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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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Development and Psychometric Evaluation of the Symptom Assessment Scale for Turkish Children with Cancer

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ABSTRACT

Objective: The present study was conducted to developed the Symptom Assessment Scale for Children with Cancer (SAS-CC) and assessed its reliability and validity for Turkish children.

Methods: This research was conducted among in 497 children with cancer who were between 7 and 18 years old. The data were collected with a demographic form and SAS-CC. Descriptive statistics, reliability and validity analysis were used to analyze the data.

Results: The mean age of children with cancer was 12.02 ± 3.38 years. The scale consists of 16 items and 3 sub-dimensions. Total factor loads were more than 0.30 in factor analyses. The confirmatory factor analysis revealed all fit indexes as higher than 0.91, and the root mean square error of approximation (RMSEA) was less than 0.080. Cronbach's alpha values of total was 0.96. According to the split-half analysis, α values of the first and second halves were 0.94 and 0.93, respectively.

Conclusions: This study demonstrated that SAS-CC is the first study to develop and test and a valid scale to evaluate symptoms in children with cancer. Effective strategies of coping with symptoms in children with cancer are required to improve prognosis, increase survival, and improve the quality of life. Therefore, assessing symptoms and their frequency in children with cancer is an majority initiative of nurses working in the pediatric oncology clinic.

Keywords: symptom; cancer child; psychometric properties; validity; reliability; SAS-CC

1. INTRODUCTION

Children with cancer suffer several symptoms caused by both cancer and the treatment used. Symptoms here refer to cancer-specific stressors perceived by the patient and those perceived by the patient's parents. Multiple symptoms negatively affect patients' biopsychosocial well-being, decreasing their quality of life. The mean number of multiple symptoms recorded in pediatric oncology patients is 11 to 13 (1).

Although treatment modalities have increased the recovery rates, they can result in adverse side effects such as chemotherapy or radiotherapy as well as symptoms resulting from the illness process itself in the child and the parents (2–4) . Symptoms such as loss of energy, vomiting, oral mucositis, constipation, nutritional problems, and sleep problems are more common than other symptoms (5,6). Children receiving cancer treatment rarely have a single symptom; instead, they experience multiple symptoms and concomitant distress. Multiple symptoms increase the severity of the distress by increasing the effects of each other and resulting in new symptoms (6).The increase in the number and severity of symptoms experienced may put the treatment on hold, reduce the dose of medication, and even discontinue the treatment. Reduced success rates of treatment may decrease the survival of children (7).

Symptom assessment can be used to evaluate symptom relief, compare treatment responses, and increase comfort (8). It is important that nurses carefully evaluate the symptoms experienced by pediatric oncology patients and plan effective interventions (9).This will help to determine the symptom frequency in pediatric oncology patients, identify priorities, and plan appropriate ways to manage symptoms (5,6).Several instruments were created to measure the symptoms of pediatric cancer patients, including the Memorial Symptom Assessment Scale (MSAS), the MD Anderson Symptom Inventory and Checklist for Symptoms in Rotterdam (10,11). As symptoms and distress experienced by pediatric patients with cancer vary significantly from those of adults, there is a need to recognize and describe the several symptoms faced by children in the pediatric oncology community. Extreme symptom discomfort can interrupt designed treatments, the efficacy of chemotherapy protocols, and the processes of recovery (5,6).The MSAS is the only scale available

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for determining the symptoms experienced by pediatric oncology patients in the age of 7-12 years and 10-18 years (12).However, technological and therapeutic developments in recent years and the processes experienced by children with cancer also change. Although treatments are planned so that the side effects are minimal, the use of high-dose drugs in chemotherapy causes children to experience many symptoms. For this reason, measurement tools appropriate for the new situation should be developed by following the changes in treatment and prognosis over time. In addition, it has been reported that other available scales measure the symptoms individually (mucositis, fatigue, etc.) or several symptoms together, with no scale covering all common symptoms (13,14).Therefore, a valid and reliable tool that measures symptoms experienced by children in the other age group of is required. In addition, more valid and reliable tools are required to increase these studies, which are limited in our country.

This study developed the SAS-CC and assessed its validity and reliability in Turkey.

2. METHODS

2.1. Study Design

This methodological, descriptive and correlational study determined the reliability and validity of the SAS-CC scale. The methods used in the study are summarized (Figure 1).

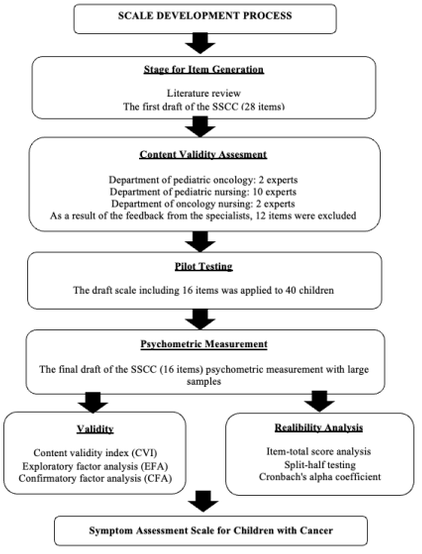

Figure I. *The Development and Validation Process of the SAS-CC*

2.2. Sample Population and Sampling

This study used convenience sampling. According to the literature, a sample size is adequate up to 300 participants and considered excellent up to 1,000 participants for scale development research (15).This study was conducted with children with cancer who were treated at a university hospital in the west of the country. A total of 502 children with cancer who were receiving therapy were interviewed. Five of them did not want to participate in the research. The final group comprised 497 patients with cancer (inpatient *n* = 335, outpatient *n* = 162) who were between 7-12 years (n=284) and 13-18 years (n=213) old, and could read, write, and communicate in Turkish. The patients were recruited from outpatient and inpatient clinics of a hospital in Turkey.

2.3. Ethics Committee Approval

Approval from the Ethics Non-Interventional Study Committee was established at the outset. Institutional permissions were obtained to conduct the study. It received verbal and written approval from children and their parents. Parents and children were also informed about the study before the data were collected. The informed consent form was signed to the parents of the children who agreed to participate.

2.4. Research Stages

Item Generation

The scale items constitute and represent all dimensional aspects of the to be measured variables. Scale items covering all symptoms experienced by children with cancer were created from information obtained from both general and child-specific descriptive and qualitative studies. Because of this literature review, we developed item pools to measure the prevalence of symptoms (5,6,16–21).

Forming Specialist Opinions

At least 10 expert opinions are needed to ensure content validity (17). The content validity of each element was checked in terms of its suitability, importance, and semantic clarification. The panel of experts included those in the field of oncology and child health. First, the 28-item pool was developed. We obtained feedback on the scales from fourteen experts (two professors from the department of pediatric oncology, three professors and seven associate professors from the department of pediatric nursing, and two associate professors from the department of oncological nursing). Experts were asked to rate them between 1 and 4 to determine the compatibility of items on the scale (1 = needs substantial improvement, 4 = very convenient). Twelve items were excluded because they had a validity index of 0.78 for item-based content (I-CVI). Consequently, the last version of the SAS-CC consists of 16 items for three sub-dimensions: general symptoms, gastrointestinal symptoms, and other symptoms.

Preliminary Testing

It is recommended that the scale be administered to a group of 20 or 30 people with similar characteristics, but who are not included in the study sample (18). Twenty children with cancer were invited to evaluate the face validity of the items and rate them for clarity and sentence fluency.

The scale was applied to 40 children who aged 7-12 years (n=20) and 13-18 years (n=20) old and meet the sampling inclusion criteria, but were not involved in the sample. As a result of the preliminary test, the children stated that the items were understandable and clear. In the test, the scale's comprehensibility was calculated to be adequate and then extended to the full sample (7-12 aged and 13-18 aged). The validity and accuracy were evaluated after the application of the scale to a large population.

2.5. Data Measurement Tools

A demographic form and SAS-CC were used for collect of data.

The Demographic Form: This form consisted of four questions, for instance age, sex, diagnosis, treatment place (ambulatory or hospitalized patients).

Symptom Assessment Scale for Children with Cancer (SAS-CC): As previously described, the SAS-CC was developed by researchers depending on the literature to measure the frequency of 16 symptoms experienced (5,6,16).This scale measured the symptoms experienced by children with cancer during the past week. It consisted of three sub-dimensions: general symptoms, gastrointestinal symptoms, and other symptoms. It is a scale of Likert type, with every item in the scale scored from 1 = never, 2=rarely, 3=sometimes, 4 = often. From the scale they received a minimum of 16 and a maximum of 64 points. Higher scores demonstrated more symptoms.

2.6. Statistical Analysis

The data were analyzed using the IBM SPSS Statistics 22.0 (Chicago, IL). Reliability analysis were used to determine the internal consistency of the scale and its sub-dimensions. The content validity index (CVI) and factor analysis were conducted for validity analysis. Using linear structural relations (LISREL), version 10.0 (Scientific Software International, 2019), we carried out a CFA with a complete calculation of the maximum possibility of information. The database was divided into two halves and on the first and second halves, respectively, EFA and CFA analyzes were performed. In addition, Tukey's test and Hotelling's T-square test were used. A margin of error of $p = 0.01$ was used.

3. RESULTS

The mean age of children with cancer in the 7-12 age group was 8.98 ± 1.45, and the mean age of children with cancer in the 13-18 age group was 15.63 + 4.02. Among them, 50.3% ($n = 250$) were girls, and 38.2% ($n = 19$) were diagnosed with acute lymphoblastic or myeloblastic leukemia and 34.2% were diagnosed with central nervous system tumor ($n = 170$), with 67.4% ($n = 335$) receiving treatment in the clinic. Table 1 represents the frequency of symptoms that children suffer in the study.

Table 1. *Symptoms Frequency Experienced by Children with Cancer in the 7-12 age group (n=284) and 13-18 age group (n=213)*

Semptom	Never		Little		Medium		Often	
	n	%	n	%	n	%	n	%
1. Nausea	59	11.9	74	14.9	177	35.6	187	37.6
2. Vomiting	59	11.9	75	15.1	184	37.0	179	36.0
3. Intestinal Changes	56	11.3	77	15.5	183	36.8	181	36.4
4. Change in Taste	53	10.7	82	16.5	183	36.8	179	36.0
5. Difficulty in Swallowing	49	9.9	86	17.3	182	36.6	180	36.2
6. Mucositis	133	26.8	43	8.7	127	25.6	194	39.0
7. Fatigue	47	9.5	103	20.7	145	29.2	202	40.6
8. Energy Loss	55	11.1	105	21.1	139	28.0	198	39.8
9. Weight Loss	76	15.3	126	25.4	135	27.2	160	32.2
10. Loss of Appetite	69	13.9	109	21.9	159	32.0	160	32.2
11. Pain	43	8.7	106	21.3	148	29.8	200	40.2
12. Sweating	298	60.0	39	7.8	115	23.1	45	9.1
13. Dizziness	325	65.4	18	3.6	113	22.7	41	8.2
14. Skin Changes	194	39.0	72	14.5	171	34.4	60	12.1
15. Difficulty in Sleeping	132	26.6	101	20.3	127	25.6	137	27.6
16. Difficulty to Get Attention	268	53.9	63	12.7	63	12.7	103	20.7

3.1. Validity Analyses

Results of content validity analysis; the I-CVIs ranged from 0.80 to 0.98, the scale-level content validity index (S-CVI) was 0.94 and were coherent.

Exploratory factor analysis was used to determine construct validity. Factor analysis revealed the KMO coefficient and Bartlett test X2 value were in Table 2 ($p < .01$). The minimum factor load were be 0.30 and above for the 7-12 aged, 13-18 aged and overall scale (Table 2).

Table 2. *Results of Explanatory Factor Analysis*

Sub-Scale	Items	7-12 aged (n=284)	13-18 aged (n=213)	Overall
First Sub-dimension (Gastrointestinal Symptoms Sub Dimension)	**1**	0.925	0.954	0.941
	2	0.921	0.958	0.944
	3	0.891	0.939	0.927
	4	0.918	0.954	0.937
	5	0.926	0.922	0.927
Second Sub-dimension (General Symptoms Sub-Dimension)	**6**	0.669	0.712	0.747
	7	0.867	0.905	0.910
	8	0.884	0.880	0.899
	9	0.705	0.830	0.737
	10	0.747	0.842	0.758
	11	0.863	0.881	0.893
Third Sub-dimension (Other Symptoms Sub-Dimension)	**12**	0.913	0.880	0.920
	13	0.932	0.895	0.925
	14	0.684	0.709	0.606
	15	0.679	0.702	0.621
	16	0.870	0.865	0.858
Kaiser-Meyer-Olkin		0.907	0.897	0.892
Bartlett's Test of Sphericity		8992.096	6066.388	7086.191

The scale consisted of three sub-dimensions, namely gastrointestinal symptoms, general symptoms, and other symptoms. The total variance for the gastrointestinal symptoms, the general symptoms, the other symptoms and total for 7-12 aged scale accounted for 66.94%, 13.88%, 8.10% and 88.92%, respectively. The total variance for the gastrointestinal symptoms, the general symptoms, the other symptoms and total for 13-18 aged scale accounted for 60.34%, 19.17%, 8.67% and 88.18%, respectively. The total variance for the gastrointestinal symptoms, the general symptoms, the other symptoms and total overall scale accounted for 62.24%, 16.30%, 9.53% and 88.08%, respectively.

Table 3. *Model Fit Indices of the Symptom Assesment Scale for Children with Cancer*

	X^2	DF	X^2/ DF	RMSEA	GFI	CFI	IFI	RFI	NFI	TLI
Three Factor Model	201.04	81	2.481	0.077	0.91	0.99	0.99	0.97	0.98	0.98

The database was divided into two halves and on the first and second halves, respectively, EFA and CFA analyzes were performed. The CFA results showed a three-factor model for SAS-CC items (Figure II). The fit indices and factor loading were presented in Table 3 and Figure II.

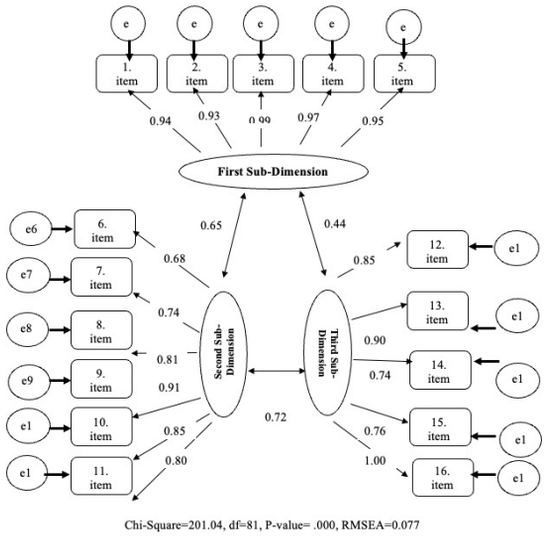

Figure 2. *Confirmatory Factor Analysis of Three Factor Model*

The additivity of the scale was measured using Tukey's additivity test as F = 1.806 and p = .179. The scale was found to be collectable. Hotelling's T-square value was 1117.300 (F = 77.715 and p < .01). No response bias was found in the scale.

Table 4. *Results of the Reliability Analyses of the Scale and Sub-Dimensions (N=497)*

	Overall Cronbach α	7-12 aged Cronbach α	13-18 aged Cronbach α	First half of Cronbach α	Second half of Cronbach α	Spearman-Brown	Guttman split-half	Correlation between two halves	M ± SD (Min-Max)
Scale Total	0.96	0.96	0.95	0.94	0.93	0.86	0.86	0.76	42.55 ± 13.55 (16-64)
First Sub-dimension	0.99	0.98	0.98						14.86 ± 4.93 (5-20)
Second Sub-dimension	0.96	0.96	0.96						17.39 ± 5.83 (6-24)
Third Sub-dimension	0.94	0.94	0.94						10.28 ± 5.08 (5-20)

Table 5. *Correlation of the Item–Total and Item-Sub-Scale Score (N=497)*

Items	Mean + SD	Item-Total Score Correlation *(r)**	Item-Subscale Total Score Correlation (r)*
1. Nausea	2.98 + 1.00	0.74	0.98
2. Vomiting	2.97 + 0.99	0.76	0.99
3. Intestinal Changes	2.96 + 0.99	0.76	0.98
4. Change in Taste	2.96 + 0.99	0.76	0.99
5. Difficulty in Swallowing	2.96 + 0.99	0.75	0.97
6. Mucositis	2.76 + 1.22	0.76	0.77
7. Fatigue	3.01 + 0.99	0.81	0.93
8. Energy Loss	2.97 + 1.02	0.79	0.91
9. Weight Loss	2.77 + 1.06	0.82	0.88
10. Loss of Appetite	2.83 + 1.03	0.84	0.90
11. Pain	3.02 + 0.97	0.81	0.91
12. Sweating	1.81 + 1.07	0.73	0.89
13. Dizziness	1.73 + 1.07	0.71	0.90
14. Skin Changes	2.19 + 1.08	0.78	0.79
15. Difficulty in Sleeping	2.54 + 1.15	0.72	0.75
16. Difficulty to Get Attention	2.00 + 1.22	0.73	0.89

** p<0.001*

3.2. Reliability Analyses

The reliability analyzes results of this study are presented in Table 4.

The correlation value of scale items and item–sub-dimensions with the total scale scores were presented in Table 5.

4. DISCUSSION

We obtained opinions from 14 experts to determine the consistency of items on the scale regarding. Further, we discussed the advices of experts on the phrase and content of items and subsequently excluded certain items from the scale. Minimum values for the number of experts mean the significance of the item. Both I-CVI and S-CVI should be above 0.80, which indicates that the experts agreed (19).Both I-CVI and S-CVI rates were detected above 0.80 in this analysis. The results of I-CVI and S-CVI demonstrated; the scale adequately assessed the subject matter, and the quality of the material was assured.

In this study, a 16-item scale was developed to determine the symptoms experienced by children with cancer. In the literature, it is stated that Memorial Symptom Scale (7-12 years old) has eight items and Memorial Symptom Scale (10-18 years old) has 30 items (12,20).The scale developed in this study has 16 items, which makes it practically feasible and can identify the important symptoms experienced by children with cancer in detail.

In the light of the literature, The Bartlett sphericality test should be statistically relevant and the KMO value should be at least 0.60 for factor analysis (21).Barlett's sphericity test value in this study was $p < 0.05$, and the KMO value was higher than 0.60 for 7-12 aged, 13-18 aged and overall scale. Additionally, the database and sample size is sufficient to evaluate factor (21).

The explained variance in multidimensional scales should be above 40%; higher the overall variance, the greater the validity of the construct. The total variance of 7-12 aged, 13-18 aged and overall scale obtained in this study was above 50%. The tool had a high explained variance. According to this study, the construct validity of the scale is suitable. In general, the minimum factor load should be 0.30 and above and items should be excluded from the scale below this value (21).This result showing that the scale had a strong factor construct in this study.

CFA was used to determine whether the original scale structure was clarified by items and sub-dimensions. CFA evaluates the construction obtained by EFA (22).For the three-factor CFA, factor loadings of the scale were greater than 0.30, the fit indexes were greater than 0.90, and the RMSEA was less than 0.080. We observed a good and significant relation between the tool and its sub-dimensions. In the literature, normal value and acceptable value have been determined for RMSEA, GFI, CFI and NFI. The normal values for these indices are <0.05,> 0.95,> 0.95 and> 0.95, respectively. The acceptable values are <0.08,> 0.90,> 0.90 and> 0.90, respectively. In this analysis, the CFA findings indicated that the data were coherent with the model, the three-factor construct was confirmed, the sub-dimensions were associated with the scale, and the items in each sub-dimensions efficiently defined their factors.

In this analysis, all the factor analysis findings accepted the construct's scale validity, supporting the scale's validity.

α values should be as close to 1. α value between 0.60 and 0.80 indicates a consistent scale. α value between 0.80 and 1.00 indicates that the scale is extremely accurate (23).In this analysis, the alpha values of the α values 7-12 aged, 13-18 aged and overall scale were higher than 0.70, and the α values of the scale and its s sub-dimensions of α values were highly reliable. In addition, The Cronbach's alpha values of the Memorial Symptom Scale (7-12 aged) and Memorial Symptom Scale (10-18 aged) were found to be 0.70 or greater were considered to demonstrate adequate internal consistency. Such results showed that the items measured the subject adequately, the items specific to the subject (23).

α values obtained from the split-half method were more than 0.70. In addition, a clear and important relationship between the halves was established, and the split-half coefficients of both the Spearman-Brown and Guttman were greater than 0.70 (23). These results proved the reliability of the scale.

The T-square test at Hotelling was used to evaluate the scale to assess the presence of bias in response (23). The test revealed that the respondents responded to the items according to their opinions, the participants' responses were different and the scale had no bias in the response, proving the scale was reliable.

Performing an item-total score analysis is a recommended step in the assessment process to determine the extent to which the individual items within the scale accurately measure the targeted variable (21).The correlation coefficients of both the item–total score and the item–sub-scale total scores were positive and greater than 0.20, in this study. Thus, all scale items showed a high correlation with the overall score and the overall score of their sub-dimensions. In our study, the obtained results surpassed the 0.20 threshold, affirming a favorable relationship. The reliability of the scale for the item was high.

4.1. Limitations

Although this study has several strengths, there is three limitation. This analysis used convenience sampling, which can affect the study's generalizability. Another limitation affecting generalizability is single institution. The Parallel Forms Reliability method was not used. In addition, this scale was carried out on the Turkish population. This may limit universal adaptation to locations speaking different languages suggested

5. CONCLUSION

This study demonstrated that SAS-CC is the study to develop and test and a valid scale to evaluate symptoms in children with cancer. This tool can be used in future studies and in pediatric oncology clinics to evaluate symptoms by nurses. Using results obtained from this scale, nurses may improve symptom management practices for children with cancer. Furthermore, it can be used for conducting comparative cross-cultural studies.

Effective strategies of coping with symptoms in children with cancer are required to improve prognosis, increase survival, and improve the quality of life. Therefore, assessing symptoms and their frequency in children with cancer is an majority initiative of nurses working in the pediatric oncology clinic. It is recommended to use this scale in symptom control based drug studies and epidemiological studies. Thus, it will lead the planning of initiatives that will increase the quality of life of children, reduce the burden of caregiving of parents and increase the status of biopsychosocial well-being. They must not only assess symptoms in children with cancer but must evaluate the efficiency of educational and interventional nursing practices providing symptom management using the SAS-CC. In addition, it is recommended that SAS-CC should be used in initiative-based studies to reduce future symptoms, as it helps to clearly demonstrate the symptoms experienced by children with cancer.

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Acquisition of data for the study: AAK
Analysis of data for the study: AAK, MB
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Clinical and Experimental Health Sciences

Efficacy of Bihemispheric tDCS in Rehabilitation of Non-Fluent Aphasia: A Single-subject Pilot Study

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ABSTRACT

Objective: Transcranial direct current stimulation (tDCS) has emerged as a potentially effective complementary tool in rehabilitation of aphasia. However, there is no consensus regarding the optimal tDCS montage to augment language outcomes in aphasia. Against this background, the present study aimed to examine efficacy of tDCS combined with language therapy in aphasia rehabilitation and to compare two different montages.

Methods: A right-handed participant suffering from chronic, non-fluent aphasia following stroke affecting the left hemisphere underwent a 5-week procedure involving tDCS coupled with language therapy. The procedure comprised two 5-day treatments of bihemispheric tDCS (over inferior frontal and posterior temporal sites determined using the international 10-20 EEG system). As part of both treatments, the left hemispheric targets were excited through anodal tDCS while simultaneously inhibiting their right-hemispheric homologues through cathodal tDCS. Baseline, post-treatment and follow-up assessments were obtained using a comprehensive language assessment tool.

Results: An increase in language outcomes, particularly in repetition, was observed following the treatments. It was also found that therapy gains were more robust following bihemispheric stimulation of the posterior temporal sites compared to the inferior frontal targets.

Conclusion: Bihemispheric tDCS coupled with language therapy appears to be effective in remediating language symptoms, particularly in terms of the repetition ability, in aphasia.

Keywords: Aphasia, stroke, tDCS, brain stimulation, rehabilitation

1. INTRODUCTION

Aphasia is an acquired neurogenic language disorder resulting from damage to specific brain regions especially in the left hemisphere and affecting oral and/or written, expressive and/or receptive language skills (1,2). Speech and language therapy offered to persons with aphasia (PWA) is the gold standard in aphasia rehabilitation and there is evidence for its efficacy in improving functional language, language comprehension and production (3). However, it is not clear how long these benefits last (3). In addition, positive therapeutic outcomes have generally been obtained in studies with longer hours of therapy or with an intensive therapy program (4–7). Moreover, PWA often do not fully recover and continue to suffer from a certain level of language difficulty even after therapy (5,8). These limitations highlight the need to complement and support speech and language therapy with other available techniques in aphasia rehabilitation.

Non-invasive brain stimulation (NIBS) techniques including transcranial direct current stimulation (tDCS) have recently been investigated as a potential complement to speech and language therapy. NIBS attempts to trigger neuromodulation of cortical activity associated with language processing. Using tDCS, depending on the montage of the two electrodes (anode and cathode), between which a weak current passes, a target region can be excited (anodal stimulation), inhibited (cathodal stimulation), or both (bihemispheric stimulation) (4). Previous research reported improvement in aphasic symptoms following excitation of the inferior frontal gyrus (IFG) in the lesioned left hemisphere through anodal tDCS (9–11) or inhibition of IFG in the intact right hemisphere through cathodal tDCS (12). Recent reviews also provided support for the effectiveness of tDCS in aphasia rehabilitation (13–15).

Despite the evidence supporting efficacy of tDCS in aphasia intervention, several studies provided little or no support for therapeutic benefits of tDCS in aphasia (16–19). Moreover, there is no agreement on the most ideal montage of the tDCS electrodes (14,20). Also, relatively limited number

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of studies directly compared bihemispheric stimulation of inferior frontal (Broca's area and its right-hemispheric homologue) and posterior temporal (Wernicke's area and its right-hemispheric homologue) sites within the same participant(s). Finally, to our knowledge, no previously published study investigated effectiveness of tDCS treatment in a PWA whose native language is Turkish, a language which is distinguished through its morphosyntactic differences (e.g., rich morphology, free word order) from English, in which most of this line of research was conducted. To fill this gap in the literature, the current study recruited a PWA suffering from chronic, post-stroke non-fluent aphasia, who underwent 5-week intervention encompassing two 5-day treatments of bihemispheric tDCS (to inferior frontal and posterior temporal sites) coupled with concurrent language therapy in addition to pre – and post-treatment assessments and follow-up assessments using a standardized language assessment test.

2. METHODS

Before commencing the study, approval was obtained from the Ethics Committee of Istanbul Medipol University. The participant provided oral consent and her custodian gave written informed consent prior to implementation.

2.1. Participant / Case History

The participant was an 81-year-old, right-handed woman with chronic aphasia following stroke in the left hemisphere 14 months prior to the commencement of the study. She suffered from hemiparesis affecting the right side of her body. She had received 8 years of formal schooling. Before the commencement of the present study, she reported having received speech and language therapy for aphasia for a period of 6 months with 2-3 sessions per week. Language assessment performed by an SLT (first author) using the Language Assessment Test for Aphasia (21) revealed severely impaired expressive skills (repetition and naming) while her receptive skills (auditory comprehension) were relatively spared consistent with non-fluent aphasia (please refer to 2.4.Language Assessments section and Table 1 for further details).

2.2. Study Design

A single-subject, crossover (ABACA) design was adopted. This study design involves a baseline where no treatment is provided (A), followed by a treatment (B), then by a return to the pretreatment baseline level (second A), then by another treatment condition (C), and finally a return to the baseline level again (third A) (22). This study design was adopted to compare two tDCS treatments by investigating the effects of their introduction and removal successively. Two tDCS treatments were coupled with a language therapy and administered to the patient in separate phases of the research. The language therapy was identical in the two treatments, while only the location of the tDCS stimulation differed between the treatments. As illustrated in Figure 1, a baseline language assessment was obtained one day before commencing Treatment 1. Treatment 1 was provided for five consecutive days during week 1. The second language assessment was performed on the next day of completion of Treatment 1, for which it served as a posttest. After a washout period of one week when no treatment was given, the third language assessment was conducted and this served as a follow-up for Treatment 1 and as a baseline for Treatment 2, which commenced on the day following the third assessment. Treatment 2 was administered for five consecutive days, and the fourth language assessment was carried out the day following completion of Treatment 2, for which it served as a posttest. Finally, the fifth language assessment was administered following a washout period of two weeks. This fifth assessment was originally planned one week after Treatment 2, as was the case for the follow-up of Treatment 1, but had to be postponed one week to accommodate the patient's availability. All treatment and assessment procedures were performed in the same setting (a quiet room) in the participant's home.

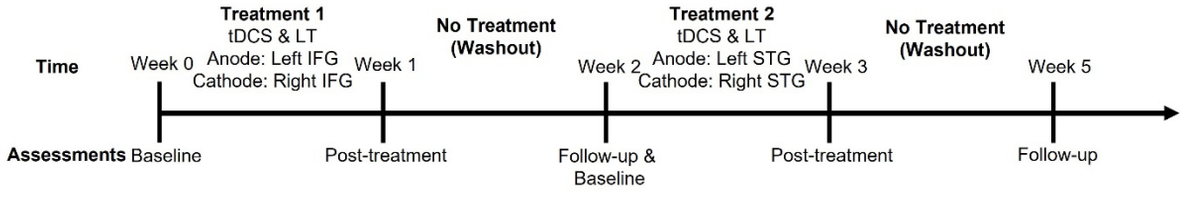

Figure 1. *Study design [LT: Language therapy].*

2.3. tDCS and Language Therapy

tDCS was delivered with a battery-driven direct current stimulator (neuroConn GmbH, Ilmenau, Germany) at 2mA using 5x7cm electrodes inside saline-soaked pads of 7x8cm. In Treatment 1, the anode was placed over Broca's area (left IFG) and the cathode over the homologous region in the right hemisphere (right IFG). In Treatment 2, on the other hand, the anode was placed over Wernicke's area (left posterior STG) and the cathode over its homologue in the right hemisphere (right posterior STG). The stimulation sites were determined using the international 10-20 system with an EEG electrode cap, as illustrated in Figure 2. Specifically, the midpoint of F7-FC5 and of F8-FC6 corresponded to the left IFG and the right IFG, respectively, while the electrodes CP5 and CP6 corresponded to the left posterior STG and the right posterior STG, respectively (15). Each tDCS treatment was administered with a daily dosage of 20 minutes for five consecutive days. The same tDCS parameters were used in Treatments 1 and 2 except for the stimulation sites. No adverse events were observed during or after the completion of the study.

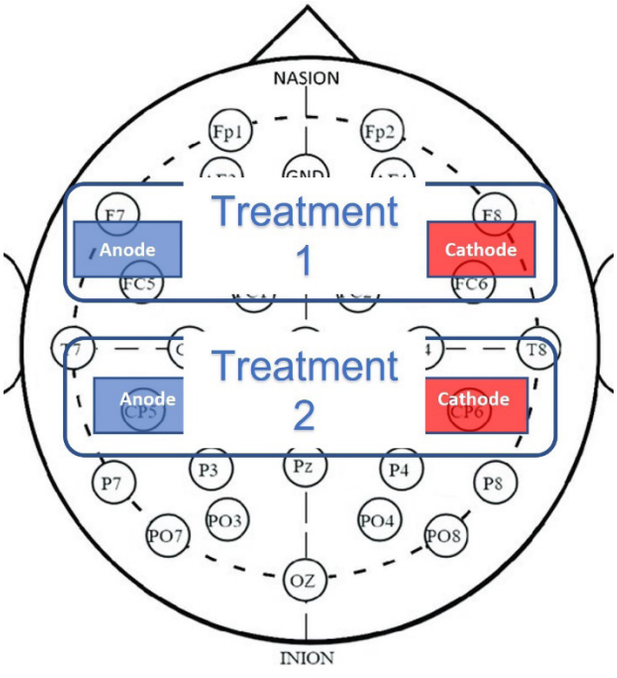

Figure 2. *tDCS montages used in Treatments 1 and 2.*

Language therapy was administered by a speech and language therapist (SLT, first author) to PWA. The therapy was primarily based on the semantic feature analysis approach, which aims to facilitate retrieval of conceptual information through access to semantic networks (23,24). To that end, visual and auditory cues were provided to PWA to support making associations with the relevant word. Specifically, PWA was trained in semantic categories (e.g., fruits, vegetables, furniture) using real-world photographs and drawings of the relevant items. The first 20 minutes of the 40-minute therapy session was provided concurrently with tDCS stimulation. Following completion of the tDCS phase of each therapy session, the electrodes were removed, and the therapy resumed until after forty minutes of therapy had been offered.

2.4. Language Assessments

The Language Assessment Test for Aphasia (ADD) (25) was used to aid diagnosis of the aphasia type and to obtain measures of linguistic performance throughout the study. ADD is a standardized, valid, and reliable language assessment tool commonly used in Turkey to aid diagnosis of aphasia and consists of eight subtests: spontaneous speech and language, auditory comprehension, repetition, naming, reading, grammar, speech acts (pragmatics), and writing (21). The assessment test was administered by the SLT who also provided the language therapy. The test was conducted at baseline, post-treatment and follow-up phases for Treatments 1 and 2, as illustrated in Figure 1. Except for the writing section, all subtests were conducted as part of the current study. However, the speech fluency subtest could not be completed at Times 2 and 3, and two parts of the auditory comprehension subtest could not be administered at Time 2.

The scores on each item of ADD were coded as follows. If the participant answered an item incorrectly or failed to provide an answer, it was coded as a zero. Partial/incomplete answers were coded as 1 if the answer was given using gestures and as 2 if it was given using speech. Likewise, correct answers were coded as 3 if it was given using gestures and as 4 if it was given using speech. The sum of the points taken from each subtest and tasks within each subtest are shown in Table 1 (for the raw data please refer to the online data repository).

2.5. Statistical Analyses

The items which could not be tested at one or more time points (the entire speech fluency subtest and two tasks within the auditory comprehension subtest) were excluded from the analyses. The results were visualized using graphs. In addition, inferential statistics were performed using nonparametric statistical tests, as the collected data were ordinal. The overall and subtest language scores obtained at the five assessment time points were subjected to Friedman tests to examine any differences in test scores across the assessment time points. Any significant effect was then followed up in post-hoc pairwise comparisons using Wilcoxon rank-sum tests with Bonferroni correction to identify specifically which time points differed significantly. Further Wilcoxon rank-sum tests were conducted to compare the change from the baseline to posttests for each treatment. The statistical analyses were conducted in R version 4.1.0 (26) using Friedman and Wilcoxon tests (the analysis code is available in the online data repository).

***Table 1**. Language assessment scores*

Tests	Tasks	Time 1	Time 2	Time 3	Time 4	Time 5
		Pre-Tx1[1]	Post-Tx1	Tx1 Follow-up & Pre-Tx2[2]	Post-Tx2	Tx2 Follow-up
ADD[3] Subtests						
Speech fluency	Spontaneous language, speech and cognition	16/40	-	-	23/40	24/40
	Automatic speech and language	0/24	-	-	8/24	4/24
Auditory comprehension	Understanding commands	9/12	-	9/12	12/12	12/12
	Understanding yes-no questions	15/20	-	16/20	17/20	16/20
	Understanding objects	18/24	15/24	18/24	18/24	18/24
	Understanding categories	12/15	15/15	15/15	15/15	15/15
	Understanding details within categories	6/15	12/15	9/15	9/15	12/15
	Simple sentence-picture matching	9/12	12/12	12/12	9/12	12/12
	Complex sentence-picture matching	9/12	12/12	12/12	9/12	12/12
Repetition	Repetition of words, phrases and sentences	0/40	0/40	0/40	10/40	20/40
Naming	Spontaneous naming of words in a category	0/8	0/8	0/8	0/8	0/8
	Confrontation naming	0/40	2/40	6/40	4/40	10/40
	Naming in response to a question – nouns	6/20	3/20	3/20	3/20	9/20
	Naming in response to a question – verbs	6/20	0/20	0/20	0/20	2/20
Reading	Silent reading of commands	0/16	0/16	0/16	0/16	0/16
	Reading numbers and letters	0/20	0/20	6/20	4/20	12/20
	Reading words	0/20	0/20	0/20	0/20	0/20
	Word-picture matching	1/15	0/15	0/15	0/15	0/15
	Reading paragraphs	0/4	0/4	0/4	0/4	0/4
Grammar	Sentence completion	0/40	2/40	10/40	8/40	14/40
Pragmatics / Speech acts	Giving appropriate responses in context	0/40	0/40	0/40	6/40	12/40

1 Treatment 1
2 Treatment 2
3 Language Assessment Test for Aphasia
Tx1
Tx22

3. RESULTS

3.1. Comparison of Language Scores Across Assessment Times

The language scores obtained at the five time points of assessment are shown in Table 1 above. For illustration purposes, the percentage of the correct responses were calculated using the following formula: (points taken / maximum points possible) * 100 for the overall scores from the entire assessment test (Figure 3a) and for the subtest scores (Figure 3b).

A Friedman test was carried out to compare the overall language scores at the five assessment time points. As summarized in Table 2, the results revealed a significant difference among the overall scores across the five assessment time points, $p < .001$. Post-hoc analyses were conducted using Wilcoxon rank-sum tests with Bonferroni correction to compare each pair of the five time points, resulting in ten pairwise comparisons. Significant differences were found between Time 1 (M = 0.70, Mdn = 0) and Time 5 (M = 1.54, Mdn = 2), $p < .001$, Time 2 (M = 0.76, Mdn = 0) and Time 5, $p = .002$, while marginally significant differences were obtained between Time 3 (M = 0.95, Mdn = 0) and Time 5, $p = .067$. Time 4 (M = 0.99, Mdn = 0) did not differ significantly from any one of the other time points. None of the other pairwise comparisons produced significant results ($p > .104$).

Further Friedman tests were performed on the scores obtained from each subsection of the assessment test. As summarized in Table 2, the results showed that the scores obtained at the five time points differed significantly in the subsections of repetition ($p < .001$), naming ($p = .029$), grammar ($p = .015$) and pragmatics ($p = .035$), while the difference was marginally significant in the auditory comprehension subsection ($p = .064$). There was no significant difference between the scores of the reading subtest across the time points, $p = .105$. Post-hoc analyses were conducted using Wilcoxon rank-sum tests with Bonferroni correction to compare each pair of the five time points for each subtest. Significant differences were found only for the repetition subtest, where Time 5 (M = 2.0, Mdn = 2) was significantly greater than Time 1 (M = 0.0, Mdn = 0), Time 2 (M = 0.0, Mdn = 0) and Time 3 (M = 0.0, Mdn = 0), $p = .004$. None of the other comparisons yielded significant results ($p > .095$).

