research.

2.1. Study Population

The study population was selected from patients aged 0-12 years who had physician-diagnosed FA according to the guidelines (5, 13), required allergen avoidance, and were admitted to or being followed at the tertiary Pediatric Allergy and Immunology Unit at a university hospital The minimum sample size was initially calculated as 67 with a significance level (α) of 5% and a power of 95% using the G*Power 3.1 software system. To enhance reliability, 100 patients were ultimately included in this study. Patients without a definitive diagnosis of FA, those who were not on an allergen avoidance diet by the time of questionnaires, or those who required food elimination due to another condition were excluded from the study.

2.2. Demographic and clinical data

Demographic and clinical characteristics of the patients, including biological sex, current age, age at onset, age at diagnosis, follow-up period (<1 year, >1 year), type of FA (IgE-mediated, non-IgE-mediated, or mixed), food allergen(s) avoided (multiple, single), number of systems/organs involved (multiple, single), history of anaphylaxis, and outcome (full, partial, null tolerance) were obtained from medical records. The demographic characteristics of the parents were recorded as those of the mother or father of the patient.

2.3. HRQL Questionnaires

Mothers and/or fathers as parents, were asked to respond to two specific questionnaires, FAQLQ-PF (14) was validated and has been used in the Turkish population [13]. On the other hand, the FAPQ questionnaires were translated into Turkish by a bilingual medical professional fluent in English and Turkish, and then independently translated back into English by another bilingual medical professional, which was confirmed for their concordance. To ensure that the questionnaire was comprehensive for Turkish participants, it was administered to five non-medical volunteers and then to the parents of fivee children with FA who were followed in our clinic. Preliminary validation of the HRQL questionnaires was checked prior to the questionnaires being distributed to the parents of all participants.

The FAQLQ-PF, an FA-specific scale, assesses the child's HRQL from the family's perspective. It is a questionnaire administered to families of children with FA aged 0-12 years to evaluate the patient's QoL. The FAQLQ-PF consists of three subgroups: emotional impact (13 items), food-related anxiety (8 items), and social and dietary limitations (9 items). Parents were asked a number of questions that varied depending on age: 12 questions were asked for children under the age of 3, 26 questions for children between the ages of 3-8, and 30 questions for children over the age of 8. The FAQLQ-PF total score and three subdomains (emotional impact, food-related anxiety, and social and dietary limitations) were converted into percentages. (Formula used: (Patient's score – lowest possible score) / (highest possible score – lowest score)*100) The average of the 3 subdomains was determined as the total score. In this questionnaire, higher scores indicated worse HRQL, whereas lower scores reflected better HRQL for the patient. The FAPQ was developed to assess parents' adaptation to their children's FA and how they cope with the condition. Family concerns, coping skills and support levels were evaluated. The survey consisted of 18 questions without any sub-domains (15). FAPQ scores were converted into percentages. (Formula used: (patient's score – lowest possible score) / (highest possible score – lowest score)*100), which resulted in a similar pattern: a high score indicating negative and a low score for positive scale for the patient. For the FAPQ, the total score was calculated and divided by the number of questions. For questions 1, 2, 3, 5, 7, 9 in the FAPQ survey, 0 points = 100%, 1 point: 75%, 2 points: 50%, 3 points = 25%, 4 points = 0%, for questions 4, 6, 8, 10-18, 0 points = 0%, 1 point: 25%, 2 points: 50%, 3 points = 75%, 4 points = 100%. The total score percentage was taken as the average of 18 questions in percentage (total % of 18 questions/18). In this questionnaire, higher scores indicated worse HRQL, whereas lower scores reflected better HRQL.

2.4. TSQM Questionnaire for Treatment Satisfaction

Nine questions in the treatment satisfaction questionnaire (TSQM) used for medication treatment, were modified by altering the word medication to allergen avoidance to assess individual treatment satisfaction for elimination diet, where higher scores indicate more satisfaction (16, 17). The TSQM has been validated and used in the Turkish population (18, 19). For the TSQM-9 questionnaire, three subgroups were separately converted into percentages (formula used: (Patient's score – lowest possible score)/(highest possible score – lowest score)*100). The total score percentage was taken as the average of three subdomains: (sum of three subgroups/3). In this questionnaire, higher scores indicate more positive treatment satisfaction.

2.5. MMS Questionnaire for Treatment Adherence

The Modified Morisky Scale is a commonly used questionnaire for medication adherence in chronic illnesses and consists of six questions. The MMS has also been validated and used in the Turkish population (20). The questions were modified in

line with elimination diet adherence instead of medication. For questions 1, 3, 4, and 6 in the MMS questionnaire, yes = 0%, no = 100%; for questions 2 and 5, yes = 100%, no = 0%. Total score is the average of the percentage of six questions (sum of the % value of six questions/6). In this questionnaire, higher scores indicate more positive treatment adherence. For MMS, the total score of the questions was calculated and divided by the number of questions.

2.6. Statistical Analyses

After data collection, analyses were performed using the SPSS software (version 19, SPSS, Inc. Chicago, IL, USA) and Jamovi (version 2.3.26, The Jamovi Project, Australia). Graphs and figures were generated using of GraphPad Prism (version 9.5.1, for MacOS, GraphPad Software, Boston, Massachusetts USA) and Adobe Illustrator 2023 (version 27.9.0, for MacOS, Adobe Inc., California, USA). Continuous variables between groups were not normally distributed, and are presented as median and IQR 25-75%. Groups were compared using the Mann-Whitney U test, and a p value <0.05 was considered significant. For the subgroup analysis, the results of the questionnaires were compared based on parents and patients' sex, current age of patients (≤5 years, >5 years), age at symptom onset (≤6 months, >6 months), follow-up duration (≤1 year, >1 year), food allergen avoidance (single, multiple), presence of anaphylaxis, number of clinically affected systems (single or multiple), and outcome (full, partial, null tolerance).

Spearman correlation analysis was used to evaluate the relationship between questionnaire scores and nominal and continuous predictors, as well as the correlations among different questionnaires. Differences were considered significant at $p < .05$, and the strength of the correlation was evaluated according to Spearman's rho value (00-0.19 "very weak," 0.20-0.39 "weak," 0.40-0.59 "moderate," 0. 60-0.79 "strong", 0 .80-1.0 "very strong'')

3. RESULTS

3.1. Patient Characteristics

The study included 100 patients, with a majority being male (59.0%, n=59) and a median age of 18.6 months (IQR 11.5-44.3). Most patients (86.0%, n=86) were diagnosed after six months of age, with a median onset age of 3.7 months (IQR 1.3-6). They were followed for an average of 18.6 months (IQR 11.5-43.3), with over half (65.0%, n=65) followed-up for more than a year.

The patients' immunologic FA phenotypes included mixed-type (61.0%, n=61), IgE-mediated (21.0%, n=21), and non-IgE-mediated (18.0%, n=18). Atopic dermatitis affected 65.0% (n=65) of patients, with urticaria (43.0%, n=43), proctocolitis (28.0%, n=28), and anaphylaxis (12.0%, n=12). Multisystemic involvement was seen in 63.0% (n=63) cases, with the skin being the most affected system (90.0%, n=90). Commonly avoided foods were hen's eggs (73.0%, n=73) and cow's milk (55.0%, n=55), with only 13.0% (n=13) achieving full tolerance.

The demographic and clinical characteristics of the patients included in the study are shown in Table 1 and Figure 1A.

3.2. HRQL, Treatment Satisfaction and Adherence Questionnaires

The FAQLQ-PF and FAPQ were used to assess HRQL, while the TSQM-9 and MMS were used to evaluate treatment satisfaction and compliance, respectively. These questionnaires were administered to the mothers of 78 patients, fathers of 32 patients, and both parents of 7 patients (Table 1).

Table 1. *Characteristics of children with food allergies and parent's questionnaires (n=100).*

Current age (mo) median and IQR 25-75%	18.6 (11.5-43.3)
Age at onset (mo) median and IQR 25-75%	3.7 (1.3-6)
Follow-up duration (mo) median and IQR 25-75%	7.5 (3.4-21.5)
Questionnaires replied by n (%)	
Mothers	78 (78)
Fathers	32 (32)
Both Parents	7 (7)
FAQLQ-PF Median and IQR 25-75%	40 (26-54)
FAQLQ-PF-emotional	50 (33-65)
FAQLQ-PF-anxiety	25 (0-50)
FAQLQ-PF-social dietary limitations	43 (20-60)
FAPQ Median and IQR 25-75%	38 (29-49)
TSQM-9 Median and IQR 25-75%	57 (41-71)
TSQM-9 effectivenes	67 (50-100)
TSQM-9 convienence	17 (0-60)
TSQM-9 global satisfaction	75 (57-93)
MMS Median and IQR 25-75%	83 (67-100)

n: number, mo: months-old, IQR: Interquartile Range, FAQLQ-PF: Food Allergy Quality of Life Questionnaire Parent Form, FAPQ: Food Allergy Parent Questionnaire,TSQM-9: Treatment Satisfaction Questionnaire a Medication, MMS: Modified Morinsky Score

The median FAQLQ-PF total score was 39 (IQR 26-54). The median overall scores on the FAQLQ-PF subscales evaluating emotional, anxiety and social dietary limitations were 50 (IQR 33-65), 25 (IQR 0-50), and 43 (IQR 20-60), respectively (Table 1).

When comparing FAQLQ-PF scores based on patients' clinical characteristics, parents of children> 5 years of age at the time of evaluation and those with multiple FA reported higher total scores compared to parents of younger children and those with a single FA (p=.043 and p=.007, respectively). The anxiety subscale scores were also higher for children aged > 5 years, those under observation for > 1 year, and those with a history of anaphylaxis (p<.001, p=.001 and p=.026, respectively). Social/dietary limitation subscale scores were significantly higher for patients with multiple food allergies, multi-system/organ involvement, and those unable to consume whole allergens (p=.005, p=.018, and p=.021, respectively). Furthermore, comparing the total and subscale scores of the FAQLQ-PF between mothers and fathers who responded to the questionnaire, mothers had notably higher scores on the emotional subscale (p=.021). The FAQLQ-PF total and subscale scores are presented in Table 2 and Figure 1B.

Table 2. *Factors affecting quality of life, treatment satisfaction and adherence in children with food allergy. Data is presented with median and IQR: 25-75%.*

	Current Age		Follow-up Duration		Parent Gender		Food Allergy		Organ(s)/system(s) Involved		Anaphylaxis History		Outcome			
													Whole allergen Consumption		Baked allergen Consumption	
	≤5 yo	>5 yo	≤1 y	>1 y	Father	Mother	Single	Multiple	Single	Multiple	No	Yes	Yes	No	Yes	No
FAQLQ-PF	35 25-51	51* 41-62	36 25-44	39 32-51	31 23-42	43 28-55	29 22-48	46* 30-55	34 24-50	44 28-55	36 25-51	52 33-61	28 7-54	39 27-53	39 26-54	37 25-53
Emotional impact	47 33-63	63 39-69	50 33-63	48 37-67	39 18-56	53* 37-68	40 27-63	53 42-67	47 31-63	53 37-66	49 33-65	51 32-67	42 20-65	50 33-64	46 39-63	53 37-69
Food-related anxiety	17 0-42	56* 36-69	8 0-33	36* 15-67	25 0-42	25 0-50	10 0-45	25 0-50	19 0-43	25 0-50	18 0-43	40* 21-77	0 0-43	25 0-50	27 0-50	9 0-40
Social and dietary limitations	43-22 60	49-20 50	40 20-60	50 24-60	30 20-53	48 23-66	33 13-50	53* 28-66	35 20-59	52* 32-66	42 20-60	56 44-62	23 0-54	47* 23-63	45 21-60	43 23-63
FAPQ	37 27-47	40 31-60	36 25-44	39 32-51	37 33-47	38 27-49	36 26-44	37 27-49	37 26-44	40 29-51	37 27-47	46 34-52	35 21-40	37 29-49	36 29-49	37 27-47
TSQM-9	57 41-71	61 42-70	53 42-71	62 41-71	62 46-74	56 41-70	58 45-72	56 40-69	51 41-69	62 45-75	54 41-71	64 53-69	58 32-85	57 42-69	67 42-76	52 41-63
Effectiveness	67 50-100	78 62-86	67 50-100	78 50-100	75 50-100	67 51-99	75 56-100	67 50-100	67 50-89	89* 67-100	67 50-100	83 49-100	72 50-100	61 33-100	78 56-100	67 50-99
Convenience	17 0-53	42 0-69	17 0-56	17 0-64	39 0-67	14 0-50	25 0-67	17 0-50	22 0-61	14 0-50	17 0-56	22 0-62	25 0-79	17 0-53	22 0-61	14 0-56
Global satisfaction	79 57-93	83 43-100	71 55-93	79 54-100	71 57-86	79 52-100	79 57-93	79 50-100	71 50-86	82 57-100	71 50-93	86 73-100	82 45-100	79 57-93	86 64-100	68* 45-86
MMS	83 67-100	83 62-87	83 62-100	83 67-100	83 50-100	83 67-100	83 67-100	83 67-100	67 50-100	100 83-100	83 67-100	100* 83-100	75 67-100	83 67-100	83 50-100	83 67-100

*yo: years-old, IQR: Interquartile Range, FAQLQ-PF: Food Allergy Quality of Life Questionnaire Parent Form, FAPQ: Food Allergy Parent Questionnaire, TSQM-9: Treatment Satisfaction Questionnaire a Medication, MMS: Modified Morinsky Score, *p<0.05, Mann-Whitney U Test*