Table 2. *Results of Friedman tests examining differences across the five assessment time points for overall and subtest language scores*

Language scores	Friedman tests
Overall language scores	**$\chi^2(4) = 54.879, p < .001$**
Subtest scores	
Auditory comprehension	$\chi^2(4) = 8.889, p = .064$
Repetition	**$\chi^2(4) = 25.857, p < .001$**
Naming	**$\chi^2(4) = 10.806, p = .029$**
Reading	$\chi^2(4) = 7.667, p = .105$
Grammar	**$\chi^2(4) = 12.421, p = .015$**
Pragmatics / Speech acts	**$\chi^2(4) = 10.341, p = .035$**

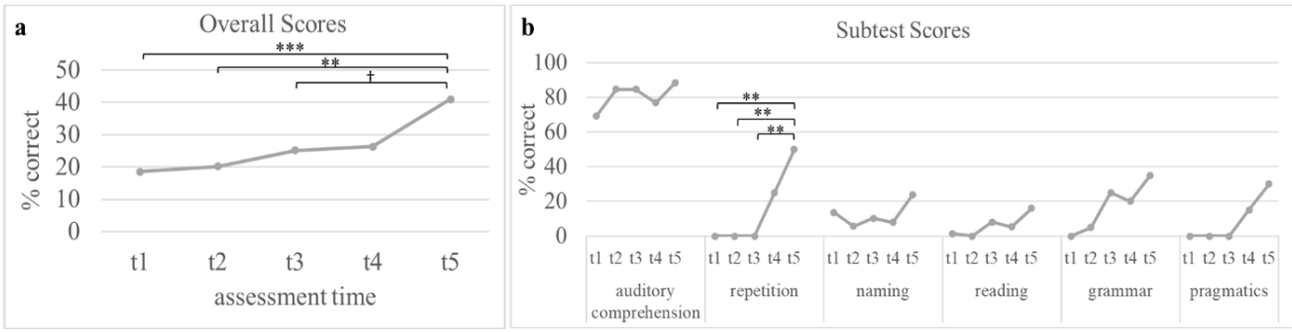

Figure 3. *Percentage of the correct responses at each assessment time for the entire assessment tool (a) and for each subtest (b) [Asterisks indicate statistical significance* (*** p < .001, ** p < .01, † p = .067)].

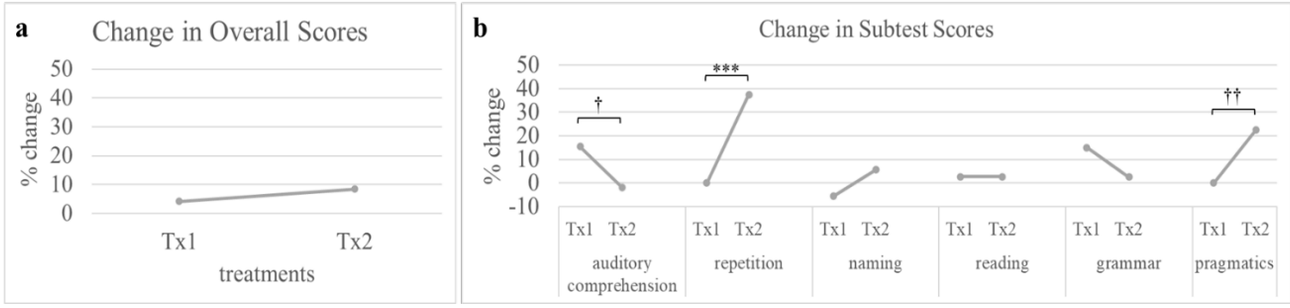

Figure 4. *Percentage of pre-to-post change in language scores associated with Treatments 1 and 2 for the entire assessment tool (a) and for each subtest (b)* [Asterisks indicate statistical significance (*** p < .001, † p = .063, †† p = .078)].

3.2. Comparison of Change in Scores Associated with Treatments

To directly compare the pre-to-post change or gain in language scores associated with Treatment 1 and Treatment 2, difference scores were calculated for each treatment using the following formula: Average of two posttest – pretest scores. In other words, the assessment scores at Time 1 (baseline) were subtracted from the average of the scores at Times 3 and 4 (posttests) to estimate the change in scores associated with Treatment 1, and the scores at Time 3 (baseline) were subtracted from the average of the scores at Times 4 and 5 (posttests) for Treatment 2. Therefore, for each treatment, the difference scores were obtained through comparison of the baseline and post-treatment assessment scores both for the overall scores from the entire assessment test (Figure 4a) and for the subtests (Figure 4b).

The analysis of the difference scores using a Wilcoxon rank-sum test did not produce a significant difference between the changes in the overall scores associated with Treatment 1 (M = 0.16, Mdn = 0) and Treatment 2 (M = 0.32, Mdn = 0), p = .12. Further Wilcoxon tests were conducted on the difference scores from the subtests of the assessment tool to compare the changes associated with the two treatments for different language skills. These analyses showed that the pre-to-post change for Treatment 2 (M = 1.50, Mdn = 1) was significantly greater than that for Treatment 1 (M = 0.0, Mdn = 0) in the repetition subtest (p < .001). Likewise, the change for Treatment 2 (M = 0.90, Mdn = 0) was greater than Treatment 1 (M = 0.0, Mdn = 0) in the pragmatics subset, albeit with marginal significance (p = .078). Also, the change in auditory comprehension following Treatment 1 (M = 0.50, Mdn = 0) was more than the change in the same domain after

Treatment 2 (M = – 0.06, Mdn = 0) with marginal significance (p = .063). The comparisons of the pre-to-post changes in the scores of the other subtests did not produce significant results (p > .13).

4. DISCUSSION

The present study compared efficacy of two bihemispheric tDCS treatments with frontal versus posterior montages paired with language therapy. We observed a significant increase in overall language outcomes following both treatments, particularly in the repetition subtest. However, the rehabilitative effect was greater for Treatment 2 with the posterior montage, and some differences were observed between the two treatments in terms of the subset scores. These findings suggest that bihemispheric tDCS treatment combined with language therapy is effective in remediating language performance in non-fluent aphasia, and that treatment gains in particular linguistic subskills may vary depending on the montage used.

We found that combining language therapy with tDCS resulted in improvement of language performance by a non-fluent PWA. This improvement was observed in the overall language scores measured in the final follow-up assessment (Time 5) when compared to the first baseline measurement (Time 1) as well as the second and third assessment times. This finding is consistent with a large body of research on the use of brain stimulation including tDCS and repetitive transcranial magnetic stimulation in rehabilitation of aphasia, which has shown promising, positive effects (4,13–15,27,28). However, in contrast with these previous studies and the findings of the present study, several studies failed to show a significant effect of tDCS on language outcomes in aphasia, or showed only weak evidence for its efficacy (16,18,29,30). The inconsistent findings might be related to variable stimulation parameters including electrode montage (anodal only, cathodal only, or bihemispheric), number and length of stimulation sessions, language outcomes assessed, as well as patient characteristics (e.g., aphasia type, severity, lesion location). Indeed, a recent meta-analyses of randomized controlled trials utilizing non-invasive brain stimulation interventions found differences among tDCS electrode montages, with the bihemispheric tDCS outperforming only anodal and only cathodal montages in terms of naming and repetition gains (28). Utilizing bihemispheric tDCS montages, our study also yielded similar findings. That is, although examination of the subset scores showed a tendency for all subtest scores (auditory comprehension, repetition, naming, reading, grammar, pragmatics) to increase from the initial tests to the final assessment; we found that only the increase with treatment in the repetition subtest remained significant after correction for multiple comparisons. This indicates that the gain in the outcomes across the treatments was more robust in the repetition task. This finding is consistent with previous research implicating tDCS treatment with improvement in performance of PWA in repetition (28,31–34).

To investigate any differences between the two tDCS montages, the pre-to-post change in language scores associated with each treatment was compared. Although Treatment 2 with the posterior temporal montage was associated with numerically greater overall gain in language performance than Treatment 1 with the inferior frontal montage, this difference was not statistically significant. This suggests that the two different montages adopted in the present study did not differ to a great extent in terms of the overall language outcomes. However, certain differences were observed between the two treatments in terms of the subset scores. In particular, the analyses of the subset scores showed that Treatment 2 brought about significantly greater pre-to-post change than Treatment 1 in the repetition subtest. Although Treatment 2 was also associated with numerically greater improvement in the naming and pragmatics subtests, this difference did not reach statistical significance. Finally, the change following Treatment 1 was numerically, but not significantly, greater than that following Treatment 2 in the auditory comprehension and grammar subsections. Overall, the greater improvement in language outcomes was observed in Treatment 2 compared to Treatment 1 in terms of the repetition performance. These findings suggest that therapy gains in particular language subskills may vary as a function of the specific montage used. In parallel with these findings, several previous studies associated excitation of the left posterior temporal cortex or inhibition of the right posterior temporal cortex with improved outcomes in aphasia (35–37). However, a few other studies failed to reveal significant improvement in language outcomes following stimulation of posterior compared to frontal sites (9,10). These differences might stem from differences across studies in terms of participant characteristics and stimulation parameters, as suggested in the preceding paragraph.

The present finding that bihemispheric tDCS with excitatory anodal stimulation of left-hemispheric language centers and inhibitory cathodal stimulation of their right-hemispheric homologues is associated with improvement in aphasic symptoms is consistent with the interhemispheric inhibition framework (4,8,38–40). Within this framework, while normally the left and the right cerebral hemispheres strike a desirable balance through mutual inhibition, this balance may be lost through a neurologic insult to the typically language-dominant left hemisphere, which may result in decreased inhibition of the right hemisphere and, hence, maladaptive overcompensation of the latter in linguistic functions. Arguably, this imbalance can be modulated either through excitation of the intact, language-related areas in the left hemisphere or through inhibition of their homologues in the right hemisphere, or both. The present study has provided evidence that combining language therapy with bihemispheric tDCS to simultaneously excite the left-hemispheric language-related areas and inhibit the homotopic regions in the right hemisphere facilitates improvement in language outcomes in chronic, non-fluent aphasia.

Although the current study allowed us to test effectiveness of tDCS coupled with language therapy in aphasia rehabilitation and to compare two different bihemispheric montages, several limitations need to be kept in mind while interpreting the results. First, although initially planned, we could not include a sham control condition in the study since the participant declined it. Inclusion of a sham control condition with a similar tDCS setup but without therapeutic stimulation could have minimized potential confounds due to a placebo effect. However, we believe that a placebo effect alone cannot explain the current results, because the participant did not exhibit a steady increase in assessment scores throughout the study phases, and, instead, there were differences between the gains associated with the two tDCS treatments. Moreover, these differences in gains between the treatments were not uniform across different subtests. A second limitation concerns the design and sample size. Since only a single subject was included in the present pilot study, it is difficult to make strong conclusions regarding generalizability of the study findings. However, there are certain advantages of using a within-subject, crossover design over between-subject, case-control designs, as the former reduces heterogeneity (e.g., differences in language symptoms and other participant characteristics) (14). Nevertheless, to establish causality between tDCS intervention and improvement in aphasic symptoms, randomized controlled trials that also consider participant characteristics and differences are needed. Third, in the current study, tDCS treatments were coupled with language therapy; therefore, it is not possible to attribute the improvement observed following the treatments solely to tDCS. However, since we found a difference between the two tDCS treatments, which comprised identical language therapy, tDCS appears to have provided unique contribution to the improvement in language outcomes. More importantly, we do not consider this as a limitation, since speech and language therapy remains to be the gold standard in aphasia rehabilitation (3) and tDCS may serve as an inexpensive and convenient complementary, adjuvant tool for speech and language therapy (4,36).

5. CONCLUSION

The current study investigated efficacy of two bihemispheric tDCS treatments coupled with language therapy, one targeting the inferior frontal cortex (Treatment 1) and the other targeting the posterior temporal cortex (Treatment 2). In both treatments, the left-hemispheric, language-related areas were excited through anodal tDCS while simultaneously inhibiting their right-hemispheric homologues through cathodal tDCS. The findings revealed an increase in overall language outcomes following the treatments, most markedly in the repetition subtest. This therapeutic effect was greater for Treatment 2 with a posterior montage. Since the rehabilitative effect was observed following anodal, excitatory stimulation in the lesioned left hemisphere with simultaneous cathodal, inhibitory stimulation of the corresponding regions within the intact right hemisphere, the findings support the interhemispheric inhibition account of neuromodulation in aphasia. Overall, the findings suggest that tDCS may be a convenient adjuvant tool that can support speech and language therapies in aphasia rehabilitation.

Data Availability Statement: *The data and statistical analysis codes that support the findings of this study are openly available in the online data repository at https://doi.org/10.17605/OSF.IO/ANCUX.*
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Research idea: DGA, TB
Design of the study: DGA, TB, ÇK
Acquisition of data for the study: DGA
Analysis of data for the study: TB
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Clinical and Experimental Health Sciences

Choice of Empirical Treatment in Patients with Wound Infection

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ABSTRACT

Objective: We aimed to determine the distribution of infectious agents in wound culture specimens, their resistance rates, and to evaluate empirical treatment choices in wound infections.

Methods: Wound culture results of adult patients between 2016-2020 were retrospectively investigated. Determination of bacteria and antibiotic sensitivity tests were done using conventional methods and automatized systems.

Results: A total of 2576 wound specimens were sent, and significant bacterial growth was detected in 1254 (48.7%). Most frequently isolated agent was *Escherichia coli (E.coli)* (24.2%), followed by *Staphylococcus aureus (S.aureus)* (16.8%). The highest rate of resistance in *Enterobacterales* species was against amoxicillin-clavulanate (AMC), except *Proteus mirabilis.* Antibiotics that *Enterobacterales* species were most sensitive were amikacin and carbapenems, while it was trimethoprim – sulfamethoxazole (TMP-SXT) for *Acinetobacter baumannii,* and amikacin for Pseudomonas aeruginosa. The highest rate of resistance in S.aureus strains was against penicillin, with a methicillin resistance rate of 22.9%, while no resistance was found against vancomycin.

Conclusion: Initial treatment in wound infections is empirical, and the range of treatment is narrowed when results of culture and sensitivity tests are obtained. Clindamycin, AMC, TMP – SXT and ciprofloxacin seem to be appropriate for outpatients, while TMP-SXT or vancomycin for gram-positive cocci, and TMP-SXT and amikacin combination for gram – negatives, and carbapenems as a last resort.

Keywords: Wound infection, empirical treatment, antibiotic resistance

1. INTRODUCTION

Wound infections (WI), which result from infection of the skin and soft tissues (SST) with pathogenic microorganisms, are frequently encountered. They may be seen in a wide spectrum, ranging from a clinically mild infection to life-threatening serious necrotizing infections. The responsible agents may differ according to the site of infection and patient risk factors, which include age, co-morbidities, immune deficiency, circulatory disorders, long – term hospitalization, trauma and contact with animals (1-4). Clinical features and risk factors of the patient are considered in the diagnosis and treatment of SST infections, culture samples are obtained from the wound for antibiotic sensitivity tests, and empirical treatment is initiated (5). Penicillins effective against gram-positive microorganisms, penicillins resistant to penicillinase, cephalosporins, macrolides, linkosamides, fluoroquinolons and trimethoprim – sulfamethoxazole (TMP-SXT) are preferred for empirical treatment. Vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitalized patients, and carbapenems for resistant gram-negative bacteria are also used (4,6).

In our hospital, oral amoxicillin-clavulanate, ciprofloxacin or TMP-SXT are used empirically in outpatients with SST infections, while intravenous (iv) vancomycin or teicoplanin, piperacillin-tazobactam or carbapenem are used alone or in combinations in hospitalized patients. We aimed to determine the distribution of microorganisms growing in wound culture samples of outpatients and hospitalized patients in our center, to determine the antibiotic resistance rates, and provide guidence for choosing empirical antibiotics.

2. METHODS

Our study was approved by the Ethics Committee of Clinical Investigations of Balıkesir University (21.10.2020, approval Nr 2020/188). Samples for culture obtained from wounds of ambulatory or hospitalized adult patients with injector or

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swab between 2016-2020 were evaluated at the Microbiology Laboratory. Our hospital is a secondary care state hospital with 400 patient beds and 54 intensive care unit beds.

All wound samples were stained with Gram stain on slides, examined with direct microscopy, and the results were entered into the laboratory data management system (LDMS). Leukocyte density, presence of epithelium, and presence of mixed flora bacteria were evaluated by the microscopic examination. Since the localization of wound samples is not always specified when sending them to the laboratory, such a classification was not made in the study.

All samples were inoculated in blood agar (RTA, Türkiye), Eosin Methylene Blue (EMB) agar (RTA, Türkiye) and chocolate agar (RTA, Türkiye), and were incubated at 37°C for 24-48 hours. Strains that were isolated were identified by conventional methods (colony morphology, gram staining, catalase, coagulase, oxydase, urease tests) and by BD Phoenix 100 automated identification system (BD Phoenix System, Beckton Dickinson, US). Culture samples in which mixed skin flora bacteria grew were considered as skin contamination. In vitro susceptibility tests of isolates which were considered as infectious agents were determined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) (7) criteria, using the Phoenix TM 100 automated identification and antibiotic susceptibility test system (BD Phoenix System, Beckton Dickinson, US). The first isolate was evaluated in patients with multiple samples. Possible results obtained from the automated system were reported in detection of extended spectrum beta lactamase (ESBL), without confirmation tests. Methicilline resistance in *S.aureus* strains were investigated by using the MIC value of cefoxitin at the automated system.

2.1. Statistical Analysis

All the data were recorded and statistical analysis was performed with SPSS 22.0 (SPSS INC, Chicago, IL, US) software. Categorical data were presented as percents. Chi-Square test was used in comparison of independent groups containing categorical variables. p values below .05 were considered as statistically significant.

3. RESULTS

A total of 2576 wound specimens were sent to our laboratory in five years, in 1185 of which (46.0%) microbial growth was not detected, and mixed skin flora bacteria grew in 137 (5.3%). A significant, pure growth was detected in 1254 samples (48.7%), and these were included in this study. Also 65.6% of these 1254 wound samples were taken from hospitalized patients (clinics and intensive care unit), and 34.4% were obtained from outpatients (Table 1). The clinics were general surgery, orthopedic surgery, plastic surgery and internal medicine. Among the wound cultures, 71.5% were Gram negative bacteria, 28.3% were Gram positive bacteria and 0.2% were fungi. The most frequently isolated organism among all samples was *Escherichia coli (E.coli)* (24.2%), followed by *Staphylococcus aureus* (*S.aureus)* (16.8%). When samples obtained from ambulatory and hospitalized patients were separately evaluated, *S.aureus* was the most frequently isolated microorganism in outpatients while *E.coli* was the most frequently isolated microorganism in hospitalized patients (both surgical/medical clinics and intensive care units). The other microorganisms isolated from 1254 wound samples included *Pseudomonas aeruginosa (P.aeruginosa)* (11.5%), *Klebsiella pneumonia (K.pneumoniae)* (10.3%), *Acinetobacter baumannii (A.baumannii)* (8.8%), *Proteus mirabilis (P.mirabilis)* (8.6%), *Streptococcus pyogenes (S.pyogenes)* (8.3%), other *Enterobacterales* species (7.1%), other Gram positive bacteria (3.2%), other nonfermenter Gram negative bacteria (1%) and *Candida* spp. (0.2%), respectively (Table 2).

Table 1. *Distribution of wound culture samples in which growth was detected according to clinic and years (n/%)*

Year	Outpatients		Hospitalized patients in clinics		ICU* patients		Total
	n	%	n	%	n	%	
2016	95	36.3	107	40.8	60	22.9	**262**
2017	88	34.4	102	39.8	66	25.8	**256**
2018	94	34.3	119	43.4	61	22.3	**274**
2019	90	37.3	78	32.4	73	30.3	**241**
2020	65	27.0	63	26.1	93	46.9	**241**
Total	**432**	**34.4**	**469**	**37.4**	**353**	**28.2**	**1254**

**ICU:Intensive care unit*

Table 2. *Distribution of agents isolated from wound cultures (n/%)*

Microorganism*	Outpatients		Hospitalized patients in clinics		ICU** patients		Total	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
E.coli	59	13.7	158	33.7	86	24.3	303	24.2
S.aureus	127	29.4	76	16.2	7	2.0	210	16.8
P.aeruginosa	42	9.7	40	8.5	62	17.4	144	11.5
K.pneumoniae	20	4.6	51	10.9	58	16.4	129	10.3
A.baumannii	19	4.4	31	6.6	60	17.0	110	8.8
P.mirabilis	36	8.3	27	5.8	45	13.4	108	8.6
S.pyogenes	82	19.0	19	4.1	3	0.8	104	8.3
Other *Enterobacterales*	37	8.6	40	8.5	12	3.2	89	7.1
Other gram positive bacteria	7	1.6	23	4.9	10	2.7	40	3.2
Other NFGN bacteria	1	0.2	2	0.4	9	2.5	12	1.0
***Candida* spp.**	2	0.5	2	0.4	1	0.3	5	0.2
Total	432	34.4	469	37.4	353	28.2	1254	48.7

**Microorganisms; E.coli: Escherichia coli, S.aureus: Staphylococcus aureus, P.aeruginosa:Pseudomonas aeruginosa, K.pneumoniae: Klebsiella pneumoniae, A.baumannii:Acinetobacter baumannii, P.mirabilis: Proteus mirabilis, S.pyogenes: Streptococcus pyogenes, Other NFGN bacteria: Other non-fermenting Gram negative bacilli*

***ICU:Intensive care unit*

The highest rate of resistance in *Enterobacterales* species, except *P.mirabilis* was against amoxicillin-clavulanate (AMC), whereas the highest rate of resistance in *P.mirabilis* was against trimethoprim-sulfametoxazole (TMP-SXT). *Enterobacterales* species showed the highest sensitivity to amikacin and carbapenems. A statistically significant increase was observed in resistance rates of *E.coli* strains against AMC, ceftriaxon and carbapenem, while piperacillin-tazobactam (TZP) resistance showed a significant decrease (p< .001, p< .001, p= .003, p< .001). Significant changes were observed over the years in resistance rates of *K.pneumoniae* against AMC, gentamicin, ceftriaxon, ciprofloxacin, TZP and TMP-SXT (p< .001, p< .001, p= .015, p= .014, p< .001). ESBL rates were 55.1% in *E.coli* strains, 52.7% in *K.pneumoniae*, showing a statistically significant increase in years (p< .001, p< .001) (Table 3).

The highest resistance rates in *A.baumannii* and *P.aeruginosa* were against ciprofloxacin. TMP-SXT was the antibiotic that *A.baumannii* was most susceptible, while amikacin was the antibiotic that *P.aeruginosa* was most susceptible. Carbapenem resistance rates showed a significant increase for both organisms in time (p= .001, p< .001) (Table 4).

Highest resistance rate in *S.aureus* strains was against penicilline, with a methicilline resistance rate of 22.9%, and a significant increase was detected in resistance in time (p=.033) (Table 5).

Table 3. *Antibiotic resistance profiles of bacteria of Enterobacterales species growing in wound cultures according to years (%)*

		AMC	AK	GEN	CRO	CIP	CARB	TZP	TMP-SXT	ESBL
E.coli **(n=303)**	**2016**	50.0	6.3	29.7	42.2	42.2	1.6	43.8	46.9	40.6
	2017	72.9	6.8	32.2	50.8	52.5	0.0	23.7	50.8	50.8
	2018	84.5	5.2	36.2	50	48.3	3.4	17.2	53.4	63.8
	2019	65.8	8.9	40.5	54.4	53.2	7.6	19.0	48.1	53.2
	2020	69.8	2.3	25.6	74.4	53.5	0.0	11.6	51.2	74.4
	***Total**	68.0	6.3	33.7	53.1	49.8	3.0	23.8	49.8	55.1
	p	**< .001**	**= .297**	**= .201**	**< .001**	**= .395**	**= .003**	**< .001**	**= .916**	**< .001**
K.pneumoniae **(n=129)**	**2016**	73.7	10.5	52.6	73.7	68.4	21.1	52.6	68.4	20.9
	2017	89.7	13.8	48.3	72.4	65.5	17.2	58.6	72.4	58.6
	2018	100	20.6	47.1	52.9	47.1	17.6	38.2	41.2	35.3
	2019	80.0	16.0	32	68.0	60.0	20	60	60	64
	2020	54.5	9.1	27.3	63.6	54.5	9.1	50	68.2	54.4
	***Total**	82.2	14.7	41.9	65.1	58.1	17.1	51.2	60.5	52.7
	p	**< .001**	**= .129**	**< .001**	**= .015**	**= .017**	**= .173**	**= .014**	**< .001**	**< .001**
P.mirabilis **(n=108)**	**2016**	12.5	0.0	37.5	6.3	37.5	6.3	0.0	50	-
	2017	28.6	4.8	42.9	4.8	61.9	0.0	0.0	71.4	-
	2018	47.1	5.9	47.1	0.0	76.5	0.0	0.0	76.5	-
	2019	29.4	29.4	64.7	29.4	76.5	5.9	11.8	76.5	-
	2020	18.9	2.7	40.5	10.8	56.8	0.0	2.7	51.4	-
	***Total**	25	7.4	45.4	10.2	61.1	1.9	2.8	63.0	-
	p	**< .001**	**< .001**	**= .001**	**< .001**	**< .001**	**= .001**	**< .001**	**< .001**	**-**
Other *Enterobacterales* **Species (n=83)**	**2016**	16.7	3.3	3.2	13.3	16.7	3.3	10.0	6.7	**-**
	2017	60	0.0	10.0	10.0	5.0	0.0	5.0	15	-
	2018	73.3	0.0	0.0	0.0	13.3	0.0	0.0	13.3	**-**
	2019	80	0.0	0.0	0.0	20.0	10.0	0.0	20.0	**-**
	2020	100	38.5	53.8	92.3	53.8	23.1	15.4	46.2	**-**
	***Total**	59	7.2	12	21.7	20.5	6.0	7.2	13.9	**-**
	P	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**	**-**

**E.coli: Escherichia coli, K.pneumoniae: Klebsiella pneumonia, P.miarbilis: Proteus mirabilis*

AMC:Amoxycilline-Clavulanate, AK:Amikacin,GEN:Gentamicin, CRO:Ceftriaxon, CIP: Ciprofloxacin, CARB:Imipenem-Meropenem, TZP: Piperacillin-Tazobactam, TMP-SXT:Trimethoprim-Sulphametoxazole, ESBL: extended spectrum beta lactamase

Table 4. *Antibiotic resistance profiles of Acinetobacter baumanni and Pseudomonas aeruginosa strains growing in wound cultures according to years (%)*

Antibiotic	*A.baumannii* (n=110)							*P.aeruginosa* (n=144)						
	2016	2017	2018	2019	2020	*Total	*p*	2016	2017	2018	2019	2020	*Total	*p*
AK	70.4	50.0	64.5	90.0	86.4	**70.0**	**< .001**	13.9	9.1	0.0	0.0	16.0	**8.3**	**< .001**
GEN	96.3	75.0	90.3	100	95.5	**90.9**	**< .001**	19.4	12.1	12.0	24.0	36.0	**20.1**	**< .001**
CARB	92.6	85.0	83.9	100	86.4	**88.2**	**= .001**	19.4	6.1	8.0	12.0	28.0	**14.6**	**< .001**
CAZ	-	-	-	-	-	**-**		36.1	18.2	36.0	8.0	24.0	**25.0**	**< .001**
FEP	-	-	-	-	-	**-**		33.3	18.2	36.0	8.0	24.0	**24.3**	**< .001**
TZP	-	-	-	-	-	**-**		33.3	21.2	28.0	8.0	32.0	**25.0**	**< .001**
TMP-SXT	66.7	60.0	67.7	100	68.2	**69.1**	**< .001**	-	-	-	-	-	**-**	
CIP	100	85.0	87.1	100	90.9	**91.8**	**< .001**	36.1	24.2	12.0	32.0	52	**31.3**	**< .001**

* *AK:Amikacin,GEN:Gentamicin, CARB:Imipenem-Meropenem,CAZ:Ceftazidim, FEP:Cefepim, TZP: Piperacillin – Tazobactam, TMP-SXT:Trimethoprim-Sulphametoxazole, CIP: Ciprofloxacin,*

Table 5. *Antibiotic resistance profiles of Staphylococcus aureus strains growing in wound cultures according to years (%)*

Antibiotic	*S.aureus* (n=210)						
	2016	2017	2018	2019	2020	*Total	*P*
GEN	17.0	15.6	8.3	15.4	7.0	**12.9**	**= .105**
PEN	100	82.2	94.4	94.9	88.4	**91.9**	**< .001**
CC	4.3	15.6	11.1	17.9	11.6	**11.9**	**= .028**
E	8.5	8.9	8.3	20.5	14.0	**11.9**	**= .026**
TE	6.4	26.7	41.7	41.0	79.1	**38.1**	**< .001**
CİP	8.5	6.7	8.3	10.3	4.7	**7.6**	**= .725**
TMP-SXT	4.3	02.2	8.3	5.1	0.0	**3.8**	**= .039**
VA	0.0	0.0	0.0	0.0	0.0	**0.0**	**-**
TEI	0.0	0.0	0.0	0.0	0.0	**0.0**	**-**
DAP	0.0	2.2	2.8	0.0	0.0	**1.0**	**= .089**
LIN	0.0	0.0	0.0	0.0	0.0	**0.0**	**-**
MET	17.0	22.2	31.3	28.2	34.9	**22.9**	**= .033**

* *GEN:Gentamicin, PEN:Penicillin, CC: Clindamycin, E: Erythromycin, TE: Tetracycline, CIP: Ciprofloxacin, TMP – SXT:Trimethoprim-Sulphametoxazole, VA: Vancomycin, TEI: Teicoplanin,DAP:Daptomycin, LIN:Linezolid, MET: Methicillin*

4. DISCUSSION

Wound infections affect the skin and soft tissues, and cause morbidity and mortality. Wound culture samples are important both for supporting the diagnosis and guidence of treatment. It is important to be careful in obtaining samples for culture in order to avoid contamination with skin flora bacteria (8-10). A significant growth was detected in 48.7% of culture samples in our study, with growth of mixed skin flora bacteria in 5.3%. This reflects an acceptable level of contamination in our hospital, while it also reflects the fact that we have to pay still more attention for antisepsis in obtaining wound samples.

While SST infections may more frequently be seen in outpatients as non-complicated forms, they may be encountered as complicated forms in hospitalized patients. Gram positive bacteria such as *S.aureus* and *Streptococcus* spp. are frequently isolated in non-complicated infections, and Gram negatives such as *E.coli*, *P.aeruginosa, K.pneumoniae* and *P.mirabilis* are more frequently found in complicated infections (4,8,11,12). The most frequently isolated agent in SST infections in a study conducted in 28 countries was *S.aureus,* according to European Antimicrobial Resistance Surveillance (EARS) data. In another study, it was reported that gram-negative microorganisms were frequently associated with surgical site infection, and *E.coli* and *P.aeruginosa* were frequently isolated (13).

Avcıoğlu et al (14) have isolated 53.0% gram negative bacteria, 46.4% gram positive bacteria and 0.6% fungi from wound samples of outpatients and hospitalized patients between 2016 – 2018. When compared with our study, the rate of Gram negative bacteria was found to be higher in our study. We think that this may reflect the fact that most of the samples in the study by Avcıoğlu et al (14) was from

outpatients, while most of the samples in our study were from hospitalized patients.

In the 1-year study by Eren et al (15) evaluating neurological intensive care unit (ICU) patients, *A.baumannii* and *S.aureus* were most frequently isolated as the pathogen agent of SST infection. Sisay et al (8) have detected 36% *S.aureus*, 13% *E.coli,* 9% *P.aeruginosa*, 9% *K.pneumoniae,* and 8% *P.mirabilis* in wound samples in their analysis of 21 studies conducted in Etiopia between 2000-2018. Tanrıverdi Çaycı et al (16) have found *E.coli* (20.5%), *S.aureus* (12.7%) and *P.aeruginosa* (11.6%) as the most frequent three microorganisms in their study evaluating outpatients and hospitalized patients between 2015-2017.

We detected *E.coli*, *S.aureus* and *P.aeruginosa* as the most frequent three microorganisms among 1254 culture specimens included in our study. On the other hand, when the patients were evaluated in three separate group consisting of outpatients, hospitalized patients and ICU patients, the most frequently isolated organisms were *S.aureus* (29.4%) and *S.pyogenes* (19.0%) in outpatients; *E.coli* (33.7%) and *S.aureus* (16.2%) in hospitalized patients, and *E.coli* (24.3%) and *P.aeruginosa* (17.4%) in ICU patients. Also, *E.coli*, *P.aeruginosa, A.baumannii, K.pneumoniae and P.mirabilis* was detected in 88.5% of wound culture specimens of ICU patients. First, it is necessary to know the distribution of pathogen agents in a certain clinic, in order to determine a rational empirical treatment. In this respect, the choice of empirical treatment for ICU patients may aim on Gram negative agents, while a combination treatment aiming both Gram negative and positive agents may be more beneficial for ambulatory and hospitalized patients.

When SST infections develop, culture specimens are obtained, after which empirical treatment is initiated with an antimicrobial agent with the highest probability to be effective against this organism, and the spectrum of treatment is narrowed after culture and antibiogram results are obtained. With this approach, success rate of treatment is increased and emergence of resistant bacteria is prevented with rational use of antimicrobials (17-19).

AMC is used against gram positive bacteria in outpatients in our hospital for the empirical treatment of SST infections, and ciprofloxacin or TMP-SXT is used against gram negatives. We found *S.aureus* as the most frequently isolated agent in outpatients, and penicillin resistance was 91.9%. This resistance rate shows that penicillin is not an appropriate choice in empirical treatment, even when gram staining shows gram positive cocci. Also, resistance against clindamycin was 11.9% in *S.aureus* strains. In *Enterobacterales* species, resistance against AMC was 25-82.2%, while resistance was 10.2 – 65.1% against ceftriaxon, 10.2-65.1% against ciprofloxacin, and 13.9-63% against TMP – SXT. Considering that the antibacterial activity of AMC against gram positive microorganisms is stronger than ceftriaxone, AMC is a better choice for infections suspected to be due to gram positives in outpatients, while clindamycin is better for clinical anaerobic infection suspicion, and ciprofloxacin or TMP-SXT for suspicion of gram negative infections, as their resistance rates are similar. The oral form of these four antimicrobial agents may be preferred for empirical treatment in outpatients. Also, as treatment have to be initiated in outpatients mostly before results of microscopic examinations are obtained, the procedure used in our hospital seem to be appropriate.

Resistance rates reported in different studies evaluating wound cultures of outpatients and hospitalized patients are 81.4-100% against penicillin, 8.9-24% against ciprofloxacin, 3.5 – 11% against TMP-SXT, and 7.6-23.1% against clindamycin in *S.aureus.* Also methicillin resistance was between 16.7-36%, while resistance against vancomycin and teicoplanin was not reported. In *Enterobacterales* species, resistance against AMC was 44-72%, and TZP was 9 – 61%, ciprofloxacin was 18-72.9%, TMP-SXT was between 10-65.2%, similar to our study (14,20-22).

Vancomycin or teicoplanin (iv) and TZP (iv) are used alone or in combination in the treatment of SST infections in hospitalized patients (4), which is also used commonly in our hospital. Vancomycin and teicoplanin are medications that are used as a last resort in the treatment of MRSA, and they are not appropriate for empirical treatment (23). We did not find resistance against vancomycin or teicoplanin in our study, but we found resistance against daptomycin, which also has a place in MRSA treatment. It is obvious that resistance against these agents will develop in the future, if they are not used rationally. As *S.aureus* is a frequently encountered agent in hospitalized patients, we believe that the decision to include vancomycin in empirical treatment should be based on the results of gram staining.

Another empirical treatment option is TZP, which is a broad-spectrum antimicrobial also having anti-pseudomonal activity (17). In our study, resistance was below 24% in *Enterobacterales* species, except *K.pneumoniae,* which was 51.2%, while resistance rate was 25% in *P.aeruginosa*. For this reason, considering the distribution of Gram negative agents, it seems to be the appropriate choice for hospitalized patients.

Carbapenems are important in the treatment of resistant Gram negative infections. As the patients in ICU were frequently treated with broad-spectrum antibiotics in initiation of empirical treatment, and shifting to an agent with narrower spectrum according to culture– antimicrobial sensitivity test results seem to be more rational. Therefore, iv carbapenem is used in the empirical treatment of UTI in hospitalized patients (17,24).

Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR), one of the members of which is our country, determine infectious agents in blood and spinal fluid samples and find their resistance rates. According to their data, carbapenem resistance in *E.coli* have increased from 2% – 3% between 2015-2019, while increasing from 30% to 39% in *K.pneumoniae*, from 32% to 38% in *P.aeruginosa*, and from 89% to 90% in *A.baumannii* at the same period. Methicilline resistance in *S.aureus* was reported to increase from 25% in

2015 to 31% in 2019 (25,26). In our study, it was observed that carbapenem resistance reached 23.1% in *Enterobacterales* species, 28% in *P.aeruginosa*, and varied between 83.3-100% in *A.baumannii* over a five-year period. According to this observation, carbapenems seem to be the most probable choice in empirical treatment of patients with a history of broad-spectrum antibiotic use in the past or history of microbial growth of resistant Gram negative microorganisms, as in ICU patients. Although the evaluated specimens were not the same as ours in CAESAR data, a similar resistance problem in especially *K.pneumoniae,* similar to *P.aeruginosa* is obvious. Thus, carbapenem use requires great care. On the other hand, carbapenems are not effective against infections with *A.baumannii*. Amikacin and TMP-SXT combination also does not seem to be appropriate, in light of resistance rates that we have found. While colistin is an important antibiotic in the treatment of these infections, it was not included in the evaluation because it could not be studied with the broth microdilution method as in EUCAST recommendations in our hospital. This is a limitation of our study.

As resistance to antibiotics may change in time, the change in resistance rates according to years were also evaluated in our study, and statistical analysis were done. Methicilline resistance in *S.aureus* strains seem to increase significantly over the years. TZP resistance in *E.coli* strains seems to decrease significantly in time, resistance to ceftriaxone seems to increase, and a significant decrease for AMC was seen between 2018-2019. The rates of resistance to AMC, ceftriaxone and TMP-SXT in *K.pneumoniae* have changed significantly over the years; an increase was seen in resistance of *A.baumannii* to carbapenem, and a significant change in *P.aeruginosa* for both carbapenem and TZP resistance. Thus, it appears that detection of frequently seen infectious agents at periodical examinations, and determination of the antibiotic resistance distribution will be helpful in decision on selection of empirical treatment (27).

5. CONCLUSION

Skin and soft tissue infections are diagnosed clinically, after which empirical treatment is initiated and treatment is narrowed after results of culture-antibiograms are obtained. Gram stain slides prepared on the same days with cultures are important, as they yield results on the same day and they are helpful to the clinician in antibiotic selection. Collaboration between the microbiology laboratory and the clinicians is very important in this respect. Clindamycin, AMC, TMP-SXT and ciprofloxacin for outpatients, TMP-SXT or vancomycin for gram positive cocci, TZP, TMP-SXT and amikacin combination for the therapy of gram negative microorganisms caused infections, and carbapenems as a last resort seem to be appropriate choices in empirical treatment.

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Clinical and Experimental Health Sciences

Prevalence and Associated Factors of Mental Health Problems Among Peripartum Women During the COVID-19 Pandemic

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ABSTRACT

Objective: This study aimed to determine the prevalence and associated factors of probable depression and probable anxiety in early postpartum women during the COVID-19 pandemic.

Methods: This cross-sectional study was conducted with early postpartum women who applied to a maternity hospital to give birth in Turkey between March-June 2021. Women aged 19-45 years, with 23–42 weeks of gestation, with a singleton pregnancy, and negative for the SARS-CoV-2 polymerase chain reaction test were included in the study. The Hospital Anxiety and Depression Scale (HADS) was used to assess the presence of probable depression (HADS depression score >7) and probable anxiety (HADS anxiety score >10). The associations between women's sociodemographic and obstetric characteristics and depression and anxiety were evaluated using univariate and multivariate analyses.

Results: A total of 450 women were included in the study. Of these, 50.2% (n=226) had probable depression, and 28% (n=126) had probable anxiety. Multivariate analysis revealed that while perceived poor income level increased the odds for the presence of probable depression, unintended pregnancy, anemia, and SARS-CoV2 infection during pregnancy were associated with probable anxiety.

Conclusion: The presence of probable depression and probable anxiety were considerably high among women who had given birth during the pandemic. This study identified the most vulnerable groups in terms of mental health problems among women who were in the early postpartum period during the pandemic. It is essential to develop strategies to prevent and control the mental health problems of these risk groups for future emergency health crises.

Keywords: Anxiety, COVID-19, depression, pandemic, early postpartum

1. INTRODUCTION

Pregnant and postpartum women are vulnerable in terms of psychiatric problems, including depression and anxiety. Preserving mental well-being in the perinatal (it covers the 21st week of pregnancy and one month after birth) period is essential to prevent the negative impact of these problems on health that may develop in both the mother and the child (1). Studies showed that risks to children of postpartum depressed mothers include problems such as excessive crying, poor cognitive function, behavioral inhibition, feeding and sleep problems, and psychiatric and medical disorders in adolescence (2). In addition, it has been reported that postpartum anxiety may cause negative effects on breastfeeding, mother-infant interaction, infant temperament, sleep, and mental development (3).

A growing body of research has reported that psychosocial stressors (such as cultural differences, low income, discrimination, and lack of social support) increase the risk of developing postpartum anxiety and depression, along with demographic factors and a history of psychiatric and psychological problems (3, 4). The COVID-19 pandemic that emerged in Wuhan, China, led to a global crisis, and measures such as social distancing and isolation/quarantine were taken to control the pandemic. The deprivation of social and family

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support due to these procedures applied during the pandemic and the unpredictability of COVID-19 significantly impacted perinatal mental health (5). Perinatal depression and anxiety rates increased compared to the pre-pandemic period, associated with mothers' fear of transmitting the virus to their infants, limited access to health care, and lack of social support (6-8).

The early postpartum period is critical, as maternal mental problems may significantly affect the health and development of the infant. Psychiatric screening of new mothers before they are discharged from the hospital is essential for early detection of those prone to developing mood disorders and anxiety and for providing them psychological support. It is important to explore how the pandemic affects women's mental health in the early postpartum period, when depression and anxiety symptoms are most likely to occur (9, 10). This study aimed to find the probable depression and probable anxiety prevalences in the early postpartum period of women who got pregnant and gave birth during the pandemic and determine the factors that increased the risk. We hypothesized that different sociodemographic, medical, and obstetric factors would impact the occurrence of probable depression and probable anxiety in the early postpartum period.

2. METHODS

2. 1. Study Permissions and Ethical Statement

The study was approved by the Ethics Committee of Zeynep Kamil Women and Children Disease Training and Research Hospital (Number: 65, Dated: 03/2021). All procedures in our study were carried out per the 1964 Declaration of Helsinki and subsequent amendments. Informed written consent was obtained from the participants.

2. 2. Setting and Study Participants

This cross-sectional study was carried out between 01 March 2021 and 01 June 2021 at a Gynecology and Pediatrics Training and Research Hospital in Istanbul, Turkey. Our hospital is a leading referral hospital that continued to provide uninterrupted services to low – and high-risk pregnant women during the pandemic. The study population consisted of women who had given birth in the hospital and were followed up in the postpartum clinic. The inclusion criteria were early postpartum women aged 19-45 years, between 23-42 weeks of gestational age, with a singleton delivery, and a negative SARS-CoV-2 polymerase chain reaction (PCR) test upon admission to the delivery room. Exclusion criteria were any previously diagnosed psychiatric disease, multiple pregnancies, and pregnancies with a fetal anomaly, suspected SARS-CoV-2 infection, or a positive SARS-CoV-2 PCR test at the time of admission, stillbirth or termination of pregnancy, and birth at ≤22 weeks of gestation.

Gestational age was calculated using the first day of the last menstrual period and confirmed by the first-trimester crown rump length (CRL). Sociodemographic and medical data concerning the birth process were obtained from the hospital's electronic records and patient files. For evaluating depression and anxiety, the Hospital Anxiety and Depression Scale (HADS) was used as a self-administered questionnaire (11). The questionnaire was conducted 48-72 hours after birth, before discharge from the hospital.

The SARS-CoV-2 PCR test includes real-time PCR (qPCR) (RT-qPCR) analysis by targeting the RdRp gene fragment. During the study period, all pregnant women who had applied to our hospital for delivery were screened for SARS-CoV-2 regardless of symptoms. Swab samples from the oropharynx and nasopharynx were used for PCR analysis. The laboratory had been authorized by the Ministry of Health of the Republic of Turkey, General Directorate of Public Health, Microbiology Reference Laboratory. We also checked the results of the SARS-CoV2 PCR tests performed previously to identify the women who had contact with a COVID-19 case during their pregnancy through electronic patient records.

The sample size was calculated as 379, assuming a depression prevalence of 56%, a margin of error of 0.05, and a 95% confidence level (12).

2. 3. Definition and Measurement of the Variables

2. 3. 1. Outcomes

The Hospital Anxiety and Depression Scale (HADS) was used to assess the presence of probable anxiety and probable depression. The HADS was developed by Zigmond and Snaith in 1983 and has been used to determine the risk and severity of anxiety disorder and depression (11). The 4-point Likert-type scale consists of 14 questions scored between 0 and 3. Seven questions (with odd numbers) on the scale measure anxiety, and the rest evaluate depression. The lowest score of both subscales is zero, and the highest is 21. The validity and reliability study of the Turkish version of the HADS was performed by Aydemir et al., and Cronbach's alpha value of the scale was determined as 0.81 (13). The cut-off values were 10 for the anxiety subscale and 7 for the depression subscale. Those who score above these points are considered at risk for anxiety and depression. The purpose of the scale is not to diagnose, but to identify risk groups by scanning anxiety and depression in a short time (14, 15). Those with scores above the threshold for the anxiety and depression subscales in HADS were referred to a psychiatrist for clinical evaluation.

2. 3. 2. Independent variables

Participants' sociodemographic, medical, and obstetric characteristics and the COVID-19 history (symptoms and hospitalization due to COVID-19) were evaluated.

The definitions of perinatal outcomes (gestational diabetes mellitus [GDM], preeclampsia, small for gestational age [SGA], low birth weight [LBW], and intrahepatic gestational cholestasis) were determined according to the international criteria (16-20).

2. 4. Statistical Analysis

Statistical analyses were performed using SPSS Statistics version 17.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). Descriptive statistics were presented as medians (interquartile ranges [25th-75th percentiles]) for the continuous variables

and frequencies and percentages for the categorical variables. The associations between individual characteristics and anxiety and depression were evaluated with univariate and multivariate logistic regression analysis (backward stepwise method). Variables with p< .1 or those shown as associated in the literature were included in the multivariate model. The strengths of the associations were defined by Odds Ratios (OR) and 95% confidence intervals (95% CI). p< .05 was considered the statistical significance level.

3. RESULTS

A total of 450 early postpartum women were included in the study. Fifteen women were excluded due to SARS-CoV-2 PCR positivity upon admission, six due to suspected infection, eight due to multiple pregnancy, 30 due to a fetal anomaly, and six due to insufficient data.

Table 1. *Sociodemographic and medical characteristics of the participants and their spouses (n=450)*

		n (%)
Age (years)	19-24	80 (17.8)
	25-34	268 (59.6)
	35-45	102 (22.7)
Body mass index (kg/m^2)	Obese (≥30 kg/m^2)	257 (57.1)
	Non-obese (<30 kg/m^2)	193 (42.9)
Educational level	Primary school	98 (21.8)
	Middle school	114 (25.3)
	High school	144 (32.0)
	University or higher	94 (20.9)
Perceived income level	Low	67 (14.9)
	Middle	341 (75.8)
	High	42 (9.3)
Parity	Nulliparity	136(30.2)
	Multiparity	314 (69.8)
Working status	Employed	96(21.3)
	Unemployed	354 (78.7)
Loss of job during the pandemic*		5 (5.2)
Smoking during pregnancy		55 (12.2)
Chronic diseases		126 (28.0)
In vitro fertilization (IVF)		8 (1.8)
Unintended pregnancy		93 (20.7)
Attending routine prenatal care	Yes	353 (87.3)
	No	57 (12.7)
Characteristics of spouse		
Age (years)	19-24	19 (4.2)
	25-34	235 (52.2)
	≥35	196 (43.6)
Educational level of spouse	Primary school	99 (22.0)
	Middle school	132 (29.3)
	High school	134 (29.8)
	University or higher	85 (18.9)
Smoker		260 (57.8)
Spouse lost his job during the pandemic		45 (10.0)
**n=96*		

Table 2. *Obstetric and neonatal outcomes of the participants*

Obstetric outcomes		n (%)
Birth	Vaginal delivery	159 (35.3)
	Cesarean section	291 (64.7)
Cesarean section*	Emergency cesarean section	182 (62.5)
	Elective cesarean section	109 (37.5)
Indication of cesarean section	Primary cesarean section	77 (26.5)
	Previous cesarean history	214 (73.5)
At least one antenatal risk factor during current pregnancy		153(34.0)
Spontaneous preterm birth		36 (8.0)
Preterm premature rupture of the membranes (PPROM)		17 (3.8)
Gestational diabetes mellitus		78 (17.3)
Hypertensive disease	No hypertension	399 (88.7)
	Gestational hypertension	11 (2.4)
	Preeclampsia-Eclampsia	35 (7.8)
	Chronic hypertension	1 (0.2)
	Chronic hypertension superimposed preeclampsia	4 (0.9)
Neonatal outcomes		
Infant gender	Girl	231 (51.3)
	Boy	219 (48.7)
Neonatal intensive care unit admission		107 (23.8)
APGAR <7		6 (1.3)
SGA infant**		42 (9.3)
LGA infant**		95 (21.1)
Low birth weight (<2500 grams)		58 (12.9)
Macrosomia (≥4000 grams)		22 (4.9)
Neonatal death		2 (0.4)
**n=291; ** according to intergrowth2; SGA: small for gestational age; LGA: large for gestational age*		

The sociodemographic and health data of the participants and their spouses are presented in Table 1. The median age (25th and 75th percentiles) was 30.0 (26.0-34.0). The obstetric and neonatal period characteristics of the participants are shown in Table 2. Cronbach's alpha value for the HADS scale was 0.807. The medians (25th and 75th percentiles) of the HADS-depression and HADS-anxiety scores were 8.0 (5.0-10.0) and 8.0 (5.0-11.0), respectively. Of the participants, 50.2% (95,0% CI: 45.5-54.9, n=226) had probable depression (HADS depression score >7), and 28% (95,0% CI: 23.9-32.4, n=126) had probable anxiety (HADS anxiety score >10).

3. 1. Regression Analysis

Univariate analysis revealed that probable depression was more prevalent in those who had primary school education compared to those with university and higher degrees, those with a perceived low-income level compared to those with a high-income level, and those whose spouses had primary or middle school education compared to those with higher education (Table 3 and 5).

Table 3. *Sociodemographic and medical factors associated with depression and anxiety, univariate analysis (n=450)*

		Depression OR [%95 CI]	**Anxiety** OR [%95 CI]
Age (years)	35-45	0.895 [0498-1.609] p= .710	1.033 [0.542-1.968] p= .922
	25-34	0.823 [0.499-1.357] p= .445	0.928 [0.533-1.614] p= .791
	19-24	*reference*	*Reference*
BMI ≥ 30 kg/m^2		1.384 [0.951-2.013] p= .089	1.147 [0.755-1.743] p= .519
Educational level	Primary school	**1.804** [1.018-.197] **p= .043***	0.997 [0.525-1.891] p= .992
	Middle school	1.681 [0.969-2.919] p= .065	0.986 [0.531-1.830] p= .964
	High school	1.334 [0.790-2.254] p=0.282	1.255 [0.704-2.235] p= .442
	University or higher	*reference*	*reference*
Unemployment		1.023 [0.597-1.472] p= .832	0.377 [0.700-1.880] p= .587
Loss of job during the pandemic		0.936 [0.256-10.047] p= .753	5.625 [0.042-1.689] p= .348
Perceived income level	Low	**2.722** [1.210-6.125] **p= .016***	**2.933** [1.075-8.001] **p= .036***
	Middle	0.843 [0.444-1.601] p=.602	2.420 [0.988-5.925] p= .053
	High	*reference*	*reference*
Multiparity		1.056 [0.706-1.580] p= .789	1.116 [0.710-1.755] p= .634
Smoking		1.442 [0.814-2.551] p= .209	1.558 [0.861-2.818] p= .143
Having chronic disease		0.828 [0.548-1.250] p= .369	1.221 [0.778-1.916] p= .385
Unintended pregnancy		1.325 [0.448-1.124] p= .179	**2.241** [1.390-3.613] **p< .001***
Having an obstetric risk factor in the previous pregnancy		0.954 [0.0863-2.468] p= .646	**1.739** [1.129-3.320] **p< .001***
Age of the spouse (years)	19-24	***reference***	***reference***
	25-34	0.619 [0.236-1.628] p= .331	3.461 [0.778-15.389] p= .103
	≥35	0.527 [0.199-1.394] p= .197	3.400 [0.760-15.201] p= .109
Educational level of the spouse	Primary school	**1.891** [1.049-3.407] **p= .034***	1.552 [0.807-2.987] p= .188
	Middle school	**1.891** [1.086-3.293] **p= .024***	1.266 [0.675-2.374] p= .503
	High school	1.576 [0.907—2.737] p= .107	1.240 [0.661-2.323] p= .753
	University or higher	*reference*	*reference*
Spouse lost his job during the pandemic		1.553 [0.829-2.809] p= .169	**2.257** [1.204-4.230] **p= .011***

**p< .05. BMI: Body mass index*

Table 4. *Obstetric and neonatal factors associated with depression and anxiety, univariate analysis (n=450)*

		Depression OR [%95 CI]	**Anxiety** OR [%95 CI]
At least one obstetric risk factor during current pregnancy		1.180 [0.812-1.713] p= .386	1.501 [0.993-2.271] p= .054
Spontaneous preterm birth		1.455 [0.730-2.901] p= .287	1.012 [0.473-2.165] p= .975
Hypertensive disease	No hypertension	-	-
	Gestational hypertension	1.194 [0.359-3.976] p= .773	1.520 [0.436-5.296] p= .511
	Preeclampsia-Eclampsia	0.940 [0.471-1.876] p= .860	1.219 [0.578-2.573] p= .603
	Chronic hypertension	0.995 [0.139-7.137] p= .996	0.887 [0.091-8.617] p= .918
	Chronic hypertension superimposed preeclampsia	*Reference*	*Reference*
Gestational diabetes mellitus		1.023 [0.646-1.715] p= .837	1.360 [0.806-2.296] p= .250
Anemia		1.130 [0.682-1.871] p= .636	**2.781** [1.655-4.673] **p< .001***
Did not breastfeed within one hour of birth		1.401 [0.960-2.046] p= .081	**1.595** [1.053-2.418] **p= .028***
At least one complication during postpartum period		1.384 [0.752-2.548] p= .297	1.233 [0.643-2.365] p= .528
Gestational week at birth		0.997 [0.986-1.008] p= .594	0.990 [0.979-1.002] p= .105
Neonatal intensive care unit admission		0.772 [0.499-1.194] p= .244	0.998 [0.615-1.617] p= .992

**p< .05*

Probable anxiety was more common among women with a perceived low-income level compared to those with high-income, whose spouses lost their job during the pandemic, whose pregnancy was unintended, who had obstetric risk factors in the previous pregnancy, who had anemia during pregnancy, who did not breastfeed within the first hour of birth, who had COVID-19 during pregnancy, and who had a contact with a COVID-19 case during pregnancy (Table 3-5).

Table 5. *The association of depression and anxiety with COVID-19 related features in early postpartum women, univariate analysis (n=450)*

Risk factors	Depression OR [%95 CI]	Anxiety OR [%95 CI]
COVID-19 during pregnancy	0.657 [0.414-1.041] p= .074	**1.874** [1.158-3.030] **p= .010***
Contacted with a COVID-19 case during pregnancy	0.867 [0.553-1.359] p= .534	**2.056** [1.282-3.298] **p= .003***
Having a relative died due to COVID-19	1.100 [0.582-2.078] p= .769	1.865 [0.970-3.587] p= .062
Symptoms due to COVID-19 during pregnancy	0.670 [0.291-1.541] p= .346	0.629 [0.270-1.461] p= .281
Hospitalization due to COVID-19 during pregnancy	2.971 [0.516-17.110] p= .223	3.437 [0.596-19.829] p= .167

**p< .05*

Multivariate analysis showed that probable depression was more frequent in those with a perceived low-income level compared to those with a high-income level. Having a SARS-CoV2 infection during pregnancy did not increase the risk of depression (Table 6). In addition, probable anxiety was more common in those whose pregnancy was unintended, who had anemia during pregnancy, and who had SARS-CoV2 infection during pregnancy (Table 6).

Table 6. *Factors associated with depression and anxiety, multivariate analysis*

Risk factors		Depression[a] positive, *n*=226 negative, *n*=224	Anxiety[b] positive, *n*=126 negative, *n*=326
BMI ≥ 30 kg/m^2		1.386 [0.943-2.035] p= .096	-
Perceived income level	Low	**2.666** [1.178-6.034] **p= .019***	-
	Middle	0.829 [0.434-1.582] p= .569	-
	High	*Reference*	-
Unintended pregnancy		-	**2.218** [1.362-3.615] **p= .001***
Anemia during pregnancy		-	**2.514** [1.475-4.288] **p= .001***
COVID-19 during pregnancy		0.630 [0.392-1.012] p= .056	**1.658** [1.005-2.735] **p= .048***

**p< .05*

[a]Age of early postpartum women, obesity, perceived income level, educational level of spouse, and COVID-19 during pregnancy were included in the analysis.

[b]Age of early postpartum women, obesity, perceived income level, women whose spouse lost job during the pandemic, unintended pregnancy, anemia during pregnancy, COVID-19 during pregnancy, and having a relative died due to COVID-19 were included in the analysis.

4. DISCUSSION

In this study, we determined the presence of probable depression in 50.2% and probable anxiety in 28% of early postpartum women. Multivariate analysis revealed that while perceived poor income level was related to probable depression; unintended pregnancy, anemia during pregnancy, and SARS-CoV2 infection during pregnancy were associated with probable anxiety.

Perinatal depression, which was 10-20% before the pandemic, reached higher rates during the pandemic. In the study by Zhang et al. in China during the COVID-19 period, perinatal depression was reported as 34% (21). In two reviews evaluating the frequency of postpartum depression, most of which used online questionnaires and included participants with middle-high incomes, the prevalences of postpartum depression were reported as 28% and 34% (22, 23). In another study conducted in Spain, postpartum women who had given birth during the pandemic had higher depression rates than the ones who had given birth previously (37.3% vs. 22.4%, respectively) (24). Two web-based online surveys conducted during the first wave of the pandemic in Turkey revealed that the prevalences of depression in pregnant women were 35.4% and 56.3% (12, 25). Our study observed that one out of every two women had probable depression. The variation in the prevalence reported in the literature might be related to the differences in the sampling and evaluation methods used. We have determined a higher depression prevalence compared to the literature, possibly because our participants had just given birth and were concerned about being infected before leaving the hospital. Also, companions were not allowed to the hospital during the pandemic period, so pregnant women were alone during and after the delivery. They could not get social support from their family members.

Women with low income have been at high risk for increased stress and psychiatric symptoms during the pandemic. In a meta-analysis conducted by Chen et al., the prevalence of postpartum depression was reported as 9.5% in high-income countries, 20.8% in middle-income countries, and 25.8% in low-income countries during the COVID-19 pandemic (22). In the study of Moyer et al., loss of income was determined as a psychosocial risk factor associated with COVID-19 (26). In our study, consistent with the literature, the OR of probable depression was 2.67 times higher in participants with a low-perceived income compared to those with a high income.

Anxiety experienced by pregnant women during the pandemic was more pronounced than in the general population. In a meta-analysis conducted before the pandemic, covering 34 countries, the prevalence of perinatal anxiety was reported as 15.2%(27). In a meta-analysis by Yan et al., in which 23 studies related to the COVID-19 pandemic were evaluated, the prevalences of psychological stress and anxiety in pregnant women were reported as 70% and 37%, respectively (1). In another study with a limited sample, the frequency of anxiety was higher in SARS-CoV-2 positive pregnant women compared to the negative participants (41.7% vs. 23.3%, p<.05) (28). In our study, probable anxiety

was 28% regardless of a previous diagnosis of COVID-19. The variation in the prevalence reported in the literature might be related to sample characteristics, assessment methods, and the pandemic wave in which the study was conducted. However, there is an increased risk of anxiety compared to the pre-pandemic period. Also, we determined that having had a COVID-19 infection during pregnancy increased the OR of the presence of probable anxiety 1.66 times.

Unintended pregnancies during an unpredictable pandemic may increase the level of anxiety. We determined in the multivariate analysis that unintentional pregnancy increased the OR of the presence of probable anxiety 2.22 times. In a study conducted in Türkiye, anxiety scores were higher in pregnant women who did not plan their pregnancy (29). Another study showed that unintended pregnancy was a determinant of the level of postpartum anxiety (30).

In addition to all the stressors of the pandemic, experiencing a pregnancy-related problem further increases anxiety (31). Kochan et al. showed that the problems experienced during pregnancy influenced the level of postpartum anxiety (30). In our study, we could not find a relationship between obstetric problems and anxiety or depression. However, in the multivariate analyses, we showed that the OR of probable anxiety increased 2.51 times in those with anemia during pregnancy.

4. 1. Strengths and Limitations

Our study was one of the first to evaluate the depression and anxiety of early postpartum women with and without a history of infection during the COVID-19 outbreak. Most previous surveys were conducted with high-income and educated participants using social media and the internet. The fact that we conducted our surveys face-to-face allowed us to access early postpartum women with medium-to-low income and education. Another strength of our study was the confirmation of SARS-CoV-2 infection during pregnancy by PCR test positivity and the exclusion of early postpartum active SARS-CoV-2 infected cases, which have the potential to affect mental status.

Our study also has some limitations. The cross-sectional design is the major limitation in evaluating causality. The fact that our research was conducted only in a single center might bring further limitations regarding the generalizability of the findings. In addition, since our study was conducted in a large and well-equipped referral hospital in the region, our population consisted of relatively high-risk women with additional co-morbidities. Also, we used scales to determine the presence of probable depression and probable anxiety. So, the outcomes were determined based on self-report, and the participants were not evaluated through a clinical assessment. We also did not evaluate sleep, appetite, physical activity status, or personality traits, which were reported to be associated with depression in the literature. In addition, many individuals infected with COVID-19 experienced the disease asymptomatically. The diagnostic value of the PCR test is limited to approximately 60%, and it may lead to missed diagnoses at a significant rate. This might have led to a misclassification bias in the assessment of the infection. Finally, although the presence of any previous psychiatric disorders was determined as one of the exclusion criteria, the fact that this was based on participants' reporting was considered a limitation.

5. CONCLUSION

Our study revealed that probable depression and probable anxiety were prevalent among women in the early postpartum period during the pandemic. Women with a low-income level, who had an unintentional pregnancy, anemia, and COVID-19 infection during their pregnancy, were vulnerable to postpartum mental health problems. Therefore, we need to implement strategies and measures to prevent and control the mental health problems among these most vulnerable pregnant women during the COVID-19 pandemic.

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Clinical and Experimental Health Sciences

Improvement of Drug Stock Management Using ABC-VED Analysis in a University Hospital

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ABSTRACT

Objective: Hospitals allocate approximately 35% of their budgets to pharmaceutical and material stocks in order to provide uninterrupted health services. For this reason, hospital managers spend a lot of time and effort in order to keep a minimum level of stock so as not to disrupt health service production and thus to better manage the limited resources of the enterprise. The aim of this study is to group the drugs according to their financial value and vital importance. In this way, it is aimed to perform stock management in the least costly, error-free and effective way.

Method: In the study, data corresponding to one-year (2021) consumption of a total of 1327 items of drugs were analyzed. The total cost is approximately 83 million ₺. In this study, an ABC-VED cross-analysis was performed on the one-year drug consumption data of a university hospital with 853 beds in order to provide an example of a more controlled inventory structure.

Results: According to the results, it is seen that the ratio of drugs in the first category, that is, drugs that are both high cost and vital for the patient, to the total drug item is 38%. The cost of these drugs constitutes 87% of the total cost.

Conclusion: Taking into account the classifications put forward in the study, strict monitoring of a small number of drugs and regular weekly counts will make drug stock management much easier for managers.

Keywords: Drug stock management, university hospital, pharmacy, ABC analysis, VED analysis.

1. INTRODUCTION

Hospitals are complex organizations consisting of units with many different functions such as pharmacy, laboratory, operating room, laundry, and human resources. Each department has its own products with different conditions in terms of high/low cost, high turnover/rare use, durability/perishability, storage conditions and vitality. This diversity challenges hospital managers to procure before the time of need, to use them in place and on time, to invoice correctly, and to prevent loss and waste. Therefore, the inventory management system should be well organized (1).

Successful inventory management is the key to improving service quality, preventing waste and increasing efficiency in hospitals. Health is a sector where the slightest mistake can cause irreversible situations for human health. Therefore, it is very important to store medical supplies and drugs continuously and in sufficient quantity and to monitor stocks. The obligation of hospitals to have the necessary materials ready for use at any time creates the need for stocking (2). However, this need should be adjusted in appropriate quantities. Keeping more than the needed adversely affects the financial structure of hospitals due to the capital tied to stocks. Insufficient stock is not recognized in time and increases the risk of exhaustion and may cause irreparable consequences such as death and disability. Compensations to be paid in such cases damage both the budget and the image of the hospital (3). As a result, in order for the hospital to survive and prevent financial losses, it is vital to monitor the warehouse stocks accurately.

When it comes to inventory management, drugs are the first thing that comes to mind in hospitals. Effective management of pharmaceuticals, which brings a burden of 10-20% to the budget, has a significant impact on the financial structure and efficiency of the hospital. However, it is often difficult to determine the correct consumption amount of medicines. In order to avoid the uncertainty arising from the use of drugs that may vary according to patient profile, reimbursement conditions, treatment protocols and physician preferences, it is necessary to keep some safety stock. This leads to stocking costs. Therefore, drugs should be strictly monitored,

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especially at the item level, and subjected to continuous control (3).

Health service production costs in Türkiye have increased significantly in recent years. Increases in the cost of medical supplies and pharmaceuticals, rising labor wages, and limited availability of outpatient and surgical services during the pandemic have exacerbated financial challenges. Public hospitals could not make payments to the companies from which they receive services. For this reason, drug inventory tracking has become even more important in order to manage limited financial resources well, prevent waste and ensure efficiency in service delivery (4).

When the literature is examined, different analyses are used in the literature for drug inventory management. ABC (Always, Better, Control), VED (Vital, Essential, Desirable), SDE (Scarce, Difficult, Easy), SOS (Season-Off Season), HML (High, Medium, Low), FSN (Fast-moving, Slow-moving, On-moving) are some examples (5, 6). The ABC-VED cross-analysis, which utilizes the advantages of both analyses, is the most widely used among them.

ABC analysis is an easy, low-cost and fast way to identify products. The aim is to classify and control materials with different prices and frequency of use according to their amounts (7). It is one of the most commonly used analysis methods in hospitals for the procurement of drugs and medical supplies.

In short, since the cost of group A drugs is high, the stock level should be kept low and monitored carefully. They constitute 15-20% of the annual stock amount and 70-80% of the stock value. Group B drugs do not require continuous control because they have a medium cost. They constitute 30-40% of the stock amount and 15-20% of the stock value. Group C drugs have low costs and require a low level of control. They constitute 40-50% of the stock amount and 5-10% of the stock value (8, 9). The characteristics of ABC groups are detailed in Table 1 (10).

Table 1. *ABC classification system features*

Group	Numbers (%)	Cost (%)	Features
A	% 10-20	% 70-80	Strict control No safety stock / very low Frequently ordering Follow-up by senior officials Individual submission
B	%30-40	% 15-20	Monthly check Safety stock low level One order every three months Moderate follow-up authorization Submission in small groups
C	% 40-50	% 5-10	Quarterly controls Security stock level high Batch order every six months Lower level employees authorization Bulk shipping

In VED analysis, products/drugs are classified into three main groups according to their vital importance: Vital, Essential and Desirable. V group drugs are of urgent and vital importance for patients and must be available in hospitals. E group drugs are drugs of moderate importance for patients, which are desirable to have in the hospital but for which there are alternatives. Group D drugs are not vital for patients and may be preferably necessary. These drugs do not directly affect service quality (11, 12).

While the price of the drug is important in ABC analysis, drugs with high vital importance are at the forefront in VED analysis, even though they are cheap. For example, drugs such as potassium ampoules and adrenaline may rank high in the VED analysis because they are of vital importance although their cost is very low (13, 14). In recent pharmaceutical inventory management practices, these two analysis methods alone are not considered reliable. Especially in university hospitals where drug variability is high, using the ABC-VED matrix instead of applying these methods alone is considered a more accurate method for purchasing (10).

In the ABC and VED Matrix, both analyses were divided into three main categories by combining the crossover. Drugs in the first category were categorized as AV, AE, AD, BV, CV (Vital-expensive), drugs in the second category were categorized as BE, CE, BD, (Moderately vital and costly) and drugs in the third category were categorized as CD (Non-vital-low costly) (15, 16).

Recently, due to reasons such as the pandemic and exchange rate variability, drug supplies of drug suppliers in Türkiye may be interrupted (17). In this case, it has become much more important for managers and pharmacists of university hospitals with a wide patient profile and drug variety to categorize stock management according to cost and vitality. Starting from this point, the study aims to classify and stock drugs according to their financial value and vitality in a university hospital with a capacity of 853 beds. A review of the literature shows that many studies in Türkiye and around the world have classified pharmaceuticals with the help of ABC and VED analyses. In this way, it is stated that stock management can be done more flexibly and effectively with fewer personnel (2, 3, 7, 8, 10, 18-21).

2. METHODS

In the study, ABC-VED matrix analysis was used for more efficient inventory management. Drugs classified according to their material and vital importance are controlled more regularly and losses, leakages and deficiencies are minimized. It has been reported that the analysis benefits the hospital budget in the long term (22). The procedure to be followed during the study is given below:

- Drugs in the hospital pharmacy will be categorized according to their costs (ABC).
- Drugs in the hospital pharmacy will be classified according to their vital importance (VED).
- Drugs will be cross-grouped according to the ABC-VED matrix.
- According to the ABC-VED matrix obtained, the rates of drugs will be compared with other existing drug order rates in the literature and the reliability of the analysis will be questioned.

Data on the drugs of Pamukkale University Hospitals for 2021 were used for the analysis. We aimed to reach the most accurate result in ABC and VED analysis by including all drugs used in the pharmacy for this study. Data were extracted from Probel Hospital automation system databases using Structured Query Language. The data extracted from the database were transferred to the Excel program and analyses were performed.

In the verification, examination, cleaning and classification stages of these data, expert opinions of the staff of pharmacy, purchasing, nursing services and drug planning units were consulted and joint studies were carried out on the tables. First of all, drugs were transformed into a unique table and submitted to the control of the pharmacist responsible for medication planning. Here, some blood products that should not be included in the table were identified and excluded. Subsequently, the drugs were grouped by the researchers as A, B and C. For this process, the costs derived from annual usage and purchase prices were used. The list with A, B, and C groups was submitted to the opinion of the procurement officer and necessary corrections were made. In the VED analysis, both the chief pharmacist and the assistant chief nurse were consulted, as vital importance information is a more subjective assessment than cost. The list was evaluated separately by both managers. The researchers finalized the medication table by resolving the differences in lists in a meeting with both managers present.

3. RESULTS

In the study, firstly ABC analysis was performed on 1327 items of drugs obtained by compiling one-year consumption data. According to the results, the value of drugs consumed for a year is over 83 million ₺. In accordance with the literature, these drugs are divided into the A group approximately 70%, B group approximately 20% and C group approximately 10%. In terms of quantity, group A is approximately 5.5%, group B is approximately 11% and group C is 83.5%. Detailed data are given in Table 2. From this point of view, it is concluded that 72 drugs among 1327 drugs should be strictly controlled in terms of cost.

Table 2. *ABC analysis results*

Group	Numbers (n)	Numbers (%)	Cost (₺)	Cost (%)
A	72	5.43%	58,916,145	70.26%
B	148	11.15%	16,586,168	19.78%
C	1107	83.42%	8,350,645	9.96%
Total	1327	100%	83,852,958	100%

As a result of the analysis, the ratio of group V drugs in terms of quantity was found to be approximately 37%, group E approximately 43.5% and group D approximately 19.5%. In terms of cost, group V was calculated as 79%, group E as 17% and group D as 4%. Detailed data are given in Table 3. From this point, it is seen that the number of drugs (group V) for which stock follow-up should be carried out at a high level in order to prevent interruption of vital health services is 494.

ABC-VED cross-analysis was performed in the rest of the study. As explained in the method section, three categories are formed as a result of crossing two analyses. According to the results of the analysis, there are a total of 513 drugs in Category I, which includes drugs of great importance in terms of both cost and vital. These drugs account for 87.5% of the total cost of drugs for a year. The ratio of the number of drugs that the pharmacy should keep control and follow-up at a high level to the total number of drugs is approximately 38%. The number of category II drugs, drugs of medium cost and vital importance, is 566. Their ratio to the total number is approximately 43%. The ratio of drugs in this category to total cost is approximately 12%. The number of Category III drugs, which are non-essential and low-cost drugs, is 248. The cost of these drugs, which constitute 19% of the total number, is below 1%. Detailed data are given in Table 4.

Table 3. *VED analysis results*

Group	Number (n)	Number (%)	Cost (₺)	Cost (%)
V	494	37.23%	66,242,621	79.00%
E	575	43.33%	14,173,616	16.90%
D	258	19.44%	3,436,720	4.10%
Total	1327	100%	83,852,958	100%

Table 4. *ABC-VED analysis results*

Group	Number (n)	Number (%)	Cost (₺)	Cost (%)
Category I	513	38.66%	73,382,231	87.51%
Category II	566	42.65%	9,934,903	11.85%
Category III	248	18.69%	535,824	0.64%
Total	1327	100%	83,852,958	100%

Table 5. *Comparison of the results with the literature*

	Current Study		Yılmaz, 2018		Khurana, Chhillar & Gautam, 2013		Wandalkar, Pandit & Zite, 2013	
	Number	Cost	Number	Cost	Number	Cost	Number	Cost
A	5.43%	70.26%	5.05%	70.08%	3.45%	70.50%	13.40%	69.10%
B	11.15%	19.78%	10.11%	19.88%	6.90%	19.68%	16.50%	19.20%
C	83.42%	9.96%	84.84%	10.04%	89.65%	9.83%	70.10%	11.70%
V	37.23%	79.00%	29.12%	44.42%	32.41%	70.90%	50.90%	55.20%
E	43.33%	16.90%	51.32%	47.06%	61.38%	28.72%	40.20%	41.50%
D	19.14%	4.10%	19.56%	8.52%	6.20%	0.38%	8.90%	3.30%
I	38.66%	87.51%	32.75%	82.55%	33.80%	92.33%	57.00%	85.30%
II	42.65%	11.85%	49.01%	15.66%	60.00%	7.29%	35.00%	14.20%
III	18.69%	0.64%	18.24%	1.79%	6.20%	0.38%	8.00%	0.50%
	Vaz et al., 2008		**Yeşilyurt, Sulak & Bayhan, 2015**		**Devnani, Gupta & Nigah, 2010**		**Uygun & Yiğit, 2016**	
	Number	Cost	Number	Cost	Number	Cost	Number	Cost
A	12.93%	69.45%	8.26%	70.38%	13.78%	69.97%	4.78%	69.61%
B	19.54%	20.48%	14.13%	20.02%	21.85%	19.95%	11.29%	20.28%
C	67.53%	10.07%	77.61%	9.60%	64.37%	10.08%	83.93%	10.11%
V	12.36%	15.67%	28.26%	52.91%	12.11%	17.14%	40.77%	80.90%
E	47.12%	70.02%	58.48%	29.18%	59.38%	72.38%	54.71%	16.52%
D	40.52%	14.31%	13.26%	17.91%	28.51%	10.48%	4.52%	2.59%
I	22.99%	74.80%	31.74%	85.93%	22.09%	74.21%	41.96%	85.97%
II	41.67%	21.68%	57.39%	12.92%	54.63%	22.23%	54.58%	13.95%
III	35.34%	3.52%	10.87%	1.15%	23.28%	3.56%	3.45%	0.08%

Table 6. *Distribution of expenditure items for 2021*

Budget Account Name	Percentage (%)
Personnel Expenses	39.66%
Energy Purchases	3.75%
Laboratory Material Purchases	
Medical Equipment Purchases	**34.53%**
Medical Drug Purchases	
Other Material Purchases (Clothing, Cleaning, Stationery, etc.)	1.58%
Service Purchases	11.03%
Maintenance and Repair Expenses	4.11%
Current Transfers	4.44%
Capital Expenditure	0.90%

4. DISCUSSION

The results obtained at the end of the study were compared with examples in the literature. According to the results of a study conducted in a university hospital in India using 348 drug data, the share of drugs in group A was found to be 12.93% in quantity and 69.45% in expenditures. Drugs in group V were found to be approximately 12% of the total amount and 15.67% of the annual drug expenditure (23). Again, in a study conducted in a tertiary care teaching hospital in India, the share of group A drugs was found to be 13.78% in quantity and 69.97% in drug expenditures in an analysis using 421 drug data. Group V was found to be 12.11% in quantity and 17.14% in cost (8).