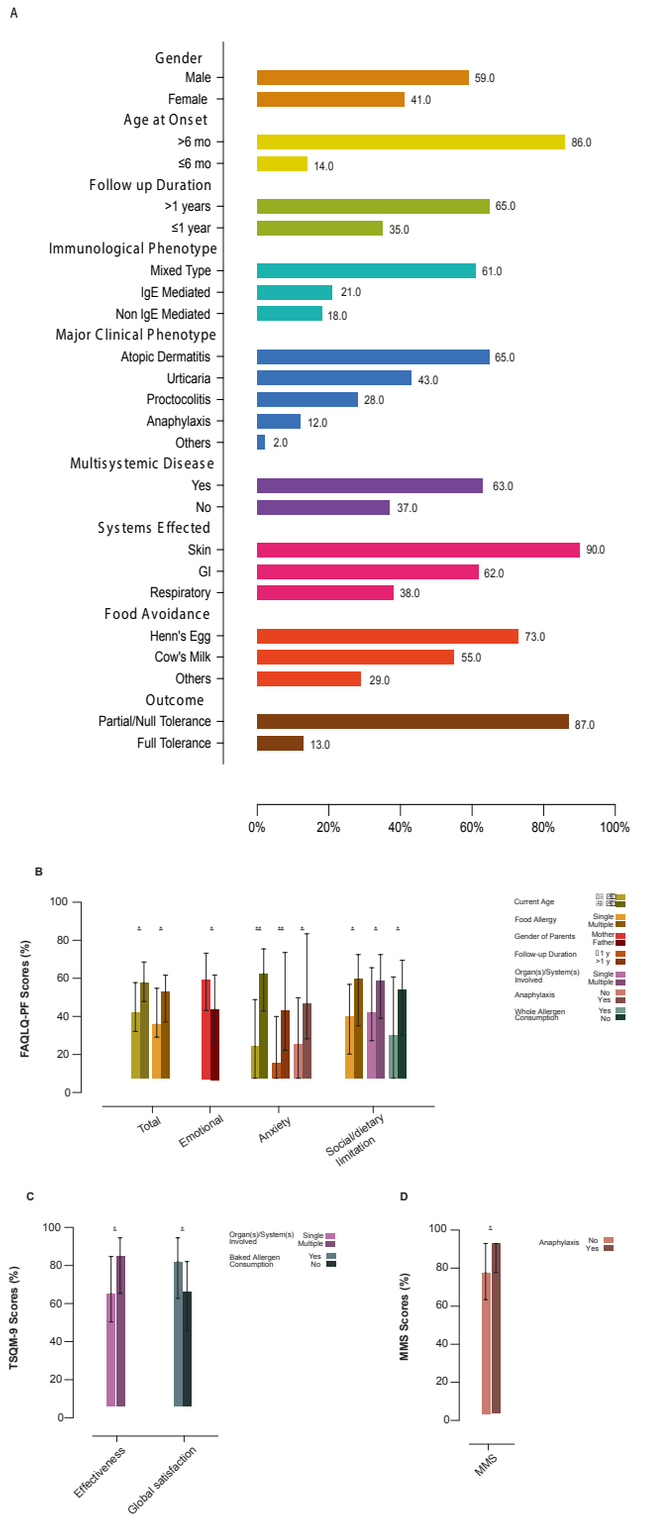
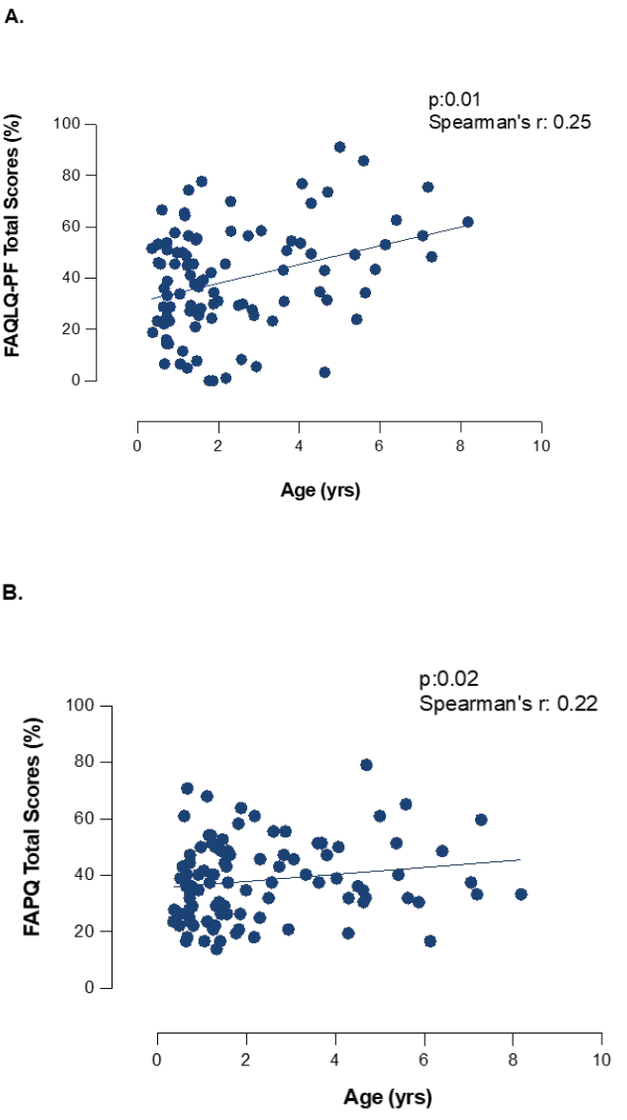


Figure 1. *(A). Clinical characteristics and (B-D). differences between total and subscales scores of the questionnaires according to the clinical characteristics of patients . Data is shown with bars and lines representing percentages and median, IQR 25-75%.p<0.05, Mann-Whitney U Test.*

The median total score of the FAPQ was 38 (IQR 29-49). When the FAPQ scores of the parents were analyzed in relation to the clinical characteristics of the patients and parental sex, no statistically significant disparity was observed (Table 2).

The TSQM-9 questionnaire showed a median total score of 57 (IQR 41-71). The TSQM-9 subscales' effectiveness, convenience, and global satisfaction had median scores of 68 (IQR 50-100), 17 (IQR 0-60), and 75 (IQR 57-93), respectively. When analyzing the TSQM-9 total and subscale scores for patients' clinical characteristics, it was found that higher effectiveness scores were reported by parents of children with multisystem involvement (p=0.011) and lower global satisfaction scores were reported by parents of children unable to consume baked allergens (p=.03) (Table 2, Figure 1C).

The median MMS score was 83 (IQR 67-100), with higher adherence scores among parents of children with a history of anaphylaxis (p=.013) (Table 2, Figure 1D).



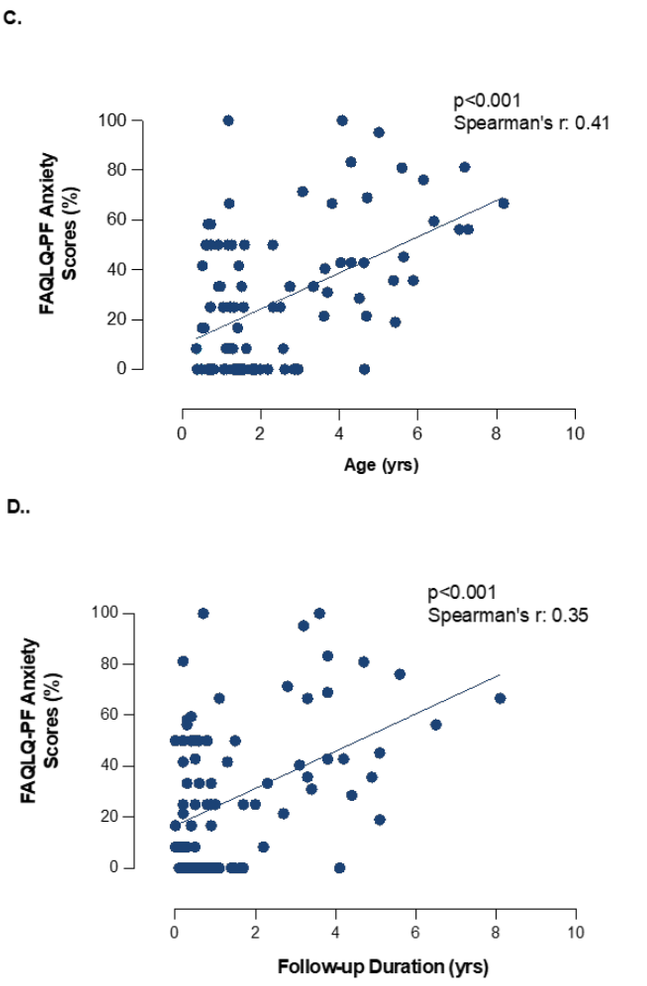


Figure 2. *Scatter plot images demonstrate a correlation between (A) total scores of FAQLQ-PF and (B) FAQLQ questionnaires and the age of patients; (C) anxiety subscale scores of FAQLQ-PF and the age; (D) anxiety subscale scores of FAQLQ-PF and follow-up duration of patients with food allergies.*

3.3. Correlation Analyses of HRQL, Treatment Satisfaction and Adherence Questionnaires' Scores

Patient age revealed a weak correlation between FAQLQ-PF (p=0.01, Spearman's r:0.25) (Figure 2A) and FAPQ total scores (p=0.02, Spearman's r:0.22) (Figure 2B), whereas a moderate correlation was found between FAQLQ-PF anxiety subscale scores (p<.001, Spearman's r:0.41) (Figure 2C). Additionally, the FAQLQ-PF anxiety subscale scores demonstrated a weak correlation between follow-up duration. (p<.001, Spearman's r:0.35) (Figure 2D). Furthermore, a weak correlation was identified between FAQLQ-PF social and dietary limitation subscale scores and the number of systems affected (p=0.024, Spearman's r:0.22).

There was a moderate correlation between the FAQLQ-PF and FAPQ total scores (p<.001, Spearman's r: 0.53). The TSQM total scores were weakly negatively correlated with FAQLQ-PF and FAPQ total scores (p=.006, Spearman's r:-0.27; p=.001, Spearman's r:-0.32, respectively). The scatter plots that depict the correlation among the questionnaire scores are presented in Figure 3A-C.

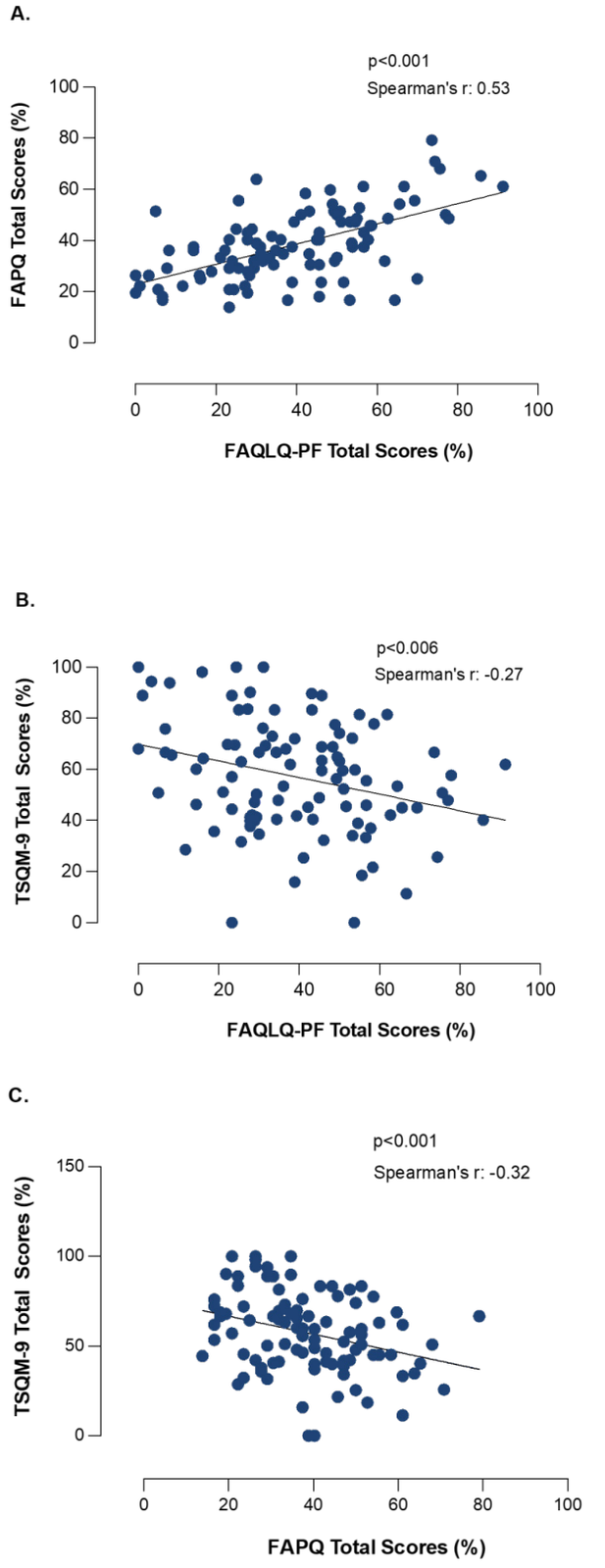


Figure 3. *Scatter plot images demonstrate correlation between total scores of; (A) FAQLQ-PF and FAPQ questionnaries, (B) FAQLQ and TSQM-9 questionnaries, (C) FAPQ and TSQM-9 questionnaries.*

4. DISCUSSION

The current investigation examined HRQL, adherence to allergen avoidance, and treatment satisfaction among a pediatric cohort of patients diagnosed with FA, who were referred to a tertiary care center for ongoing monitoring and management. The majority of patients exhibited a mixed-type FA phenotype starting earlier than 6 months, lasting more than 1 year and were characterized by AD, along with frequent multisystemic involvement. Notably, avoidance of egg and cow's milk was prevalent among patients, with only a small fraction achieving complete tolerance to these foods. The data obtained from our cohort are highly valuable in this respect, as the study encompasses a group of patients with severe FA who have been referred to an expert opinion for further follow-up and evaluated for the impact of allergen avoidance on HRQL, treatment satisfaction, and adherence. The clinical conditions that could influence these outcomes were also analyzed. Based on these data, a strategic plan for nutrition, education, and psychological counselling should be provided to children with FA and their families during follow-up.