Another research was conducted in a university hospital in Türkiye using 753 drug data and according to the results of the analysis, the share of group A drugs in the total number was 4.78% and their share in the annual drug expenditures was 69.61%. The share of group V drugs in the total number was found to be 40.77% and the share in the annual drug expenditures of the hospital was found to be 80.90%. In the ABC-VED matrix, 41.96% of the total amount of Category I and 85.97% of the hospital's annual pharmaceutical expenditure (19).

In a study conducted at the Post Graduate Institute of Medical Education and Research in Chandigarh, India, the percentage of drugs in group A in terms of quantity was found to be 13.4% and the percentage of drugs in terms of cost was found to be 69.1%. In the VED analysis, the percentage of

drugs in group V was found to be 50.9% in terms of quantity, and 55.2% of their share within the annual drug expenditures of the hospital. Category I was found to be 57% total quantity and 85.3% of the annual drug expenditures of the hospital (20).

In a study conducted on 145 drug data in a neuropsychiatric hospital in Delhi, group A drugs were found to be 3.45% of the total quantity and 70.5% of the total cost. Group V drugs were found to be 32.41% of the total quantity and 70.90% of the total cost. Category I drugs were found to be 33.8% of the total quantity and 92.33% of the total cost (24). Furthermore, a study conducted in 2013 in a public hospital in Türkiye using data on 460 medicines found that group V medicines accounted for 28.26% and 52.91% of the cost (25). Details of the comparisons between the present study and the literature are given in Table 5.

When compared with other previous studies in the literature, it is seen that the results are very similar to some studies (19, 24). Differences were observed in the VED analysis with other studies examined. This is thought to be the result of the differentiation of the patient profile served by the hospitals in the region. However, the results of category I, II, and III are similar in all examples. Category I provides tracking of a large cost with few items. It is seen that the first category, which stands out in terms of both cost and vitality, accounts for approximately one-third of the pharmaceutical items and more than 85 percent of the total cost. Therefore, according to the results of the analyses presented in the study, strict monitoring of a small number of drugs and regular weekly counts will make drug inventory management much easier for managers. The second category of medicines accounts for about half of the total pharmaceutical items and a small part in terms of cost. The results show that the organization of these drugs is in accordance with the conditions stated in the literature. In other words, the level of monitoring and delegation can be kept at a moderate level. Purchases can be made on quarterly orders and sent to the units.

Hospital managers try to find the most appropriate solution between spending as little as possible of the already limited financial resources on stocks and the disruption of health service production as a result of not having sufficient stocks. Studies indicate that giving equal importance to all drugs in the hospital inventory in planning is an inefficient approach (5, 26). As in our study, multidimensional analyses should be performed by considering the methods that are important for the hospital. Thus, more time can be allocated to the follow-up and control of a small number of drugs that should be given more importance.

In this study, ABC-VED cross-analysis was used both because it is frequently used in the literature and because it is thought to best fit the planning structure of the existing hospital. The data for the analysis to be applied should be easily identifiable or extractable from the database. At the same time, it is easy to understand and easy to use, which will be preferred by hospital administrators. Some methods suggested by academics in the literature may not be practically suitable for the hospital supply chain. Of course, this may vary depending on many variables such as the size of the institution, financial situation, and patient profile (10, 26).

Another point to be mentioned is the financial importance of well-organized inventory management. As seen in many examples in the literature, 35% of total expenditures are due to drug and material purchases (14, 21, 22). The annual expenditures of the analyzed hospital also support this conclusion (Table 6). In a study conducted with the ABC-VED method for inventory control of high-cost drugs in two different hospitals in Ethiopia, it was reported that nearly 20% savings were achieved. The rate of expired drugs was reduced from 4.5% to 0.27% in the first hospital and from 10% to 2% in the second hospital (27). At this point, it is clear that the study will benefit the relevant hospital. The majority of the pharmaceutical procurement of public hospitals in Türkiye is provided through the State Supply Office. A similar study can be applied on this platform on a hospital basis. By creating special order suggestions for hospitals, a high amount of benefit can be achieved at the national level.

The analysis was conducted on more than 4 million consumption data of 1327 medicines with an annual cost of approximately 85 million ₺. Therefore, it is exemplary in terms of having more comprehensive data than similar studies in the literature. The study data examined in the literature is not at a level to serve as an example of a full-fledged hospital. In addition, while performing ABC and VED analyses in the study, experts were consulted to the extent not seen or specified in the literature (8, 13, 19, 20, 23-25) and the method followed was specified. The aim is to present the correct results more clearly.

5. CONCLUSION

This study has provided guidance to hospital managers by categorizing medicines according to their cost and vital importance. However, this analysis may need to be improved according to changing policies and patient profiles. Multidimensional analyses can be developed according to variables such as supply requirement, ease of availability, fast-slow stock movement and seasonal usage changes. For example, chemotherapy drugs constitute a significant portion of Group A drugs. The reason for this is the Health Implementation Communiqué rule for the procurement of inpatient medicines in Türkiye. Inpatient medicines must be supplied by the hospital where the patient is hospitalized. If the medication is obtained from outside with a prescription, the amount of the medication will be deducted from the patient's package bill. In another country where this practice does not apply, the drug item in group A may be higher. This may require prioritizing other criteria in the analysis.

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Clinical and Experimental Health Sciences

Effect of Tooth Bleaching on The Color Change of Laminate Veneer Restorations: A Pilot Study

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ABSTRACT

Objective: This pilot study aimed to evaluate the effect of tooth bleaching on the long-term color change of laminate veneers restored with different translucency CAD/CAM materials.

Methods: In this study, 20 upper central teeth extracted due to periodontal, orthodontic problems and trauma were used. The teeth were embedded in acrylic blocks and divided into 4 groups of 5 teeth each. Groups A and B were bleached with a vital bleaching agent for two 20-minute sessions before preparation and teeth were prepared for laminate veneer restoration following bleaching. Groups C and D were prepared without bleaching treatment. Groups A and C were restored with high translucent A1 IPS Emax CAD material and Groups B and D were restored with low translucent A1 IPS Emax CAD material. For all restorations adhesive cementation was applied and aged for 2 years by thermal cycling. The color of the restorations was measured using a spectrophotometer after cementation and the measurement was done again after 2 years aging. The Kruskal Wallis test was used to compare data and multiple comparisons were tested with Dunn's test.

Results: The translucency of the blocks and tooth bleaching caused a significant difference between the groups according to the Kruskal Wallis test. Color changes (ΔE00) of Group A, B, C and D was 0.89±0.01, 0.87±0.01, 0.81±0.01, 0.8±0.01 respectively.

Conclusion: Tooth bleaching causes a greater color change in laminate veneer restorations and the translucency of the material affects the color change of the laminate veneer restorations after aging.

Keywords: Laminate veneer, bleaching, translucency, IPS Emax CAD

1. INTRODUCTION

One of the most important goals of modern dentistry is to restore the tissue integrity, function and phonation of patients and natural dental aesthetics. Currently, it is possible to produce restorations with optical properties similar to those of natural teeth (1).

Owing to the complex optical properties of natural teeth, it is very difficult for dentists to achieve color harmony in aesthetic restorations. To ensure color harmony for aesthetic restorations, it is necessary to know the optical properties of the tooth and materials (2). Translucency is one of the main factors that affect material selection and aesthetics. All dental ceramics contain crystals such as lithium disilicate, leucite, and fluorapatite. An increase in the crystal content increases the opacity of the material and may negatively affect its aesthetics (3). In terms of the lens effect, the high translucency of the material improves the natural appearance of the restoration owing to the reflectance of the natural teeth. However, translucency decreases the covering capacity so that discolorations may be visible through restoration (4).

Porcelain laminate veneers are minimum thickness restorations. The low thickness and high translucency of the material increases the optical property, which is an important criterion for aesthetics and provides a natural tooth appearance (5). Tooth discoloration is an important esthetic problem and can be treated by bleaching and laminate veneer restorations. Following bleaching procedures, it is possible to choose more translucent materials and contribute to aesthetics (6).

Color is a complex concept that includes both subjective and objective phenomena. Since Sigried Forsius first descripe color in 1611, many systems and approaches have been developed to explain it (7). Although there are many different color determination systems, Munsell and CIE color systems are the most preferred in terms of international acceptability, reliability and ease of application (8,9).

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Although there are many studies on the color changes of laminate veneer restorations in the literature, there are not enough studies on laminate veneer restorations applied to bleached teeth. This pilot study aimed to evaluate the effect of tooth bleaching on the long-term color change of laminate veneer restorations produced with different translucency CAD/CAM materials. The first null hypothesis was that bleaching does not affect the long-term color stability of lamina veneer restorations after aging. The second null hypothesis was that translucency would not affect the long-term color stability of laminate veneer restorations after aging.

2. METHODS

Ethical approval was obtained from the Ethics Committee of Marmara University Faculty of Medicine 09.2021.418 protocol number was obtained for this pilot study.

In this study, 20 maxillary central teeth (A4 shade) which kept in 10% thymol solution, without caries extracted due to periodontal, orthodontic problems or trauma were used. Teeth were kept in 10% thymol solution at the time of extraction. The specimens were embedded in acrylic blocks and divided into 2 main groups (n = 10) according to the bleaching procedure applied. Each of these groups were divided again into subgroups (n=5) according to the translucency of the lithium disilicate glass ceramic material as high and low translucency. The study groups are shown in Figure 1.

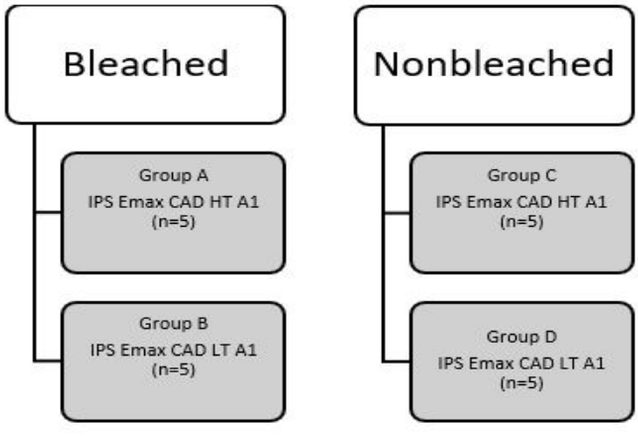

Figure 1. *Study groups*

2.1. Tooth Bleaching Procedures

Groups A and B were bleached before preparation of laminate veneer restorations. Groups C and D were prepared without any bleaching procedure. Office type whitening agent containing 40% hydrogen peroxide (Opalescence Boost, Ultradent Products, USA) was applied to the labial surfaces of the specimens with the help of a brush in accordance with the manufacturer's recommendations and activated by 460 nm wavelength LED light device (Flash Whitening Lamp; WHITEsmile GmbH, Birkenau, Germany) for 20 minutes. The bleaching agent was then removed with the help of cotton pellets and the same application was repeated once more after one week. As a result, two bleaching sessions were performed on the specimens.

2.2. Tooth Preparation

All specimens were prepared for all-ceramic laminate veneer restorations one week after the bleaching procedure. Self-limiting depth-cutting burs (Laminate Veneer Kit, Meisinger USA, LLC) of 0.3 and 0.5 mm were used initially to standardize the depth-cut and the facial surface was reduced 0.3 mm at the cervical third and 0.5 mm at the middle and incisal thirds. A chamfer diamond bur was used for final refinement of the preparation. A total of 1 mm reduction was performed at the incisal edge with a fissure diamond bur under a water coolant. Proximal finish lines were located at the proximal contact areas of the specimens. The cervical finishing lines were prepared 1 mm above the cemento-enamel junction without sharp line angles. The labial surface of each specimen was smoothened with a fine diamond-finishing bur.

2.3. Impression of Specimens

A Cerec laboratory scanner inEos X5 (Sirona, Dentsply, Bensheim, Germany) was used to obtain digital impressions of all the prepared specimens.

2.4. Fabrication of CAD/CAM Laminate Veneers

High and low translucent A1 CAD blocks (Emax, Ivoclar Vivadent, Schaan, Liechtenstein) were used for the laboratory production of 20 laminate veneer restorations using the Cerec inLab CAD/CAM (Dentsply Sirona, Germany, Bensheim) system. The restoration designs were made using inLab Software 18.1 (Dentsply Sirona, Germany, Bensheim). All restorations were designed 0.6 mm at the gingival region and 1 mm at the incisal region. Crystallization and glazing of lithium disilicate laminate veneer restorations were achieved using the IPS Ivocolor Glaze Powder (Ivoclar Vivadent) with Programat P310 (Ivoclar Vivadent) in Programme number 81 (crystallization/glaze mode).

2.5. Cementation

All laminate veneer restorations were etched with hydrofluoric acid (Ultradent porcelain etch, USA) for 20 seconds followed by washing with water and an air jet. The G-Multi Primer agent was then applied to the restorations and dried in oil-free air for 5 seconds. The prepared tooth surfaces were etched with 37% phosphoric acid (d'line phosphoric acid gel, Lithuania) for 30 seconds followed by washing with water and air jet. The filler-free bonding agent (G-Premio bond, Japan) was applied homogeneously with a brush for 10 seconds and diluted by 5 seconds of air. Laminate veneer restorations were cemented with GC Veneer (Japan) transparent cement which was applied to the inner surface of the lamina veneer restorations and placed on the extracted teeth with finger pressure. Excess cement was removed using a probe. Led Rainbow Curing Light (Liang Ya, Guangdong, China) with a 430 nm wave length was used for the

polymerization process. The curing light was first applied to the palatal surfaces of the specimens for 90 seconds and then to the buccal surfaces of the specimens for 90 seconds to ensure light polymerization.

2.6. Thermocycle

After 24 hours after cementation, all specimens were subjected to thermocycling procedures (Thermocycling TC-3; SD Mechatronik GMBH), 20.000 thermal cycles per specimen with temperature changes from 5° to 55° every 30 seconds. There are literature reviews concluded that 10.000 cycles corresponds to approximately 1 year of in vivo functioning (10,11,12), therefore, the specimens were aged for 2 years with 20.000 thermal cycles.

2.7. Evaluation of the color stability

Color measurements of the specimens were performed after cementation and again after 2 years of aging using a spectrophotometer (Vita Easyshade, Vident, Brea, California, USA) and measurements were taken at the middle third of the restorations. Each measurement was performed three times and the average of all three scores was calculated. The CIEDE2000 formula (ΔE_{00}) was used to calculate color change.

Currently, the CIEDE2000 formula, which belongs to the CIE color system, is used in the literature. $\Delta L'$, $\Delta C'$ and $\Delta H'$ in the CIEDE2000 formula (ΔE_{00}) represent the lightness, brightness and hue, respectively that match the samples. The RT rotation function calculates the differences between the color and hue in the blue region. The weighting function (SL, SC, SH) regulates the variations between the total color differences of the pairs in the L', a', and b' coordinates and the parametric factors (KL, KC, KH) (8). In the literature, the perceptibility limit in the CIEDE2000 color difference formula is specified as $\Delta E_{00} \leq 0.8$, and the acceptability limit is $\Delta E_{00} > 1.8$ (9).

$$\Delta E_{00} = [(\Delta L/kLSL)^2 + (\Delta C/kCSC)^2 + (\Delta H/kHSH)^2 + RT\ (\Delta C/kCSC)\ (\Delta H/kHSH)\ ]^{1/2}$$

2.8. Statistical Analysis

IBM SPSS V23 was used to evaluate and compare data. Conformity to normal distribution was evaluated using the Shapiro-Wilk test. An independent samples t-test was used to compare normally distributed data according to the paired groups. The Mann Whitney U test was used to compare the data that were nonnormally distributed according to the paired groups. One-way analysis of variance was used to compare normally distributed data according to groups of three or more and multiple comparisons of the results were analyzed using Tukey HSD and Tamhane's T2 tests. The Kruskal Wallis test was used to compare nonnormally distributed data by groups of three or more and multiple comparisons were tested with Dunn's test. Analysis results are presented as the mean ± standard deviation and median (minimum – maximum). The significance level was set at $\alpha < .05$.

3. RESULTS

The shade of all groups was changed and became darker, more red and more yellow but all the ΔE_{00} values were within the acceptability limit as the ΔE_{00} value was below the 1.8 threshold (Figure 2).

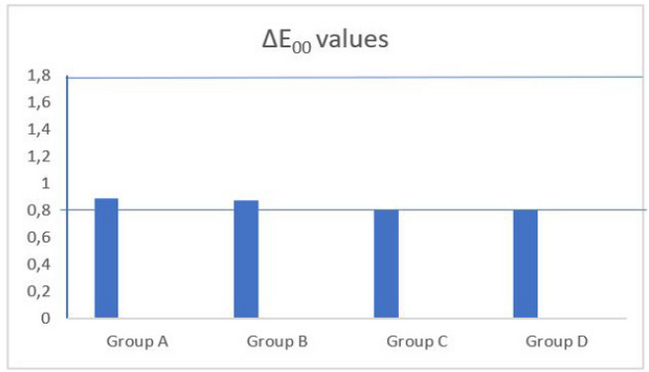

Figure 2. *ΔE_{00} values of all groups*

Table 1 shows the means of ΔL, ΔC, ΔH, and ΔE_{00}, test statistics and the p values.

Table 1. *Mean, standard deviation and median (minimum – maximum) values of color coordinates and color change of the samples as a result of thermal aging*

	Group A	Group B	Group C	Group D	Test statistic	p value
ΔL	0.8 ± 0.1	0.8 ± 0.07	0.7 ± 0	0.66 ± 0.05	10.664	**.014**
	0.8 (0.7 – 0.9)	0.8 (0.7 – 0.9)	0.7 (0.7 – 0.7)	0.7 (0.6 – 0.7)		
ΔC	0.68 ± 0.15	0.68 ± 0.11	0.7 ± 0	0.7 ± 0.1	0.259	0.968
	0.7 (0.5 – 0.9)	0.7 (0.5 – 0.8)	0.7 (0.7 – 0.7)	0.7 (0.6 – 0.8)		
ΔH	0.86 ± 0.18	0.82 ± 0.25	0.82 ± 0.08	0.9 ± 0.19	0.214	0.885
	0.9 (0.6 – 1.1)	0.8 (0.6 – 1.2)	0.8 (0.7 – 0.9)	0.9 (0.6 – 1.1)		
ΔE_{00}	0.89 ± 0.01	0.87 ± 0.01	0.81 ± 0.01	0.8 ± 0.01	16.368	**.001**
	0.89 (1 – 1)	0.87 (1 – 1)	0.81 (1 – 1)	0.80 (1 – 1)		

ΔL, ΔC and ΔH= Color coordinates, ΔE00= color change

Table 2. *Mean, standard deviation and median (minimum – maximum) values of color change of bleached and nonbleached groups*

	Bleached Group	Nonbleached Group	Test statistic	p value
ΔE_{00}	0.88 ± 0.01	0.81 ± 0.01	0.000	**< .001**
	0.88 (1 – 1)	0.81 (1 – 1)		

ΔE00=Color change

Statistically significant differences were found between the medians of the ΔL values of the groups according to the Kruskal Wallis test (p= .014). This difference was due to the difference between the low translucent lithium disilicate glass ceramic (bleached) group and the low translucent lithium disilicate glass ceramic (nonbleached) group. The median ΔL value for the high translucent lithium disilicate glass ceramic (bleached) group and low translucent lithium disilicate glass ceramic (bleached) group was 0.8, and the median ΔL value for the high translucent lithium disilicate glass ceramic (nonbleached) group and low translucent lithium disilicate glass ceramic (nonbleached) group was 0.7.

There was no statistically significant difference between the medians of ΔC values between the groups according to the Kruskal Wallis test (p= .968) and there was no statistically significant difference between the means ΔH values of the groups according to one-way analysis of variance (p= .885).

Statistically significant differences were found between the medians of the ΔE_{00} values of the groups according to the Kruskal Wallis test (p= .001). The highest median value was 0.89 in high translucent lithium disilicate glass ceramic (bleached) group followed by low translucent lithium disilicate glass ceramic (bleached) group with 0.87, while the lowest median value was 0.80 in low translucent lithium disilicate glass ceramic (nonbleached) group and followed by high translucent lithium disilicate glass ceramic (nonbleached) group with 0.80.

Statistically significant differences were found between the medians of ΔE_{00} values according to their bleaching status according to the Mann Whitney U test (p< .001) (Table 2). The median ΔE_{00} value of the bleached groups (Group A and Group B) was 0.88 while the median of those nonbleached groups (Group C and Group D) was 0.81. The color change was higher in the bleached group.

4.DISCUSSION

The first null hypothesis that there would be no difference in the long-term color stability of laminate veneer restorations after aging depending on bleaching was rejected. A significant color change was observed in the bleached groups. The second null hypothesis that there would be no difference in the long-term color stability of laminate veneer restorations after aging depending on translucency was also rejected. High translucent materials changed color significantly more in both bleached and nonbleached groups. According to this pilot study, it can be concluded that more color change was obtained at laminate veneer restorations that were constructed on bleached teeth and the translucency of the material affected the color change after aging.

Meireless et al (13) in their in vitro study, investigated the effect of the concentration of bleaching agents containing 10% and 16% carbamide peroxide on the reversal of whiteness and reported that the original concentration of the bleaching agent was not effective in restoring whiteness. Therefore, in this pilot study, office bleaching agent which containing 40% hydrogen peroxide was preferred.

The preparation depth and thickness of ceramic restorations may affect the overall shade (2). Self-limiting depth-cutting burs were used for the standardization of the tooth preparation and the thickness of 0.6 mm at the gingival region and 1 mm at the incisal region were selected for the manufacturing of all laminate veneer restorations to standardize color measurements (10).

Currently, CAD/CAM systems are frequently used for the production of laminate veneer restorations because of their advantages of digital workflow and time saving (14). More translucent ceramic systems, such as lithium disilicate, increase natural appearance by allowing greater light transmission (1). Therefore, IPS Emax CAD blocks containing lithium disilicate crystals were used in this pilot study.

It is possible to use light-cure or dual-cure resin cements for the cementation of laminate veneer restorations. Dual-cure resin cement may change color over time owing to presence of amine groups. Light-cure resin cements are frequently preferred for the cementation of laminate veneer restorations because they do not change color, offer different translucency and color alternatives and have advantages such as a long working time (15). In this pilot study, a light-cure resin cement (G-Cem Veneer) was used for cementation.

Subjective and objective methods have been used to evaluate color changes after bleaching procedures and aging (16). Spectrophotometers, colorimeters and computer software programs are the objective methods. Colorimeters measure only three wavelengths of reflected light whereas spectrophotometers measure reflections in the entire visible spectrum of the light. Colorimeters provide inconsistent results, especially on curved surfaces. A spectrophotometer was used to obtain error-free and consistent results (17). In this pilot study a spectrophotometer (VITA Easyshade) was used to evaluate color change values.

Gómez-Polo et al (18) compared the CIELab and CIEDE2000 formulas to determine differences in color perception. Within the limits of this study, it was concluded that the CIEDE2000 formula reflects the color difference perceptible by the human eye better than the CIELab formula. The amount of color change is interpreted according to two different threshold values: the color change value that can be noticed by 50% of the observers is defined as the 'perceptibility threshold' and the acceptable color change by 50% of the observers is defined as the 'acceptability threshold' value. Paravina et al (9) reported a perceptibility threshold of 0.8 and the acceptability threshold of 1.8 for ΔE_{00}. In this pilot study the color change due to aging was calculated using the new CIEDE2000 formula. The detectability threshold value was used as ΔE_{00}=0.8 and the acceptability threshold value was ΔE_{00}=1.8.

In the literature, reversal of whitening efficacy has been reported at a rate of 41% at 1 year for in-office bleaching (19) and a return rate of 26% was reported at 18 months for

at-home bleaching (20). In this pilot study, the color change of the bleached groups was significantly higher than the nonbleached groups after 2 years of bleaching procedures. Therefore, the amount of color change may be higher in the bleached groups, because the whiteness is reversed.

Several studies have been conducted on the color stability of ceramics after aging (5,21 – 23). Different results have been reported in these studies, such as increasing or decreasing L, C and H values. In their study in 2012, Bagis and Turgut investigated the color change of laminate veneer restorations produced from ceramic materials using different production techniques and reported that ceramics become darker and more red and yellow after aging (2). In 2017, Alghazzawi compared the optical properties of 7 different translucent zirconia and lithium disilicate materials after aging. All materials got darker and more red, more yellow after aging in this study too (21). Parallel to the results of these two studies, after 2 years of aging L, C and H values were also affected in this pilot study. All groups became darker, red and yellow, the L values decreased and the C and H values increased.

Azer et al (22) investigated the effect of translucency of leucite glass and composite resin laminate veneer restorations on color and concluded that the translucency of the tested materials affected the color of the restorations and that the translucent ones changed color more than the opaque ones. Lee et al (23) investigated the color stability of laminate veneer restorations using different ceramic and resin systems after aging and used high and low translucent forms of nanofluorapatite and lithium disilicate materials. They reported that the color change values were higher for materials with high translucency. The results of this study were consistent with those of Azer and Lee's studies. High translucent forms of lithium disilicate glass ceramic exhibited more color change than low translucent forms.

Dalloo et al (24) examined the impact of bleaching before and after veneer preparation on the color masking ability of laminate veneer restorations manufactured using high translucent lithium disilicate glass ceramic in 3 groups as nonbleached, bleaching before and bleaching after preparation. They concluded that the bleached group exhibited a significant ΔE value compared with the nonbleached group. These results were consistent with those of this pilot study. The bleached after preparation group exhibited the highest ΔE_{00} value.

Yuan et al (25) compared the color stability of lithium disilicate and zirconium materials after brushing and thermal aging. 5, 10 and 15 years of brushing and thermal aging were applied to the specimens and the color changes were calculated using the CIELab formula. They concluded that the color change of both materials was within the clinically acceptable values. The results of this study are consistent with those of Yuan et al and the color changes of the lithium disilicate material groups were within clinically acceptable values.

In 2019, Kim and Kim investigated the color stability and translucency parameters of lithium disilicate and monolithic zirconia. The materials were aged by autoclave for 1, 3, 5 and 10 hours. While the color change of the monolithic zirconia material was above the acceptable threshold value, that of the lithium disilicate material was below the detectability threshold value (26). In this pilot study, the color change of lithium disilicate materials was below the acceptability threshold and above the perceptibility threshold. This difference may be due to the fact that thermal aging was used as the aging method in this pilot study, whereas Kim and Kim used autoclave aging.

One of the limitations of this pilot study was that only lithium disilicate glass ceramic materials were tested. Different CAD-CAM materials used for laminate veneer restorations may differ in their color masking ability. In this study, office bleaching agent containing 40% hydrogen peroxide was used. Different results can be obtained with bleaching agents with a different contents and percentages. Although the purpose of in vitro studies is to simulate the clinical situation, clinical trials are needed to investigate the extent and detection of color changes under clinical conditions.

5. CONCLUSION

The application of bleaching process before preparation for laminate veneer restorations affects the long-term color stability of laminate veneer restorations but within the acceptability limit.

The translucency of the material used in the production of laminate veneer restorations effects the long-term color stability of the laminate veneer restorations. More translucent material changes color more.

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Clinical and Experimental Health Sciences

Increased Expression of the *Actin-Related Protein 2 (ACTR2)* Gene in Pterygium

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ABSTRACT

Objective: Pterygium is a fibrovascular conjunctival degeneration whose pathogenesis remains unclear, although many risk factors have been identified. In our study, we purposed to find the level of *Actin Related Protein 2 (ACTR2)* gene expression in healthy conjunctiva tissues and pterygium to increase our understanding of the pathogenesis of pterygium.

Methods: The study included 27 patients who underwent pterygium excision. *ACTR2* mRNA expression level in healthy conjunctiva tissues and pterygium were determined by the Real-Time PCR method.

Results: According to the results we obtained, *ACTR2* gene expression was increased in 74% (20/27) of our cases, while *ACTR2* gene expression was decreased in 26% (7/27). *ACTR2* mRNA expression was detected to be remarkably higher in pterygium in proportion to conjunctiva tissue ($p < 0.05$).

Conclusion: Our findings show that the *ACTR2* gene and cell migration mechanism may play a role in the development of pterygium. However, supplementary research is requirement to determine the efficacy of the *ACTR2* gene in pterygium disease and to better understand the relationship of the *ACTR2* gene with pterygium.

Keyword: Actin cytoskeleton, *ACTR2*, Cell migration, Expression, Pterygium

1. INTRODUCTION

Pterygium is an ocular surface disease caused by abnormal growth of the conjunctiva and defined by conjunctivalization, inflammation, and connective tissue remodeling (1,2). Although the pathogenesis of pterygium is not clearly known, many risk factors such as Ultraviolet (UV), sex, age increase, inflammatory mediators, viral infections, epithelial-mesenchymal cell transition, apoptotic and oncogenic proteins, oxidative stress, lymph angiogenesis, and DNA methylation play a role in the pathogenesis of pterygium (3,4).

Pterygium is a benign disease with neoplastic-like features such as proliferation, angiogenesis, cell migration, and recurrence (5). In many recent studies, data supporting that pterygium may be a neoplastic condition has been obtained. For example, it has been shown that the *Mitogen-activated protein kinase (MAPK*) signaling pathway, which is active in all cancer types, is also active in pterygium tissue (6). A significant relationship was found between *Kirsten rat sarcoma viral oncogene (K-RAS)*, one of the most frequently observed proto-oncogenes in tumor proliferation, and pterygium. A correlation between postoperative recurrence and younger age has been demonstrated (7). Tumor-associated genes *P63* and *P16* were determined to be remarkably higher in the pterygium epithelial tissue compared to normal conjunctival tissue (8). *P53* is a tumor suppressor protein responsible for regulating cell cycle arrest, and mutations in *P53* are used as the most common genetic markers in human neoplasms (6,9). It has been reported that the function and expression of *P53* in pterygium tissues are irregular and mutant *P53* has been shown to be overexpressed in pterygium tissues (6,10). Although many genes, risk factors, and molecular mechanisms associated with pterygium have been identified, its pathogenesis still remains unclear (3,11).

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The best available treatment option is excision with conjunctival autograft due to the low recurrence rate (12). However, recurrence usually occurs in surgically resected pterygium and post-relapse dysplasia is observed (11,13). Therefore, the identification of mechanisms that activate or support the proliferation and cell migration of pterygium is important for the treatment and prevention of primary pterygium and recurrent pterygium (5).

The actin cytoskeleton *(ARP2/3 complex)* is a dynamic complex with well-known functions in cell morphogenesis, cell migration, inflammation, cell division, and signal transduction (14,15). Actin-Related Protein 2 *(ACTR2-ARP2)* is a member of the *ARP2/3 complex*, which consists of seven proteins. *ACTR2*-mediated actin regulation is thought to drive lamellipodia generation and act as a control center for actin-based cell migration (16). Cell migration, which is a fundamental cellular process, can promote intravascular proliferation and metastasis of cancer cells by using microtubule and actin dynamics (17). Thus, the *ARP2/3 complex* has been associated with promoting the migration and invasion of diverse cancers, and ARP subunits have been shown to be abnormally expressed in tumors (18,19).

Considering this information, we think that there may be a relationship between *ACTR2*, which plays a central role in actin-based cell migration, and pterygium, which is thought to be a tumor precursor. However, there is no publication documenting the relationship between the *ACTR2* and pterygium proliferation, migration, or invasion. In our study, we purposed to determine the *ACTR2* gene expression in pterygium and healthy conjunctival tissues and to reveal the role of actin-based cell migration in the pathogenesis of pterygium.

2. METHODS

2.1. Subjects

Twenty-seven volunteers who applied to Tokat Gaziosmanpasa University Faculty of Medicine, Department of Ophthalmology, and were diagnosed with pterygium were included in the study. While forming the study groups, pterygium tissues taken during the surgical operation were used as the patient group, and conjunctival tissues from the same eye of the same patients were used as the control group. Necessary permission was obtained for the study by the Clinical Research Ethics Committee of Tokat Gaziosmanpasa University Faculty of Medicine (approval number 19 KAEK-024). Written informed ethical consent was obtained from all participants before the study. The Declaration of Helsinki was complied with while conducting the study. Tokat Gaziosmanpasa University Scientific Research Projects (project number 2019/110) helped to finance our study.

Patients without glaucoma, corneal disease, and uveitis were added to the project. The pterygium diagnosis of the patients added in our study was confirmed histopathologically. Information such as age, gender, disease history, and family history was recorded using the hospital information system. Tissues were obtained from 16 right and 11 left eyes. In the power analysis performed with G. Power 3.1.9.6 Statistical Software, the minimum sample size in the groups was determined as 20.

2.2. Identifying ACTR2 Gene Expression

Following the manufacturer's instructions, total RNA isolation from conjunctival and pterygium tissues were carried out (Thermo, USA). Reverse Transcription Polymerase Chain Reaction method cDNA synthesis (GeneAll, Korea) was performed using isolated total RNAs. The cDNA concentrations were detected with the Qubit dsDNA Assay Kit (Invitrogen, USA). The quantity of cDNA required for PCR was calculated separately for each sample. The *ACTR2* expression level was detected utilizing SYBR Green-based Real-Time PCR (qRT-PCR) (Applied Biosystem StepOnePlus). The PCR reaction (total volume 20 µL) included 2X SYBR Green Master Mix (10 µL), cDNA (3 µL), primers (1 µL), 1X ROX dye (0.4 µL), Nuclease Free Water (NFW) (4.6 µL), and NFW was used as the negative control. The PCR program consisted of 2 minutes (1 cycle) at 50 °C, followed by 40 cycles at 95 °C for 10 minutes, at 95 °C for 15 seconds, and at 55 °C for 1 minute. *ACTR-2* gene expression level was normalized with the Actin-Beta *(ACTB)* housekeeping gene and the relative expression was detected by the $2^{-\Delta\Delta Ct}$ value (20).

2.3. Statistical Analysis

SPSS software version 16.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. Values are given as Mean ± SD. T-test was used to determine *ACTR2* mRNA expression in pterygium and healthy conjunctival tissues. p values less than 0.05 were considered significant. Fold change was determined according to the range of 0.9 – 1.1. Accordingly, the *ACTR2* gene expression is decreased in pterygium tissue at values below 0.9. If it is in the range of 0.9–1.1, it does not change compared to normal conjunctival tissue. *ACTR2* gene expression at values greater than 1.1 was interpreted as increased in pterygium tissues (21).

3. RESULTS

The patients in the study were 33% (9) female and 67 % (18) male, with an age range of 43 – 78 years and a mean age ± SD 58± 8.43. According to the qRT-PCR analysis results *ACTR2* gene expression level increased by 74% (20/27) and decreased by 26% (7/27) in all pterygium tissues. When these data were analyzed, it was found that *ACTR2* gene expression increased (2.70±0.520) in pterygium tissue compared to normal conjunctival tissues. Fold change is shown in Figure 1. A range of 0.9-1.1 was used in the evaluation of the data. The increase in *ACTR2* mRNA expression level was statistically significant in pterygium tissue (p=0.005). *ACTR2* expression level is shown in Table 1.

Table 1. *Expression level of ACTR2*

	ACTR2 (mean ± SD)	*p* value
Pterygium tissue (n= 27)	2.70±0.520	* *p*=0.005
Conjunctiva tissue (n=27)	1	

*Abbreviations: SD, standard deviation, *= p < 0.05*

4. DISCUSSION

Pterygium is a conjunctival vascular growth that occurs on the cornea, causing symptoms such as redness, double vision, blurred vision and itching. Many risk factors have been identified and UV rays are shown as the strongest risk factor (22). However, its pathogenesis has not yet been clarified. Although pterygium is considered a degenerative disease and a benign tumor, it is thought that it should be considered a neoplasia due to its tumor-like properties (23). The observation of cell migration, cell proliferation, and local angiogenesis in the development of pterygium indicates uncontrolled cell proliferation (6). In addition, the fact that the pterygium shows common features with tumors such as hyperplasia, corneal invasion, uncontrolled cell proliferation, dysplasia, and recurrence after resection supports the view that it may be a premalignant tissue (6,23).

ARP2/3 complex play a role in many cellular processes such as cell activation, cellular movement, intercellular interactions, cytokinesis, vesicular trafficking, signal transduction, phagocytosis, adhesion, and mechanical processes (24,25,26). The function and dysregulation of *ACTR2* one of the two main actin-related proteins in the ARP2/3 complex have been associated with many diseases and cancer types in recent years (25). For example, Essential Thrombocytosis (ET), a myeloproliferative neoplasm, is characterized by abnormal proliferation of megakaryocytes and platelets. In this disease, low *ACTR2* expression ($p < 0.05$) has been reported to have significant prognostic value (27).

In another study, increased expression of *ACTR2* and *ACTR3* was associated with the stage of malignancy in colon cancer cells and stromal cells around the tumor (28).

Zhang et al. (29) observed that *ACTR2* expression was higher in gastric cancer tissues compared to normal gastric tissues, and *ACTR2* supported both cell proliferation and invasion. Moreover, high *ACTR2* expression has been associated with the aggressive behaviors observed in gastric cancer such as poor prognosis, tumor size, advanced tumor stage, and lymph node invasion.

Silencing *ARPC2* in breast cancer has been observed to result in a significant reduction in the invasion of breast cancer cells (30). Furthermore, high *ARPC2* expression level has been associated with low quality of life of breast cancer patients (31).

Huang et al. (19) investigated the possible association between members of the ARP2/3 complex and hepatocellular carcinoma (HCC) and reported significant upregulation of ARP2/3 complex subunits (especially *ARPC2, ACTR3*, and *ARPC5*) and correlated it with poor prognosis. Therefore, they reported that *ARPC2, ACTR3*, and *ARPC5* could be used as a biomarker and promising molecular targets for HCC therapy in the future.

Chen and Jiang (25) investigated the roles of *ACTR2* in diffuse large B-cell lymphoma (DLBCL) and found that upregulation of *ACTR2* exacerbates DLBCL malignancy by activating Wnt Signaling. All of these studies emphasize the importance of the ARP2/3 complex in the migration and invasion of many cancer cell types.

According to the literature, there is no study investigating the possible relationship between ARP2/3 complex and pterygium. However, the relationship between cell migration-related genes such as *Spermidine/Spermine N1-Acetyltransferase 1 (SAT1)* or *Calgranulin B (S100A9)* and pterygium has been investigated. In particular, pterygium migration was reduced when IPENSpm (SAT1 inhibitor) was administered. As a result, it was thought that the spread of pterygium could be prevented by using cell migration inhibitors and could be used as new treatment options (32).

Our research is the first to investigate the effects of the *ARP2/3 complex (ACTR2)* on cell migration in pterygium. According to our study, *ACTR2* gene expression in pterygium, which is thought to be a tumor analog, was significantly increased in pterygium tissues compared to normal conjunctival tissues (p=0.005). Our research results suggest that *ACTR2* may play a role in the pathogenesis of pterygium. However, although our study shows that *ACTR2* affects the migration of pterygium cells, experiments such as Western Blot, RNA interference (RNAi) and sequencing for this mutation are needed to define the function of *ACTR2* in pterygium formation and recurrence.

5. CONCLUSION

Surgical excision seems to be the best treatment option since the pathogenesis of pterygium remains unclear and effective treatments other than surgery are not sufficient. However, more effective treatment options are needed due to the astigmatism, and recurrence rates that can be observed after excision. Cell migration inhibitors affecting the *ACTR2* and perhaps other members of the *ARP2/3 complex* may provide new opportunities for therapeutic approaches to block or reduce the spread of pterygium.

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Research idea: KŞ, ÖA, NB,

Design of the study: KŞ, ÖA, NB, SŞ

Acquisition of data for the study: SOA, ÖA, HDD, RT

Analysis of data for the study: SOA, KŞ, NB, SŞ

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Clinical and Experimental Health Sciences

Evaluation of Candidemia Cases Developed in the Intensive Care Unit: A Ten-Year Analysis

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ABSTRACT

Objective: Fungal infections have been a major health problem for many years. They constitute a major cause of increased mortality and morbidity, especially in immunocompromised patients and intensive care unit (ICU) patients. In this study, we aimed to evaluate the epidemiologic characteristics, mortality and causative agent distribution of cases of healthcare-associated candidemia (HCA) in intensive care units of our hospital and to contribute to the literature.

Methods: Our study included patients diagnosed with healthcare-associated candidemia who were hospitalized in 3rd level ICUs with various complaints between November 2011 and August 2021 in Meram State Hospital.

Results: In our study, the mean age of patients who developed candida infection during intensive care unit hospitalization was 67.2±20.5 years. Of these patients, 59.5% (n:103) were men and 40.5% (n:70) women. Mean duration of hospitalization in the intensive care unit was 38.2±29.5 (min:1, max:231) days. Grouping of candida related HCAIs developed in patients according to Centers for Disease Control and Prevention (CDC) criteria shows that the most common candida related healthcare-associated infection (HCAI) was central line-associated bloodstream infection (CLABSI) at 52% and the second most common was laboratory-confirmed bloodstream infection (LCBI) at 31.2%. Cumulatively, candidemia are significantly higher to other candida related HCAIs.

Conclusion: To prevent and empirically treat candidemia, which has a very high mortality rate, the causative agent distribution of the center should be well understood. Large-scale, high-quality studies using various biomarkers in addition to clinical findings for the correct antifungal selection and to reduce mortality due to invasive candidiasis in line with these selections are warranted.

Keywords: Candidemia, mortality, healthcare-associated infection, HCAI

1. INTRODUCTION

Fungal infections have been a major health problem for many years. They constitute a major cause of increased mortality and morbidity, especially in immunocompromised patients and intensive care unit (ICU) patients (1). Candida species account for a large proportion of healthcare-associated fungal infections (HCFAIs). According to the Centers for Disease Control and Prevention (CDC), *Candida albicans* is the 7th most common nosocomial agent (2,3).

In humans, Candida is a normal flora element of the gastrointestinal and genitourinary systems. However, under appropriate conditions, it may exhibit a wide range of pathogenicity from regional mucosal infection to septicemia with multiple organ failure. The immune response of the host plays a decisive role in the development of Candida infection or determination of the type of infection (4).

In ICU patients, the risk of developing fungal infections has increased due to reasons such as sepsis-related disruption in the mucosal and/or skin barrier, advanced age, impaired production or function of neutrophils, metabolic dysfunction, prolonged surgery, prolonged exposure to broad-spectrum antibiotics, intravenous nutrition, mechanical ventilation, and use of multipath catheters (5). While the number of ICU beds constitutes approximately 5% of the total number of hospital beds, the development of more than 20% of health

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care-associated infections in ICU patients emphasizes the importance of the situation (6). In addition to the increasing number of invasive candidiasis cases worldwide, a prospective study reports an increase in non-albicans candida infections, which are highly virulent and frequently associated with treatment failure (7).

Early diagnosis and treatment of healthcare-associated candida infections that will develop in these patients becomes more important due to factors such as high morbidity and mortality in ICU patients, prolonged hospitalization and increased health care costs. However, the diagnosis of invasive candida infections is usually based on suspicion. Most of the time, the patient's clinic cannot be differentiated from a bacterial infection (8). However, there are no main criteria for empirical antifungal use in ICU patients. Meanwhile, early antifungal treatment is not recommended due to reasons such as antifungal resistance, drug toxicity and increased treatment cost (9). Antifungal treatment is frequently initiated in ICU patients after failure to respond to antibacterial treatment (7).

In our study, we aimed to examine the epidemiologic characteristics, mortality, and causative agent distribution of healthcare-associated candida infections that developed in the intensive care units of our hospital for a period of 10 years in order to evaluate the situation and contribute to the literature.

2. METHODS

The study approval was obtained from Karatay University Faculty of Medicine, Non-interventional Clinical Trials Ethics Committee (approval date/number: 21.09.2022/2022/020). Following the ethics committee approval we included patients who were hospitalized in the 3rd level ICUs (Emergency critical ICU, Neurosurgery ICU, Internal Medicine ICU, General surgery ICU, General ICU, Chest diseases and Thoracic surgery ICU, Coronary and Cardiovascular surgery ICU, Nephrology ICU, Neurology ICU, Reanimation ICU) with various complaints between November 2011 and August 2021 in Konya Meram State Hospital, who had candida growth in the samples taken, and who were diagnosed with infection with clinical or laboratory findings of these growths and who were older than 18 years of age. Surveillance data of these patients prospectively recorded by the infection control committee operating within the hospital were retrospectively analyzed. Patients who developed HCAIs but in whom no candida species grew were excluded from the study. The number of patients enrolled in the study was 173.

Microbiologic evaluation

Various samples (blood, catheter, urine, tracheal aspirate, throat, bronchoalveolar lavage culture) were collected from the patients based on clinical and physical examination results. Cultures were repeated at appropriate intervals for patients whose fever persisted above 38°C. Sterile samples were incubated in BACTEC 9240 (Becton Dickson, Diagnostic Instrument System, Spark, USA) and necessary inoculation and candida related identification procedures were performed by microbiology specialists working in our hospital. HCAIs were diagnosed and identified according to the generally accepted CDC criteria (10).

Statistical analysis

For statistical analysis, mean, standard deviation, minimum, maximum and ratio were used as descriptive statistics of the data. The t-test was used for intergroup comparisons, Mann-Whitney U test was used in the presence of data not conforming to normal distribution, and Chi-square test and Fisher's Exact test were used to analyze categorical data. For statistical significance, $p<.05$ was accepted. IBM SPSS® 23.0 program was used in the analyses.

3. RESULTS

In our study, the mean age of patients who had candida infection during intensive care unit hospitalization was 67.2±20.5 years (min: 18, max: 96). Of these patients, 59.5% (n:103) were men and 40.5% (n:70) women. The mean duration of hospitalization in intensive care unit was 38.2±29.5 (min:1, max:231) days.

Our study enrolled patients who developed candida infection in all 3rd level ICUs operating within the hospital during the determined date intervals. The distribution of patients with candida infection by the ICUs is given in Table 1.

Table 1. *The distribution of patients with candida infection by the ICUs.*

Inpatient Ward	Number of Patients (n)	Percentage (%)
Reanimation ICU	30	17.3
Neurology ICU	28	16.2
Nephrology ICU	26	15
Emergency Critical ICU	25	14.5
Chest Diseases ICU	22	12.7
Cardiovascular Surgery ICU	12	6.9
Internal Medicine ICU	10	5.8
General Surgery ICU	7	4
General ICU	6	3.5
Neurosurgery ICU	5	2.9
Thoracic Surgery ICU	2	1.2

Since we did not focus on a single ICU patient in our study, the diagnosis of patients for hospitalization also varied. The diagnoses of the patients admitted to the ICU are detailed in Table 2.

Our analysis revealed that 75.1% (n:130) of the patients who were followed up and treated in different ICUs for various reasons did not have any comorbid condition at the time of hospitalization, while 24.9% (n:43) had one or more

comorbidities. The distribution of comorbid events recorded is given in Table 3.

Table 2. *Diagnoses for ICU Admission.*

Diagnosis at Admission	Number of patients (n)	Percentage (%)
General state disorder (GSD)	27	15.6
Cerebrovascular accident (CVA)	24	13.9
Renal failure (Acute/Chronic)	19	11
Trauma	18	10.4
Pneumonia	14	8.1
Asthma/COPD exacerbation	13	7.5
Malignancy	10	5.8
Other respiratory events	9	5.2
Ischemic heart disease	8	4.6
Other	7	4
Congestive heart failure (CHF)	6	3.5
Other neurological diseases	4	2.3
Ileus and its complications	3	1.7
Postoperative follow-up	3	1.7
Pulmonary embolism	3	1.7
GI bleeding	2	1.2
Septicemia	2	1.2
COVID-19	1	0.6

Table 3. *Comorbidity status at admission*

Comorbid	Alive (n)	Dead (n)	p Value
	20	23	0.475
Comorbidity	**Number of patients (n)**		**Percentage (%)**
Chronic renal failure	13		30.2
Hypertension	9		20.9
Malignancy	8		18.6
CVA	6		14
CHF	5		11.6
Diabetes Mellitus (DM)	4		9.3
Asthma/COPD	4		9.3
Other	2		4.7
Coronary artery disease	1		2.3

CVA: Cerebrovascular accident, CHF: Congestive heart failure, COPD: Chronic obstructive pulmonary disease

Grouping of candida related HCAIs developed in patients according to CDC criteria shows that the most common candida related HCAI was central line-associated bloodstream infection (CLABSI) at 52% and the second most common was laboratory-confirmed bloodstream infection (LCBI) at 31.2%. Cumulatively, candidemia are significantly higher to other candida related HCAIs. The distribution of all candida related HCAIs is shown in table 4.

When patients with candida related HCAI were evaluated for the development of secondary candida related infection, only 9 patients (5.2%) developed secondary infection in addition to the existing candida related HCAI.

It is undeniable that ICU patients are exposed to various invasive interventions due to their treatment needs and severe clinical pictures. These invasive procedures constitute a risk factor for the development of infection in general. Thus, we analyzed the parameters that may be risk factors for the development of HCAI in our patients (Table 5). Seven (4%) of our patients did not have any risk factor, whereas 166 (96%) patients had one or more risk factors. The most common risk factor in our patients was the use of urinary catheter at a rate of 95.8%. This was followed by central venous catheter use at 88% and mechanical ventilation at 69.3%.

Table 4. *Distribution of Candida related HCAIs*

Type of HCAI (%)	Alive (n)	Dead (n)	p Value
CLABSI (52)	34	56	.639
LCBI (31.2)	20	34	
Catheter-associated urinary tract infection (CAUTI) (8.1)	8	6	
Other infections of the respiratory system (3.5)	2	4	
Primary deep incisional candida infection (2.3)	4	0	
Health care-associated pneumonia (HCAP)	1	2	
Symptomatic UTI (0.6)	1	0	
Intracranial infections (0.6)	1	0	

CLABSI:Catheter line-associated infection LCBI: Laboratory-confirmed bloodstream infection

Table 5. *Risk factors.*

Risk Factor(%)	Alive (n)	Dead (n)	p Value*
Use of urinary catheter (95.8)	62	97	.065
Use of central venous catheter (88)	55	91	*.036**
Mechanical ventilation (69.3)	39	76	*.005**
Total parenteral nutrition (61.4)	43	59	.720
Use of H_2 receptor antagonists (36.1)	30	30	.104
Blood transfusion (35.5)	24	35	.944
Use of peripheral arterial catheter (35.5)	28	31	.255
Enteral nutrition (32.5)	26	28	.243
Hemodialysis (28.3)	16	31	.299
Surgical drain (5.4)	8	1	*.003**
Chest tube/thoracentesis (2.4)	3	1	.307
Colostomy (1.8)	1	2	.784
Nephrostomy (1.8)	2	1	.569
Lumbar puncture (1.2)	1	1	1.000

**p≤.05*

Candida species that cause HCAI were also analyzed by screening the culture results of our patients. According to surveillance data, the most common causative agent was *Candida parapsilosis* (37.6%) and the second most common causative agent was *C. albicans* (24.3%) (Table 6).

Table 6. *Distribution of Candida species.*

Candida Species (%)	Alive (n)	Dead (n)	p Value
C. parapsilosis (37.6)	25	40	.294
C. albicans (24.3)	19	23	
C. tropicalis (6.9)	4	8	
C. glabrata (2.9)	1	4	
C. lusitaniae (1.7)	1	2	
C. famata (1.2)	1	1	
C. kefyr (0.6)	0	1	
C. dubliniensis (0.6)	0	1	
Candida spp. (24.3)	20	22	

In our study, we observed that the mortality in patients with candida related HCAI was 59%. In addition, we also evaluated whether age, gender, comorbidity, and development of secondary infection had an effect on mortality among the patients included in the study.

No statistically significant difference was found between gender and mortality in our patients with Candida related HCAI (p>.05). However, there was a statistically significant difference between the age of the patients and mortality (p>.05).

Patients were divided into two groups of ≤ 14 days and ≥ 15 days according to the length of hospitalization and the effect on mortality was evaluated. However, no statistically significant difference was found between the days in the ICU and mortality (p>.05).

We also evaluated the relationship between the type of infection and the development of secondary infection with mortality. No statistically significant correlation was found between the mortality of the patients and either the type of infection or the development of secondary candida related HCAI (p values .639 – .110, respectively).

We also performed an analysis to evaluate the effect of comorbidities on the mortality of candida related HCAI and found no statistically significant difference between mortality in the groups with and without comorbidities (p>.05). In addition, we also found that 7 patients had multiple comorbidities. We compared these 7 patients with patients who had only one comorbidity in terms of mortality. However, we did not find a statistically significant difference in mortality between the group of patients with one comorbidity and the group of patients with multiple comorbidity (p>.05).

Analysis of the effect of risk factors for HCAI on mortality revealed that the use of MV (p>.01), the surgical drain (p>.01) and the use of CVC (p>.05) increased mortality statistically significantly, while there was no statistically significant difference between the other factors and mortality.

Finally, the difference between the genus of the pathogen causing candida related HCAI and mortality was examined and no statistically significant difference was found between candida species and mortality (p>.05). Since the number of HCAIs caused by Candida species such as *C. famata, C. dubliniensis, C. kefyr* was very low, they were grouped under two groups as albicans and non-albicans and re-evaluated. However, no statistically significant difference was found between the albicans and non-albicans groups in terms of mortality (p>.05).

4. DISCUSSION

Critically ill patients who are treated in intensive care units are facing healthcare-associated invasive candidiasis due to the necessity of various invasive interventions, the use of broad-spectrum and multiple antibiotics, or the use of immunosuppressant drugs such as corticosteroids. Generally, cases of HCAI invasive candidiasis developing in this patient group cause high mortality and morbidity (11).

According to a review of invasive candidiasis cases worldwide, the causative agent is mostly identified as C. *albicans*. However, there has been a significant increase in the detection of non-albicans candida species in the last decades and they have been the cause of almost 50% of the cases (7, 12).

In our study, unlike most of the studies in the literature (13-15), we found that C. *parapsilosis* was the most common causative agent of candida related HCAI. C. *albicans* was the second most common causative agents with the same rates. In a study from our country, C. *parapsilosis* was the most frequent agent and C. *albicans* was the second most frequent agent isolated from blood culture (16).

C. *glabrat*a was detected as the most common non-albicans candida species throughout the world except Latin America. Similar to our study, the most common non-albicans species in Latin America was C. *parapsilosis* (15). Growth of C. *kefyr* was detected in only one of the patients in our study. Candidemia of C. *kefyr* is generally observed in patients with intensive use of corticosteroids or other immunosuppressant agents or in patients with severe malignancy causing immunodeficiency (17). We have also determined that our patient was a patient with malignancy-related immunodeficiency in parallel with the data in the literature. As these studies*, C. parapsilosis* in candidemia is posing a major threat for immunocompromised patients. The study highlights the urgent need to evaluate the possibility of development of *C. parapsilosis* candidemia in immunocompromised patients exposed to these risk factors effective should be implemented.

Classification of the candida related HCAIs that developed in our patients according to CDC criteria revealed that the most common infection was CLABSI at a rate of 52%. In a study the most common candida related HCAI was CAUTI (73.1%; n: 30) (18). This difference is speculated to be due to the time period during which the patients included in the study were hospitalized in intensive care unit. Because according to the CDC criteria published in 2016, candida growth alone in the urine sample does not diagnose UTI. Patients hospitalized between 2014 and 2016. Whereas, since the patient population in our study was from 2011 to 2021, we observed

a dramatic decrease in the number of CAUTIs since 2016 in the same study.

Rates vary depending on various factors, but HCAI invasive candidiasis infections have a high mortality rate with an attributable rate of approximately 49%. Moreover, this rate may increase up to 98% in septic shock cases in which antifungal treatment is delayed (19). In our study, we found a mortality rate of 59% in cases of HCAI invasive candidiasis developing in the ICU. Similar to our study, it was reported a 30-day post-infection mortality of 57.1% (16).

The intensive care unit patient group generally consists of patients with additional comorbidities. Previous studies have reported that DM predisposes to invasive candidiasis due to microvascular perfusion disorders, immunocompromisation, neutrophil function defects and hyperglycemia (20,21). In our study, no significant relationship was found between any comorbid condition, including DM, and mortality. It is thought that this may be related to our sample. Because only 43 of our 173 patients had comorbid conditions and only 4 of them had DM.

A meta-analysis reported that renal replacement therapy, mechanical ventilation, blood transfusion and DM were important risk factors in addition to well-known risk factors for invasive candidiasis infections (TPN use, colonization with candida, abdominal surgery, broad-spectrum antibiotic use, sepsis) (22).

Since there were no candida-negative cases in the patient group included in our study, we evaluated the effect of these risk factors on mortality. In the light of the data we obtained, we noted that the use of MV, CVC and surgical drains made a statistically significant difference in mortality. Similar to our results, it was found the relationship between MV and mortality to be significant, whereas the relationship between the use of CVC was found to be insignificant in their study (18). It was also found a significant association between MV and mortality in their study on HCAI candida infections in ICU patients (23). In a similar study, it was found no significant association between MV and the development of invasive candidiasis but found a significant association with tracheostomy (14).

Although candida species have the ability to grow even in parenteral nutrition fluids where bacteria cannot grow, in our study, no significant relationship was found between the use of TPN and mortality (24). In our study, no significant relationship was seen between gender and mortality, whereas a significant relationship was observed between age and mortality. However, in another study, no significant relationship between neither age nor gender and mortality (18).

Prolonged hospitalization in the ICU is a risk factor for invasive candidiasis due to severe disease status and invasive therapies. In addition, one-way regression analyses showed a high odds ratio (OR) for length of ICU stay (22). Patients were divided into 3 groups according to length of stay as less than 7 days, 7-14 days and more than 14 days and evaluated the relationship with the development of invasive candidiasis and found that the development of invasive candidiasis was significantly higher in the groups of less than 7 days and more than 14 days (14). In our study, since there were no candida-negative cases, we divided the patients into two groups as ≤14 days and ≥15 days and examined whether there was a difference in terms of mortality. We noted no statistically significant difference in mortality between the two groups.

Invasive infection severity, treatment strategies, virulence, infection prognosis and even clinical diagnosis of various candida species may be different (25). We therefore compared the mortality of all candida species (*Candida spp., C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. famata, C. kefyr, C. dubliniensis, C. lusitiane*) isolated from our patients. Our analysis showed that there was no significant difference between candida species in terms of mortality. Since we had a limited number of patients with some rare species, we divided the patients into two groups as albicans and non-albicans and made another comparison. Again, no significant difference was found in terms of mortality. In a study no mortality difference was found between candida species (18). However, in a study on patients hospitalized in chest diseases ICU, mortality was higher in patients with non-albicans growth than in patients with albicans growth (26). In addition, patients were devided into 3 groups (A: *C. albicans*, B: *C. parapsilosis*, *C. tropicalis*, C: *C. glabrata*, *C. krusei*) and investigated their effects on mortality. At the end of the study, they found no significant difference of mortality between group A and B but reported that group C had significantly less mortality than group A (27).

Our study has various limitations. While most of the studies in the literature involved single ICU patients and several-year episodes, our study included all 3rd level ICU patients except the pediatric ICU operating within our hospital and a 10-year period was scanned. Our study was not multicenter, which prevents the generalization of our results. In addition, our study has a retrospective design, therefore not all of the data required for the calculation of the "Candida Score" for invasive candidiasis could be obtained and the relationship between high score and mortality could not be examined (28). Another point is that the antifungal resistance pattern could not be studied for patients in every period in our hospital. Since the cases hospitalized with candidemia were analyzed retrospectively, the number of echoes that could be reached was very low (due to the recording of the echo results on a different system), and echo records were not stated respectively, because hospitalized cases with candida were analyzed.

5. CONCLUSION

Candida species are among the flora elements of our body but can cause invasive candidiasis infections of varying severity in immunocompromised patient populations such as critically ill patients in the ICU. Many factors affect the severity of infection to a greater or lesser extent. Our study suggests that the use of MV, age, use of CVC, and surgical

drains increase the mortality rate. To prevent and empirically treat candidemia, which has a very high mortality rate, the causative agent distribution of the center should be well understood. Large-scale, high-quality studies using various biomarkers in addition to clinical findings for the correct antifungal selection and to reduce mortality due to invasive candidiasis in line with these selections are warranted.

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Clinical and Experimental Health Sciences

High-Fidelity Environment Effect on Early Postpartum Haemorrhage Management

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ABSTRACT

Objective: In order to compare outcomes of a simulation of early postpartum haemorrhage management through a medium-fidelity mannequin in a high-fidelity environment before clinical practicums with those after clinical practicums in nursing students.

Methods: This quasi-experimental study was performed on second-year nursing students (n=61). After the simulation, the students were asked to perform postpartum haemorrhage control in clinical practicums. Data were collected with the State-Trait Anxiety Inventory, Student Satisfaction and Self-Confidence in Learning Scale, Educational Practices Questionnaire, and Student Self-Evaluation Form.

Results: The mean satisfaction, self-confidence, and Educational Practices Questionnaire scores after the simulation were high. The mean scores for feeling competent in involution, haemorrhage control, fundus massage, perineal care, andestablishing appropriate communication with patients and their relatives were 19.18±5.70 after the simulation and 23.83±5.03 after the clinical practicums.

Conclusion: The medium-fidelity simulation in a high-fidelity environment of early postpartum haemorrhage management enhanced the students' learning satisfaction and self-confidence. This helped them to feel more competent in the clinical practicums.

Keywords: High-fidelity environment, postpartum haemorrhage, nursing student, simulation

1. INTRODUCTION

New educational techniques are needed to enable nursing students to acquire cognitive, sensorial, and psychomotor behaviour. One of these techniques is simulation used "to replace or amplify real experiences with guided experiences, often immersive in nature, that evoke or replicate substantial aspects of the real-life in a fully interactive fashion" (1). As an interactive way of learning, it can offer real-life situations in a high-fidelity learning environment. It is effective in the acquisition of cognitive, sensorial, and psychomotor knowledge and skills (2). Simulations offer learner-centred experiences and learning environments which provide support and increase confidence. They allow students to gain experience by repeating and learning from their mistakes without causing damage to individuals (3). Simulations are divided into low, medium, and high fidelity (3,4). In many cases, the fidelity is described by the sophistication of the technology of the mannequins used (4). In the low-fidelity simulation, low-fidelity mannequins (segmented clinical task trainers) are utilized to improve psychomotor skills. In the high-fidelity simulation, high and medium-fidelity mannequins and standardized patients are employed (3). The medium-fidelity mannequins can more realistically mimic reality with pulses, heart sounds, and lung sounds, but they lack the physiological display of chest rise and fall with breathing, blinking, or automated physiological responses to interventions. Most high-fidelity manikins have expanding chests that breathe and have variable heart rates and tones, measurable blood pressure, and palpable pulses (5,6). In healthcare simulation, "high fidelity" refers to the highest

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representation of reality, including mannequins as well as all details about the environment and the patient's condition (5-7). A high-fidelity environment portrays an actual setting with appropriate working equipment as in a real-life setting (4). Realism is achieved not only by choosing a simulator but also by creating a high-reality environment (e.g., wig, hospital clothing, etc.) (6). Learners need the scenario to make sense, and if it seems true to life, they are willing to accept some artificial aspects of the physical environment (8). The more realistic environment might augment learning or serve as an obstacle to attaining objectives (9). The goal of simulation in the education of nursing students is to imitate various activity areas (medium/high fidelity) in accordance with the learning objectives and to finalize the implementation of the simulation scenario in the high-fidelity environment with team participation (10,11). The evidence has shown that such simulations in nursing education have positive outcomes, primarily including self-confidence and satisfaction, improved knowledge, critical thinking, and clinical skills (12-15).

Simulation-based education is valuable in that it improves maternity nurses' knowledge, self-confidence, and clinical skills to protect women's health status from deteriorating due to postpartum haemorrhage (16).

The normal involution process in the postpartum period, physiological haemorrhage following the steps of lochia rubra, lasting for the postpartum three days, lochia alba and serosa, is expected. Postpartum haemorrhage is commonly defined as blood loss exceeding 500 ml following a vaginal birth and 1000 ml following a caesarean section (17). When diagnosed and treated early, postpartum haemorrhage becomes a preventable cause of maternal deaths. Excessive postpartum haemorrhage is a condition that must not occur. Postpartum haemorrhage accounts for 25% of maternal mortality worldwide (18,19). Early recognition and immediate intervention are of essential importance. If it occurs within the first 24 hours of labour, it is considered an early haemorrhage and if it occurs in the 24th hour-6th week after labour, it is regarded as a late haemorrhage. Bleeding that requires more than one pad per hour indicates excessive bleeding (20,21).

Using simulations in education about maternity cases allows students to encounter a higher number of real conditions and practice more in a safer environment. This interactive experience gained through simulation tools typical of maternity, helps students learn relevant practices better and improve their critical thinking skills (22). Studies including newly graduated nurses have shown that utilizing simulations in education about postpartum haemorrhage management is effective in the improvement of nurses' knowledge and practice (16,23). There have been only a few studies revealing outcomes of simulations by using a medium-fidelity mannequin before clinical practicums with those after the practicums (24,25). In addition, a high-fidelity simulation has been found to enhance nursing students' performance in postpartum haemorrhage management (26). Therefore, the main purpose of this study is to examine the results of the simulation application performed in a high-fidelity environment in the management of early postpartum haemorrhage. This study differs from other similar studies by means of its findings about the effect of simulation performed in a high-fidelity environment on clinical skills.

2. METHODS

2.1. Study Design

The study had a quasi-experimental design and was conducted between February and May in 2017.

2.2. Aim

The present study was carried out to compare outcomes of a simulation of early postpartum haemorrhage management through a medium-fidelity mannequin in a high-fidelity environment before clinical practicums with those after clinical practicums in nursing students.

2.3. Research Question

How does a simulation of postpartum haemorrhage management with a medium-fidelity mannequin in a high-fidelity environment affect self-satisfaction, self-confidence, self-evaluation, and the anxiety of students?

The PICOT format was used to summarize the research question that explores the effect of simulation (27).

(P) – Population: Second-year university students aged 18 – 24 years, studying nursing and taking the course Obstetrics and Maternal Health.

(I) – Intervention: Write a script on postpartum haemorrhage management, apply it in groups, and have students fill out the following forms. Student Satisfaction and Self-Confidence in Learning Scale, Educational Practices Questionnaire, Student Self-Evaluation Form of Clinical Practice, and State-Trait Anxiety Inventory (Figure 1).

(C) – Comparison: Comparing the students' self-evaluation scores for target skills after the simulation with those after the clinical practicums. The relation between anxiety about target skills and self-evaluation scores after the simulation and those after the clinical practicums (See Tables 3 and 4).

(O) – Outcome: Changes in students' satisfaction, self-evaluation, and anxiety related to their skills for postpartum haemorrhage management.

(T) – Time: Measurement of the outcome before simulation, after simulation, and after clinical practicums.

2.4. Participants and Setting

The study sample included 64 second-year nursing students taking the course Obstetric Nursing in the nursing department of a foundation university. Three students were

excluded from the study due to absenteeism. The study was completed on 61 students.

2.5. Data collection instruments

2.5.1. Student Satisfaction and Self-Confidence in Learning Scale

The scale was created by Jeffries and Rizzolo and composed of 13 items. It was adapted to Turkish by Unver et al. and the number of the items decreased to 12 . It is a five-point Likert scale and includes the subscales of satisfaction with current learning and self-confidence in learning. The former is composed of five items and the latter is composed of seven items. There were not any negative statements. Higher scores on the scale indicate higher satisfaction and self-confidence. Cronbach's alpha coefficients on the Student Satisfaction and Self-Confidence in Learning Scale were 0.77 and 0.85 (28). In this study Cronbach's alpha coefficients of the Student Satisfaction and Self-Confidence in Learning Scale were 0.91 and 0.92.

2.5.2. Educational Practices Questionnaire

The questionnaire was developed by Jeffries and Rizzolo and adapted to Turkish by Unver et al. . It is composed of 16 items and four subscales; namely, active learning, collaboration, diverse ways of learning, and high expectations. Active learning had ten items, collaboration had two items, diverse ways of learning had two items and high expectations had two items. The scale is divided into two sections. The first section determines whether the best simulation design elements are implemented and is called presence. The second section determines to what extent simulation design elements are important for students and is called importance. Cronbach's alpha coefficient of the Educational Practices Questionnaire was 0.91 (28). In this study Cronbach's alpha coefficient is 0.91.

2.5.3. Student Self-Evaluation Form of Clinical Practice

Student Self-Evaluation Form of Clinical Practice, which allowed the students to self-rate their performance after the simulation and the clinical practicums, was developed by the researchers. The opinions of five experts were obtained for the self-evaluation form. There were no revisions by the experts. The form was tested by conducting a pilot study with 10 students. The students involved in the pilot study were not included in the main study.

During both the simulation and the clinical practicums, the students were expected to perform three skills (i.e. 1. uterine involution; checking fundal level, vaginal tonus, vaginal bleeding and fundal massage, 2. perineal care; perineal evaluation and perineal wash and 3. establishing an appropriate communication with the women and their relatives) as required by the scenario. A visual analogue scale was used to determine how the students perceived their competencies in the abovementioned three skills. At one end of the scale is the score of zero corresponding to very incompetent and at the other end is the score of ten corresponding to very competent. The students were given the scale without numbers on it. Students marked a point indicating their perceived competency. One of the researchers measured the point with a ruler to determine its numerical value.

2.5.4. State-Trait Anxiety Inventory

Developed by Spilberger, Gorsuch, and Lushene, the inventory was adapted into Turkish by Öner and Le Compte . It includes two scales: state anxiety and trait anxiety. The total score for each scale of the inventory ranges from 20 to 80. Higher scores indicate more severe anxiety (29). Cronbach's alpha coefficient ranged from 0.83 to 0.92 for the State Anxiety Scale, and from 0.86 to 0.92 for the Trait Anxiety Scale (29). In this study Cronbach's alpha coefficient is 0.46 for the State Anxiety Scale, and from 0.61 for the Trait Anxiety Scale.

2.6. Data analysis and study process

Data analysis was performed by using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) for Windows version 21.0. Kolmogorov–Smirnov test was utilized to determine whether the data were normally distributed. Descriptive statistics including mean and standard deviation, minimum–maximum values, frequencies, and percentages were used for the analysis. The results obtained by the descriptive statistical tests were compared by using the Pearson Correlation Test and Paired Samples t-test. $p < .05$ was considered statistically significant.

2.7. Procedure

All the students participating in the study were given a two-hour theoretical course about postpartum haemorrhage management. Thereafter, they attended eight-hour skill training in the laboratory. Students filled out the Trait Anxiety Scale after the skills training.

A scenario about postpartum haemorrhage involving the target three skills uterine involution evaluation (fundal level, vaginal tonus, vaginal bleeding, and fundal massage), perineal care (perineal evaluation, perineal wash), and feeling competent in communication with women and their relatives, was created by the researchers in light of the literature (30) (See Box 1).

After taking the theoretical course and the skill training, the students participated in the scenario. Before taking part in the scenario, the students were administered the State Anxiety Inventory.

The scenario was carried out in a "Centre of Advanced Simulation and Education". The environment in which the simulation took place was designed as a high-fidelity environment. This environment included the equipment

of a standard patient room (bed, basic medical equipment, patient monitor and alarm system, bathroom and toilet). Laerdal Nursing Anne Mannequin was given the appearance of a bleeding puerperal woman with the application of mullage (bloody pad soaked in red paint, pale skin image, weak and moaning voice on the mannequin). In the scenario, a lecturer played a role as a patient's relative among the scenario characters.The facilitator, who was a relative of the patient, had the profile of an anxious patient.

Students were expected to manage bleeding by integrating three skills in the scenario and using time management, teamwork, and communication skills. The faculty members evaluated whether the scenario was carried out correctly through the checklist containing these parameters. This checklist was prepared by the scenario developers as a guide for instructors to follow the flow of the scenario in line with the learning objectives of the scenario. It was used within the scope of the research not as a data collection tool but to manage the discussion in debrifing. As moderators, two lecturers monitored the flow of the scenario from the control room and the students' performances and took notes for the debriefing session via checklist.

Sixty-one students in the sample were divided into two stations of thirty-one and thirty students. Groups consisting of two students each were formed at these stations. The scenarios were implemented simultaneously at both stations. Students were given 10 minutes to realize the scenario. A debriefing session was held with the students who completed the scenario application. These sessions lasted 30 minutes and included eight students each. Following the debriefing, the students completed Student Satisfaction and Self-Confidence in Learning Scale, Educational Practices Questionnaire, and Student Self-Evaluation Form of Clinical Practice at face-to-face interviews. It took about ten minutes to fill in them. After the simulation, the students attended clinical practicums lasting 80 hours in total. They first implemented the three skills on the woman as required by the scenario and then filled in the Student Self-Evaluation Form of Clinical Practice and the State-Trait Anxiety Inventory.

Box 1. *The simulation scenario*

S.Y. giving a normal spontaneous birth to a healthy female infant does not receive any treatment and is followed in her postpartum period. Due to postpartum cramps, she has been given a sedative and a medication that has a hypnotic effect (5mg Zolpidem Tartrate tablet). She is accompanied by her older sister, who has high anxiety. The scenario starts with the sentence uttered excitedly by her older sister "My sister is bleeding and changes her pad more than once every hour. The scenario is directed towards achieving the following objectives:
The students will observe and evaluate women based on their information about the women.
The students determine priorities based on their evaluations of the women and then plan, implement evaluate postpartum care.
The students establish effective communication with members of the healthcare team.
The students establish therapeutic communication with the women and their families.

The steps followed in the study are presented in a flow chart in Figure 1.

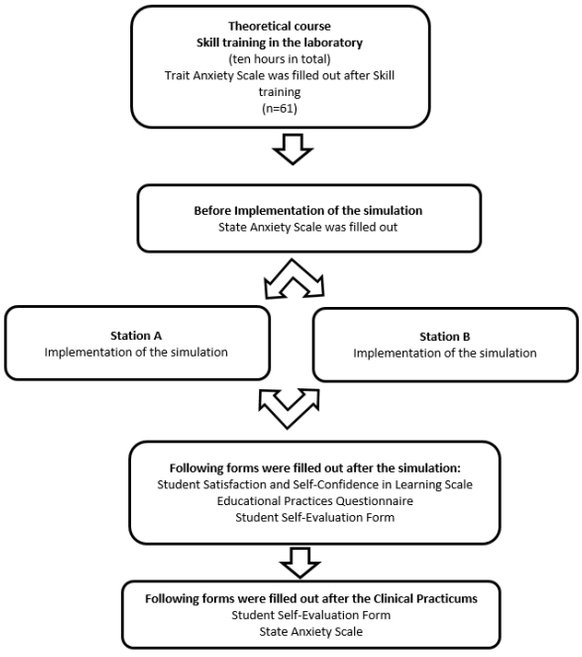

Figure 1. *Flow chart of the study*

2.8. Ethical Consideration

Approval for the study was obtained from the ethical review boards at the authors' institution (2017-3/2). The researcher gave written information to the students participating in the study and explained the purpose of the research, confidentiality of the data collected, protection of anonymity, and the right of refusal to participate. The participants were assured that their education would not be affected when they could not participate in the study or wanted to quit the study. All the participants gave written informed consent. They were informed that the training given in the scope of the course was part of a study and the evaluations would not affect the evaluation of their performance in the course. Data were gathered by the researcher who was not responsible for offering the course.

3. RESULTS

Of 61 participants, 88.7% were female and 11.3% were male. The mean age was 19.85±0.89 years in the female students (range: 18-24 years) and 19.57±0.53 years in the male students (range: 19-20 years). The mean scores for state anxiety were 38.74±4.96 before the simulation and 42.56±4.83 after the clinical practicums. The mean score for trait anxiety was 47.69±5.29.