In our study population, we found that children with multiple FA and those older than 5 years experienced a significant decline in their HRQL scores. In contrast, another study by Morou et al. demonstrated that the severity of the allergy and a history of anaphylaxis significantly influenced HRQL(21). Specifically, among the subdomains of HRQL, the emotional subdomain of the FAQLQ-PF was particularly impaired in mothers compared to fathers. As noted by Gupta et al., and Warren et al. managing a child's FA often leads to marital strain and emotional burden, particularly for mothers (22, 23). Walkner et al. and Springston et al. reported that FA leads to heightened parental vigilance and stress, affecting family dynamics and social interactions (24, 25). A systematic review by Cheon et al. emphasized the effectiveness of educational interventions in improving the HRQL of children with FA and their parents (26). Despite the heterogeneity and limited number of studies, the analysis highlighted that support and educational materials significantly contributed to better HRQL outcomes.

The anxiety subdomain of HRQL exhibited higher scores, indicating poorer outcomes for FA patients older than five years, those with a follow-up duration exceeding one year, and those with multisystemic involvement in our study group. Additionally, social and dietary limitations were found to be influenced by factors such as multiple FA, a history of anaphylaxis, and an inability to develop full tolerance to allergens. Consistent with our findings, Le Bovidge et al. and Shaker et al. also reported elevated parental anxiety and psychosocial impact in patients with FA (15, 27). Moreover, Shemesh et al. and Lieberman et al. found that children with FA often face bullying and social isolation, further exacerbating their stress and anxiety levels (28, 29). In such a condition, routine daily activities such as grocery shopping, meal preparation, and dining out, may pose significant limitations and additional emotional stress for parents. Tailored interventions addressing these factors individually for patients with FA and their families may lead to improvements in HRQL. Furthermore, examining specific subdomains of HRQL can guide physicians in providing targeted support to enhance their overall HRQL experience.

The participants in the present study illustrated that as the age and follow-up duration of patient with FA increased, their HRQL declined. Additionally, decreased HRQL correlated with impaired treatment satisfaction. As reported by other studies, a notable finding is the strong correlation between parental anxiety and children's QoL scores. Parents' perceptions of their children's allergy severity significantly influenced their anxiety levels and overall family QoL (22, 23). The Chinese FAQL-PB used by Leung et al. also supports the robustness of these findings across different cultures (30). It is apparent that enduring and severe food allergies adversely affect various aspects of HRQL, particularly exacerbating anxiety and imposing social and dietary restrictions. Over the course of follow-up for FA, it is essential to develop effective coping mechanisms and life skills to manage this chronic condition for both pediatric patients and their parents, and to incorporate these strategies into therapeutic approaches.

The literature on treatment satisfaction and adherence in FA management is relatively sparse, although these have recently been evaluated as outcomes in interventional research focused on tolerance development in FA (31). A meta-analysis by Cheon et al. highlighted that educational interventions have the potential to improve treatment adherence and satisfaction (26). Additionally, DunnGalvin et al. and LeBovidge et al. reported similar findings on psychosocial burden and the importance of support systems in improving treatment adherence (15, 32). Our study contributes significantly to this area by highlighting that treatment satisfaction is impaired in FA patients with multiple allergies and allergen avoidance and is promoted in those who are able to consume baked. allergens. Our study was constrained by the relatively small sample size of pediatric patients compared to the prevalence of FA in the general population. This limitation arises from our study population comprising referred severe or persistent food allergy patients from second – or third-level healthcare facilities, thereby restricting the generalizability of our data, which can be attributed to the strength of the current data to recommend the routine evaluation of the HRQL, treatment satisfaction, and adherence during follow-up in such FA patients to improve the tailored management strategies for each patient.

5. CONCLUSION

this study underlines the importance of integrating HRQL assessments, treatment adherence evaluations, and satisfaction surveys into comprehensive management strategies for FA, targeting both patients and parents. These outcomes and their subdomains are closely linked to clinical parameters associated with the severe and persistent phenotypes of FA.

Funding: *This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors*

Conflicts of interest: *Authors have no conflict of interest to disclose.*

Ethics Committee Approval: *The study was approved by the Ethics Committee of Marmara University, School of Medicine. (Date: 04.03.2022 and protocol number 09.2022.389)*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: EKA, APS, OG

Design of the study: EKA, APS, OG

Acquisition of data for the study: EKA, APS, OG, BU, MYA, EYG, RB, SBE, SB, AO

Analysis of data for the study: EKA, APS, MYA, EYG

Interpretation of data for the study: EKA, APS, MYA, EYG

Drafting the manuscript: EKA, APS

Revising it critically for important intellectual content: EKA, APS, SBE, SB, AO

Final approval of the version to be published: EKA, APS, SBE, SB, AO

REFERANCES

[1] Antolin-Amerigo D, Manso L, Caminati M, de la Hoz Caballer B, Cerecedo I, Muriel A, Rodriguez-Rodriguez M, Barbarroja-Escudero J, Sanchez-Gonzalez MJ, Huertas-Barbudo B, Alvarez-Mon M. Quality of life in patients with food allergy. Clin Mol Allergy. https://doi.org/2016;14:4. 10.1186/s12948.016.0041-4.

[2] Sampath V, Abrams EM, Adlou B, Akdis C, Akdis M, Brough HA, Chan S, Chatchatee P, Chinthrajah RS, Cocco RR, Deschildre A, Eigenmann P, Galvan C, Gupta R, Hossny E, Koplin JJ, Lack G, Levin M, Shek LP, Makela M, Mendoza-Hernandez D, Muraro A, Papadopoulous NG, Pawankar R, Perrett KP, Roberts G, Sackesen C, Sampson H, Tang MLK, Togias A, Venter C, Warren CM, Wheatley LM, Wong GWK, Beyer K, Nadeau KC, Renz H. Food allergy across the globe. J Allergy Clin Immunol. 2021;148(6):1347-1364. https://doi.org/10.1016/j.jaci.2021.10.018.

[3] Warren CM, Turner PJ, Chinthrajah RS, Gupta RS. Advancing Food Allergy Through Epidemiology: Understanding and Addressing Disparities in Food Allergy Management and Outcomes. J Allergy Clin Immunol Pract. 2021;9(1):110-118. https://doi.org/10.1016/j.jaip.2020.09.064.

[4] Protudjer JL, Jansson SA, Ostblom E, Arnlind MH, Bengtsson U, Dahlen SE, Kallstrom-Bengtsson I, Marklund B, Middelveld RJ, Rentzos G, Sundqvist AC, Akerstrom J, Ahlstedt S. Health-related quality of life in children with objectively diagnosed staple food allergy assessed with a disease-specific questionnaire. Acta Paediatr. 2015;104(10):1047-1054. https://doi.org/10.1111/apa.13044.

[5] Allergy TNGoF. Turkish National Guadline of Food Allergy. Asthma Allergy Immunology. 2017;15.

[6] Aksoy AG, Boran P, Karakoc-Aydiner E, Gokcay G, Tamay ZU, Devecioglu E, Baris S, Ozen A. Prevalence of allergic disorders and risk factors associated with food allergy in Turkish preschoolers. Allergol Immunopathol (Madr). 2021;49(1):11-6. https://doi.org/10.15586/aei.v49i1.23.

[7] Cummings AJ, Knibb RC, King RM, Lucas JS. The psychosocial impact of food allergy and food hypersensitivity in children, adolescents and their families: a review. Allergy. 2010;65(8):933-945. https://doi.org/10.1111/j.1398-9995.2010.02342.x.

[8] Knibb R, Halsey M, James P, du Toit G, Young J. Psychological services for food allergy: The unmet need for patients and families in the United Kingdom. Clin Exp Allergy. 2019;49(11):1390-1394. https://doi.org/10.1111/cea.13488.

[9] Cummings AJ, Knibb RC, Erlewyn-Lajeunesse M, King RM, Roberts G, Lucas JS. Management of nut allergy influences quality of life and anxiety in children and their mothers. Pediatr Allergy Immunol. 2010;21(4 Pt 1):586-594. https://doi.org/10.1111/j.1399-3038.2009.00975.x.

[10] Avery NJ, King RM, Knight S, Hourihane JO. Assessment of quality of life in children with peanut allergy. Pediatr Allergy Immunol. 2003;14(5):378-382. https://doi.org/10.1034/j.1399-3038.2003.00072.x.

[11] Flokstra-de Blok BM, van der Velde JL, Vlieg-Boerstra BJ, Oude Elberink JN, DunnGalvin A, Hourihane JO, Duiverman EJ, Dubois AE. Health-related quality of life of food allergic patients measured with generic and disease-specific questionnaires. Allergy 2010;65(8):1031-1038. https://doi.org/10.1111/j.1398-9995.2009.02304.x.

[12] Primeau MN, Kagan R, Joseph L, Lim H, Dufresne C, Duffy C, Prhcal D, Clarke A. The psychological burden of peanut allergy as perceived by adults with peanut allergy and the parents of peanut-allergic children. Clin Exp Allergy 2000;30(8):1135-1143. https://doi.org/10.1046/j.1365-2222.2000.00889.x.

[13] Santos AF, Riggioni C, Agache I, Akdis CA, Akdis M, Alvarez-Perea A, Alvaro-Lozano M, Ballmer-Weber B, Barni S, Beyer K, Bindslev-Jensen C, Brough HA, Buyuktiryaki B, Chu D, Del Giacco S, Dunn-Galvin A, Eberlein B, Ebisawa M, Eigenmann P, Eiwegger T, Feeney M, Fernandez-Rivas M, Fisher HR, Fleischer DM, Giovannini M, Gray C, Hoffmann-Sommergruber K, Halken S, Hourihane JO, Jones CJ, Jutel M, Knol E, Konstantinou GN, Lack G, Lau S, Marques Mejias A, Marchisotto MJ, Meyer R, Mortz CG, Moya B, Muraro A, Nilsson C, Lopes de Oliveira LC, O'Mahony L, Papadopoulos NG, Perrett K, Peters RL, Podesta M, Poulsen LK, Roberts G, Sampson HA, Schwarze J, Smith P, Tham EH, Untersmayr E, Van Ree R, Venter C, Vickery BP, Vlieg-Boerstra B, Werfel T, Worm M, Du Toit G, Skypala I. EAACI guidelines on the diagnosis of IgE-mediated food allergy. Allergy 2023;78(12):3057-3076. https://doi.org/10.1111/all.15902.

[14] DunnGalvin A, de BlokFlokstra BM, Burks AW, Dubois AE, Hourihane JO. Food allergy QoL questionnaire for children aged 0-12 years: content, construct, and cross-cultural validity. Clin Exp Allergy 2008;38(6):977-986. https://doi.org/10.1111/j.1365-2222.2008.02978.x.

[15] Lebovidge JS, Stone KD, Twarog FJ, Raiselis SW, Kalish LA, Bailey EP, Schneider LC. Development of a preliminary questionnaire to assess parental response to children's food allergies. Ann Allergy Asthma Immunol. 2006;96(3):472-477. https://doi.org/10.1016/S1081-1206(10)60916-7.

[16] Atkinson MJ, Sinha A, Hass SL, Colman SS, Kumar RN, Brod M, Rowland CR. Validation of a general measure of treatment satisfaction, the Treatment Satisfaction Questionnaire for Medication (TSQM), using a national panel study of chronic disease. Health Qual Life Outcomes 2004;2:12. https://doi.org/10.1186/1477-7525-2-12.

[17] Atkinson MJ, Kumar R, Cappelleri JC, Hass SL. Hierarchical construct validity of the treatment satisfaction questionnaire for medication (TSQM version II) among outpatient pharmacy consumers. Value Health 2005;8 Suppl 1:S9-S24. https://doi.org/10.1111/j.1524-4733.2005.00066.x.

[18] Eltan SB, Keskin O, Deveci MF. Safety, efficiency, and treatment satisfaction in children with primary immunodeficiency receiving subcutaneous immunoglobulin treatment. North Clin Istanb. 2022;9(3):228-34. https://doi.org/10.14744/nci.2020.16870.

[19] Kaplan MD, Kasnakoüğlu BT, Yigitbasi T, Kaplan YC. Evaluation of satisfaction with over-the-counter weight loss supplements. Journal of Medical Marketing: Device, Diagnostic and Pharmaceutical Marketing. 2013;13(2):68-73. https://doi.org/10.1177/174.579.0413480521.

[20] Bekir Vural ÖrTA, Pınar Topsever, Müge Filiz. Reliability And Validity of Turkish Version of Modified Morisky Scale. Turkish Family Physician. 2012;3/4.

[21] Morou Z, Vassilopoulou E, Galanis P, Tatsioni A, Papadopoulos NG, Dimoliatis IDK. Investigation of quality of life determinants in children with food allergies. Int Arch Allergy Immunol. 2021;182(11):1058-1065. https://doi.org/10.1159/000516875.

[22] Gupta RS, Warren CM, Smith BM, Blumenstock JA, Jiang J, Davis MM, Nadeau KC. The public health ımpact of parent-reported childhood food allergies in the United States. Pediatrics 2018;142(6):e201811235. https://doi.org/10.1542/peds.2018-1235.

[23] Warren CM, Gupta RS, Sohn MW, Oh EH, Lal N, Garfield CF, Caruso D, Wang X, Pongracic JA. Differences in empowerment and quality of life among parents of children with food allergy. Ann Allergy Asthma Immunol. 2015;114(2):117-125. https://doi.org/10.1016/j.anai.2014.10.025.

[24] Springston EE, Smith B, Shulruff J, Pongracic J, Holl J, Gupta RS. Variations in quality of life among caregivers of food allergic children. Ann Allergy Asthma Immunol. 2010;105(4):287-294. https://doi.org/10.1016/j.anai.2010.08.003.

[25] Walkner M, Warren C, Gupta RS. Quality of life in food allergy patients and their families. Pediatr Clin North Am. 2015;62(6):1453-1461. https://doi.org/10.1016/j.pcl.2015.07.003.

[26] Cheon J, Cho CM, Kim HJ, Kim DH. Effectiveness of educational interventions for quality of life of parents and children with food allergy: A systematic review. Medicine (Baltimore). 2022;101(36):e30404. https://doi.org/10.1097/MD.000.000.0000030404.

[27] Shaker MS, Schwartz J, Ferguson M. An update on the impact of food allergy on anxiety and quality of life. Curr Opin Pediatr. 2017;29(4):497-502. https://doi.org/10.1097/MOP.000.000.0000000509.

[28] Shemesh E, Annunziato RA, Ambrose MA, Ravid NL, Mullarkey C, Rubes M, Chuang K, Sicherer M, Sicherer SH. Child and parental reports of bullying in a consecutive sample of children with food allergy. Pediatrics. 2013;131(1):e10-7. https://doi.org/10.1542/peds.2012-1180.

[29] Lieberman JA, Weiss C, Furlong TJ, Sicherer M, Sicherer SH. Bullying among pediatric patients with food allergy. Ann Allergy Asthma Immunol. 2010;105(4):282-286. https://doi.org/10.1016/j.anai.2010.07.011.

[30] Leung TF, Yung E, Wong YS, Lam CW, Wong GW. Parent-reported adverse food reactions in Hong Kong Chinese pre-schoolers: epidemiology, clinical spectrum and risk factors. Pediatr Allergy Immunol. 2009;20(4):339-346. https://doi.org/10.1111/j.1399-3038.2008.00801.x.

[31] Demidova A, Drewitz KP, Kimkool P, Banjanin N, Barzylovich V, Botjes E, Capper I, Castor MAR, Comberiati P, Cook EE, Costa J, Chu DK, Epstein MM, Galvin AD, Giovannini M, Girard F, Golding MA, Greenhawt M, Ierodiakonou D, Jones CJ, Khaleva E, Knibb RC, Macit-Celebi MS, Mack DP, Mafra I, Marchisotto MJ, Mijakoski D, Nekliudov N, Ozdemir C, Patel N, Pazukhina E, Protudjer JLP, Rodriguez Del Rio P, Roomet J, Sammut P, Schoos AM, Schopfer AF, Schultz F, Seylanova N, Skypala I, Sorensen M, Stoleski S, Stylianou E, Upton J, van de Veen W, Genuneit J, Boyle RJ, Apfelbacher C, Munblit D, Consortium C. Core Outcome Set for IgE-mediated food allergy clinical trials and observational studies of interventions: International Delphi consensus study 'COMFA'. Allergy. 2024;79(4):977-989. https://doi.org/10.1111/all.16023.

[32] DunnGalvin A, Treneva M, Pampura A, Grebenko A, Makatsori M, Munblit D. Quality of life associated with maternal anxiety disorder in Russian children and adolescents with food allergy. Pediatr Allergy Immunol. 2020;31(1):78-84. https://doi.org/10.1111/pai.13130.

How to cite this article: Günal Ö, Sefer PA, Utuk B, Yorgun Altunbaş M, Güngören Yalçın E, Babayeva R, Eltan Bilgiç S, Barış S, Özen OA, Aydıner Karakoç E. Cross-sectional Analysis of Health Quality, Treatment Satisfaction and Adherence in Children with Food Allergy. Clin Exp Health Sci 2025; 15: 217-225. DOI: 10.33808/clinexphealthsci.1589757

Clinical and Experimental Health Sciences

Effect of Hypochlorous Acid on Acrylic Resins in Preventing Cross-infection Between Clinic and Laboratory

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Received: September 03, 2024 **Accepted:** March 10, 2025

ABSTRACT

Objective: The COVID-19 pandemic and monkeypox disease have highlighted the importance of preventing cross-infection between dental laboratories and clinics. Thus, the aim of this study was to evaluate the effects of hypochlorous acid (HOCL), a natural disinfectant that is effective on bacteria and viruses, on polymethylmetacrylate (PMMA) to prevent cross-infection.

Methods: A total of 120 PMMA (60 heat-activated and 60 three dimensionally printed) samples were prepared. The samples were immersed in HOCL and ortophtalaldehyde (OPA) disinfectant twice for 30 minutes to simulate the travel time to clinic and the dental laboratory. 10 samples from each group were kept in deionized water as a control group. Flexural strength and surface roughness measurements of the samples were performed. T-test and Kruskal Wallis H test were used for statistical evaluation (α=.05).

Results: There was no significant difference in flexural strength or Ra values between the HOCL and OPA groups.

Conclusion: HOCL PMMA may be considered an alternative disinfectant for preventing cross-infection between dental laboratories and clinics in dentures and orthodontic appliances. Further research is required to evaluate its biological efficacy and long-term use.

Keywords: *Acrylic resin, COVID-19, Disinfectants, Hypochlorous acid, Infection control*

1. INTRODUCTION

Removable prostheses and orthodontic appliances will be sent from the clinic to the laboratory and back several times during the period from taking impressions to being applied to the patient. During this process, they may be contaminated with blood and saliva in the clinic, and with equipment such as felt, disks, pumice or dirty hands in the laboratory. (1) Therefore, it is important for patients, physicians, dental assistants and dental technicians to prevent cross-infection between dental clinics and dental laboratories.

Polymethyl methacrylate (PMMA), used in the manufacturing of prostheses and orthodontic appliances, has been widely used in dentistry for many years. The advantages of PMMA resins include ease of application, acceptable aesthetic appearance, physical and mechanical properties, low molecular weight, and a reasonable price. However, they have low thermal conductivity and low resistance to fatigue (2,3)

During the past years, three-dimensional (3D) printed materials have become popular in PMMA production, as well as in dentistry in general. 3D PMMA materials have the advantages of higher accuracy, less material waste, and lower infrastructure costs in less time. The disadvantages of the additive manufacturing process include the inability to use all materials, the staircase effect caused by layer-by-layer production, repeatability, and the need for support structures (4-5)

Various studies have investigated the use of disinfectants like sodium hypochlorite, chlorhexidine, and glutaraldehyde for disinfecting PMMA materials (6,7). However, limited research exists on the effects of orthophthalaldehyde (OPA) and hypochlorous acid (HOCl)—the disinfectants used in this study—on acrylic resin materials (8,9).

Orthophthalaldehyde is a highly effective chemical agent commonly employed for high-level disinfection, especially for medical instruments that cannot undergo heat sterilization (10).

https://doi.org/10.33808/clinexphealthsci.1552492

Hypochlorous acid (HOCl) is a versatile disinfectant renowned for its broad-spectrum microbicidal properties. As a strong oxidant, it is easy to use, cost-effective, and highly effective against a wide range of microorganisms, including bacteria and viruses (11,12). It is listed among the World Health Organization's (WHO) recommended biocides for combating coronaviruses and is also included in the U.S. Environmental Protection Agency's 'N' list of disinfectants effective against emerging pathogens, including SARS-CoV-2 (13,14). Studies have highlighted its utility in diverse applications, such as cleaning implant surfaces and disinfecting dental plaster models (15,16).

The aim of this study was to evaluate the effect of hypochlorous acid and orthophthalaldehyde disinfectants on the fracture resistance and surface roughness of heat-activated polymerization (HA) and 3D printed PMMA resins. The null hypothesis of the study was that short-term use of OPA and HOCL to prevent cross-infection would have no effect on the fracture resistance or surface roughness of PMMA.

2. METHODS

A total of 120 samples and two different disinfectant solutions (HOCL and OPA) were used in this study. For the 3D bending test, 60 samples with dimensions of 65×10×3 mm (in accordance with ADA specification no. 21) were prepared. Of these, 30 samples were made of 3D-printed PMMA, and the remaining 30 were made of HA PMMA. Similarly, for the surface roughness test, another 60 samples with dimensions of 20×20×1.5 mm were prepared, evenly split between 30 samples of HA PMMA and 30 samples of 3D-printed PMMA (17).Each group of 30 samples was further divided into three subgroups: Group I: OPA disinfectant group Group II: HOCL disinfectant group Group III: Control group (Distilled water)

2.1. Fabrication of 3D PMMA Resin Specimens

Specimens for the three-point bending test and surface roughness test were designed and saved as Standard Tessellation Language (STL) files. The 3D-printed specimens were fabricated using these STL files on a 3D printing unit (Ackuretta FreeShape 120, Taiwan) with a cross-sectional thickness of 100 microns, following the manufacturer's instructions (Dentona Denture 385, Dortmund, Germany). (Ay design, Ankara, Türkiye)

2.2. Fabrication of HA PMMA Resin Specimens

Wax specimens were prepared using 3D PMMA specimens as molds. They were polymerized using heat-polymerizable PMMA (Meliodent, Heraeus Kulzer GmbH Co, Germany) according to the manufacturer's recommendations and cooled on the bench for 30 minutes at room temperature. A precision scale (Kern ABJ 220-4 NM, Germany) was used to determine the ideal ratio of polymer (powder) to monomer (liquid).

The same finishing and polishing process was applied to both HA and 3D samples. First, they were smoothed with a tungsten carbide router at low speed. Then, each surface was finished under water with 400 grit silicon carbide papers (Met Rolls; Kemet, London, England) for 30 seconds (18) For polishing a mixture of dental pumice and water was used with felt for 90 seconds, then universal polishing paste (Universal Polishing Paste, Ivoclar Vivadent, Schaan) was applied. (Figure 1)

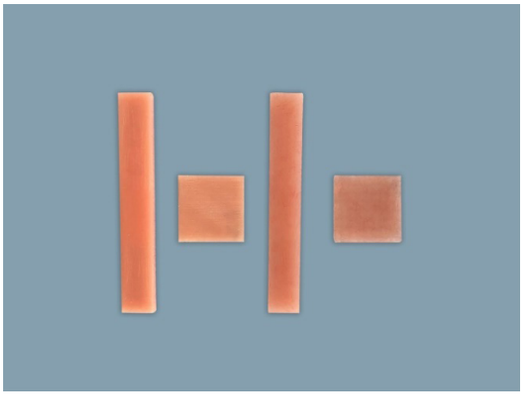

Figure 1. *3D and HA PMMA specimens.*

The dimensions of the PMMA specimens were checked using a digital caliper (Shinwa Co, Osaka, Japan). 30 specimens in each group were randomly divided into 3 groups (HA PMMA for 3D bending test, HA PMMA for surface roughness test, 3D PMMA for 3D bending test and 3D PMMA for surface roughness test) (n=10). Ten specimens from each group were used as a control group. All specimens were stored in distilled water at 37°C to maintain dimensional stability throughout the study.

2.3. Disinfection Method

Specimens were kept in disinfectant twice for 30 minutes each, simulating the circulation between dental laboratory and the clinic. They were kept in distilled water for 5 minutes between the two disinfections. Orthophthalaldehyde (OPA) (Cidex OPA, Johnson & Johnson Medical, Norderstedt, Germany) and HOCL (SuperOx, Anolit, Yenimalle, Ankara) were used as disinfectants. Specimens in the control group were additionally soaked in distilled water for 30 minutes twice and removed. In order to provide the same conditions as the disinfectant-exposed specimens, they were soaked in distilled water again for 5 minutes between the two half-hour periods (18).

2.4. Three-Point Bending Test

Flexural strength of disinfected specimens was determined using a 3-point bending testing in a universal testing machine (Lloyd LRX, Lloyd Instruments Ltd, Fareham, Hampshire, England). Each specimen was placed horizontally on two vertical supports with a 50 mm distance, and the head speed was 5 mm/min. The load was applied to the center of the specimens where fractures occurred (19) The fracture force was recorded in Newtons (N). Flexural strength values were calculated using the formula S= 3FL/2bd2 (S: flexural strength (N/mm2), F: Load recorded at break (N), L: Distance between supports (50 mm), b: Specimen width (10 mm), d: Specimen thickness (3 mm) (20).

2.5. Surface Roughness Evaluation

After exposure to the disinfectant solutions, surface roughness measurements were conducted using a profilometer (Perthometer M2, Mahr GmbH, Göttingen, Germany). Three measurements were taken from each sample, and the average of these values was calculated and recorded as the surface roughness (Ra) in micrometers (µm).

2.6. Statistical Analyses

Statistical analyses were performed using the Statistical Package for Social Science Software (SPSS, version 23.0, IBM Corp, Chicago, USA). For comparisons between three or more groups, Kruskall Wallis H test was used because the data were not normally distributed. For comparisons between groups, a t-test was used due to the normal distribution of data (α=.05).

2.7. Scanning Electron Microscopy (SEM) Analysis

In order to evaluate the surface properties, the homogeneity of the samples was examined under 1000 X magnification using a scanning electron microscope (SEM) (Evo 40, Carl-Zeiss, Oberkochen, Germany). Gold coating was applied using a sputter coating machine (Emitech K550X, Richmond Scientific, ORE, England).

3. RESULTS

The results for flexural strength, surface roughness, and SEM analysis of the samples exposed to both disinfectants are presented below.

3.1. Flexural Strength

The flexural strength (MPa) values and standard deviations (SD) of the HA PMMA and 3D PMMA resin groups are shown in Table 1. The flexural strength values of the HA PMMA resin group were statistically significantly higher than 3D PMMA group in all three groups (DW, HOCL and OPA) (P= .0001). Differences between rows within columns are displayed with lowercase superscripts in Table 1.

Table 1. *Mean flexural strength (MPa) values and standard deviations (SD)*

	Mean (MPa)-(SD) DW	Mean (MPa)-(SD) HOCl	Mean (MPa)-(SD) OPA
HA PMMA	147.92 (13.73)^{Aa}	143.09 (12.80)^{Aa}	142.03 (9.05)^{Aa}
3D PMMA	56.42 (19.55)^{Ab}	55.20 (16.26)^{Ab}	56.99 (14.13)^{Ab}

HA PMMA: Heat activated polymethyl methacrylate 3D PMMA: Three dimensionally printed polymethyl methacrylate DW: Distilled water, HOCL: Hypochlorous acid, OPA: Ortho phthalaldehyde. Superscript lowercase letters incate the statistically significant differences between studiedresin groups for disinfectants and control (p<0.05). Superscript capital letters incate the difference between disinfectant groups within each resingroup (p>.05).

No significant difference was found between the flexural strength (MPa) values of HOCL, OPA disinfectant applications and the control group for each resin group. The differences between columns were shown with a capital letter superscript in Table 1. The p values were *(p>.05)* for both HA PMMA and 3D PMMA.