Table 1 presents the mean scores for the subscales of Student Satisfaction and Self-Confidence in Learning Scale. The mean score was 4.20±0.64 for satisfaction and 4.14±0.53 for self-confidence in learning.

Table 1. *The Mean Scores for Student Satisfaction and Self-Confidence in Learning Scale (N=61)*

Subscales	Range	Mean±SD
Satisfaction with current learning	2-5	4.20 ± 0.64
Self-confidence in learning	3-5	4.14 ± 0.53

Table 2 shows the scores for the presence and importance sections of the Educational Practices Questionnaire and the relation between these scores. There was a significant positive relation between the presence and importance of the best simulation design elements (p<.001).

Table 2. *The Distribution of the Mean Scores for Subscales of the Educational Practices Questionnaire according to the Presence and Importance of the Best Simulation Design Elements (N=61)*

Educational Practices Questionnaire	Presence Mean±SD	Importance Mean±SD	r*	p
Active Learning	4.25±0.55	4.26±0.61	0.451	**.001**
Collaboration	4.33±0.63	4.12±0.87	0.430	**.001**
Diverse Ways of Learning	4.40±0.66	4.43±0.77	0.560	**.001**
High Expectations	4.32±0.68	4.39±0.68	0.542	**.001**
Total	4.31±0.50	4.28±0.56	0.486	**.001**
**Pearson Correlation Test*				

Table 3 demonstrates the mean scores for self-evaluation after the simulation and the clinical practicums. There was a significant difference in the score obtained after the simulation for feeling competent in uterus involution and haemorrhage control, fundamental massage and communication with women and their relatives, and in the score obtained after clinical practice (p<.001). There was not a significant difference in the scores for competence in perineal care (t:-1.55 p>.05)

Table 3. *The Comparison of the Students' Self-Evaluation Scores for Target Skills after the Simulation and the Clinical Practicums (N=61)*

Target Scores	After the Simulation Mean±SD	After the Clinical Practicums Mean±SD	t*	p
Feeling competent in uterine involution checking fundal level, vaginal tonus, vaginal bleeding, and fundal massage	5.90±2.55	7.24±2.42	**-3.95**	**.001**
Feeling competent in perineal care checking perineal evaluation and perineal wash	7.27±2.30	7.89±2.54	-1.55	.128
Feeling competent in communication with women and their relatives	6.00±2.43	8.74±1.59	**-2.05**	**.001**

** Paired Samples t-test*

Table 4 shows the relation between the self-evaluation scores and the anxiety scores after the simulation and the clinical practicums. There was a significant moderate negative relation between feeling competent in communication with the women and their relatives and state anxiety after the simulation (r: – 0.341; p<.05). However, there was not a significant relation between them after the clinical practicums (r: – 0.425; p<.01).

Table 4. *The Relation between Anxiety about Target Skills and Self-Evaluation Scores (N=61)*

Target Skills	After the simulation State anxiety r	p	Trait anxiety r	p	After the clinical practicums State anxiety r	p	Trait anxiety r	p
Feeling competent in uterine involution checking fundal level, vaginal tonus, vaginal bleeding, and fundal massage	-0.033	.801	-0.104	.423	-0.059	.679	-0.104	.423
Feeling competent in perineal care checking perineal evaluation and perineal wash	0.129	.363	-0.173	.179	-0.276	.064	0.048	.738
Feeling competent in communication with women and their relatives	**-0.341**	**.007**	**-0.425**	**.001**	-0.219	.115	**-0.425**	**.001**

r= Pearson correlation test

4. DISCUSSION

In the present study, the effects of a medium-fidelity simulation in a high-fidelity environment on outcomes of postpartum haemorrhage management practices before and after clinical practicums were examined. The students got very high scores for satisfaction with and self-confidence in learning. The mean scores, determined by a five-point Likert scale, were higher than four. Results of similar studies in the literature pointed out that simulation-based education enhanced student satisfaction and self-confidence (14,26,31,32). Hicks et al. examined knowledge, performance, and self-confidence in students receiving simulation-based education and those not getting this education. They found no difference in knowledge and performance between the students offered simulation-based education and those not

receiving this education; however, they reported that the students getting this education had higher self-confidence than those not getting this education (33). In a study by Mahfouz et al. using high-fidelity simulation techniques had significant effects on self-confidence compared to low-fidelity simulation (14). Alharbi et al., concluded that experiencing human patient simulation with nursing students can increase satisfaction and self-confidence (15).

In a study by Mert Karadas and Terzioglu using high-fidelity simulation and standardized patients, simulation practices increased students' self-confidence in the management of postpartum haemorrhage (26). Choi and Wong the more realistic environment might augment learning or serve as an obstacle to attaining objectives (9). A qualitative study performed by Kaddoura et al. stated that nursing students feel self-confident after simulation because they practice skills in an environment that is similar to clinical practice (34). In the present study, improved self-confidence and satisfaction of the students can be attributed to receiving simulation-based education in a high-fidelity environment which allowed the students to merge their theoretical knowledge and skills they acquired through the simulation.

The students got low scores for feeling competent in involution, haemorrhage control, fundal massage, and communication with women and their relatives after the simulation. Evaluation of student's performance by faculty members can cause students to experience stress. Many students have verbalized feeling uncomfortable with the fact that they are watched and evaluated by faculty on performance (35). Although simulation improves students' clinical skills, it can also induce anxiety (36). While some anxiety can help facilitate performance, too much can be debilitating (4,36). This is an expected result.

However, their self-evaluation scores significantly increased after the practicums compared to their scores after the simulation. Ha found students were satisfied with the simulation because it prepared them for future clinical practice (37). Martins and Pinho stated that students were satisfied with the simulation because they found an improvement in their collaboration and communication skills (38). Thomas and Marz emphasized that simulation-based education has a positive effect on acquiring professional nursing skills. Similar studies also showed that this type of education facilitated the acquisition of care skills, communication skills, and decision-making skills (39). Mert Karadas and Terzioglu evaluated students' communication skills during simulation practices and clinical practices and obtained similar results to the present study (26). It is obvious that simulation-based education will help students feel more competent in clinical practice.

Another striking finding of this study was that a negative relation between the students' self-rating scores for feeling competent in their communication with patients and their relatives after the simulation disappeared after the clinical practicums. This finding suggests that students' anxiety about their communication with patients and their relatives after the simulation decreased during the clinical practicums. There is evidence in the literature that severe anxiety due to insufficient experience in clinical environments has a negative effect on learning (40).

In addition to insufficient experience, there may be many factors causing stress during simulations. For example, attending a simulation for the first time, using an unfamiliar mannequin/standardized woman, and the presence of educators evaluating student performance during simulations can create stress (2). Regarding the effects of types of simulation practices, Mert Karadas and Terzioglu revealed no difference in the severity of anxiety (26). However, Horsley and Wambach reported that the presence of faculty members in the simulation laboratory caused an increase in anxiety levels but not significantly (41). In the present study, the faculty members were available in the simulation laboratory during the implementation of the scenario, but they were in the control room of the laboratory and the students were not aware of their presence. In accordance with principles of simulation practices, even if students are informed that faculty members who sit in the control room can only intervene when necessary, evaluation of student's performance by these faculty members can cause students to experience stress.

It has been emphasized in the literature that using simulations in early nursing education is useful in the reduction of anxiety in students (40,42). Simulation-based education before clinical practicums decreased anxiety (42-46) but did not increase self-confidence and satisfaction (30). This education provides students with an opportunity to gain experience in conditions they are likely to encounter in practice (42). Evidence from both the present study and the literature underlines the role of simulation-based education in terms of enabling students to prepare for clinical practices.

5. CONCLUSION

This study investigates nursing students' experiences of learning postpartum haemorrhage management in a simulation environment and then using these skills in clinical practice. Specifically, the aim of the study is to evaluate the impact of simulation on clinical practice. The use of simulations of medium – and high-fidelity environments in postpartum haemorrhage management increased the students' satisfaction and self-confidence.This caused the students to feel more competent in the clinical practicums. This means that simulation can help students enter clinical practice more prepared and confidently. Although anxiety about communication with women and their relatives was high during the implementation of the scenario, it decreased during clinical practicums. Higher levels of skill and confidence in post-simulation clinical practice helped students be better prepared for real-world applications of simulation. This can enable students to be more successful and effective in the clinical setting. The difference of this study from other studies is that it directly evaluates the effect of simulation on clinical practices and measures how this effect is reflected

in student satisfaction, self-confidence and skill levels. In addition, it is thought to contribute significantly to the literature since the focus of the study is the evaluation of a specific skill such as postpartum haemorrhage management. However, randomized, controlled studies are needed to reveal the effects of simulation-based education on clinical nursing practice.

The limitation of this study was that the sample did not include a control group and that the long-term effects of simulation-based education were not evaluated.

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Clinical and Experimental Health Sciences

Determining the Care Burden and Burnout Levels of Family Members Providing Care for Cancer Patients

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ABSTRACT

Objectives: Caregivers' care burden and burnout levels are projected to increase as the physical independence of their patients decreases. This study aimed to determine the care burden and burnout perception of caregivers of cancer patients.

Methods: The study was conducted with 143 patient relatives who were providing primary care for patients treated between March 1 and June 1, 2017, in the clinics of the Institute of Oncology of Istanbul University. The data were collected using the " Personal information form ", "Zarit Caregiver Burden Scale" and "Maslach Burnout Inventory". Data were analyzed using the SPSS statistical program. Descriptive statistics (frequency, percentage, mean, minimum, maximum, standard deviation) student t-test, one-way ANOVA, and Pearson correlation coefficient were used for data analysis.

Results: The mean score from the burden interview was found 27.38±17.04. In the burnout inventory results, the mean of emotional exhaustion is 19.61±9.08, the mean of depersonalization is 8.78±5.38, and the mean of decrease in personal accomplishment is 29.66±5.91. Individuals' perceptions of emotional exhaustion and depersonalization increase as their perception of care burden increases ($p<.001$). There was, however, no relationship between care burden and personal accomplishment ($p>.05$). The study demonstrated that caregivers' care burden and burnout perceptions increased as cancer patients' self-care ability worsened ($p<.01$).

Conclusion: It has been found that the perceived care burden level is low and burnout level is middle among the caregivers of cancer patients. The patients' level of dependency increases perceptions of caregiver burden and burnout increases too.

Keywords: Cancer patient, family, care burden, burnout, oncology

1. INTRODUCTION

Cancer is a life-threatening disease with increasing prevalence worldwide and is one of the most important diseases today. The process of diagnosing and managing the disease and patient care represents a very difficult period for the patients and their families. Family members providing care for the patients may feel physically and emotionally exhausted (1). As the patients lose their ability to function independently, their care is assumed by another person (2). Caregivers must also take on the responsibility of care. Caregiving starts when the patient's condition progresses, gets worse and symptoms deteriorate. Caregivers empathize with the patients, and they may experience feelings of self-respect and satisfaction with the self, but may also suffer from negative feelings due to personal or extraneous causes, as their experience, communication and personal development improve as they provide care. (3). Monetary problems, psychological problems, and patient burnout may particularly lead to an unfavorable course of events for the patient and the caregiver (4).

Care of the patients diagnosed with cancer is undertaken by their family members. Caregiving may be a financial and psychological burden for caregivers. The care burden is even greater for the relatives of terminally ill patients. The fact that the psychological state of the caregiver is affected in the caregiving of terminally ill patients adds further to the caregiving burden (5). Studies have shown that women constitute the majority of caregivers in many countries (4,5). Compassion and affection which are characteristics of women are regarded as key perceptions leading them to assume patient care. In addition, women can undertake care duties better and find solutions to any problems they may encounter faster than men. However, male caregivers have a stronger mood than female caregivers (3).

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Limitations in a Caregiver's life can increase anxiety and the perception of depression, causing the person to feel burnout. Burnout is defined as the state where varied resources the individual has are about to be depleted, and the individual is in a constant condition of being unhappy and having negative feelings in daily living. Individuals experiencing burnout syndrome are those with diminished life energy who feel desperate against life and make no effort to get their lives back on track (6). Physical and psychological problems the caregivers of cancer patients experience and the limitations to their activities of daily living may increase their anxiety and depression perception and may thus lead the caregivers to feel exhausted. The changing social relations and roles the caregivers undertake and the economic problems they experience may enhance burnout perception. This may have negative consequences on the well-being of both the caregiver and the patient. It is therefore essential to determine the caregiver's perceived burnout and to take precautions accordingly (6). This study aims to determine the care burden and burnout perception of caregivers of cancer patients.

2. METHODS

2.1. Design and Sample

The study was performed in a descriptive and cross-sectional design to determine the care burden and burnout perception of caregiving family members of cancer patients. The population of the research consists of 156 patient relatives who provide care to patients receiving treatment recorded at Istanbul University Oncology Institute clinics between March 1 and June 1, 2017. In the power analysis that has been made, it has been discovered that the sample size is α=.05, and the significance level is 1-α=.95 confidence interval β=.10 error risk is 1-β=.90 and the total number of subjects is 110. Research has been made with the 143 patients' relatives who provide primary care for the cancer patient. The patient relatives included in the study were above 18 years of age, had no communication problems, and agreed to take part in the study.

2.2. Research Questions

Is the care burden of caregivers increasing for cancer patients who cannot meet their self-care needs?

Is the perception of burnout increasing among caregivers of cancer patients who cannot meet their self-care needs?

Does the burnout of individuals caring for cancer patients increase as the care burden increases?

2.3. Data Collection

After obtaining the approvals of the ethics board and the institution, the patient's relatives were explained the purpose of the study, their written consents were received, and the personal information forms were completed using the face-to-face interviewing method. The data were collected using the " Personal information form ", "Zarit Caregiver Burden Scale" and "Maslach Burnout Inventory".

2.3.1. Personal information form

There are total of 23 questions in the personal information form including 9 questions regarding the sociodemographic characteristics of the participants, 8 questions regarding the individual characteristics of the caregiver, and 6 questions regarding the person's level of relationship with the caregiver.

There is also a question assessing 9 self-care needs. In this question, patients who perform self-care activities without support are considered independent, and patients who need support are considered dependent.

2.3.2. Zarit Caregiver Burden Scale

Developed in 1980 by Zarit et.al. to evaluate the stress of caregivers, the scale includes 22 questions. Scores from the scale increase with increasing distress experienced in caregiving (7). The internal consistency coefficient of the scale was found to range between .87 and .94. The validity and reliability of its Turkish adaptation were established the 2008 year (8). The Cronbach's alpha coefficient of the scale was .81 in the present study.

2.3.3. Maslach Burnout Inventory

The inventory comprises 22 questions. It is a Likert-type scale, scored from 0 to 5 as never, a few times a year, a few times a month, a few times a week, and every day. It has three sub-dimensions, i.e. "Emotional exhaustion" including 9 items (1, 2, 3, 6, 8, 13, 14, 16, 20), "Depersonalization" including 5 items (5, 10, 11, 15, 22) and "Reduced personal accomplishment including 8 items (4, 7, 9, 12, 17, 18, 19, 21). Emotional exhaustion and Depersonalization include negative, and reduced personal accomplishment includes positive responses. Low reduced personal accomplishment and high emotional exhaustion and depersonalization indicate a high level of burnout. To rate, three separate burnout scores are calculated for each individual. Responses were scored on a six-point likert scale (ranging from 0 = never to 6 = everyday) for each subscale and tabulated into three tiers (low, moderate, or high) based on the reference ranges provided with the Maslach Burnout Inventory: for emotional exhaustion, low (0–16), moderate (17–26) and high (≥27); for depersonalization, low (0–6), moderate (7–12) and high (≥13), and finally, for personal accomplishment, low (≤31), moderate (32–38) and high (≥39). Capri found a Cronbach's alpha internal consistency coefficient of .93. (9). Internal consistency coefficients for sub-dimensions were .83, .75, and .88 respectively. In this study, Cronbach's alpha coefficients were .82 for emotional exhaustion, .86 for depersonalization, and .85 for reduced personal accomplishment.

2.4. Ethical Considerations

This study was taken with the consent of the Marmara University Institute of Health Sciences ethics committee (No: 11, Date: 28.03.2016). It was compatible with Helsinki Declaration policies.

2.5. Statistical Analysis

Data were analyzed using the SPSS statistical program. Descriptive statistics (frequency, percentage, mean, minimum, maximum, standard deviation) student t-test, one-way ANOVA, and Pearson correlation coefficient were used for data analysis. The level of significance was set at p<.05.

2.6. Limitations of the study

Due to the long duration of cancer treatments, patient circulation in oncology clinics is low. For this reason, the study was conducted with a limited number of patients.

3. RESULTS

Of the respondents, 66.4% were female, 46.2% were aged 30 years and below, 46.9% had bachelor's degrees, 87.4% were urban residents, 60.1% had mid-level income, 73.4% were staying with the patient at night, 64.8% had a constant place of residence with the patient, and 72.7% were not responsible for providing care for another individual. Of the respondents, 54.5% were providing care for a first-degree relative, 86.7% received assistance in caregiving and 39.9% spent more than 18 hours of their time with the patient. Of the patients the respondents provided care for, 52.4% were male, 73.5% were aged 30 years and older, 33.6% had breast cancer, 59.4% had had the disease for 0-6 months and all had social security. Of them, 46.9% were dependent on others in eating and drinking, 67.1% in bathing/showering, 65% in dressing up/grooming, 60.1% in going to the toilet, 68.5% in walking/strolling, 70.6% in climbing upstairs, 73.4% in shopping, 85.3% in cooking and 87.4% in maintaining the household. Patients' and caregivers' sociodemographic data are provided in Table 1.

The mean score from the caregiver burden scale was detected as 27.38±17.04, under this circumstances care burden for the respondents is low. In the providing care scale, the situation of being dependent on the relative has the highest interval with 2.47±1.62 and the situation of taking care of someone else than the relative has the lowest interval with 0.46±0.82.

Patient relatives who were aged 31 to 45 years had a higher perception of care burden compared to others (p<.001). There is a significant difference between the occupation and the perceived care burden of the caregiver (p<.001). Workers and civil servants have a higher care burden perception than members of other occupations. There is a difference between the age of the patient cared for and the perceived care burden (p<.001). Individuals providing care for patients aged 61 years and above have higher perceptions of care burden compared to other age groups. Individuals providing care for patients who had been diagnosed 2 years ago and more also have higher perceptions of care burden. There is a difference between each of the items in self-care of the patient cared for and perceived care burden (p<.001). The care burden increases as the ability of patients to perform self-care tasks decreases. The difference between patients' ability to perform self-care tasks and the care burden perceived by the caregiver is shown in Table 2.

Table 1. *Sociodemographic Characteristics Distribution of Caregivers and Patients (N=143)*

Characteristics	n	%
Caregivers		
Gender		
Female	95	66.4
Male	48	33.6
Age		
30 years and below	66	46.2
46 years and above	49	34.2
31-45 years	28	19.6
Occupation		
Student	38	26.6
Worker	29	20.3
Housewife	28	19.6
Civil servant	19	13.3
Other	19	13.2
Retired	10	7
Social Security		
Yes	134	93.7
No	9	6.3
Patients cared for		
Gender		
Male	75	52.4
Female	48	47.6
Age		
45-60 years	39	27.3
61 years and above	39	27.3
30 years below	38	26.5
31-45 years	27	18.9
Diagnosis		
Breast	48	33.5
Lung	19	13.3
Bone	18	12.6
Testicular Tumor	17	11.9
Colon	10	7
Pancreas	10	7
Ovary	10	7
Liver	9	6.3
Ewing Sarkom	2	1.4
Disease Duration		
0-6 months	85	59.4
2 years and above	29	20.3
6-12 months	19	13.3
1-2 years	10	7

Table 2. *The difference between patients' ability to perform self-care tasks and care burden perceived by the caregiver (N=143)*

Self-care behaviors	Mean ± SD		t	p
	Dependent	Independent		
Eating/drinking	58.12±22.52	42.71±26.81	5.78	.000*
Bathing/showering	54.58±23.14	39.74±23.14	5.27	.000*
Dressing up/grooming	56.21±21.53	38.21±23.08	6.25	.000*
Going to the toilet	55.21±22.48	38.18±21.84	5.95	.000*
Walking/strolling	56.21±25.64	40.21±25.30	5.65	.000*
Climbing upstairs	59.22±23.47	41.63±26.57	6.2	.000*
Shopping	60.18±21.57	37.24±19.71	6.02	.000*
Cooking	59.61±20.39	41.68±24.93	6.1	.000*
Maintaining the household	61.24±20.74	36.14±21.66	6.21	.000*

*Note. SD = standard deviation; *p<.001.*

As for the results of the burnout inventory; the mean emotional exhaustion score was detected as 19.61±9.08, the mean depersonalization score was detected as 8.78±5.38 and the mean reduced personal accomplishment score was detected as 29.66±5.91. Caregivers have the middle perception of burnout. There is a difference between emotional exhaustion and caregiver gender (p<.001) while there is no difference between patient family member gender and depersonalization or reduced personal accomplishment (p>.05). Perception of emotional exhaustion is higher among women. There is a difference between the social security status of the caregiver and emotional exhaustion and depersonalization (p<.001), while social security status and reduced personal accomplishment are not significantly related (p>.05). Emotional exhaustion and depersonalization perceptions are higher in caregivers without social security. There was a difference between emotional exhaustion and depersonalization and income status (p<.001), but no difference between income status and reduced personal accomplishment (p>.05). Perception of emotional exhaustion and depersonalization is higher in those with low levels of income. Emotional exhaustion and depersonalization perceptions are higher in the absence of individuals assisting in patient care. There is a significant difference between each of the items in the ability of the patient cared for to perform self-care tasks and perceptions of emotional exhaustion, depersonalization, and reduced personal accomplishment (p<.001). Caregivers of patients who are dependent on others in eating/drinking, bathing/showering, dressing up/grooming, going to the toilet, walking/strolling, climbing upstairs, shopping, cooking, and maintaining the household have higher perceptions of emotional exhaustion, depersonalization, and reduced personal accomplishment. The difference between the ability of the patients cared for to perform self-care tasks and the burnout perceived by the caregivers is provided in Table 3.

Table 3. *The difference between the ability of the patients cared for to perform self-care tasks and burnout perceived by the caregivers (N=143)*

Self-care behaviors	Sub-dimensions	Mean ± SD		t	p
		Dependent	Independent		
Eating/drinking	Emotional exhaustion	22.16±8.25	17.36±6.54	3.25	.001**
	Depersonalization	9.52±5.64	8.12±4.52	3.52	.000*
	Decrease in personal accomplishment	31.63±8.52	27.43±5.63	4.35	.000*
Bathing/showering	Emotional exhaustion	23.21±6.85	16.17±7.85	4.52	.000*
	Depersonalization	10.25±4.52	7.82±5.63	4.53	.000*
	Decrease in personal accomplishment	31.57±6.54	27.38±7.45	4.52	.000*
Dressing up/grooming	Emotional exhaustion	24.17±7.52	15.24±4.31	4.63	.000*
	Depersonalization	11.25±3.54	6.54±3.82	5.65	.000*
	Decrease in personal accomplishment	30.60±7.12	26.46±8.63	4.37	.000*
Going to the toilet	Emotional exhaustion	23.18±7.64	16.21±7.64	4.51	.000*
	Depersonalization	11.62±4.63	6.22±3.71	5.61	.000*
	Decrease in personal accomplishment	32.64±8.63	26.42±6.97	4.55	.000*
Walking/strolling	Emotional exhaustion	22.51±9.12	17.54±8.25	3.65	.000*
	Depersonalization	10.20±5.28	7.80±4.23	4.32	.000*
	Decrease in personal accomplishment	31.61±7.92	27.44±8.52	4.22	.000*
Climbing up stairs	Emotional exhaustion	21.65±7.56	18.14±7.23	3.15	.000*
	Depersonalization	10.55±5.34	7.24±3.10	4.86	.000*
	Decrease in personal accomplishment	31.59±9.52	27.36±9.46	4.65	.000*
Shopping	Emotional exhaustion	24.53±5.31	15.30±3.69	4.63	.000*
	Depersonalization	9.54±4.39	8.10±2.54	3.61	.000*
	Decrease in personal accomplishment	30.58±7.22	26.48±8.22	4.69	.000*
Cooking	Emotional exhaustion	25.54±4.82	16.54±5.12	5.21	.000*
	Depersonalization	9.64±3.91	8.00±3.17	3.54	.000*
	Decrease in personal accomplishment	31.62±8.46	27.43±6.17	4.29	.000*
Maintaining the household	Emotional exhaustion	25.94±9.21	16.21±4.54	5.65	.000*
	Depersonalization	10.25±3.82	7.54±2.92	4.64	.000*
	Decrease in personal accomplishment	31.58±9.11	27.37±9.38	4.36	.000*

*Note. SD = standard deviation; *p<.001; **p=.001.*

When the relationship between burnout perception and care burden perception was examined, a statistically significant relationship was found between caregivers' perceptions of burden, emotional exhaustion, and depersonalization (p<.001). Perceptions of emotional exhaustion and

depersonalization intensified as the care burden increased. Care burden and reduced personal accomplishment, however, were not significantly related. The relation between caregivers' perceptions of burnout and care burden is presented in Table 4.

Table 4. *The relation between caregivers' perceptions of burnout and caregiver burden (Pearson correlation test) (N=143)*

Burnout subdimensions		
Emotional exhaustion	r	0.816
	p	.000 *
Depersonalization	r	0.571
	p	.000 *
Decrease in personal accomplishment	r	0.049
	p	.573

*Note. *p<.001.*

4. DISCUSSION

Our study demonstrated that the majority of the people assuming the care of cancer patients were women, which was consistent with the literature (Table 1) (10,11). Because of the compassion and affection inherent to the nature of women, women undertake the caregiving responsibility in our society. Given the responsibilities taken by women within the family, it is possible to consider them as being better fitting for the caregiving task. Caregivers of cancer patients were aged between 40 and 55 in many studies in the literature (11) ,whereas the corresponding age group was 30 years and below in our study (Table 1). The caregiver within a family is likely chosen from the younger and more vigorous members considering the complicated nature of cancer and the difficulties in its care, which may require a more quick-acting individual in the face of an unusual situation. Of the patients cared for, 52.4% were male 27.3% were aged between 45 and 60 and 27.3% were above 61 years. Based on the American Cancer Society data , cancer has a higher incidence after 40 years of age. It is also estimated that more than half of cancer patients are 65 years and above. Breast cancer, the most common type of cancer, was seen in 33.6% of the patients receiving care (12). Patients who are being given care are dependent on others to perform self-care tasks. According to the Family Caregiver Alliance trial, cancer patients fall short in self-care and have high care needs (4).

It was determined that the burden of care perceived by the patient's relatives who participated in the survey was low. Similarly, in the literature, the perceived care burden for caregivers of cancer patients was found to be low. (13). We believe that the perception of caregiver burden was low because caregivers were young and the patients cared for were mostly newly diagnosed. Individuals providing care for 2 years or more have a higher care burden perception. The caregiver's struggle together with a patient increases the perception of care burden. Consistent with our study, several studies have reported increased care burden with longer disease duration (3,14,15)

There is a difference between each of the items in self-care of the patient cared for and the perception of care burden (p<.001) (Table 2). Perception of care burden is higher in caregivers of patients who were dependent on others in eating/drinking, bathing/showering, dressing up/grooming, going to the toilet, walking/strolling, climbing upstairs, shopping, cooking, and maintaining the household. We believe that caregivers experience further difficulties when the conditions of the patients cared for progress and deteriorate and their symptoms worsen.It has been detected that the burnout perception of caregivers is middle. We believe that burnout perception was middle because patients cared for were mostly newly diagnosed patients and caregivers assumed this role not too long ago. In the literature, it is seen that caregivers of patients experience psychological problems (3). Perceptions of emotional exhaustion, depersonalization, and reduced personal accomplishment differ by whether the caregiver shares the same house with the patient as well as the daily time the caregiver spends with the patient. It has been determined in the literature that caregivers' inability to spare enough time for themselves because they spend too much time on patients increases the perception of burnout (15). In addition, not having someone to assist in patient care and assuming the care of the sick relative alone also increases perceived emotional exhaustion and depersonalization. It has been determined in the literature that individuals who do not receive support in meeting their care needs are more psychologically affected (11). There is a difference between the ability of the patient to perform self-care tasks and the perception of burnout (p=.001) (Table 3). Caregivers of dependent patients had higher perceptions of emotional exhaustion, depersonalization, and reduced personal accomplishment. We believe that caregivers not only experience physical difficulties as a result of the patient not being able to maintain self-care but also perceive burnout because of the sadness of seeing the dependency of the patients who once carried out all their tasks on their own.

There was a relationship between perceived care burden emotional exhaustion and depersonalization. Increased care burden led to an increased perception of emotional exhaustion and depersonalization. On the other hand, care burden and reduced personal accomplishment were not significantly related (Table 4). We believe that caregivers' perceived personal accomplishment is not affected by the positive experiences including their empathy for the patient, personal satisfaction, increased experience, communication and personal development going further as they provide care, and their growing self-respect. There are many studies in the literature describing the negative impact of increased care burden on caregivers' psychology. As the care burden increases, perceptions such as burnout, stress, fatigue, and weariness intensify as well (3,11,14,16).

5. CONCLUSION

It has been found that the perceived care burden level is low and burnout level is middle among the caregivers of cancer patients. Caregivers' care burden and burnout levels increase as patients' dependency increases. Caregivers' perceptions of emotional exhaustion and depersonalization also increase with increasing care burden whereas perceived personal accomplishment is not altered. Caregivers should not be forgotten while providing care for the cancer patient. Caregivers should be informed about the care, general health condition, and the use of resources. Parents and group meetings can increase the care quality by alleviating the caregivers' burden. This research has been done in the university hospital. The differences can be discovered by doing studies in private and public hospitals.

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Clinical and Experimental Health Sciences

Study About the Stages of Recovery with Patients Hospitalized in Psychiatric Clinic According to Sociodemographic and Clinical Factors

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ABSTRACT

Objective: This study aims to describe the stages of recovery and the effect of sociodemographic and clinical factors on the recovery stages of individuals with mental illness under inpatient treatment.

Methods: 171 patients who received inpatient treatment between April 2021 and June 2021 were taken to the study. Data were collected by using the Introductory Information Form and the Stages of Recovery Instrument from participants.

Results: According to the nurse, awareness was the highest level of the recovery stages and, according to the patient, growth was the highest level of the recovery stages. The average of the subscales of stages of recovery scale was found as 12.57±7.54 for moratorium, 19.26±5.84 for awareness, 19.22±6.40 for preparation, 19.52±7.03 for rebuilding and 20.03±7.73 for growth. Social support was found effective in all stages of recovery except moratorium stage; income level was found effective in preparation, rebuilding, and growth stages; diagnosis of the illness was found effective in the awareness stage; going to the Community Mental Health Center (CMHC) was found effective in preparation stage and child presence was found effective in rebuilding stage of recovery.

Conclusion: Our findings are important for identifying the effects of situations that can be changed by intervention, such as level of social support, income level, and going to CMHC, on improvement, and for drawing attention to actions that legislators should take regarding to collaboration between mental health professionals and institutions.

Keywords: Mental illness, recovery, hospitalized, sociodemographic factors

1. INTRODUCTION

Recovery from mental illness has different meanings. The definition of “clinical recovery” used and evaluated by mental health professionals is considered more of an outcome or condition, an improvement in symptoms, and recovery does not differ between individuals (1,2). However, the definition of “personal recovery” that emerged after the 1980s (3,4) emphasizes that the person with mental illness, despite any limitations, lives a satisfying life that is subjectively self-defined and accepted as a process (1,5). Personal recovery is defined as having positive effect on well-being beyond the effects of clinical or symptomatic improvement (2). This personal recovery, is a consumer-driven and individuals during the process of personal recovery are active members of this process rather than passive care recipients (5). Although personal recovery is viewed as an individual situation, approaches including incorporate complex realities of life and its relational nature seem important (6).

The concept of recovery is controversial in the current literature and mental health care. Having uncertainties in the concept of recovery, and difficulties about understanding this concept (7-11) also brings challenges (8,12). Furthermore, it has been determined that patients and clinicians do not perceive clinical improvement and personal healing in the same way and have different perspectives (13). While traditionally used clinical measures provide important information for clinicians, they may not assess constructs important to personal recovery (14). This situation shows that it is very difficult for patients and clinicians working with the same goal to meet at the same point. Therefore,

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a common understanding about concept of recovery should be developed to be taken up seriously by both of health professionals and service users (9). However, to develop this common understanding, the concept of personal recovery needs to be well understood. In particular, determining which recovery stage the individual is in will provide a framework for determining interventions according to the characteristics of that stage (7). Additionally, it counts as another dimension of assessment that creates potential basis on better understanding of recovery process and developing targeted treatment approaches (14). Although many studies have been carried out to better explain the concept of recovery and determine the factors affecting it, it is not possible to say anything definite about the effects of these factors (15-18). It has been stated that religiosity and spirituality are related to all stages of recovery in different ways and that these concepts are values that can be used in recovery processes (19). Leith & Stein (17) found that internalized stigma predicted the moratorium stage and personal loss significantly predicted the growth stage. They stated that the scale identified these obstacles to recovery and that precautions should be taken in clinics against them (17). Since the complexity of recovery is added to the complex structure of human beings, it seems that more studies are needed to obtain clearer information on these issues. It can be said that studies using the recovery of stages scale provide important clues in the care of individuals with mental illness. For this reason, it is extremely important to try to learn how individuals with mental health problems perceive their recovery processes and the effect of sociodemographic and clinical variables on recovery. This study was conducted to see the effect of different variables on recovery in all patients hospitalized in psychiatry clinics with a general point of view, and each disease group could not be evaluated within itself. This study aims to describe the stages of recovery and the effect of sociodemographic and clinical variables on the recovery stages of individuals with mental illness under inpatient treatment. As far as we know, this is the first study in Turkey that investigate how inpatients with mental health problems perceive their recovery processes and the effects of sociodemographic and clinical variables on recovery.

The hypotheses of the study are as follows;

H_1: There is a difference between sociodemographic characteristics and the stages of recovery.

H_2: There is a difference between clinical variables and the stages of recovery.

H_3: There is a difference between nurses' and patients' evaluations of recovery.

2. METHOD

2.1. Study Design, Setting and Sample

This descriptive, correlational, cross-sectional study is conducted between April 2021 and June 2021 on inpatients in a mental health and diseases hospital in Türkiye. The study population is consisted of all inpatients in a mental health and diseases hospital between April 2021 and June 2021. The criteria for participation in the study were age over 18 years, having a diagnosis of mental illness and consent to participate in the study. Exclusion criteria of the study are illiterate has been diagnosed with mental retardation, is in the acute stage, and has difficulty understanding and answering questions. No sample selection was used in the study; 16 patients who failed to complete the forms were excluded from the study. The study was completed with 171 patients who agreed to participate. The nurses of all patients who filled out the study data were also interviewed face-to-face, the sub-dimensions of the scale were explained one by one, and they were also asked with a question at what stage their patients might be.

2.2. Measures

***Introductory Information Form*:** This questionnaire is prepared by the researchers consists of 24 questions containing information on age, gender, education and employment status, income level perception, having children or not, diagnosis of mental illness, duration of treatment for mental illness, having registration in the Community Mental Health Center or not and having regular visits on CMHC or not, having physical illness or not and thoughts about recovery. In this form, participants were asked to indicate their assessment of their recovery status and life satisfaction on a scale from 0 (lowest score) to 10 (highest score). In addition, the stages of recovery were explained to each patient's nurse, and they were asked about which stage their patient was in.

Stages of Recovery Instrument (STORI-30): The Stages of Recovery Instrument was developed by Andresen et al. (5) to assess the recovery process at specific stages. Karakaş and Gürhan (20) studied its validity and reliability for our country, and the scale is a 6-point Likert scale. The questions on the scale are ranked between 0 and 5. While "0 = I do not agree" is expressed, "5" means " I fully agree." Five subscales with five stages are indicated in the scale. "1st stage=Moratorium is the stage where there is deep loss and hopelessness, the individual withdraws to protect him/herself", "2nd stage=Awareness involves the realization that all is not lost and a fulfilling life is possible", "3rd stage=Preparation is the stage where the individual works on recovery, evaluates his/her strengths and weaknesses and starts working to improve his/her recovery skills, "4th Stage=Rebuilding involves the individual actively working toward a positive identity, setting meaningful goals, and taking control of their life," and "5th Stage=Growth involves the individual taking control of the illness itself and living a fulfilling and meaningful life that is accompanied by the resilience and positive self-esteem" (5). There is no total score on the scale. The high scores obtained in the subscales of the scale indicate the person's stage. As we move from the moratorium to the growth stage, the perception of improvement also increases. The Cronbach's alpha values of subscales of scale was found range from

0.77 to 0.92 (20). In this study, it is found that the subscales Cronbach's alpha values ranged from 0.76 to 0.89.

2.3. Data Collection

After ethical and institutional approvals were obtained, patients were informed about the study, and study forms were distributed in person to those who volunteered to participate in the study. However, because the COVID-19 epidemic was still ongoing, patients were not left with them while filling out the forms; instead, they waited in the nurses' station, located near the patients, until they completed the forms. It took 15-20 minutes for patients to complete the forms.

2.4. Data Analysis

Statistical analyses were performed using the SPSS program (IBM SPSS Statistics 24). Frequency tables and descriptive statistics were used to interpret the findings. The with kurtosis and skewness values was used to evaluate the fit of the data to the normal distribution. It was used in the comparison of data "Independent Sample t", "ANOVA", "Mann-Whitney U" and "Kruskal-Wallis H" test. Bonferroni correction was applied for pairwise comparisons of variables with significant difference for three or more groups. Variables with a value of 5 or less were not considered when looking at the difference between variables.

2.5. Ethical Considerations

The research was conducted under the Declaration of Helsinki; and written approval was obtained from the study's institution and Non-Interventional Clinical Research Ethics Committee of Gazi University (02.03.2021-E.40777). Both verbal and written consent was obtained from the patients participating in the study.

3. RESULTS

3.1. Sociodemographic variables

The mean age of the study participants was 36.32±11.05; 62% were male, 47.4% were single, 29.8% had secondary education, 48.5% had middle income, 80.1% lived with their family and 50% of the participants had good social support (Table 1).

3.2. Clinical features

It was found that 28.1% of the study participants were diagnosed with bipolar affective disorder, 88.9% had no additional mental illness, 40.4% had a treatment period less than one year, 39.8% had a duration of hospitalization as ≤7 days and 55.6% of them had 1 or 2 hospitalizations.