3.2. Surface Roughness

The surface roughness measurements of the two resin groups were compared using a t-test (Table 2). The Ra value differences between HA PMMA and 3D PMMA resins were not statistically significant when disinfected with HOCl and OPA *(p HOCl=.111, pOPA=.852).* Exposure with three different solutions did not cause a significant difference in Ra values in both the HA PMMA *(p=.464)* and 3D PMMA *(p=.307)* groups. *(p>.05).*

Table 2. *The mean surface roughness (Ra) values and standard deviations (SD)*

Ra	Mean (SD) DW	Mean (SD) HOCl	Mean (SD) OPA
HA PMMA	.64(.20)^{Aa}	.63(25)^{Aa}	.52(.21)^{Aa}
3D PMMA	.42(.14)^{Ab}	.46(.20)^{Aa}	.54 (.17)^{Aa}

HA PMMA: Heat activated polymethyl methacrylate 3D PMMA: Three dimensionally printed polymethyl methacrylate DW: Distilled water, HOCL: Hypochlorous acid, OPA: Orthophthalaldehyde. Superscript lowercase letters incate the statistically significant differences between studied resin groups for each disinfectant and control (p<.05). Superscript capital letters incate the difference between disinfectant groups within each resin group (p>.05).

3.3. (SEM) Analysis

SEM images of 3D PMMA specimens are displayed in Figure 2. DW immersed specimens have a smooth structure. The amount of roughness in the specimens disinfected with OPA was observed to be higher than the specimens disinfected with HOCL. HA PMMA specimens have a rougher appearance than 3D PMMA specimens in all three solutions (Figure 3). The findings are in parallel with the numerical values.

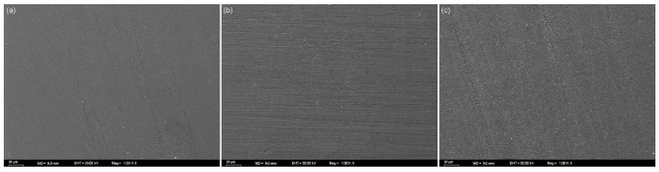

Figure 2. *SEM images of DW (a), OPA (b) and HOCL(c) of treatment of 3D PMMA original magnification 1000 X*

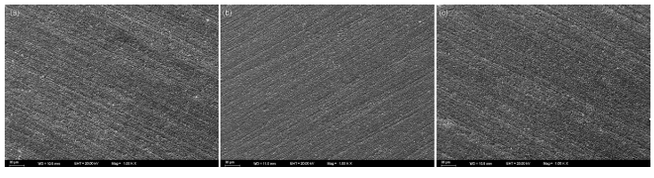

Figure 3. *SEM images of DW (a), OPA (b) and HOCL(c) of treatment of HA PMMA original magnification 1000 X*

4. DISCUSSION

In this study, the effect of two different disinfectants (HOCL and OPA) on the flexural strength and surface roughness of HA and 3D PMMA specimens were evaluated. Compared to the OPA and DW groups, HOCL was found to have no negative effect on the flexural strength and surface roughness of PMMA specimens and the null hypothesis that it can be used for a short time to prevent cross-infection between the laboratory and clinic was accepted.

Disinfection of impressions, models, orthodontic appliances and prostheses by dentists and dental technicians during transfer between clinic and laboratory is a routine procedure. Antibacterial chemicals used in spray and dip disinfection can influence mechanical properties such as flexural strength, hardness and roughness (18,21,22).

The effect of various disinfection methods and solutions on the physical properties of HA PMMAs has been evaluated in the literature (23,24). However, there are limited studies on the impact of disinfection protocols on the mechanical properties of 3D PMMAs and data on HOCL disinfection, an innovative approach (9,19,25).

In this study, the flexural strength values (DW, HOCL and OPA) of the 3D PMMA resin group were found to be significantly lower than HA PMMA group in all three immersion solutions *(P= .0001).* This result is consistent with previous studies (19,26). Akhaltham et al. concluded that 3D resins had lower flexural strength and elastic modulus than HA PMMA resins. Researchers have reported that the choice of appropriate disinfectant and material is effective on the life of the prosthesis and orthodontic appliances (19).

Although the results are similar, the difference between the immersion times should not be ignored. In the literature, the factors affecting the strength of 3D PMMA resins include water storage, immersion in chemicals and aging (27). Resin materials easily absorb water due to their polarity. The amount of water absorption is related to the amount of residual monomer and the diffusion coefficient of the resin. The diffusion coefficient of the resin depends on the time it takes for the material to saturate with water. Therefore, the immersion time of the resin material in the disinfectant has an effect on the results (28). In this study only one-hour long findings were evaluated, which simulated the travel time between the laboratory and the clinic. The lack of difference in flexural strength values between disinfectants in both resin groups can be attributed to the fact that the time was limited to only one-hour. The decreased flexural strength of 3D printed resins after disinfection was explained by the higher water absorption rate of 3D printed resins compared to HA resins (29).

Previous studies have reported that NaOCl immersion decreases flexural strength. The lower flexural strength value was associated with the absorption of NaOCl aqueous solution and the active chlorine content. It was reported that the active chlorine content increased the residual monomer solubility, which was compensated by more water absorption, affecting the PMMA strength (19,30,31). In this study, the use of hypochlorous acid for a short time to prevent cross-infection may have prevented the negative effects of active chlorine content.

Freitas et al (32) evaluated the mechanical properties and anti-biofilm formation of CAD/CAM milled, 3D printed denture base resins and conventional resins. It was concluded that 3D printed specimens had the lowest flexural strength and the highest surface roughness values. A value (57.23 ± 9.07*; p < .05*) below the required resistance (65 MPa) recommended by ISO 20795-1:2013 for denture base resins was observed for 3D printed specimens. Similarly, in this study, 3D printed resin flexural strength values were 56.42 ± 19.55, 55.20 ± 16.26 and 56.99 ± 14.13 for DW, HOCL and OPA, respectively. These results are in line with the study of Prpić et al. (26). (Max: 84.5 MPa and Min: 60.0 MPa)

The low flexural strength value of 3D PMMA resin may also be related to the polymerized material, polymerization time and printing structure (26,33). In other words, the flexural strength value is not only related to the production method of the material but also to the selected material itself. In a previous study, the lower mechanical performance of 3D printed denture resins was associated with a lower conversion rate compared to conventional PMMA resins (34).

When evaluated in terms of surface roughness values, the results of this study are similar to previous studies (32,35). Al Dwairi et al (35) concluded that HA PMMA surface roughness values were higher than three different brands of 3D PMMA materials. The difference between the values of three different brands supports the idea that the material and printing structure used are effective for mechanical properties. Freitas et al (32) similarly concluded that the surface roughness values of HA PMMA resin were higher than the 3D printed group.

Unlike previous studies on PMMA disinfection, HOCL was used as a disinfectant in this study. HOCl is an easy-to-use, cost-effective, and non-toxic disinfectant that combines most of the desired effects of an ideal disinfectant. It has been shown to inactivate a variety of viruses, including coronaviruses, in less than 1 min. The American Food and Drug Administration (FDA) has approved the use of hypochlorous acid in eye and dental applications and as a rinse-free disinfectant for fruits and vegetables, demonstrating its biological safety (10,11,12).

Gessi et al (36) found that HOCl-based disinfectant did not damage floor surfaces even after more than a thousand applications and was also safe for sensitive surfaces used as controls. They concluded that unlike bleach or chlorine solutions, HOCl is not corrosive to surfaces or equipment due to its slightly acidic pH conditions. The study achieved a satisfactory level of antimicrobial decontamination and at the same time reported CO2 emission savings of approximately 30% per square meter.

According to the data obtained from this study, hypochlorous acid can be used as an alternative disinfectant to prevent

laboratory-clinic cross-infection for 3D and HA-PMMA resin prostheses and orthodontic appliances. On the other hand, care should be taken to ensure that there is a balance between the effectiveness of disinfectants and their effect on the resin.

The current study has certain limitations. Because of the artificial travel time between the clinic and laboratory, the amount of time that PMMA materials were exposed to the disinfectant was restricted. Future studies may focus on the effects of longer exposure to HOCl on the mechanical properties and color of PMMA materials or the microbiological activity of viruses such as Sars Cov-2 and monkey pox. Thus, the use of biological disinfectants such as HOCl, which do not have negative effects on mechanical properties and the environment, may become widespread.

5. CONCLUSION

1. Immersing 3D and HA-PMMA resins in disinfectant solutions (HOCL and OPA) for 1 hour resulted in no significant difference in flexural strength values.

2. Immersion of 3D and HA PMMA resins in DW and disinfectant solutions (HOCL and OPA) for 1 hour did not produce a significant difference on Ra values.

3. 3D printed PMMA resins showed lower flexural strength values than HA PMMA resins.

4. The surface roughness values of HA PMMA resins immersed in disinfectant solutions were higher than the surface roughness values of 3D PMMA resins.

5. Hypochlorous acid showed no adverse effects on the mechanical properties of 3D and HA-PMMA resin prostheses and orthodontic appliances when used as an alternative disinfectant to prevent cross-infection between the laboratory and the clinic.

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: FB

Design of the study: FB,OUA

Acquisition of data for the study: FB,OUA,TÖ

Analysis of data for the study: FB,OUA,TÖ

Interpretation of data for the study: FB,OUA,TÖ

Drafting the manuscript: FB,OUA,TÖ

Revising it critically for important intellectual content: FB,OUA,TÖ

Final approval of the version to be published: FB,OUA,TÖ

REFERENCES

[1] Salvia AC, Matilde Fdos S, Rosa FC, Kimpara ET, Jorge AO, Balducci I, Dis Koga-Ito CY. Disinfection protocols to prevent cross-contamination between dental offices and prosthetic laboratories. J Infect Public Health. 2013; 6(5):377-382. https://doi.org/10.1016/j.jiph.2013.04.011

[2] Raszewski Z, Toporowska AN, Nowakowska D, Więckiewicz W.Update on acrylic resins used in dentistry. Mini Rev Med Chem. 2021; 21(15):2130-2137. https://doi.org/10.2174/1389557521666210226151214

[3] Zafar MS. Prosthodontic applications of polymethyl methacrylate (PMMA): an update. Polymers (Basel) 2020; 12(10):2299. https://doi.org/10.3390/polym12102299

[4] Katreva IP, Dikova T, Abadzhiev M, Tonchev T, Dzhendov D, Simov M, Angelova S, Pavlova D, Doychinova M. 3D-printing in contemporary prosthodontic treatment. Scripta Infect Public Health. 2016; 2(1):7-11

[5] Barazanchi A, Li KC, Al-Amleh B, Lyons K, Waddell JN. Additive technology: update on current materials and applications in dentistry. J Prosthodont.2017; 26(2):156-163. https://doi.org/10.1111/jopr.12510

[6] Carvalho CF, Vanderlei AD, Marocho SM, Pereira SM, Nogueira L, Paes-Júnior TJ. Effect of disinfectant solutions on a denture base acrylic resin. Acta Odontol Latinoam. 2012; 25(3): 255-260.

[7] Chau VB, Saunders TR, Pimsler M, Elfring DR. In-depth disinfection of acrylic resins. J Prosthet Dent. 1995 Sep;74(3):309-313. https://doi.org/10.1016/s0022-3913(05)80140-4

[8] Yokliant W, Leevarakarn S. Effect of ortho-phthaladehyde disinfectant on color stability of two different acrylic resin teeth using spectrophotometer. J.Dep. Med. Serv.2022; 47(3). 96-103

[9] Kadhim GF, Jabbar MK, Hameed NY. Hypochlorous acid: effects on two surface properties of denture materials. J.Oral Dent Res. 2022; 9(1):44-52

[10] Akamatsu T, Minemoto M, Uyeda M. Evaluation of the antimicrobial activity and materials compatibility of orthophthalaldehyde as a high-level disinfectant. J Int Med Res. 2005 ;33(2): 178-187. https://doi.org/10.1177/147323000503300205

[11] Block MS, Rowan BG. Hypochlorous acid: a review. J Oral Maxillofac Surg.2020; 78(9): 1461–1466. https://doi.org/10.1016/j.joms.2020.06.029

[12] Nguyen K, Bui D, Hashemi M, Hocking DM, Mendis P, Strugnell RA, Dharmage SC. The potential use of hypochlorous acid and a smart prefabricated sanitising chamber to reduce occupation-related COVID-19 exposure. Risk Manag Healthc Policy.2021; 22(14):247-252. https://doi.org/10.2147/RMHP.S284897

[13] United State Environmental Protection Agency. About list N: disinfectants for coronavirus (COVID-19). 2022. https://www.epa.gov/pesticide-registration. Accessed 23 Dec 2022

[14] World Health Organization. Cleaning and disinfection of environmental surfaces in the context of COVID-19. 2020. https://www.who.int/publications/i/item/cleaning-and-disinfection-of-environmental-surfaces-inthe-context-of-covid-19. Accessed 23 Dec 2022

[15] Jasim JM, Abass SM.The effect of hypochlorous acid disinfectant on the reproduction of details and surface hardness of type III dental stone. Cureus.2022; 14(11) 1-7. https://doi.org/10.7759/cureus.32061

[16] Chen CJ, Chen CC, Ding SJ. Effectiveness of hypochlorous acid to reduce the biofilms on titanium alloy surfaces in vitro. Int J Mol Sci. 2016; 17(7):1161. https://doi.org/10.3390/ijms17071161

[17] Srinivasan M, Kalberer N, Kamnoedboon P, Mekki M, Durual S, Ozcan M , Müller F. CAD-CAM complete denture resins: an evaluation of biocompatibility, mechanical properties, and surface characteristics. J Dent. 2021; 114:103785. https://doi.org/10.1016/j.jdent.2021.103785

[18] Savabi O, Attar K, Nejatidanesh F, Goroohi H, Badrian H .Effect of different chemical disinfectants on the flexural strength of heat-polymerized acrylic resins. Eur J Prosthodont Restor Dent. 2013; 21(3):105-108.