It was determined that 93% of the participants had no chronic physical illness, 12.3% were registered with the CMHC, and 8.8% went to the CMHC. In addition, according to the nurse of the patient in our study, the highest stage of recovery was the awareness stage with 35.7%; but according to the patient, the highest stage of recovery was the growth stage with 44.4%. It was found that patients rated their recovery status as 6.06±2.62 out of 10 points on average, and satisfaction with life was 5.35±2.91 out of 10 points on average (Table 2).

Table 1. *Sociodemographic features*

Variable	n	%
Gender		
Male	106	62.0
Female	65	38.0
Age		
18-27	44	25.7
28-37	56	32.7
38-47	45	26.3
≥48	26	15.3
Marital status		
Married	56	32.7
Single	81	47.4
Divorced	29	17.0
Widowed	5	2.9
Having a child		
Yes	72	42.1
No	99	57.9
Income level perception		
High	52	30.4
Middle	83	48.5
Low	31	18.2
Very low	5	2.9
Educational level		
Primary school	35	20.5
Secondary education	51	29.8
High school	52	30.4
Associate degree	12	7.0
Bachelor and above	21	12.3
Social support level		
Very high	14	8.1
High	87	50.9
Middle	47	27.5
Low	23	13.5
Type of social support		
Emotional support	48	28.1
Financial support	27	15.8
Information support	15	8.7
General support	74	43.3
All	7	4.1
Who do you live with		
Alone	28	16.4
With family	137	80.1
Relative	6	3.5

3.3. Mean of STORI-30 subscale and Cronbach Alpha values

The mean score of STORI-30 subscale moratorium was 12.57±7.54, the mean score of subscale awareness was 19.26±5.84, and the mean score of subscale preparation was 19.22±6.40, the mean of the rebuilding subscale was 19.52±7.03, the mean of the growth subscale was 20.03±7.73, and the Cronbach alpha values were 0.813, 0.799, 0.760, 0.825, and 0.893, respectively (Table 3).

Table 2. *Clinical features*

Variable (N:171)	n	%
Diagnosis		
Bipolar disorder	48	28.1
Depression	33	19.3
Psychosis	36	21.1
Substance abuse	44	25.7
Anxiety disorders	10	5.8
Day of hospitalization		
≤7 day	68	39.8
8-15 day	55	32.2
16-23 day	28	16.3
≥24 day	20	11.7
Number of hospitalizations		
1-2	95	55.6
3-4	36	21.1
5-6	22	12.9
≥7	18	10.4
Time of treatment		
≤1 year	69	40.4
2-5 year	37	21.6
6-10 year	28	16.4
>10 year	37	21.6
Registration a CMHC		
Yes	21	12.3
No	150	87.7
Going to CMHC		
Yes	15	8.8
No	156	91.2
Additional physical illness		
Yes	12	7.0
No	159	93.0
Additional mental illness		
Yes	19	11.1
No	152	88.9
Self recovery stage according to the patient		
Moratorium	23	13.5
Awareness	33	19.3
Preparation	14	8.2
Rebuilding	25	14.6
Growth	76	44.4
Patient's recovery stage according to the nurse		
Moratorium	52	30.3
Awareness	61	35.7
Preparation	41	24.0
Rebuilding	14	8.2
Growth	3	1.8

CMHC= Community Mental Health Center

Table 3. *The distribution of outcomes concerning scale*

Scale (N:171)		Mean	S.D.	Median	Min.	Max.	Cronbach-α
STORI-30	Moratorium	12.57	7.54	12.0	0.0	30.0	0.813
	Awareness	19.26	5.84	20.0	0.0	30.0	0.799
	Preparation	19.22	6.40	19.0	0.0	30.0	0.760
	Rebuilding	19.52	7.03	20.0	0.0	30.0	0.825
	Growth	20.03	7.73	21.0	0.0	30.0	0.893

STORI-30= Stages of Recovery Instrument

3.4. Comparison of sociodemographic features and recovery stage values

It was found that rebuilding scores (p<.05) those with a child; and preparation scores (p<.05), rebuilding scores (p<.05) and growth scores (p<.05) were significantly higher among those with high income.

Awareness scores (p<.01) and preparation scores (p<.001) for individuals with very high levels of social support compared to individuals with low levels of social support, as well as rebuilding scores for individuals with very high income compared to individuals with low income were found (p<.01) and growth scores (p<.001) were significantly higher (Table 4).

No significant relationship was found between gender, age, marital status, educational level, and the subscales of recovery stages.

3.5. Comparison of clinical features and recovery stage scores

It was found that awareness scores (p<.05) were higher in those with substance abuse than those with psychosis and anxiety disorders; and rebuilding scores (p<.05) were higher in those with mental illness than without additional mental illnesses. In addition, it was found that the preparation scores of those who went to the CMHC were higher (p<.05) than those who did not (Table 5).

On the other hand, no significant association was found between the recovery scale subscales for additional physical illnesses, time of diagnosis, number of relapses, duration of treatment, day of hospitalization, and number of hospitalizations.

3.6. The relationship between the degree of evaluation of patients' recovery status and the degree of satisfaction with life and the subscales of recovery stages

It was found that patients' self-assessment scores and recovery stages were negatively correlated with the subscale moratorium, weakly (p<.001); positively correlated with the subscale preparation, and weakly (p<.01); positive correlation with the rebuilding subscale, weakly (p<.001), and positive correlation with growth subscale with moderately statistically significant relationship. It was found that the level of life satisfaction and the stages of

recovery were negatively correlated with the moratorium subscale, weakly (p<.001) and positively correlated with the awareness subscale, weakly (p<.05); positive correlation with the subscale preparation, weakly (p<.001); positive correlation with the subscale rebuilding, weakly (p<.001) and positive correlation with the subscale growth, moderately (p<.001), statistically significant relationship were found (Table 6).

Table 4. *Comparison of sociodemographic features and recovery stage values*

Variable (N=171)	n	*Moratorium* $\bar{X} \pm S.D.$	*Awareness* $\bar{X} \pm S.D.$	*Preparation* $\bar{X} \pm S.D.$	*Rebuilding* $\bar{X} \pm S.D.$	*Growth* $\bar{X} \pm S.D.$
Gender						
Male	106	12.70±7.66	19.18±6.31	19.06±6.55	19.35±6.93	19.77±7.77
Female	65	12.35±7.39	19.38±5.03	19.49±6.17	19.80±7.24	20.45±7.71
Statistical Analysis*		t=0.289[a]	t=-0.223[a]	Z=-0.293[b]	t=0.406[a]	Z=-0.485[b]
Probability		p=.773	p=.824	p=.769	p=.685	p=.628
Age						
18-27	44	12.84±7.75	18.16±6.46	17.84±7.07	16.95±8.08	17.45±8.79
28-37	56	11.76±7.15	19.48±5.57	19.13±6.14	20.16±5.84	20.96±7.12
38-47	45	14.40±7.43	20.29±5.84	20.71±6.32	20.76±7.09	20.11±8.04
≥48	26	10.65±7.86	18.85±5.23	19.19±5.61	20.34±6.66	22.23±5.44
Statistical Analysis*		F=1.696[c]	χ^2=3.862[d]	χ^2=4.973[d]	χ^2=6.496[d]	χ^2=5.701[d]
Probability		p=.170	p=.277	p=.174	p=.090	p=.127
Marital status≠						
Married	56	12.45±7.01	19.21±6.22	19.98±6.14	20.59±6.87	21.34±6.40
Single	81	12.04±7.83	18.69±5.97	18.56±6.83	18.38±7.35	18.92±8.36
Divorced	29	13.83±7.26	21.06±4.74	19.72±5.77	20.83±6.05	20.76±7.99
Statistical Analysis*		χ^2=1.184[d]	χ^2=3.881[d]	χ^2=2.540[d]	χ^2=4.162[d]	χ^2=2.245[d]
Probability		p=.553	p=.144	p=.281	p=.125	p=.326
Having a child						
Yes	72	13.13±7.41	19.99±5.76	19.75±5.88	20.83±6.77	21.24±6.75
No	99	12.16±7.64	18.73±5.86	18.84±6.75	18.57±7.10	19.15±8.29
Statistical Analysis*		t=0.825[a]	Z=-1.931[b]	Z=-1.029[b]	Z=-2.018[b]	Z=-1.315[b]
Probability		p=.411	p=.053	p=.303	**p=.044**	p=.189
Income level ≠						
High (1)	52	12.73±8.17	20.56±5.55	20.77±5.98	21.33±7.13	21.69±7.67
Middle (2)	83	12.54±7.45	18.67±5.96	19.03±6.08	18.99±6.91	19.62±7.38
Low (3)	31	13.35±6.81	18.84±5.65	16.81±7.56	17.39±6.95	17.42±8.32
Statistical Analysis*		F=0.130[c]	F=1.820[c]	χ^2=7.166[d]	F=3.412[c]	χ^2=6.724[d]
Probability		p=.878	p=.165	**p=.028**	**p=.035**	**p=.035**
Difference				**[1-3]**	**[1-3]**	**[1-3]**
Educational level						
Primary school	35	13.40±7.79	17.95±5.76	18.49±6.92	18.94±7.53	19.45±7.25
Secondary education	51	12.84±7.72	20.51±5.12	19.76±6.14	19.31±6.11	20.53±7.00
High school	52	12.69±7.35	19.45±6.05	19.33±6.53	19.88±7.81	19.65±9.25
Associate degree	12	11.83±5.65	16.92±7.23	17.67±7.63	18.83±9.30	19.58±6.97
Bachelor and above	21	10.61±8.33	19.29±5.90	19.76±5.27	20.48±4.97	20.95±6.95
Statistical Analysis*		χ^2=2.227[d]	χ^2=3.643[d]	χ^2=0.689[d]	χ^2=1.021[d]	χ^2=0.786[d]
Probability		p=.694	p=.457	p=.953	p=.907	p=.940
Social support level						
Very high (1)	14	8.86±5.40	22.36±4.16	22.93±4.23	24.64±4.43	22.93±5.92
High (2)	87	11.97±7.85	20.09±5.85	20.37±5.91	20.24±6.70	21.97±6.81
Middle (3)	47	13.87±6.56	17.85±5.77	18.02±6.46	18.32±7.07	18.68±7.29
Low (4)	23	14.39±8.57	17.09±5.63	15.09±6.86	16.13±7.49	13.69±9.09
Statistical Analysis*		χ^2=7.181[d]	F=4.088[c]	F=6.907[c]	χ^2=16.263[d]	χ^2=20.310[d]
Probability		p=.066	**p=.008**	**p=.000**	**p=.001**	**p=.000**
Difference			**[1-4]**	**[1-4]**	**[1-3,4]**	**[1-3,4]**
						[2-3,4]

*≠(n=166) *a= Independent Sample-t; b= Mann-Whitney U; c= ANOVA; d= Kruskall-Wallis H*

Table 5. *Comparison of clinical features and recovery stage scores*

Variable (N=171)	n	*Moratorium*	*Awareness*	*Preparation*	*Rebuilding*	*Growth*
		$\overline{X} \pm S.D.$	$\overline{X} \pm S.D.$	$\overline{X} \pm S.D.$	$\overline{X} \pm S.D.$	$\overline{X} \pm S.D.$
Diagnosis						
Bipolar disorder [(1)]	48	12.44±7.62	19.10±5.83	19.90±6.55	19.54±6.67	20.29±7.62
Depression [(2)]	33	12.52±6.06	18.94±6.22	19.52±7.03	18.73±7.99	20.33±7.71
Psychosis [(3)]	36	12.81±8.12	18.19±5.29	17.89±6.65	19.42±6.60	20.33±7.30
Substance abuse [(4)]	44	13.86±7.72	21.11±5.80	19.78±5.63	19.82±7.15	19.18±7.70
Anxiety disorders [(5)]	10	6.80±7.23	16.70±5.52	17.40±5.89	21.10±7.41	20.40±10.90
Statistical Analysis*		χ^2=6.420 [d]	χ^2=11.980 [d]	χ^2=4.974 [d]	χ^2=0.824 [d]	χ^2=1.337 [d]
Probability		p=.170	**p=.018**	p=.290	p=.935	p=.855
Difference			**[4-3,5]**			
Day hospitalization						
≤7 day	68	13.06±7.66	19.79±5.76	19.78±5.91	19.63±7.39	19.53±8.25
8-15 day	55	12.18±7.36	18.94±5.98	18.75±6.54	18.89±7.06	19.89±7.78
16-23 day	28	10.57±6.96	18.11±6.36	18.75±7.40	20.18±7.28	21.42±6.70
≥24 day	20	14.75±8.16	19.90±5.04	19.30±6.41	19.95±5.47	20.15±7.36
Statistical Analysis*		F=1.367 [c]	F=0.683 [c]	χ^2=0.887 [d]	F=0.256 [c]	χ^2=0.812 [d]
Probability		p=.255	p=.564	p=.828	p=.857	p=.847
Number of hospitalizations	95	12.22±7.39	19.51±6.38	19.07±6.59	19.91±7.22	20.18±7.69
1-2	36	13.50±8.09	20.17±4.52	19.72±6.51	19.39±6.61	19.89±8.37
3-4	22	14.14±7.75	17.18±5.40	17.86±5.61	17.73±7.07	18.68±8.00
5-6	18	10.61±6.89	18.67±5.93	20.67±6.15	19.94±6.97	21.17±6.51
≥7						
Statistical Analysis*		F=0.972 [c]	χ^2=5.382 [d]	F=0.724 [c]	χ^2=1.781 [d]	χ^2=0.798 [d]
Probability		p=.407	p=.146	p=.539	p=.619	p=.850
Time of treatment						
≤1 year	69	11.84±8.06	20.17±6.57	19.75±6.97	20.08±7.35	21.04±7.57
2-5 year	37	12.10±6.46	18.54±4.81	18.70±5.71	19.16±7.26	18.92±7.75
6-10 year	28	12.89±7.30	19.14±6.05	18.93±7.00	18.71±7.29	18.61±9.26
>10 year	37	14.14±7.73	18.35±5.09	19.00±5.57	19.45±6.11	20.32±6.68
Statistical Analysis*		F=0.808 [c]	χ^2=6.225 [d]	χ^2=1.586 [d]	χ^2=1.119 [d]	χ^2=2.550 [d]
Probability		p=0.491	p=0.101	p=0.663	p=0.772	p=0.466
Going to CMHC						
Yes	15	11.87±8.60	20.40±4.95	22.40±5.62	21.60±5.37	23.73±5.69
No	156	12.63±7.45	19.15±5.92	18.92±6.39	19.32±7.15	19.67±7.82
Statistical Analysis*		Z=-0.451 [b]	Z=-0.752 [b]	Z=-2.062 [b]	Z=-1.050 [b]	Z=-1.898 [b]
Probability		p=.652	p=.452	**p=.039**	p=.294	p=.058
Additional physical illness						
Yes	12	11.83±9.85	19.42±7.57	18.50±8.12	19.92±9.38	19.17±8.10
No	159	12.62±7.37	19.25±5.72	19.27±6.27	19.49±6.86	20.09±7.72
Statistical Analysis*		Z=-0.257 [b]	Z=-0.245 [b]	Z=-0.006 [b]	Z=-0.312 [b]	Z=-0.436 [b]
Probability		p=.797	p=.806	p=.995	p=.755	p=.663
Additional mental illness						
Yes	19	15.42±7.15	21.84±5.16	21.79±5.98	22.63±6.26	20.63±8.47
No	152	12.21±7.53	18.93±5.85	18.90±6.39	19.13±7.04	19.95±7.66
Statistical Analysis*		t=1.761 [a]	Z=-1.928 [b]	Z=-1.743 [b]	Z=-1.976 [b]	Z=-0.492 [b]
Probability		p=.080	p=.054	p=.081	**p=.048**	p=.622

*CMHC= Community Mental Health Center; *a= Independent Sample-t; b= Mann-Whitney U; c= ANOVA; d= Kruskall-Wallis H*

Table 6. *Examination of the relationships between some parameters and subscales*

Correlation[1] (N:171) STORI-30		*The level of evaluation of recovery status (0-10)*	*The level of satisfaction with life (0-10)*
Moratorium	*r*	-0.417	-0.388
	p	**.000*****	**.000*****
Awareness	*r*	0.048	0.157
	p	.533	**.047***
Preparation	*r*	0.233	0.342
	p	**.002****	**.000*****
Rebuilding	*r*	0.361	0.482
	p	**.000*****	**.000*****
Growth	*r*	0.534	0.605
	p	**.000*****	**.000*****

*STORI-30= Stages of Recovery Instrument, 1Spearman correlation, *p<.05, **p<.01, ***p<.001*

4. DISCUSSION

This study is made to determine the stages of recovery and the effects of sociodemographic and clinical factors on the stages of recovery in individuals with mental illness during inpatient treatment.

It was found that there was correlation between having a child and the rebuilding subscale of the stages of recovery scale. While there is a limited evidence base for incorporating parenting as a component of recovery for adults with mental illness, recovery-oriented interventions "designed to support a parent in the context of his or her mental illness" had been shown effective on improving parent, child and family well-being (21). The family-focused practice had also shown being an effective approach to supporting individuals with mental illness (22). This study, it is thought that the significant relationship between having a child and the restructuring subscale can be due to the fact that having a child forces them to rebuild their lives and having control over their families.

It is determined that individuals with high income have significantly higher scores in preparation, rebuilding, and growth subscale than individuals with low income. Previous studies have shown that poverty plays an important role in people with mental illness who have difficulty maintaining friendships, developing old and new relationships, and re-establishing their relationships after a crisis (23). In addition, financial support in the form of "activity support" for 9 months period for people with mental illness was found to produce significant changes in quality of life, self-esteem, increasing social relationships, and decreasing symptoms (24). The high-income perception may have contributed to the decrease in symptoms of illness by increasing the quality of life that had deteriorated during the illness, restoring social ties, and increasing self-esteem.

The awareness subscale scores were significantly higher in individuals diagnosed with substance dependence than those diagnosed with anxiety and psychosis. On the other hand, Leith and Stein (17) found no significant difference between psychosis and mood disorder diagnoses on the stages of recovery scale (17). The awareness stage involves realizing that not everything is over and fulfilling life is still possible. On the other hand, Insight is explained as the awareness of one's internal state and changes at the level of insight in many psychiatric disorders is a well-known reality (25). The biggest handicap in the evaluation of these results is the heterogeneity of the sample and the fact that each patient group was not evaluated within itself. Considering that there are periodic insight differences even within the diseases themselves, it is certain that there are insight differences between the diseases. For this reason, it would not be appropriate to make a clear assessment among diseases. however, this study has not been done to determine clear differences anyway. The aim was to present a more general perspective. In this context, it is thought that one of the reasons why the scores of individuals with substance addiction are significant may be that hospitalized addicted patients are in the detox process, which is the first stage of treatment, and this process reduces the person's physical addiction and increases awareness.

It is found that individuals with an additional mental illness had significantly higher rebuilding scores than individuals without additional mental illness. The detailed analysis found that 63% of individuals diagnosed with an additional mental illness had diagnosis of substance dependence. This suggests that treatment for the additional mental illness may have contributed to the individual's rebuilding.

This study found that individuals with good social support had higher scores on the awareness, preparation, rebuilding, and growth subscales. İn the study of Gandotra et al. (16) is found similar results to our study. In the literature, it is found that perceived social support positively affects recovery (19,26), and there is a positive relationship between the level of social support and quality of life (27). In a systematic review study of personal recovery, social support is found as one of the factors supporting recovery (28). These studies can be accepted as indicators of that social support and functionality play an important role in recovery.

The preparation scores of those who went to CMHC were higher than those who did not go to CMHC. The preparation stage is a period of which the person begins to work on improving their recovery skills. Previous studies have found that services provided in CMHC have a positive impact on many factors that contribute to improvements, such as patients' insight, quality of life, and functionality (29,30). In the study by Kurt et al. (31), it was determined that individuals who used CMHC services perceived greater personal recovery than the outpatient clinic group (31). It was also found that mental health services significantly predicted process-based recovery (17). In our study, this situation can be interpreted as the patients who went to CMHC were in preparation for recovery or that CMHC services helped the patients to prepare for the recovery process. However, it

can be interpreted that either way CMHC contributes the patients to internalize their recovery processes.

In our study, scores for the preparation, rebuilding, and growth subscales were higher in patients with undergraduate and postgraduate education, but the difference was not significant. Gandotra et al. (16) found that the group with the lowest educational level had lower scores for the preparation, rebuilding, and growth subscales. In addition, it was determined that as the level of education increases in people with mental illness, functional improvement (32) and functionality (33) also increase. However, O'Shea and Salzer (34) found that individuals with higher levels of education had lower scores in recovery and quality of life (34). Although there are different results about education levels in the literature, it is thought that high education levels may bring positive features such as using effective and different coping methods, but no result confirming this view has been reached. The fact that there are different results in the literature suggests that the effect of individual development on recovery may also be an important factor.

While in our study, 44.4% of patients considered themselves to be in the growth stage, it was determined that the nurses who took care of these patients considered that only 1.8% of the same patients were in growth stage. Similarly, in other studies also was found that most patients viewed themselves as being in the growth stage (18,35). The study, the patient's nurse also assessed the patient's recovery stage, considering the same stage scales. It was found that the stage of recovery that patients set for themselves and the stages that their nurses hold for the patient were very different. Previous study has found that patients and clinicians have different perspectives on personal recovery, this finding supported our study result (13). Our third hypothesis was confirmed. These results may be an indication that both clinicians and patients may not have the same evaluation of recovery. Clinicians are advised to pay attention to this issue.

For variables such as gender, age, occupation, marital status, additional physical illness, duration of illness, day of hospitalization, number of hospitalizations, and duration of treatment, no significance was found concerning the subscales of the stages of recovery scale. In other studies, no significant relationship was determined between recovery stages and different sociodemographic variables such as age (15), age and gender (17), gender, marital status, and duration of illness (16), age, gender, marital status, and occupation (18). It was seen that our first hypothesis could not be confirmed importantly. Although many studies, including ours, have found that sociodemographic variables alone are not effective; it is believed that it may be useful to conduct more studies in-depth quantitative and addressing different variables.

Variables such as day of hospitalization, number of hospitalizations, illness, and duration of treatment were the variables thought to contribute to an individual's recovery status. In the study by Gandotra et al. (16), no significant difference was found concerning to the duration of illness and type of treatment (pharmacological-pharmacological+psychological) variables. It was seen that our second hypothesis could not be confirmed importantly. One of the significant factors in the emergence of these result may be the heterogeneous sample and the lack of a separate evaluation for the diseases. In addition, factors such as the severity of the disease, its duration, the duration of hospitalization and treatment may have been effective, such as the fact that many factors were evaluated according to this heterogeneous sample and could not be detailed. In addition to these, the absence of a significant difference in terms of these variables in our study may also be considered that these variables alone may not have a significant effect on the psychological recovery process for all patients hospitalized in the psychiatry clinic. It can be said that there is a need for more detailed studies on these variables, considering all these possible causes. Although our study has some important shortcomings, it is thought that it can provide a general perspective for patients hospitalized in psychiatry clinics.

There was a negative and weak relationship between patients' the level of satisfaction with life and the degree of evaluation of their recovery and the moratorium stage. In addition, as we go from the moratorium stage to the growth stage, the direction of the relationship is positive and gradually increased. A parallel relationship was observed between patients' level of satisfaction with life and recovery. Also, it was found that the degree of evaluation of their recovery was very similar and consistent with the scale stages. This result can be seen as an indication that the answers given by the patients are consistent.

This study has some limitations. The first one is that the study was conducted in only one institution. The second one is, data of the study was based on self-report in which patients may have perceived or reported their recovery differently. Third one is that the time interval of the study was limited for 3 months and as the number of COVID-19 cases increased during this period of the study; therefore, the number of patients had been smaller than desired. The fourth one is that patients' perceptions of recovery were measured with a single measurement tool. Fifth, there is no detailed evaluation to address each patient group separately.

5. CONCLUSION

In conclusion, in this study, between all recovery stages except the moratorium stage and social support; between preparation, rebuilding, and growth stages and income level perception; between the awareness stage and the diagnosis of the disease; It has been found that there was a significant relationship between the preparation stage and the state of going to the CMHC and between the rebuilding stage and having a child. Our results are important to see the effect on recovery by improvement of these situations, such as the level of social support, income level, and going to the CMHC, which can be modified by an intervention, and to draw attention to the actions that legislators should take regarding

to collaboration between mental health professionals and institutions. In addition, it is suggested that the basis of care interventions for patients should be formed according to the characteristics the stage of recovery in which the patient is located. In this way, rather than a general approach to patients, the factors that are specific to the individual and important for his/her recovery will be determined. In further studies, we recommend to determining the changes on recovery by providing social support and income levels with interinstitutional collaboration.

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Author Contributions:

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Design of the study: MK, AK, NG
Acquisition of data for the study: MK, AK, HE
Analysis of data for the study: AK, NG, HE
Interpretation of data for the study: MK, AK, HE, ZA
Drafting the manuscript: MK, AK, NG, ZA, HE
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Clinical and Experimental Health Sciences

Investigation of the Effect of Mindfulness-Based Psychoeducation on Defense Mechanisms and Mindful Awareness in Nursing Students

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ABSTRACT

Objective: Today, there is a growing interest in mindfulness-based therapies. Such practices can be beneficial by contributing to the personal and professional development of nursing students. In this study, the effect of mindfulness-based psychoeducation applied to Turkish nursing students on defense mechanisms and mindfulness was evaluated.

Methods: The research was designed as a quasi-experimental study with a control group. Psychoeducation was delivered online between 08.03.2021 and 17.05.2021 in 16 sessions over 8 weeks. Data were collected using the Defense Style Questionnaire and the Awareness Scale. Percentage distributions, t-test, chi-square test, and paired samples t-test were used to analyze the data.

Results: It was found that the students' use of mature defenses and mindful awareness increased after the mindfulness-based psychoeducation, and the differences between the experimental-control groups and the mean scores of the pretest-posttest data were significant ($p<.05$).

Conclusions: If mindfulness-based psychoeducation can be integrated into the curriculum of nursing students, it may help them to better understand themselves and their environment and to cope with the difficulties they encounter in their professional practice by supporting their personal development.

Keywords: Mindfulness, psychoeducation, defense mechanisms, mindful-awareness, nursing

1. INTRODUCTION

The most basic function of the self is the use of defense mechanisms. These mechanisms are used in every age period by the developmental process. It is inevitable for an individual to constantly use defense mechanisms to protect the self in its development and adaptation to the environment (1). Defense mechanisms are unconscious strategies that mediate the individual's response to internal conflicts and challenging situations (2). Defense mechanisms are classified into three categories: mature (sublimation, humor, anticipation, and suppression), neurotic (reaction development, deconstruction, intellectualization), and primitive/immature (distortion, denial, splitting) (3). Healthy people generally tend to use neurotic and mature defense mechanisms to cope with adversity in their daily lives. However, they may also use immature defenses in response to traumatic events (4-6). When defense mechanisms are not used at the appropriate time, at a proper frequency, and in a measured condition, they can negatively affect mental health (6,7). They are defense mechanisms that make negative emotions such as anxiety, guilt, shame, sadness, and anger more acceptable. It is an automatic response that occurs outside of awareness (4,8). When it comes to the existence of defense mechanisms that the person uses unconsciously, it is crucial to gain self-awareness in order to prevent mental problems and support self-development (9).

Mindful awareness is a mind-body practice that involves paying attention to what is happening in the moment and accepting what is perceived without judgment. Mindfulness involves awareness and attention; Awareness is the mechanism that constantly monitors the background of the state of consciousness (9-11). Studies have found that people with mindful awareness use more positive coping methods and mature defense mechanisms, and less negative coping methods, neurotic, and immature defense mechanisms (12-14).

It has been noted that nurses and nursing students often encounter difficult situations in their professional journey and experience mental problems such as burnout, stress, anxiety, depression, and use more neurotic and immature defenses (14-17). Many psychological strategies and therapies have been used and proven to be effective in improving defense mechanisms and coping with mental problems (7,18-20). Many

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studies indicate the effectiveness of mindfulness practices in nursing students for both individual and patient benefit (21-27).

In this research, a structured psychoeducation on defense mechanisms was prepared to help students discover the defenses they use most, get to know defense mechanisms better, learn how to develop mature defense mechanisms, support their mental health development by increasing their awareness, and test the effectiveness of the psychoeducation.

Hypotheses

H1: Mindfulness-based psychoeducation program decreases the level of immature defense mechanisms levels and increases mature defense mechanisms of the nursing students.

H2: Mindfulness-based psychoeducation program increases mindful awareness levels of the nursing students.

2. METHODS

2.1. Design and Sample

The study was conducted to determine the effect of mindfulness-based psychoeducation program applied with distance education on defense mechanisms, mindful awareness of Turkish nursing students. This study was designed as a quasi-experimental study with a control group (Trial Registration: BLINDED).

It was conducted with 96 nursing students enrolled in the Faculty of Health Sciences of a state university in the east of Türkiye in the 2020-2021 academic year. 86 students who met the research criteria (being a first-year student, being over 18 years old, not having any psychiatric diagnosis, volunteering to participate in the study, not having received mindfulness training before, not a foreign national) were included in the study. It was considered appropriate to include in the study by first-year students who were at the beginning of their nursing education and had not yet taken a course related to personal development (such as introduction to psychology, interpersonal relations and communication). Among these students, the intervention and control groups were formed without randomization after detailed information about the research process was given to the students at their request. Those who wanted to receive training and believed that they could attend the training continuously were determined as the intervention group (45), and those who did not want to participate in the training and were unsure were determined as the control group (41). However, the research was completed with 33 students in the intervention group and 39 students in the control group. 12 students from the intervention group left the research because they could not attend the sessions regularly, while 2 students in the control group dropped out of the research without stating a reason for their request. In the power analysis of the 72 students who participated in the research, it was determined that the margin of error of the study was .05, the effect size was .5, and the power of to represent the universe was .80 (28). These values indicate that the sample is adequate.

2.2. Data Collection and Program Application

Due to the Covid-19 pandemic, nursing education in our institution was continued in the 2020-2021 academic year with a distance education called DEP (Distance Education Platform). This situation necessitated online psychoeducation to be given as part of the research. The Mindfulness-Based Psychoeducation program was conducted via DEP between 08.03.2021 and 17.05.2021 after obtaining the necessary permission and support from the institution. The scales created by the researcher through "Google Form" were shared with the students in the Mindfulness-Based Psychoeducation Program at DEP, and the pretest data were collected in the first session, while the posttest data were collected two weeks after the last session. On the first page of this form, the purpose of the research was explained, the confirmation icon indicated that they were voluntarily agreeing to participate in the research, why they had to provide name or code information, and the response time (30 minutes) for the forms was stated..

2.3. Data Collection Tools

In this study Personal Information Form, Defense Style Questionnaire (DSQ-40), and Mindful Attention Awareness Scale (MAAS) were used.

Personal Information Form: It consists of questions about the socio-demographic characteristics of the students (age, gender).

Defense Style Questionnaire (DSQ-40): The scale was developed by Andrews et al. (3) and adapted into Turkish by Yılmaz et al. (29). In the scale, which determines the occurrence of defense mechanisms at the level of consciousness and behavior, and consists of 40 items, 20 defenses, and 3 sub-dimensions (mature, neurotic, and immature), each item is rated between 1-9 (absolutely inappropriate – absolutely appropriate). Mature defenses are; repression, anticipation, sublimation, and humor. Neurotic defenses; subversion, counter-reaction, idealization, and artificial altruism. Immature defenses are; passive aggression, expression, projection, isolation, denial, division, devaluation, autistic fantasy, displacement, dissociation, rationalization, and somatization. The scale is scored on defenses or sub-dimensions, not on the total score. The total score or arithmetic mean is used in calculating each defense or sub-dimension. As the score increases, the use of the defense/sub-dimension also increases. In the Turkish adaptation study of the scale, the Cronbach's Alpha values were .70, .61, and .83, respectively, according to the mature, neurotic, and immature sub-dimensions (29), while in this study, it was .65, .71, and .82 respectively.

Mindful Attention Awareness Scale (MAAS): Developed by Brown and Ryan (30) and adapted to Turkish by Özyesil et al. (11). The scale has a 15-items and 6-point Likert scale that measures the differences in the ability to be aware of

what is happening in the moment and to pay attention to them. Scores on the scale range from 15 to 90 points. A high score indicates a high level of mindful awareness. While the Cronbach Alpha value of the scale in the Turkish adaptation study (11) was .80, in this study it was .82.

2.4. Mindfulness-based Psychoeducation Program

Mindfulness-based cognitive therapy is evidence-based psychoeducation. Psychoeducation was organized in 2 consecutive sessions once a week for 8 weeks, and each session lasted 60 minutes, for a total of 16 sessions. The content of the psychoeducation program was explained in detail, and techniques such as presentation and question-answer narrative techniques, video, audio recording, mindfulness assignments for the next session were used in the training. A WhatsApp group was created to remind and share materials with students participating in psychoeducation. It was stated that the students should be very careful, observant, and patient while doing the exercises in order to get to know themselves, be aware of their emotions, thoughts, physical sensations and focus. The applications were performed online-live in DEP and recorded by the system. The researcher's camera was turned on and the student's camera was left on as well. The privacy of all participants was emphasized and it was stated that the existing recordings would not be used elsewhere, that the participants would not be able to make recordings, and that the sharing in the sessions would be kept confidential. Students had access to the recordings at any time during the day the psychoeducation began and for 15 days after the psychoeducation ended. On the days when psychoeducation was not taking place, reminder messages were sent twice a week via DEP and WhatsApp applications for home applications. The content of Mindfulness-based psychoeducation program is shown in Table 1.

Table 1. *Content of mindfulness-based psychoeducation program*

Week 1 **Session 1** ***(First meeting)***	-Meeting, informing about how the group will work, expressing expectations and providing information about the concept of mindfulness, applying pretests.
Session 2 ***(Awareness and me)***	-Raisin exercise and body scan -Home practice: mindful eating and body scan -Talking about timing and barriers to home practice.
Week 2 **Session 3** ***(Devil, me and angel)***	-Concepts related to the defense mechanism (what is the self, its characteristics, and how it works, the functioning of defense mechanisms against obstacles and conflicts, giving examples from the conflicts we experience and the defenses we make), recognizing defenses, and thinking about other defenses,
Session 4 ***(Life begins with a breath)***	-3 minutes of breath awareness and body scanning -Sharing experiences about mindful eating and body scanning -Home practice: breath awareness, body scan, choosing an enjoyable routine in everyday life and doing it mindfully
Week 3 **Session 5** (***Awareness and autopilot)***	-What are our automatic thoughts? Gaining the ability to make connections between automatic thoughts and defense mechanisms, to understand the relationship between emotion, thought, and behavior, and to recognize cognitive errors
Session 6 ***(Awareness and everyday life)***	-3 minutes of breath awareness and body scanning without direction, noticing the automatisms in our lives, can this be an emotion, thought, behavior and sensation? -Sharing experiences of breathing awareness, body scanning, choosing an enjoyable routine in daily life, and doing it with mindfulness -Home practice: breath awareness, body scan, sitting meditation, choosing an unpleasant routine and doing it with mindfulness
Week 4 **Session 7** ***(Fight or run away)***	-Learning the importance, functions, types of defense mechanisms, and primitive defense mechanisms. -Explanation of the usage with examples -Sharing experiences observed or lived by the participants
Session 8 **(Living in our minds*)***	-30 minutes of sitting meditation with breathing, practicing thoughts and feelings, being in the moment, noticing the floating mind, gathering, seeing and hearing exercises -Sharing experiences with breathawareness, body scanning, seated meditation, choosing an uncomfortable routine, and mindful action -Home practice: breath awareness, body scan, sitting meditation, being in the moment, and recognizing obstacles to being in the moment
Week 5 **Session 9** ***(Ideal me)***	-To recognize *mature* defense mechanisms, to realize how much we use them, to see where we are by comparing our real selves with our ideal selves. Connecting dreams, reality and ideals
Session 10 ***(Tidying up the scattered mind)***	-Breath awareness, sitting meditation, body scanning, mindful movement, how to recover the dispersed mind, what are my supports when I have difficulty. -Sharing feedback about being present in the home practices, and those who prevent being present -Home practice: breath awareness, body scan, seated meditation, mindful movement, thoughts and feelings in response to a pleasant or unpleasant situation, recording body sensations

Week 6 **Session 11** ***(Healthy or Not?)*** **Session 12** ***(Noticing the stress)***	-Recognizing neurotic defense mechanisms, giving examples of their relationship to mental illness, questioning ourselves, reviewing the situations we have difficulty with, looking at how these situations affect our feelings, thoughts and behaviors, noticing physical sensations
	-Sitting meditation with breathing, 30 minutes of body scanning, coping with challenging situations, endurance, observing happening -Sharing feedback about thoughts, feelings and body sensations in the face of difficulties during the home practice -Home practice: 30-40 minutes of sitting meditation, breath-body awareness while doing this practice, body sensations, feelings, thoughts and reactions, noticing difficulties, thinking that all difficulties are temporary,
Week 7 **Session 13** ***(To allow)*** **Session 14** ***(Finding supports)***	-3 minutes of breath awareness, body scan, sitting meditation, what could be alternatives to strains, what could be different, what would you feel, what would you think, what would you do, what would you sense. -Sharing experiences of being able to stay with a difficult situation while doing the home practice, sharing a supportive emotion, thought, if any, thinking of being a guest -Home practice: 30-40 minutes of sitting meditation, breath-body awareness while doing this practice, noticing the difficulties, implementing the practice of noticing the alternative that will make you feel better, not what you always do
	-3-minute breath awareness, body scan, seated meditation, mindful movement, mindful eating -Sharing feedback on supports and alternatives for home practices -Home practice: breath awareness, body scan, seated meditation, mindful movement, mindful eating, and what are the practices that make you happy. Making any activity that makes you happy mindful, (compassion meditation) comparing these practices with previous experiences, seeing if there are any differences,
Week 8 **Session 15** ***(Start the change)*** **Session 16** ***(Closure)***	-Body scan, gracious compassion, closing meditation, -Reviewing home practices, recognizing how we feel, thinking, and responding to repeated challenges, sharing experiences about seeing the difference from the previous one -Home practice: What will sweeten you up or make you happy? Notice, think, feel and perform, a good word, writing, drawing, sharing, doing activity, breathing-body scanning, compassion meditation
	Evaluation, summarizing, receiving feedback from group members and applying posttests.
Operational plan of the sessions – Clarification of the session agenda – Assessment of home practices from the previous session – Sharing what can and cannot be done, obstacles, benefits, expectations, challenges and experiences. – Implementing the session agenda and sharing experiences afterwards – Indication of what is required to be implemented until the next session, sharing of training materials (presentation, audio, video),	

References (Frey and Totton, 2016; Atalay, 2019; Harris, 2019; Hayes and Smith, 2021).