[19] Alkaltham NS, Aldhafiri RA, Al-Thobity AM, Alramadan H, Aljubran H, Ateeq IS, Khan SQ, Akhtar S, Gad MM. Effect of denture disinfectants on the mechanical performance of 3D-printed denture base materials. Polymers. 2023; 15(5):1175. https://doi.org/10.3390/polym15051175

[20] Ellakwa AE, El-Sheikh AM. Effect of chemical disinfectants and repair materials on the transverse strength of repaired heat-polymerized acrylic resin. J Prosthodont. 2006; 15(5):300-305. https://doi.org/10.1111/j.1532-849X.2006.00131

[21] Ahila SC, Thulasingam C. Effect of disinfection on gypsum casts retrieved from addition and condensation silicone impressions disinfected by immersion and spray methods.SRM J Res Dent Sci. 2014; 5(3):163-169. https://doi.org/10.4103/0976-433X.138724

[22] Bensel T, Bock JJ, Kebernik A, Arnold C, Mansour S, Boeckler AF. Effect of disinfectants on mechanical properties of orthodontic acrylics. Int.J.of Biomater. 2019; 24:1-10. 1096208. https://doi.org/10.1155/2019/1096208

[23] Sartori EA, Schmidt CB, Walber LF, Shinkai RS .Effect of microwave disinfection on denture base adaptation and resin surface roughness. Braz Dent J. 2006; 17(3):195-200. https://doi.org/10.1590/S0103-64402006000300004

[24] Bulut AC, Yurksayar AAD. Effect of eight different disinfection methods on the surface roughness and flexural strength properties of acrylic resins: In vitro study. Turkiye Klinikleri J Dental Sci.2023;29 (1): 139-146. https://doi.org/10.5336/dentalsci.2022-92010

[25] Koujan A, Aggarwal H, Chen PH, Li Z, Givan DA, Zhang P, Fu CC.Evaluation of candida albicans adherence to CAD-CAM milled, 3D-printed, and heat-cured PMMA resin and efficacy of different disinfection techniques: an in vitro study. J Prosthodont.2023; 32(6): 512-518. https://doi.org/10.1111/jopr.13583

[26] Prpić V, Schauperl Z, Ćatić A, Dulčić N, Čimić S. Comparison of mechanical properties of 3D-printed, CAD/CAM, and conventional denture base materials. J Prosthodont. 2020 ;29(6):524-528. https://doi.org/10.1111/jopr.13175

[27] Gad, MM, Fouda SM. Factors affecting flexural strength of 3D-printed resins: a systematic review.J Prosthodont.2023; 11:1-15.32(S1):96-110. https://doi.org/10.1111/jopr.13640

[28] Bettencourt AF, Neves CB, de Almeida MS, Pinheiro LM, Oliveira SA,Lopes, LP, Castro MF. Biodegradation of acrylic based resins: a review. Dent. Mater.2010; 26(5): 171-180. https://doi.org/10.1016/j.dental.2010.01.006

[29] Gad MM, Alshehri SZ, Alhamid SA, Albarrak A, Khan SQ, Alshahrani FA, Alqarawi FK. Water sorption, solubility, and translucency of 3D-printed denture base resins. Dent. J.2022; 10(3):1-13. https://doi.org/10.3390/dj10030042

[30] Kurt A, Erkose-Genc G, Uzun M, Sarı T, Isik-Ozkol G. The effect of cleaning solutions on a denture base material: elimination of candida albicans and alteration of physical properties. J. Prosthodont.2018;27(6): 577–583. https://doi.org/10.1111/jopr.12539

[31] Gad MM, Abualsaud R, Fouda SM, Rahoma A, Al-Thobity AM, Khan SQ, Akhtar S, Al-Harbi FA. . Effects of denture cleansers on the flexural strength of PMMA denture base resin modified with ZrO2 nanoparticles. J. Prosthodont 2021; 30(3): 235–244. https://doi.org/10.1111/jopr.13234

[32] Freitas RFCP, Duarte S, Feitosa S, Dutra V, Lin WS, Panariello BHD, Carreiro ADFP. Physical, mechanical, and anti-biofilm formation properties of CAD-CAM milled or 3D printed denture base resins: in vitro analysis. J. Prosthodont.2023; 32(S1):38-44. https://doi.org/10.1111/jopr.13554

[33] Gad MM, Fouda SM, Abualsaud R, Alshahrani FA, Al-Thobity AM, Khan SQ, Akhtar S, Ateeq IS, Helal MA, Al-Harbi FA. Strength and surface properties of a 3D-printed denture base polymer. J. Prosthodont.2022; 31(5),412–418. https://doi.org/10.1111/jopr.13413

[34] Alifui-Segbaya F, Bowman J, White AR, George R , Fidan I. Characterization of the double bond conversion of acrylic resins for 3D printing of dental prostheses. Compend Contin Educ Dent.2019; 40(10):7-11.

[35] Al-Dwairi ZN, Al Haj Ebrahim AA, Baba NZ. A Comparison of the surface and mechanical properties of 3D printable denture-base resin material and conventional polymethylmethacrylate (PMMA). J.Prosthodont.2023; 32(1): 40–48. https://doi.org/10.1111/jopr.13491

[36] Gessi A, Formaglio P, Semeraro B, Summa D, Tamisari E, Tamburini E. Electrolyzed hypochlorous acid (HOCl) aqueous solution as low-impact and eco-friendly agent for floor cleaning and sanitation. Int. J. Environ. Res. Public Health. 2023; 20(18):6712. https://doi.org/10.3390/ijerph20186712

How to cite this article: Basmacı F, Uslu Akçam Ö, Özcivelek T. Effect of Hypochlorous Acid on Acrylic Resins in Preventing Cross-infection Between Clinic and Laboratory. Clin Exp Health Sci 2025; 15: 226-231. https://doi.org/10.33808/clinexphealthsci.1552492

Clinical and Experimental Health Sciences

Recent Developments in Adrenergic Receptor Polymorphisms in Essential Hypertension

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Received: January 6, 2025 **Accepted:** January 21, 2025

ABSTRACT

Objective: This review's goal is to provide an overview of the most recent data about the genetic foundations of adrenergic receptor polymorphisms in connection with essential hypertension (EH). Since EH is idiopathic, research is centered on its genetic underpinnings and significant interindividual differences in response to various therapies. Polymorphisms as an important element affecting individual disease susceptibility processes, are therefore, area of research interest for especially for genes that modulate variety of metabolic processes

Methods: A comprehensive, systematic literature search was conducted using a number of databases, including PubMed, Google Scholar, and Web of Science (WOS). Recent research in the field that looked into the connections between blood pressure, heart disease, hypertension, and vascular problems was taken into account. Only studies with common polymorphisms, uniform criteria and statistics were included in order to assess consistent information and provide a broad perspective.

Results: There are a limited number of studies in the literature after 2010 related to the adrenergic system polymorphisms, blood pressure, and/or essential hypertension. Genome-wide studies and meta-analyses reveal that there are several variants whose roles were supported by independent studies. ADRA1 Arg347Cys (rs1048101), ADRA2 C-1291G variant (rs1800544), ADRB1 Arg38Gly, ADRB2 Arg46Gly and ADRB3 Trp64ARg (rs4994) can be counted as major polymorphisms with their role verified by multiple researches.

Conclusion: Despite being supported by numerous research, the association between adrenergic system polymorphisms and essential hypertension cannot be conclusively established due to the unpredictability of study patient numbers, side effects, and inconsistent findings. Larger and more controlled population-based studies are required to provide a clear picture of the disease's variability and treatment responses.

Keywords: Adrenergic receptors, blood pressure, essential hypertension

1. INTRODUCTION

Catecholamines, a class of molecules with an amine chain and a catechol ring, are important components of the sympathetic nervous system. These consist of dopamine, adrenaline, and noradrenaline, the latter two are also known as epinephrine and norepinephrine. This family of molecules regulates the body's neurological and metabolic activities and serves as essential targets for a variety of pharmaceutical medications.

Catecholamine polymorphisms have drawn attention since they play a significant role in the pharmacological effects of certain diseases. Sympathetic denervation directly relates to elevated blood pressure (BP) and heart rate, two characteristics of hypertension, one of the most common disorders in the world, especially in the elderly (1). Essential hypertension (EH) is idiopathic, and there are a number of risk factors to take into account. The primary causes involve genetic basis in personal family history, dietary regime, aging, and obesity.

Studies linking more than 50 genes to hypertension have been conducted, and the number is continuously increasing (2). Currently, however, little is known about the hereditary component of hypertension. The genetic foundation of EH includes gene-environment interactions, epigenetic variables, and the interplay of several genes, each of which has a minor impact. Three main groups of protein families have emerged as a result of genetic research: adrenergic receptors, the renin-angiotensin-aldosterone system, and sodium reabsorption-regulating channels.

Through a current analysis of clinical findings, this review will briefly address the role of adrenergic receptor polymorphisms in EH. We examined the significance of

https://doi.org/10.33808/clinexphealthsci.1614451

these SNPs in the generation and treatment responses of EH by comparing published results. The role of adrenalin and adrenergic receptors, directly or indirectly correlated with the regulation of blood pressure (BP) and heart rate, will be overviewed in this perspective based on the current knowledge of the polymorphisms of the relevant elements. It is believed that this compilation will help to integrate current accumulated knowledge in the field, provide a preliminary perspective for the design of future studies, and increase our understanding of the SNP-related genetic basis of adrenergic system components in this one of the most prevalent and complexly structured disorders.

2. ADRENERGIC RECEPTOR POLYMORPHISMS

One essential part of the autonomic nervous system is the adrenergic system. In addition to controlling cardiovascular, respiratory, and metabolic processes and regulating blood pressure through its effects on the central nervous system, it also contributes to the regulation of renal sodium through renin-aldesterone-angiotensin system (3, 4). It operates primarily through adrenergic receptors, which react to the hormon/neurotransmitters adrenaline and noradrenaline. The adrenergic system orchestrates wide range of physiological responses to stress and activity. Adrenaline is secreted mainly by the adrenal medulla and it enhances systemic effects like elevated heart rate, vasodilation, and energy mobilization during stressful situations. Noradrenaline is a neurotransmitter that is released by sympathetic nerve terminals, whereas adrenaline is mostly classified as a hormone. Two main classes of adrenergic receptors are distinguished by their pharmacological and functional characteristics: a-adrenergic receptors (α1 and α2); β-adrenergic receptors (β1, β2, β3). Additionally, each a group has also been subdivided into α1A, B and D; α2A, B and C (5). The receptor classes are schematically represented in Figure 1, together with the most extensively studied polymorphisms linked to hypertension and blood pressure. An overview of studies examining the relationship between AR polymorphisms and blood pressure or hypertension that were discussed in this review is presented in Table 1.

Table 1. *A summary of recent studies on adrenergic receptor polymorphisms in relation to blood pressure and hypertension.*

Reference (Ref. No)	SNP	Ethnicity	Sample size (HT/NT)	Association/ significance	Parameter
Adefurin et al. 2017 (10)	ADRA1B rs10070745	Caucasians and African Americans	105	Yes	Arterial pressure
Eldeeb et al. 2022 (18)	ADRA2B rs1800888 (301-303 I/D)	Saudi population	200 HT/100 NT	No	Hypertension with Type 2 diabetes mellitus
Wu et al. 2015 (25)	ADRB1 rs1801253 (Arg389Gly)	Chinese	93	No	Essential hypertension
Chen et al. 2018 (26)	ADRB1 rs1801253 (Arg389Gly)	Not specified	261/261	No	Essential hypertension
Varakantham et al. 2018 (30)	ADRB1 rs1801252 (Ser49Gly) rs1801253 (Arg389Gly)	South Indian	292/324	No	Essential hypertension
Cai et al. 2015 (35)	ADRB2 rs11168070 (-468 C/G)	Chinese Kazakh	150/150	No	Essential hypertension
Yan et al. 2020 (37) Meta-analysis	ADRB2 rs1042713 (Arg16Gly)	Chinese	3390/2528	Yes	Essential hypertension
Maamor et al. 2024 (39) Meta-analysis	ADRB2-rs1042713 rs1042714	East Asian	7269/7615	Yes	Hypertension
Li et al. 2018 (40) Meta-analysis	ADRB3 rs4994 (Trp64Arg)	Chinese Japanese German Italian	5088/4467	Yes	Essential hypertension

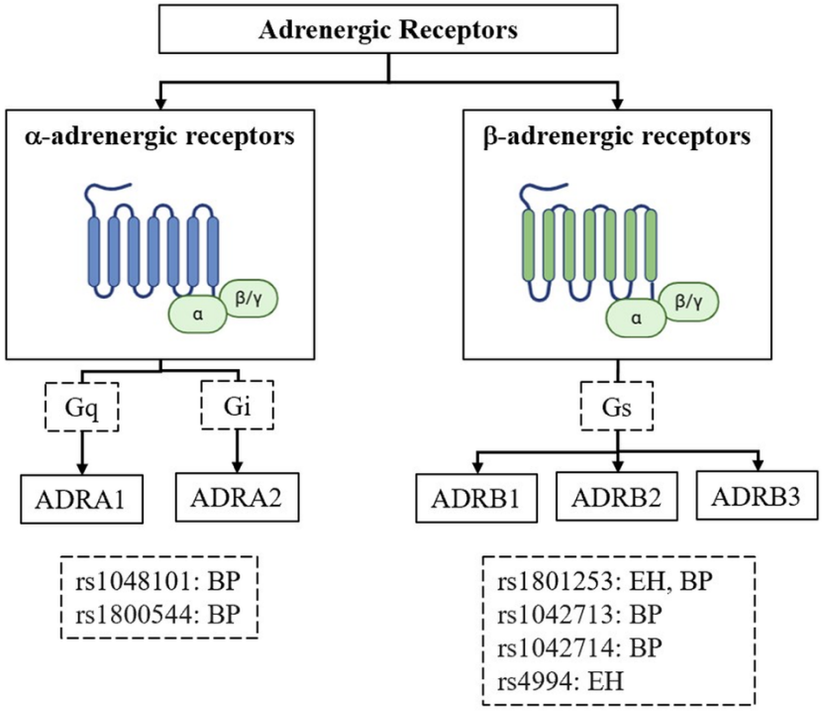

Figure 1. *The receptor classes, together with the most extensively studied polymorphisms linked to hypertension and blood pressure.*

2.1. α-adrenergic receptor polymorphisms

The α-adrenergic receptors are G-protein-coupled receptors, and they activate second messenger systems through the activation of G-proteins (G_q or $G_{i/o}$). α1 mediates vasoconstriction and is crucial for controlling vascular tone, whereas α2 controls the release of noradrenaline from presynaptic terminals (6).

α1-AR activation causes smooth muscle contraction in blood vessels (e.g., arteries, veins), leading to vasoconstriction. This increases vascular resistance, which raises blood pressure. The route proceeds via Gq-mediated activation of phospholipase C, which cleaves phosphoatidylinositol-4,5-bisphopshate (PIP2) to create diacylglycerol (DAG) and inositol trisphosphate (IP3). IP3 is a known messenger for Ca^{2+} release from intracellular stores. In addition to blood vessels, α_1-AR activation causes contraction of smooth muscles in other organs, such as the bladder, prostate, intestinal tract, and uterus.

The α2-ARs are found on pre – and post-synaptic neurons of the central and peripheral nervous systems and blood vessels. They play a significant role in regulating sympathetic tone through both central and peripheral sympathetic inhibition. They make substantial contributions to the homeostatic regulations, controlling contraction and relaxation of vascular smooth muscle, as well as other functions such as anxiety and stress-related behaviors, pain perception, platelet aggregation, and lipolysis.

The α2-ARs act through the G_i/G_o family of G-proteins in their physiologic functions, including vascular, cardiac, and metabolic systems, as well as the central and peripheral nervous systems. When agonists attach to receptors, the receptor couples with associated G-proteins, triggering effector reactions such as phospholipase C activation or adenylyl cyclase (AC) inhibition. However, their main role is G_i-mediated AC inhibition, modulating sympathetic activity to produce lower blood pressure and decreased heart rate.

The studies investigated the relation between adrenergic system polymorphisms and essential hypertension, blood pressure, and treatment response was compiled in our earlier work up to 2010 (7). Therefore, the interest of this review is limited only to the clinical articles published after this date.

In 2012, a genome-wide study found that among 28 pathways with biological relevance to hypertension, only ADRA1 pathway showed significant association with hypertension (8). However, this analysis included SNPs associated with genes implicated in the whole ADRA1 pathway, including those involved in the synthesis of norepinephrine and adrenaline, such as PNMT, MAO, COMT, GNAQ, GNA11 etc. Below is a summary of a small number of SNP research on α1-AR subtypes.

2.1.1. α1 (ADRA1)-adrenergic receptor polymorphisms

The human α1-AR is the predominant α1-AR subtype in vascular smooth muscle, the heart, and the liver. Considering its role in smooth muscle contraction, early research examined the connection between previously identified SNPs and hypertension. Compared to other adrenergic receptors, the relationship between ADRA1 and EH seems to be modest.

Given that α1A-ARs play a crucial role in controlling vascular resistance, multiple studies assessed the possible impact of the rs1048101 polymorphism with blood pressure readings both in healthy subjects or subjects with arterial lypertension. Three genotypes for the α-adrenergic receptors ADRA1A Arg347Cys (rs1048101, previously known as Arg492Cys), ADRA2A 1780 C>T (rs553668), and ADRA2B Del 301–303 (rs28365031) were investigated for exercise capacities, heart-rate recovery, and systolic and diastolic blood pressures in a healthy Brazilian population without any known cardiac issues. The maximum systolic blood pressure was found to be linked to rs1048101 in men and rs28365031 in women (9). Another variant was rs10070745 mutation, which contributed to the ethnic differences in phenylephrine sensitivity, a selective α1-AR agonist, and was significantly linked to vasoconstrictor responses to adrenergic stimulation (10). One study looked into whether α-AR polymorphisms affect how the body reacts to α-AR blockers. Based on the observation that interindividual variations remarkably affect blood pressure and side effects, a wide range of α1 – and α2 – variants were genotyped in 116 patients (11). The study examined a wide range of SNPs for α1A-variants, including variants of rs1048101 and rs2229125 linked to hypertension. Only two associations were discovered: the rs10515807 variant of α1B and the ADRA2A SNPs rs553668/ rs521674, which are both associated with higher dosages of α-adrenergic receptor blockers ($p < 0.05$) and a higher incidence of adverse effects ($p = 0.005$).

2.1.2. α2 (ADRA2)-adrenergic receptor polymorphisms

The function of α2-AR polymorphisms in BP regulation or essential hypertension has been the subject of numerous investigations. However, the majority of research examining the connection between hypertension and distinct polymorphic locations shows no correlation across ethnic groups. Although it has a clear role in controlling blood pressure, heart rate, and cardiovascular function, research examining the relationship between BP, EH, and genetic α2-AR variations yielded mixed findings (12-14). Kurnik et al (15) conducted a study in which they examined the cardiovascular responses of nine SNPs of ADRA2A (rs11195418, rs1800544, rs2484516, rs1800545, rs1800035, rs1800038, rs34303217, rs553668, and rs3750625) to the selective α2-AR agonist dexmedetomidine. Seventy-three healthy black and white American individuals, ages 18 to 45, participated in the study. A placebo group and a group receiving an infusion of dexmedetomidine participated in the trial. Out of all the variations, the rs553668 variant responded more strongly to the α2-AR agonist dexmedetomidine, whereas the others had no discernible impact. However, this study's small sample size led to some tiny genotype groups and broad confidence intervals; as the author noted, the results are preliminary and need to be confirmed in larger, clinical cohorts. Yağar et al (16), on the other hand, reported a promising association between (ADRA2A) C-1291G gene polymorphism (rs1800544) and response to dexmedetomidine.

Since Black Americans are known to have a higher risk of hypertension, a study looked at how their cardiovascular responses to stress may contribute to the development of hypertension. In normotensive individuals subjected to cold or psychological stress, the genotype analyses of ADRA1A Arg347Cys (rs1048101), ADRA2A C-1291G (rs1800544), and ADRA2B Insertion/Deletion (Ins/Del 301-303, rs1800888), showed that vascular reaction was more strongly associated with rs1800544, whereas heart rate reactivity was more closely linked to rs1048101 (17). A study carried out in Saudi population with 200 subjects, however, found no correlation between rs1800888 and hypertension (18).

Unfortunately, there are very few publications on this topic. It can be said that two polymorphisms associated with hypertension stand out in common from these studies:

α1A-AR polymorphisms Arg347Cys, especially linked to blood regulation, and a promoter variant of α2A-AR, C-1291G. The other SNPs in α1A, α1B, and α1D subtypes are the subject of ongoing research, and further studies are required to properly evaluate their contributions to hypertension.

2.2. β-adrenergic receptor polymorphisms

The β-adrenergic receptors (β-AR) couple to either G_s or G_i (heterotrimeric stimulatory and inhibitory G-proteins) proteins. Similar to α-AR, they are classified into three-subtypes: β1, β2 and β3. They act on the sympathetic control of heart rate and myocardial contraction. A mixed population of α-ARs are expressed in the human heart, with about 80% of the receptors being of the α1-AR subtype and 20% being of the β2-AR subtype (19). Any inhibitory effect on especially β1 receptor disrupting this approximate ratio whether in the gene regulation level, or in the protein level result in pathological heart problems (20). The receptor protein undergoes a conformational shift when β-AR agonist is present, which impacts the heterotrimeric G protein's ability to dissociate into its constituent subunits. A primary effect of the β-AR is stimulation of adenylyl cyclases, of which human cardiac tissues express several subtypes. Adenylyl cyclases catalyze the conversion of ATP to the second messenger cAMP, whereupon this interaction active catalytic PKA subunits are released. PKA alters a variety of cellular functions, from contractility to patterns of global gene expression, via phosphorylating serine and threonine residues on many proteins. Several important PKA targets are β-ARs themselves, L-type Ca^{2+} channels, the sarcoplasmic reticular Ca^{2+} /ATPase (SERCA) inhibitory protein (21).

β2-ARs induce bronchodilation and relaxation of smooth muscles. Defective β2-mediated vasodilation could result

in both increased arterial resistance and reduced venous compliance. β-ARs are useful targets for exogenously delivered inhibitory drugs, known as β-blockers. Mostly located in brown adipose tissue, the relatively new β3 receptor subtype contributes to the increase of lipolysis in this tissue and is also in charge of thermogenesis in skeletal muscles. There are many SNPs identified in the gene of β-ARs corresponding to different parts in structure (22).

2.2.1. ADRB1

The earliest studies on β1-adrenergic receptor polymorphisms dated back to 1999, where Mason et al (23) showed that replacement of Gly389 with Arginine resulted in a high affinity receptor-G_s complex, which in turn could result in pathological responses, especially in cardiovascular disorders. Similarly, Maqbool et al (24) reported two polymorphisms in a short communication (Ser40Gly and Arg389Gly), but they especially emphasized the substitution of the positively charged arginine for the neutral glycine for its effect in receptor / G protein coupling, thereby reducing efficiency of therapies based on β-AR antagonists.

Since then, numerous studies have examined the genetic variations of β-adrenoceptors and their connection to blood pressure and disorders of the cardiovascular system. Only work from the last 10 to 15 years was covered in order to provide an overview of the most recent advancements.

In a Chinese population study, Arg389Gly (1165 G>C) polymorphism was examined in 93 patients. Based on prior reports on the differential responses of patients with ADRB1 variant to β-blockers, the study analyzed reactions to metoprolol and shown that individuals with CC genotype had better outcomes than those with heterozygous GC mutations (25). A larger cohort with 261 EH patients treated with metoprolol through the same regimen were similarly investigated. Gly/Gly polymorphism in Arg389Gly ADRB1 was found to have a significantly improved metoprolol antihypertensive effect than those with heterozygous ADRB1, Arg389Gly (26).

An early meta-analysis of 5088 EH patients that included case-control trials prior to June 2012 revealed that the Gly allelic frequency of the Arg389Gly polymorphism was substantially lower in EH patients than in controls (27). Similarly, another meta analysis looked into systolic blood pressure, diastolic blood pressure, and hypertension in 29 136 people from 6 cohort studies in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. There was a significant linkage for rs1801253 in ADRB1 (Arg389Gly), with the Gly allele associated with a lower mean systolic blood pressure, diastolic blood pressure and prevalence of hypertension (28). In 2015, Ma et al. also collected and analysed reports on Arg389Gly for heart failure, involving 1736 participants. They were unable to demonstrate any link for this genotype, further confirming that larger cohorts, carefully selected sample population (family history, environmental characteristics, genetic etiology, etc.), and unbiased evaluations are necessary for conclusive interpretations (29).

An intriguing work compared protein mRNA expression levels rather than DNA sample variabilities to investigate the Arg389Gly (rs1801253) and Ser49Gly (rs1801252) polymorphisms in EH patients from the South Indian population. Contrary to earlier findings, this study found that Gly49Gly mRNA levels influenced antihypertensive medication responsiveness; however, they were unable to show any association with the risk of EH or a comparable effect on Arg389Gly (30). Although ADRB1 polymorphisms did not increase the genetic risk of EH, the increased Gly49Gly mRNA levels would indicate a possible contribution to the interindividual variations in drug response. According to a recent study including 147 individuals with hypertrophic cardiomyopathy, the Ser49Gly polymorphism can affect how well a patient responds to beta-blocker metoprolol, however the Arg389Gly polymorphism had no significant effect (31).

2.2.2. ADRB2

ADRB2 plays a potential role in blood pressure regulating by their action on vascular resistance, renin release, and renal sodium excretion (32). At 1998, Timmerman et al (33) reported four intragenic variants at the promoter region and N-terminus of the β2-AR in a study involving the offspring of 23 hypertensive and 22 normotensive European families. The position – 47 variant was substantially more common in children of hypertensive parents, and Arg46Gly at +46 was strongly linked to parental hypertension and elevated blood pressure in this sample pool. All variants were shown to be in linkage disequilibrium. The Arg16Gly and Gln27Glu alterations, which alter the extracellular portion of the receptor, were the main focus of later research; however, conflicting results also emerged as data collected from that time. A study with a large Northern Han Chinese population included 390 healthy participants and 747 hypertension individuals (34). Genotyping was performed to identify the C-47T, A46G and C79G polymorphisms of the ADRB2 gene. Compared to controls, hypertension participants had a substantially higher G allelic frequency of the A46G polymorphism. Linkage disequilibrium was detected between the C-47T, A46G and C79G polymorphisms. According to haplotype analysis, the T-47-A46-C79 haplotype protected against EH, but the T-47-G46-C79 haplotype raised the risk. A small study with a special cohort involving 150 individuals from Chinese Kazakh ethnic group has investigated 5'-UTR in six loci (35). Only the genotype and allele frequency distribution of the rs11168070 (-468C/G) locus showed a significant difference between the normotensive and EH groups. A46G (rs1042713, Arg16Gly) is one of the most studied genetic polymorphism found in ADRB2, and it has been demonstrated that the Arg16→Gly substitution amplifies the agonist-mediated receptor downregulation (36). A recent meta-analysis on the subject showed significant association with the risk of EH. This meta-analysis involved a total of 16 studies containing 3390 cases and 2528 controls (37). Another recent meta

analysis searched for the ADRB2 rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu). These polymorphisms were selected based on their documented impact on the augmentation of vascular resistance and their potential association with raised aldosterone levels, as essential hypertension is a salt-sensitive phenomenon (38). This meta-analysis displayed that ADRB2-rs1042713A allele carriers exhibited significantly lower basal blood flow and attenuated elevation in forearm blood flow as opposed to the G allele, similar to the results of Yan et al (39).

2.2.3. ADRB3

Mainly located in adipose tissue, β3 subtype is implicated in lipolysis, obesity, thermogenesis. The gallbladder, bladder, and brown adipose tissue all contain β3 receptors. Several studies have examined its connection to essential hypertension. One of the most recent papers was written by Li et al (40), who conducted a meta-analysis of 16 research with a total of 95555 patients to examine Trp64Arg polymorphism. Most of these studies focused on Chinese and Japanese ethnic backgrounds, with a smaller percentage of Caucasian people. Some of these studies found favorable connections, while others found none at all.

3. CONCLUSION

If predictors were appropriately categorized, blood pressure regulation may be improved and associated cardiovascular damage could be decreased. The genetic variants identified in the catecholamine pathways in connection with blood pressure regulation and hypertension have been the exclusive focus of this review. Because there aren't many studies released in the recent ten years, the selected works mostly featured meta-analyses on the issue of interest. Most of the studies in the field should be re-evaluated using larger and more controlled cohorts, and there are gaps that need to be filled. Ethnicity, sample power, sex, polygenetic variables or linkage effects, durations and consistencies of applied treatments, the reliability of control groups, etc. are some of the issues that always plague polymorphism investigations and lead to conflicting results.

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *An ethics committee approval was not required for the study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: BE, İA, OO
Design of the study: BE, İA, OO
Acquisition of data for the study: BE, İA, OO
Analysis of data for the study: BE, İA, OO
Interpretation of data for the study: BE, İA, OO
Drafting the manuscript: BE, İA, OO
Revising it critically for important intellectual content: BE, İA, OO
Final approval of the version to be published: BE, İA, OO

REFERENCES

[1] Krushkal J, Xiong M, Ferrell R, Sing CF, Turner ST, Boerwinkle E. Linkage and association of adrenergic and dopamine receptor genes in the distal portion of the long arm of chromosome 5 with systolic blood pressure variation. Hum Mol Genet. 1998;7(9):1379-1383. DOI: 10.1093/hmg/7.9.1379

[2] Morris BJ, Benjafield AV, Lin RC. Essential hypertension: genes and dreams. Clin Chem Lab Med. 2003;41(7):834-844. https://doi.org/10.1515/CCLM.2003.127

[3] Geevarghese M, 3rd, Patel K, Gulati A, Ranjan AK. Role of adrenergic receptors in shock. Front Physiol. 2023;14:1094591. https://doi.org/10.3389/fphys.2023.1094591

[4] Raymond JR, Hnatowich M, Lefkowitz RJ, Caron MG. Adrenergic receptors. Models for regulation of signal transduction processes. Hypertension 1990;15(2):119-131. https://doi.org/10.1161/01.hyp.15.2.119

[5] Wu Y, Zeng L, Zhao S. Ligands of adrenergic receptors: A structural point of view. Biomolecules 2021;11(7): 936. https://doi.org/10.3390/biom11070936

[6] Badino P, Odore R, Re G. Are so many adrenergic receptor subtypes really present in domestic animal tissues? A pharmacological perspective. Vet J. 2005;170(2):163-174. https://doi.org/10.1016/j.tvjl.2004.05.015

[7] Orun O, Nacar C, Cabadak H, Tiber PM, Dogan Y, Guneysel O, Fak AS, Kan B. Investigation of the association between dopamine D1 receptor gene polymorphisms and essential hypertension in a group of Turkish subjects. Clin Exp Hypertens. 2011;33(6):418-421. https://doi.org/10.3109/10641963.2011.561898

[8] Reder NP, Tayo BO, Salako B, Ogunniyi A, Adeyemo A, Rotimi C, Cooper RS. Adrenergic alpha-1 pathway is associated with hypertension among Nigerians in a pathway-focused analysis. PLoS One 2012;7(5):e37145. https://doi.org/10.1371/journal.pone.0037145

[9] Nunes RA, Barroso LP, Pereira Ada C, Krieger JE, Mansur AJ. Gender-related associations of genetic polymorphisms of alpha-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B2 receptor with treadmill exercise test responses. Open Heart. 2014;1(1):e000132. https://doi.org/10.1136/openhrt-2014-000132

[10] Adefurin A, Ghimire LV, Kohli U, Muszkat M, Sofowora GG, Li C, Levinson RT, Paranjape SY, Stein CM, Kurnik D. Genetic variation in the alpha(1B)-adrenergic receptor and vascular response. Pharmacogenomics J. 2017;17(4):366-371. https://doi.org/10.1038/tpj.2016.29

[11] Berends AMA, Bolhuis MS, Nolte IM, Buitenwerf E, Links TP, Timmers H, Feelders RA, Eekhoff EMW, Corssmit EPM, Bisschop PH, Haak HR, van Schaik RHN, El Bouazzaoui S, Wilffert B, Kerstens MN. Influence of receptor polymorphisms on the response to alpha-adrenergic receptor blockers in pheochromocytoma patients. Biomedicines 2022;10(4):896. https://doi.org/10.3390/biomedicines10040896

[12] Sõber S, Org E, Kepp K, Juhanson P, Eyheramendy S, Gieger C, Lichtner P, Klopp N, Veldre G, Viigimaa M, Döring A, Putku M, Kelgo P, Shaw-Hawkins S, Howard P, Onipinla A, Dobson RJ, Newhouse SJ, Brown M, Dominiczak A, Connell J, Samani N, Farrall M, Caulfield MJ, Munroe PB, Illig T, Wichmann HE, Meitinger T, Laan M. Targeting 160 candidate genes for blood pressure regulation with a genome-wide genotyping array. PLoS One 2009;4(6):e6034. https://doi.org/10.1371/journal.pone.0006034

[13] Li JL, Canham RM, Vongpatanasin W, Leonard D, Auchus RJ, Victor RG. Do allelic variants in alpha2A and alpha2C adrenergic receptors predispose to hypertension in blacks? Hypertension 2006;47(6):1140-6. https://doi.org/10.1161/01.HYP.0000217972.80731.ef

[14] Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet. 2009;41(6):666-676. https://doi.org/10.1038/ng.361

[15] Kurnik D, Muszkat M, Li C, Sofowora GG, Friedman EA, Scheinin M, Wood AJ, Stein CM. Genetic variations in the alpha(2A)-adrenoreceptor are associated with blood pressure response to the agonist dexmedetomidine. Circ Cardiovasc Genet. 2011;4(2):179-187. https://doi.org/10.1161/CIRCGENETICS.110.957662

[16] Yagar S, Yavas S, Karahalil B. The role of the ADRA2A C1291G genetic polymorphism in response to dexmedetomidine on patients undergoing coronary artery surgery. Mol Biol Rep. 2011;38(5):3383-3389. https://doi.org/10.1007/s11033-010-0446-y

[17] Kelsey RM, Alpert BS, Dahmer MK, Krushkal J, Quasney MW. Alpha-adrenergic receptor gene polymorphisms and cardiovascular reactivity to stress in Black adolescents and young adults. Psychophysiology 2012;49(3):401-412. https://doi.org/10.1111/j.1469-8986.2011.01319.x

[18] 18. Eldeeb HM, Elgharabawy RM, Abd Elmoniem AE, Ahmed AA. Alpha-2 beta-adrenergic receptor (301-303 I/D) gene polymorphism in hypertension and type 2 diabetes mellitus diseases among Saudi cases in the Qassim region. Sci Prog. 2021;104(2):368504211012162. https://doi.org/10.1177/00368504211012162

[19] Bristow MR, Anderson FL, Port JD, Skerl L, Hershberger RE, Larrabee P, O'Connell JB, Renlund DG, Volkman K, Murray J. Differences in beta-adrenergic neuroeffector mechanisms in ischemic versus idiopathic dilated cardiomyopathy. Circulation 1991;84(3):1024-1039. https://doi.org/10.1161/01.cir.84.3.1024

[20] Bristow MR, Hershberger RE, Port JD, Gilbert EM, Sandoval A, Rasmussen R, Cates AE, Feldman AM. Beta-adrenergic pathways in nonfailing and failing human ventricular myocardium. Circulation. 1990;82(2 Suppl):I12–I25.

[21] Zhu H, Poole J, Lu Y, Harshfield GA, Treiber FA, Snieder H, Dong Y. Sympathetic nervous system, genes and human essential hypertension. Curr Neurovasc Res. 2005;2(4):303-317. https://doi.org/10.2174/156720205774322575

[22] Taylor MR, Bristow MR. The emerging pharmacogenomics of the beta-adrenergic receptors. Congest Heart Fail. 2004;10(6):281-288. https://doi.org/10.1111/j.1527-5299.2004.02019.x

[23] Mason DA, Moore JD, Green SA, Liggett SB. A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. J Biol Chem. 1999;274(18):12670-12674. https://doi.org/10.1074/jbc.274.18.12670

[24] Maqbool A, Hall AS, Ball SG, Balmforth AJ. Common polymorphisms of beta1-adrenoceptor: Identification and rapid screening assay. Lancet 1999;353(9156):897. https://doi.org/10.1016/s0140-6736(99)00549-8

[25] Wu D, Li G, Deng M, Song W, Huang X, Guo X, Wu Z, Wu S, Xu J. Associations between ADRB1 and CYP2D6 gene polymorphisms and the response to beta-blocker therapy in hypertension. J Int Med Res. 2015;43(3):424-34. https://doi.org/10.1177/0300060514563151

[26] Chen L, Xiao T, Chen L, Xie S, Deng M, Wu D. The association of ADRB1 and CYP2D6 polymorphisms with antihypertensive effects and analysis of their contribution to hypertension risk. Am J Med Sci. 2018;355(3):235-239. https://doi.org/10.1016/j.amjms.2017.11.002

[27] Wang H, Liu J, Liu K, Liu Y, Wang Z, Lou Y, Niu Q, Gu W, Wang L, Li M, Zhu X, Wen S. beta1-adrenoceptor gene Arg389Gly polymorphism and essential hypertension risk in general population: A meta-analysis. Mol Biol Rep. 2013;40(6):4055-4063. https://doi.org/10.1007/s11033-012-2483-1

[28] Johnson AD, Newton-Cheh C, Chasman DI, Ehret GB, Johnson T, Rose L, Rice K, Verwoert GC, Launer LJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, Caulfield M, van Duijn CM, Ridker PM, Munroe PB, Levy D; Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium; Global BPgen Consortium;, Women's Genome Health Study. Association of hypertension drug target genes with blood pressure and hypertension in 86,588 individuals. Hypertension 2011;57(5):903-910. https://doi.org/10.1161/HYPERTENSIONAHA.110.158667

[29] Ma ST, Zhao W, Liu B, Jia RY, Zhao CJ, Cui LQ. Association between beta1 adrenergic receptor gene Arg389Gly polymorphism and risk of heart failure: A meta-analysis. Genet Mol Res. 2015;14(2):5922-5929. https://doi.org/10.4238/2015.June.1.9

[30] Varakantham V, Kurakula Sailoo AK, Nagalla B, Bharatraj DK. mRNA expression profile in peripheral blood mononuclear cells based on ADRB1 Ser49Gly and Arg389Gly polymorphisms in essential hypertension - A case-control pilot investigation in South Indian population. Clin Chem Lab Med. 2018;56(8):1230-1237. https://doi.org/10.1515/cclm-2017-0882

[31] Raimoglou D, Izgi C, Enar R, Karpuz MH, Karadag B, Iktimur B, Raimoglu U, Soysal AU, Kargin OA, Guven M, Malikova N, Citak E, Yurtseven E, Durmaz E. Structural and functional impact of adrenoceptor beta-1 gene polymorphism in patients with hypertrophic cardiomyopathy and response to beta-blocker therapy. Anatol J Cardiol. 2024;28(3):150-157. https://doi.org/10.14744/AnatolJCardiol.2023.3898

[32] Liggett SB. Molecular and genetic basis of beta2-adrenergic receptor function. J Allergy Clin Immunol. 1999;104(2 Pt 2):S42-46. https://doi.org/10.1016/s0091-6749(99)70272-1

[33] Timmermann B, Mo R, Luft FC, Gerdts E, Busjahn A, Omvik P, Li GH, Schuster H, Wienker TF, Hoehe MR, Lund-Johansen P. Beta-2 adrenoceptor genetic variation is associated with genetic predisposition to essential hypertension: The Bergen Blood Pressure Study. Kidney Int. 1998;53(6):1455-1460. https://doi.org/10.1046/j.1523-1755.1998.00926.x

[34] Lou Y, Liu J, Li Y, Liu Y, Wang Z, Liu K, Wu H, Niu Q, Gu W, Guo Y, Li Z, Wen S. Association study of the beta2-adrenergic receptor gene polymorphisms and hypertension in the Northern Han Chinese. PLoS One 2011;6(4):e18590. https://doi.org/10.1371/journal.pone.0018590

[35] Cai W, Yin L, Cheng J, Wang S, Wei Y, Cao W, Cheng J. Relationship between the single nucleotide polymorphisms of β_2-adrenergic receptor 5'-regulatory region and essential hypertension in Chinese Kazakh ethnic minority group. International Journal of Clinical and Experimental Pathology.2015;8(7):8358-8366. ISSN:1936-2625/IJCEP0003427

[36] Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor

impart distinct agonist-promoted regulatory properties. Biochemistry1994;33(32):9414-9419. https://doi.org/10.1021/bi00198a006

[37] Yan L, Wang H, Liu P, Wang M, Chen J, Zhao X. Association between the A46G polymorphism (rs1042713) in the beta2-adrenergic receptor gene and essential hypertension susceptibility in the Chinese population: A PRISMA-compliant meta-analysis. Medicine (Baltimore). 2020;99(46):e23164. https://doi.org/10.1097/MD.0000000000023164

[38] Pojoga L, Kolatkar NS, Williams JS, Perlstein TS, Jeunemaitre X, Brown NJ, Hopkins PN, Raby BA, Williams GH. Beta-2 adrenergic receptor diplotype defines a subset of salt-sensitive hypertension. Hypertension 2006;48(5):892-900. https://doi.org/10.1161/01.HYP.0000244688.45472.95

[39] Maamor NH, Ismail J, Malek KA, Yusoff K, Boon-Peng H. AGT, CYP11B2 & ADRB2 gene polymorphism & essential hypertension (HT): A meta-analysis. Indian J Med Res. 2024;159(6):619-626. https://doi.org/10.25259/ijmr_520_23

[40] Li YY, Lu XZ, Wang H, Zhou YH, Yang XX, Geng HY, Gong G, Kim HJ. ADRB3 Gene Trp64Arg polymorphism and essential hypertension: A meta-analysis including 9,555 subjects. Front Genet. 2018;9:106. https://doi.org/10.3389/fgene.2018.00106

How to cite this article: Erdat B, Atlıhan İ, Orun O. Recent Developments in Adrenergic Receptor Polymorphisms in Essential Hypertension. Clin Exp Health Sci 2025; 15: 232-239. DOI: 10.33808/clinexphealthsci.1614451