2.5. Data Assessment

The coding and analysis of the data was done using the SPSS-23 package program, and the significance level was accepted as p<.05. Percentage distributions were used to analyze the data, t-test and chi-square test were used to compare the intervention and control groups, and paired samples t-test was used to compare the intervention and control groups within the group.

2.6. Ethical Considerations

The necessary institutional approval and support for the research was obtained from the Deanery of the Faculty of Health Sciences. In addition, ethical approval was obtained from the Ethics Committee of the Institute of Health Sciences of the University. (Decision dated 27.01.2021 and numbered 2021/18). Online with the informed consent sent on the DEP platform was obtained from the students participating in the research and the research was conducted according to the Principles of the Declaration of Helsinki.

3. RESULTS

The demographic information of the students who participated in the research is presented in Table 2. Looking at Table 2, there are 24 (61.5%) females and 15 (38.5%) males in the control group and 24 (72.7%) females and 9 (27.3%) males in the intervention group. The average age of the students in the intervention group is 20.46 years, while the average age of the students in the control group is 20 years. The study found that there was no statistically significant difference between the groups in terms of control variables, and the groups were similar to each other (p> .05, Table 2).

Table 2. *Comparison of control variables of intervention and control groups*

Characteristics	Intervention Groups		Control Groups		Test	p
Age ($\bar{x}$ ±SD)	20.46±2.349		20±2.716		t=-.773	P= .442
Gender	**n**	**%**	**n**	**%**		
Female	24	61.5	24	72.7	X^2=1.007	P=.316
Male	15	38.5	9	27.3		

n:number, %: percent, x̄: average, SD: Standard deviation, t: t test, X^2 : Chi square, p: significance

In Table 3, the pretest mean scores of the students' "Defense Style Questionnaire (DSQ-40)" sub-dimensions are presented. It was determined that the students in the pre-psychoeducational intervention group scored 9.81±2.443 from the 'Immature Defense' sub-dimension and 12.56±2.045 on the 'Mature Defense' sub-dimension. It is seen that the students in the control group got 9.01±1.775 and 12.18±2.278 points in the same order.

It was determined that there was no significant difference in the pretest mean scores between the groups (p> .05). It was determined that the students in the intervention group scored 11.62±2.403 points from the "Neurotic Defense" sub-dimension, and the students in the control group scored 10.26±2.076 points, and the difference between the pre-test mean scores between the groups was statistically significant (p< .05).

Table 3. *Comparison of students' defense style questionnaire and mindful attention awareness scale pretest mean scores between groups*

Scales		Pre-test			
		Intervention Groups (n:33)	Control Groups (n:39)	Test t	p
		x̄ ±SD	x̄ ±SD		
DSQ-40 Sub-dimensions	Immature	9.81±2.440	9.01±1.775	1.565	.123
	Neurotic	11.62±2.401	10.26±2.076	2.587	.012*
	Mature	12.56±2.045	12.18±2.278	.743	.460
MAAS		54.66±10.595	57.25±8.321	-1.161	.249

**p< .05, p: significance t: the paired-samples t testi, x̄: average, SD: standard deviation, DSQ-40: Defense Style Questionnaire, MAAS: Mindful Attention Awareness Scale*

When the pretest mean scores of the "Mindful Attention Awareness" scale of the students are evaluated in Table 3; It was determined that the total mean score of the Mindful Attention Awareness Scale in the intervention group students was 54.66±10.593 and 57.25±8.321 in the students of the control group, and the difference in the pretest mean scores between the groups was not statistically significant (p> .05).

When the posttest mean scores of the students in the "Defense Style Questionnaire (DSQ-40)" are examined in Table 4; It was found that the students in the "Immature Defense" sub-dimension scored 9.41±2.053, while the students in the control group who did not receive psychoeducation scored 8.95±1.774, and there was no significant difference between the posttest mean scores (p> .05). The students who received psychoeducation got 11.77±2.035, 13.40±1.781 points, respectively, from the sub-dimensions of "Neurotic Defense" and "Mature Defense" respectively, while the students who did not receive psychoeducation scored 10.02±2.099, 11.69±2.445 points, respaectively, and the difference between the posttest means scores of the intervention and control groups was statistically significant (p<.05).

Table 4. *Comparison of students' defense style questionnaire and mindful attention awareness scale posttest mean scores between groups*

Scales		Post-test			
		Intervention Groups (n:33)	Control Groups (n:39)	t	p
		x̄ ±SD	x̄ ±SD		
DSQ-40 Sub-dimensions	Immature	9.41±2.053	8.95±1.774	1.012	.315
	Neurotic	11.77±2.035	10.02±2.099	3.568	.001*
	Mature	13.40±1.781	11.69±2.445	3.349	.001*
MAAS		63.75±7.578	56.46±9.083	3.659	.000*

**p<.05, p: significance t: the paired-samples t testi, x̄: average, SD: standard deviation, DSQ-40: Defense Style Questionnaire, MAAS: Mindful Attention Awareness Scale*

When the students' "Mindful Attention Awareness" scale posttest mean scores are evaluated in Table 4; It was determined that the total mean score of the Mindful Attention Awareness scale of the students who received psychoeducation was 63.75±7.578, the students who did not receive psychoeducation was 56.46±9.083, and the difference between the posttest mean scores was statistically significant (p< .05).

When the pretest-posttest mean scores of the psychoeducation-receiving students' in the sub-dimensions of the "Defense Style Questionnaire (DSQ-40)" were examined; It was found that the students scored 9.81±2.440 points in the pretest and 9.41±2.053 points in the posttest from the "Immature Defense" sub-dimension, and the difference between the mean scores within the group was statistically significant (p< .05). It was determined that the students receiving psychoeducation who participated in the study scored 11.62±2.403 points in the pretest and 11.77±2.035 points in the posttest from the "Neurotic Defense" sub-dimension, and the difference between the mean scores of the group was not statistically significant (p> .05). It was determined that the students who received psychoeducation scored 12.56±2.045 points in the pretest and 13.40±1.781 points in the posttest from the "Mature Defense" sub-dimension, and there was a statistically significant difference between the mean scores of the group (Table 5, p< .05). When the pretest-posttest mean scores of the "Mindful attention awareness" scale of students receiving psychoeducation were evaluated; It was determined that the total mean score of the Mindful attention awareness scale was 54.66±10.593 in the pretest and 63.75±7.578 in the posttest, and the within-group difference was statistically significant (Table 5, p< .05).

Table 5. *Comparison of the pretest-posttest mean scores of the intervention group students' defense style questionnaire and Mindful attention awareness scale*

Scales		Intervention Groups (n:33)			
		Pre-test x̄ ±SD	Post-test x̄ ±SD	t	p
DSQ-40 Sub-dimensions	Immature	9.81±2.440	9.41±2.053	4.621	.000*
	Neurotic	11.62±2.403	11.77±2.035	-1.279	.210
	Mature	12.56±2.045	13.40±1.781	-7.032	.000*
MAAS		54.66±10.593	63.75±7.578	-11.819	.000*

**p<.05, p: significance t: Independent Samples t testi, x̄: average, SD: standard deviation, DSQ-40: Defense Style Questionnaire, MAAS: Mindful Attention Awareness Scale*

When the students in the control group were evaluated in the sub-dimensions of the "Defense Style Questionnaire (DSQ-40)" were evaluated; the scores of the students in the "Immature Defense" sub-dimension of the students were 9.01±1.775 in the pre-test, 8.95±1.774 in the post-test, from the ''Neurotic Defense'' sub-dimension 10.26±2.076 in the pre-test, 10.02±2.099 in the post-test and from the ''Mature Defense'' sub-dimension 12.18±2.278 in the pre-test, 11.69±2.445 in the post-test, and there was no statistically significant difference between in-group mean scores (Table

5, p> .05). Considering the pretest-posttest mean scores of the "Mindful Attention Awareness" scale of the control group students; The total mean score of the Mindful Attention Awareness scale was found to be 57.25±8.321 in the pretest and 56.46±9.083 in the posttest, and the within-group difference was not statistically significant (Table 6, p>.05).

Table 6. *Comparison of the pretest-posttest mean scores of the control group students' defense style questionnaire and mindful attention awareness scale*

Scales		Control Groups (n:39)			
		Pre-test	Post-test	t	p
		$\bar{x}$ ±SD	$\bar{x}$ ±SD		
DSQ-40 Sub-dimensions	Immature	9.01±1.775	8.95±1.774	0.229	.820
	Neurotic	10.26±2.076	10.02±2.099	1.028	.310
	Mature	12.18±2.278	11.69±2.445	1.574	.124
MAAS		57.25±8.321	56.46±9.083	1.363	.181

**p<.05, p: significance t: Independent Samples t testi, x̄: average, SD: standard deviation, DSQ-40: Defense Style Questionnaire, MAAS: Mindful Attention Awareness Scale*

4. DISCUSSION

This research was conducted to evaluate the effect of mindfulness-based psychoeducation on nursing students' use of defense mechanisms and mindfulness. Recognizing the defense mechanisms that people use increases their awareness (7, 13). Today, it is seen that there is a growing interest in mindfulness-based therapies (34). A review of the literature reveals studies of mindfulness practices with nursing students (9,34-37). However, mindfulness practices for defense mechanisms were not found. Studies showing that mindfulness practices used in different populations affect defense mechanisms are quite limited (13, 18). The results of this research were attempted to be discussed in line with the relevant literature with similar studies.

Pretest mean scores of immature and mature defense sub-dimensions of DSQ-40 of the students participating in the study were similar between the intervention and control groups, and the mean score of the neurotic defense subdimension was higher in the intervention group than in the control group. It is known that individuals who have psychological difficulties in coping with problems in daily life use more neurotic defenses (1, 4). Voluntary participation of students who wanted to receive psychoeducation in the study was crucial for the sustainability of the research.. It may be that these students use neurotic defenses more in the context of their willingness to find a solution to cope with the difficulties they encounter in daily life and their volunteer work.

The mindfulness pre-test mean scores of the students participating in the research were similar in the intervention and control groups. The literature indicates that the results obtained from studies examining mindfulness in health/nursing students are consistent with the results of the research (16, 35-37).

While the students' mean scores on the immature defense sub-dimension did not differ between the intervention and control groups on the post-test measures, the mean scores on the Neurotic and Mature Defense sub-dimensions were higher for the students who received psychoeducation. However, one should not be misled by the fact that the use of neurotic defenses seems to have increased in the intervention group in the post-test measures, since the use of neurotic defenses in pre-test measurements is significantly higher. In other words, this difference was already present when the intervention and control groups were compared before psychoeducation. The increase in the use of mature defense mechanisms in the students in the intervention group after psychoeducation is an expected result. It can be seen that the results of this research are supported by the results of the studies in the literature (7, 13, 18).

In the study, it was observed that after psychoeducation, students in the intervention group used immature defenses less, and mature defenses more. Studies have shown that immature defenses decrease and mature defenses increase in individuals after different practices such as mindfulness/cognitive behavioral therapy/psychotherapy (7, 13, 19). In Hersoug et al.'s (20-21) study of various occupational groups who were actively working, a non-directive meditation program was found to reduce immature defenses (13).

In this study, it was determined that mindfulness practices had no effect on the neurotic defenses used by students. The use of defense mechanisms is a necessity of life and operates automatically as it involves perceiving and processing information and responding to stressful situations (4). This makes it difficult for individuals to focus on immature defenses (20, 38). It is noted that individuals are patient, able to observe, and improved their attention with awareness practices (39-41). In this case, being careful and patient, being able to observe makes it easier to focus on immature and mature defenses (38). Recognizing and changing neurotic defenses is not easy, but it is stated that they can be changed with long-term psychotherapies (5, 19). Literature information supports the research findings.

Mindful awareness has been found to increase in students receiving psychoeducation. Similar studies with nursing students have found that different mindfulness practices at different times are effective in increasing awareness. In these studies, it is stated in addition to increasing awareness, improves the quality of life (42), decreases stress and anxiety (16, 42, 43), reduces stress, anxiety, and depression (37, 41, 44, 45), increases self-care and self-efficacy (46-48), decreases negative thoughts (39), influences ethical decision making (49), increases self-knowledge, insight development, internal coping mechanisms and self-care (34, 49), that the increase in student's self-awareness leads to the development of their skills and causes them to provide better care to patients (34, 47).

5. CONCLUSION

Mindfulness-based psychoeducation has influenced nursing students' mindfulness and use of defense mechanisms. Based on the results of this study, the following conclusions were reached:

- Mindfulness-based psychoeducation program may be beneficial in reducing perceived immature defense mechanisms levels and can contribute to increase the level of mature defense mechanisms.
- Mindfulness-based psychoeducation program can be effective in increasing the level of mindful awareness.

Nursing educators should consider implementing mindfulness interventions to improve the mental health of nursing students. Mindfulness practices can contribute to the personal, educational and professional development of nursing students. It is recommended that more research be conducted on the impact of mindfulness in educational and healthcare settings in our country, and that the long-term effects of these studies be examined. This research can be used as a method of mindfulness-based psychoeducation to increase the use of defense mechanisms and mindfulness in nursing students. If it can be integrated into nursing curricula, it can contribute to the personal development of students, to better understand themselves and their environment, and to cope with the difficulties they encounter in their professional practice. The results obtained from this study may contribute to future studies.

5.1. Strengths and Limitations of the Study

The implementation of online psychoeducation in the research contributes to the field. Nursing students may be reluctant to participate in such practices, because of the time commitment; therefore, online implementations may be preferable. There are studies showing that mindfulness practices are delivered online and are effective, and there are positive opinions about online training (21, 34, 48, 49).

The limited number of studies in the literature evaluating the effect of mindfulness practices on defense mechanisms limits the comparability of this study with similar studies. Another limitation is that the study was conducted through DEP, and repeated measurements could not be made in the long term, as the system would be shut down after a while when the practices were over. Therefore, it is unknown how long the effectiveness of the practices continues. In this study, the most common difficulties were the feedback in the sessions, the students' different expectations, getting bored with the sessions, having trouble maintaining, and thinking of inadequacy in practice. These problems caused some students to stop practicing. While the number of students participating in the sessions was 45 at the beginning of psychoeducation, this number gradually decreased, and the number of students participating in all sessions became 33. These students, on the other hand, stated that they were able to get used to and understand the sessions towards the end of the process. The research results can be generalized to the students who participated in the study.

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Clinical and Experimental Health Sciences

Effects of Postpartum Web-Based Breastmilk and Breastfeeding Education: A Quasi-Experimental Study

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ABSTRACT

Objective: The aim of the study was to examine the effect of web-based breastfeeding education applied to postpartum primiparous women on breastmilk, breastfeeding knowledge and self-efficacy.

Methods: This quasi-experimental study was conducted with 73 participants (experimental group=37; control group=36) at the Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital in Istanbul, Türkiye. The study was at postpartum services between June 2018 and October 2019. The participants in the experimental group attended the internet-based breastmilk and breastfeeding education program; where as those in the control group received routine care. Data were collected pre and post training using the demographic information form, Breastmilk and Breastfeeding Knowledge Test and Breastfeeding Self-Efficacy Scale.

Results: Our result showed that there was no significant difference found between the experimental and control groups on post-training on Breastmilk and Breastfeeding Knowledge Test ($p>.05$). On the other hand, there was significant difference between the experimental and control groups on post-training on Breastfeeding Self-Efficacy Scale ($p<.05$).

Conclusion: The data obtained showed that web-based breastmilk and breastfeeding education increased breastfeeding and breastmilk knowledge in the experimental group, however this increase was not at a level that would make a difference between the groups. But it had a positive effect on breastfeeding self-efficacy. In nursing practices, web-based breastmilk and breastfeeding education can be used to increase breastfeeding self-efficacy in women in the postpartum period.

Keywords: Breastfeeding, breast milk, distance education, online learning, postpartum period

1. INTRODUCTION

Breastfeeding ensures the mental, physical, psychological and social development of the baby. It protects babies from diseases, it is easily digested and is a reliable food source. In order for babies to get enough breastmilk, women should be supported in breastfeeding (1, 2). According to the results of the Turkey Demographic and Health Survey (TNSA) the rate of breastfeeding after birth, in the first hour is 71.00%, and the exclusive breastfeeding percentage in the first 6 months is 41.00 % (3).

One of the most important steps to take is to encourage mothers to breastfeed and support breastfeeding is to educate women on correct breastfeeding technique, breast care and how to deal with breastfeeding problems, and increase their self-efficacy (2, 4). Breastfeeding self-efficacy is the determination which a woman feels during the breastfeeding process. It has been reported that the breastfeeding duration of mothers who has high breastfeeding self-efficacy is longer than the others (4).

Since 1991, the Promotion of Breast Milk and Baby-Friendly Health Organizations Program has been implemented in Türkiye. Within the scope of the program, mothers are encouraged to breastfeed within the first hour, mothers and babies stay in the same room, health workers are trained about breastfeeding regularly, and training booklets, brochures, etc. are given to mothers in hospitals as education materials (5). In the current literature, it is stated that group

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or individual breastfeeding and breastfeeding trainings in the antenatal or postpartum period are not effective enough in maintaining breastfeeding (6), and that current education technologies (message, teleconference, e-mail, web-based education, etc.) has been reported to increase effective breastfeeding and contribute to the country's economy (7,8).

Developing internet technology has accelerated the access to information. Web-based education method which icludes enriched with videos, animations, spot information and pictures rather than verbal narration is preferred more than traditional education methods, especially by individuals under the age of 30. The rate of women using computers and internet in Türkiye is reported to be 76.30% (9).

Web-based breastfeeding education may be an effective option for nurses (6). Web-based breastfeeding education, which is independent of time and place and based on the individual's learning needs, can enable women to access information independently and quickly in the postpartum period. In studies using web-based education, it was determined that breastfeeding knowledge and attitudes of mothers increased (6).

This can be the first study to examine the effect of web-based breastfeeding education programme in Türkiye. The study was carried out to determine the effect of web-based breastfeeding training applied to postpartum primiparous women on breastfeeding knowledge and self-efficacy.

Hypotheses

H^1. After the training, the participants in the experimental group will be higher Breast Milk and Breastfeeding Knowledge Scores and Breastfeeding Self-Efficacy Scores than before the training.

H^2. After the training, the Breast Milk and Breastfeeding Knowledge Scores and Breastfeeding Self-Efficacy Scores of the participants in the experimental group will be higher than the control group scores.

2. METHODS

2.1. Design

Design of this study was quasi-experimental.

2.2. Participants

The study was conducted with postpartum mother between June 2018 and October 2019 at the Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital in Istanbul, Türkiye.

The inclusion criteria set as: being primipara, over the age of 18, giving birth to a singleton and mature baby, at least primary school graduate, having a smart phone or a computer, having no problems that may cause breastfeeding problems in breast evaluation (crack, redness, drooping), wound, etc.) and not having received breastfeeding education in the antenatal period. 100 mothers who met our criteria were involved in the study.

Sample Size Calculation

Sampling was calculated by using the $P^{0.05}$ power analysis program. It was determined that web-based breastfeeding education would create a 30.00 % variation between the knowledge scores of the women in the experimental and control groups, and that at least 31 people should be included in each group in the sample calculation made by accepting the power of the study as 80.00 % and α= .05.

In our study, convenience sampling method was used (10). One hundred motheres who met the sampling criterias were divided into two groups. The experimental group had 50 women and the control grup consisted of 50 women. 27 participants (Experimental Group=13; Control Group=14) whose babies were admitted to the intensive care unit, did not enter the training modules and did not fill in the forms withdrew/were excluded from this study. Hence, a total of 73 participants (Experimental Group=37; Control Group=36) completed the study (Figure 1. Research Scheme).

2.3. Data Collection and Instruments

2.3.1. Demographic Information Form (DIF)

The researchers prepared the DIF based on a review of the literature. The DIF included 12 questions designed to evaluate the participants' (6,11) sociodemographic characteristics (age, educational status, employment status, etc.), birth and infant characteristics (week of birth, birth weight of the baby, how many minutes after birth she started breastfeeding, etc.).

2.3.2. Breastmilk and Breastfeeding Knowledge Test (BBKT)

There are 10 multiple-choice questions in the Breast Milk and Breastfeeding Knowledge Test, framed by the researchers in line with the current literature (12). The test aims to evaluate the participants' level of knowledge about breast milk and breastfeeding. A minimum of 0 and a maximum of 100 points can be gained from the scale.

After the BBKT was prepared, it was submitted for expert review. When the test's difficulty index (p) was evaluated, it was determined that 5 questions were very easy (50%), 2 questions were very difficult (20%), and 3 questions were acceptable (30%). When the discriminative index was evaluated, 8 items were found to be excellent (80%), 1 item was acceptable (10%), and 1 item was weak (10%). The KR-20 reliability coefficient of the test was calculated to be 0.61, and it was determined that our measurement tool is quite reliable (13).

2.3.3. Breastfeeding Self-Efficacy Scale (BSES)

It was firstly created by Dennis to determine women's breastfeeding self efficacy (14). It had a total of 33 items. Nonetheless, a 14-item short form version was evolved to be used instead of the 33-item form (14). The Turkish version of the scale were accomplished by Aluş Tokat, Okumuş, and Dennis (15). To each item of the 5-point likert-type scale based on self-report, participants respond as 1= Not at all sure, 2= Not so sure, 3= Sometimes I am sure, 4= I am sure, or 5=Very sure. An increase in the score obtained is interpreted as an increase in breastfeeding self-efficacy. The score of the breastfeeding self-efficacy scale differs from 14 to 70 points. When the participant has between 14 and 32 points, she is advised to have low efficacy; between 33 and 51, moderate; and between 52 and 70, high efficacy. In our study, the Cronbach's Alpha value was found to be 0.86 in our study (15).

2.3.4. Web Based Breastmilk and Breastfeeding Education Program

The program consisting of three modules was prepared by the researchers by using the current literature (6,12) and two experts were asked for their opinions. Adjustments were made in line with expert recommendations. Then, a pilot study was conducted with five postpartum women and their feedback on the "Web-Based Breastmilk and Breastfeeding Education Program" was received. In line with the feedback, the "Web-Based Breastmilk and Breastfeeding Education Program" was finalized. The final version of the program was integrated into the website www.kadinvehastaliklari.com, prepared by one of the researchers. Participants can enter the program with their own computer, mobile phone, etc. with the user name and password given to them was able to login using devices.

Module 1 (Breastmilk and Breastfeeding); the content of breast milk, the factors that increase and decrease breastmilk, the benefits of breastmilk for the baby, etc. topics are included. The module consists of 27 audio slides and visual materials.

Module 2 (Effective Breastfeeding); it consists of a total of 12 voiced slides and 2 videos showing the correct breastfeeding positions about effective and correct breastfeeding, the frequency and duration of breastfeeding, breastfeeding positions, the mother's nutrition, sleep and rest during breastfeeding, and the situations that the mother should pay attention to before and after breastfeeding.

Module 3 (Breastfeeding and Breast Problems); it consists of 22 audio slides, 1 video and visual material that includes breastfeeding and breast problems encountered during breastfeeding and solution suggestions.

After completing one module, the participant was able to move on to the next module. After the participant completed the training modules, the researchers received a completion e-mail from. Participants could enter the modules as many times as they wanted.

2.3.5. Web Based Breastmilk and Breastfeeding Education Program Brochure

The brochure included information on how to enter the web-based breastfeeding training program, the content of this program, how the modules progressed, how to watch videos and animations, and basic information about the research process.

Procedure

1. Researchers introduced themselves, introduced in the purpose of the study, and invited mothers to participate in this study. Women who agreed to engage in this study signed a consent form.
2. Participants completed the DIF, BBKT and BSES scales.
3. Participants in both the experimental and control groups attended rousetine breastfeeding training by a breastfeeding nurse before discharge.
4. The participants were given a Web-Based Breastmilk and Breastfeeding Education Program Introductory Brochure in the experimental group. If the participant did not enter the program within two days, a reminder was sent by sending an e-mail.
5. After 15 days, the experimental group participants who completed the Web-Based Breastmilk and Breastfeeding Training were sent to the e-mails of the BBKT and BSES transferred to Google Drive and asked to fill in. Simultaneously, the post-test was administered to the participants in the control group.

2.4. Data Analysis

Data was analysed using the IBM SPSS 22 software. Data normality was decided using the Kolmogorov-Smirnov test. Independent groups t-test ve paired sample t-test were used in the analysis of data that conformed to normal distribution. Chi-square test was used for comparison of categorical data. An ANCOVA test was used to determine the differences between the experimental and control group in order to measure the effectiveness of the web-based breastmilk and breastfeeding education program. Statistical significance was accepted as $p < .05$.

2.5. Ethical Considerations

Permission for the trial was gained from the Ethics Committee of Ministry of Health Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital Clinical Research Clinical Research Ethics Committee (23.12.2016 /178). All women enrolled in the study were ask to read and sign the informed consent form. The study was guided by the criteria set by the declaration of Helsinki.

3. RESULTS

The mean age of the mothers in this study was 27.80±5.30 (min:19-max:42) years. There were no differences found in mothers' age, employment status, gestational week, mode of delivery, birth weight of the baby and time of first breastfeeding between the groups (p > .05). Only statistically significant difference was found in the educational status of the participants between the two groups (p< .05) (Table 1).

It was found that the post-training BBKT scores of the experimental group participants improved statistically significantly compared to the pre-training scores (pre-training= 51.89±20.39, post-training=61.35±19.74) (p<.05). The pre-training and post-training BBKT scores of the control group were similar (p>.05) (Table 2).

The pre-training BBKT scores of the control group (62.77±13.22) were statistically higher than the experimental group (51.89 ± 20.39) (p<.05). In order to find the actual conclusion of breastmilk and breastfeeding education on BBKT, the post-training BBKT scores of the experimental and control groups were compared with the ANCOVA test. The independent variable was the study group, and the dependent variable was postpartum 15th day BBKT scores. Participants' scores pre-training of BBKT as covariate. After settling for pre-training scores, there was no significant difference between the two groups on post-training on BBKT (p>.05) (Table 2).

It was found that the post-training BSES scores of the experimental group participants improved statistically significantly compared to the pre – training (pre-training= 52.89±9.72, post-training= 60.58±8.66, respectively, p<.001). Pre-training and post-training BSES scores of the control group were similar (p>.05) (Table 2).

The post-training scores of the two groups were compared with the ANCOVA test. The independent variable was the study group, and the dependent variable was the post-training BSES scores. Mothers' scores pre-training of BSES as covariate. The suitability of the data for analysis was evaluated with the Custom Hypothesis Test. After settling for pre-training scores, there was a difference between the both groups on post-training on BSES (p<.05) (Table 2).

Table 1. *Comparison of demographic, obstetric and breastfeeding characteristics of the participants in the groups*

	Experimental Group (n=37)		Control Group (n=36)		t	p
Characteristics	**X ± SD**		**X ± SD**			
Maternal age (year)	28.45 ± 6.23		27.16 ± 4.26		1.03	.30
Gestational week	37.72 ± 2.68		38.33 ± 2.17		-1.05	.29
Newborn birth weight (gr)	2919.18 ± 467.31		3136.11 ± 651.81		-1.63	.10
First breastfeeding time (min.)	240.67 ± 544.87		429.58 ± 1012.20		-0.99	.32
	n	**%**	**n**	**%**	**X^2**	**p**
Educational Status						
• Primary education	19	51.40	7	19.40	8.1	***.01***
• High Scool	9	24.30	14	38.90		
• University	9	24.30	15	41.70		
Employment status						
• Working	5	13.50	12	33.30	4.01	.056*
• Not working	32	86.50	24	66.70		
Mode of delivery						
• Cesarean Section (S/C)	21	56.80	19	52.80	0.11	.73
• Vaginal delivery (VD)	16	43.20	17	47.20		

*SD. Standard Deviation; Independent groups t-test, Chi-square test, *Fisher Exact Test*

Table 2*. Comparisons of participants' BBKT and BSES scores*

		Pre-training		Post-training					
Scores	**Groups**	**X ± SD**	**t ; p^1**	**X ± SD**	**t ; p^2**	**t ; p^3**	**F**	**ηp 2**	**p^4**
BBKT Scores	**Experimental Group (n=37)**	51.89 ± 20.39	-2.71; *.009**	61.35 ± 19.74	-2.14; .83	2.22; *.032**	0.29	0.00	.86
	Control Group (n=36)	62.77 ± 13.22		62.22 ± 14.56		.17 ; .86			
BSES Scores	**Experimental Group (n=37)**	52.89 ± 9.72	-1.35; .17	60.58 ± 8.66	1.46; .14	-4.90; *.00**	5.70	0.76	*.019**
	Control Group (n=36)	55.83 ± 8.73		57.66 ± 8.39		-1.32; .19			

BBKT. Breastmilk and Breastfeeding Knowledge Test; BSES. Breastfeeding Self Efficacy Scale

p1 = Comparison of pre-training scores between groups (Independent sample t test)

p2 = Comparison of post-training scores between groups (Independent sample t test)

p3 = Comparison of pre-training and post-training scores within the group (Paired sample t test)

p4 = ANCOVA

**p< .05*

The participant rate with high BSES score in both experimental and control groups were similar (Experimental group n=26, 70.30%; Control group n=27, 75.00 %). After the training, while the participant rate with high BSES scores increased in the experimental group (n=32, 86.60%), only one participant in the control group had an increase in the BSES score (n=28, %77.80) (Table 3).

Table 3. *Distribution of BSES scores of participants in groups*

	Pre-training		Post-training	
	n	%	n	%
Experimental Group				
• Low BSES (14-32)	1	2.70	-	-
• Modarate BSES (33-51)	10	27.00	5	13.50
• High BSES (52-70)	26	70.30	32	86.60
Total	**37**	**100.00**	**37**	**100.00**
Control Group				
• Low BSES (14-32)	1	2.80	1	2.80
• Modarate BSES (33-51)	8	22.20	7	19.40
• High BSES (52-70)	27	75.00	28	77.80
Total	**36**	**100.00**	**36**	**100.00**

BSES. Breastfeeding Self Efficacy Scale

4. DISCUSSION

This may be the first study in Türkiye to evaluate the effect of web-based breastmilk and breastfeeding training applied to postpartum primiparous women on breastfeeding knowledge and self-efficacy based on the current literature. Findings from the study revealed that web-based breastmilk and breastfeeding education increased breastfeeding self-efficacy, but had no effect on BBKT scores. Our findings are important in terms of demonstrating that web-based breastmilk and breastfeeding training can be a new approach that can be used by healthcare professionals to increase breastfeeding self-efficacy.

The homogeneity of the groups was evaluated considering that web-based breastmilk and breastfeeding education might be affected by variables such as maternal age, educational status, employment status, week of birth, mode of delivery, birth weight of the baby and time of first breastfeeding (16). It was determined that the other characteristics of the participants in the groups were similar except for the education level, and other studies in the literature were similar to our findings (11,17,18).

The education level of the women in the experimental group was found to be lower than that of the control group. It may be a result of the women in the experimental group showing more interest in web-based breastfeeding education in order to obtain information. Because as the education level of women increases, it becomes easier for them to access information by using different sources. Our finding may be important in showing that web-based breastmilk and breastfeeding education can be an important option for women with low education levels.

There are several studies reveal that the two weeks following the birth is an important period for breastfeeding. Women suffer from lack of milk, swelling of breasts, babies crying, baby refusal to drink milk, fatigue, insufficient support, etc. which may cause stoping breastfeeding (19). Breastfeeding education increases mothers' knowledge, attitudes and self-efficacy, increasing both the percentages of initiation of breastfeeding and the duration of breastfeeding (16,20-22). Studies that used breastfeeding education, short motivational interview, and short-term training methods have also shown that the level of breastfeeding knowledge increases (6). Internet-based educations are one of the effective ways to deliver a good intervention.

In our study, it was determined that the post-training BBKT scores and BSES scores of the participants in the experimental group of web-based breastfeeding education increased statistically significantly compared to the pre-training, confirming our H1 hypothesis. When the effect of web-based breastfeeding training on BBKT was tested with ANOVA, which is one of the advanced analysis methods; no statistically significant difference found between the post-training BBKT scores of the experimental and control groups and our H2 hypothesis was not accepted.

Web-based breastfeeding training provides an environment that women can learn by taking responsibility regardless of time and place during education. The videos, pictures, animations, and written information it contains have shown in many studies to increase women's knowledge of breastfeeding (6, 23, 24). Unlike other's study, in our study, it was determined that web-based breastmilk and breastfeeding education increased the breastfeeding knowledge level of the experimental group compared to the pre-training, but there was no significant difference between the groups. In support of our study finding, it was reported in Abuibdihal's (2021) study that web-based breastfeeding education was not effective at the desired level in increasing the level of knowledge (11). The difference between the studies may be caused by many factors such as the content difference of the web-based breastfeeding education programs used, and the duration of the trainings.

In fact, with web-based training, women can learn breastfeeding knowledge by working on their own. They can arrange the learning program themselves, as there are no time and place restrictions. The self-learning process can positively affect self-efficacy and this can encourage breastfeeding practice. It has been reported that the breastfeeding duration of mothers with high breastfeeding self-efficacy is longer than others (4).

When the effect of web-based breastmilk and breastfeeding education on BSES was tested with ANCOVA; there was a statistically significant difference found between the post-training BSES scores of the two groups so our H2 hypothesis was accepted. Similar to our findings, it was found that breastfeeding education increased BSES in studies where different education methods were applied (24-26). Different from Abuibdihal's study, the significant increase in BSES in

our study can be explained by the fact that the studies were conducted with different cultures (11).

Limitations

Our study was conducted in one hospital. The findings cannot be generalized to all women. The study was planned as randomized. However, randomization could not be achieved due to case losses. The high number of case losses is unfortunately a general problem of research conducted in our country. Another limitation of the study is that the education levels of the participants in the experimental and control groups were not similar. One of the limitation of the study is that we do not have information about how many times participants entered the web-based breast milk and breastfeeding training, how long they took to complete and how many times they repeated the modules. It is recommended that future researchers add software to their websites that will allow them to evaluate them.

5. CONCLUSION

The data we obtained showed that web-based breastmilk and breastfeeding education increased breastfeeding and breastmilk knowledge in the experimental group, however this increase was not at a level that would make a difference between the groups. But it had a positive effect on breastfeeding self-efficacy. With the rapid increase in the use of technology, nurses have to adapt to new teaching methods and use them in nursing care. In nursing practices, web-based breastmilk and breastfeeding education can be used to increase breastfeeding self-efficacy in women in the postpartum period. It is recommended that future researchers conduct randomized controlled, multicenter studies with more participants on the subject.

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Acquisition of data for the study: BY, FEY, ATB, BYD
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Clinical and Experimental Health Sciences

Determining the Effects of Nasopharyngeal Suction with Negative and Positive Pressure: Randomised Controlled Trial

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ABSTRACT

Objective: The study aims to determine the effects of nasopharyngeal suction with negative and positive pressure on pain level, respiratory parameters, and mucosal irritation in infants.

Methods: This study was conducted as a posttest randomized controlled experimental research. The data was collected at Göztepe Training and Research Hospital between January and November 2020. While the positive pressure suction method was applied to the experimental group, the negative pressure suction method was applied to the control group.

Results: It is determined that the pain level mean scores at the 1st and 5th minutes after the suction procedure are lower in the experimental group than in the control group (p=0.01). SpO2 values at the 1st, 5th, and 15th minutes after the procedure are significantly higher in the experimental group than in the control group (p=0.01). There is no statistically significant difference in the respiratory rate values between experimental and control groups (p>0.05).

Conclusion: It is determined that the post-procedure pain level is lower and the SpO2 value is higher in the experimental group (positive pressure suction) compared to the control group (negative pressure suction). Both methods do not cause mucosal irritation, as well.

Keywords: Infant, mucosal irritation, pain, respiratory parameters, suction

1. INTRODUCTION

Nurses are responsible for the suction function to clear the airway from secretions for effective breathing. Suction is defined as the removal of secretions from the respiratory system with a negative-pressure vacuum device (1, 2). In patients who are unable to extract respiratory secretions independently, suction is essential to maintain the oxygen demand and ventilation at the desired level and remove these secretions (1, 3).

The infant's respiratory and circulatory systems may be adversely affected as a result of the suction procedure, and several complications such as trauma, hemorrhage, and pain may develop. Suction is therefore one of the procedures that should be applied with caution in infants. Suction procedures for the respiratory tract in infants include oro/nasopharyngeal and endotracheal suction and suction methods include open and closed system suction methods (4, 5).

Oro/nasopharyngeal suction is a method that requires the use of negative pressure to remove secretions from the oropharynx, nasopharynx, or both (6, 7). When a foreign body penetrates the trachea from the pharynx, when respiratory secretions are too much, or when the secretion cannot be removed by normal cilia movement, coughing holds an important role. The inability to cough leads to atelectasis, pneumonia, and respiratory failure during infection of the respiratory tract. The cough reflex matures around the age of five in children. Adults can quickly remove existing airway secretions, but children with excessive airway

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secretions before this age are unable to do so easily. These secretions may be removed through either nasopharyngeal or oropharyngeal suction (8).

There are several risks and complications associated with the suction procedure. The most common ones are hypoxia, bradycardia, tachycardia, hypotension, hypertension, cardiac arrhythmia, cardiac arrest, atelectasis, bronchospasm, elevated intracranial pressure, nosocomial infection, tracheobronchial damage, and pain. Hypoxemia is the most prevalent and serious complication among them. To avoid suction-induced hypoxemia, different suction methods are being developed and novel devices are being employed (9, 10). Suction, a painful procedure, has been reported to negatively impact the physiological parameters, comfort, sleep, growth, and hospital stay of infants. The primary goal of pain management in infants is to minimize the pain experienced by infants due to various medical procedures and allow them to cope with the pain (11-13).

When the literature was examined, it was seen that signs and symptoms such as respiratory rate, heart rate, hypoxia, retractions (subcostal, intercostal, supracostal), nasal flaring, and irregular breathing were examined as respiratory parameters of newborns. (14).

Trauma is another complication of negative pressure suction. Suctioning of the tracheal, oral, and nasopharyngeal mucosa caused by negative pressure may result in hemorrhage and ulceration. As a result of the increased vacuum pressure generated during suction, the mucosal fragments are displaced from the catheter holes and absorbed (15,16).

When the literature was examined, no study was found regarding positive pressure aspiration in newborns. However, it is known that nasopharyngeal aspiration performed with negative pressure causes pain in babies and also causes negative consequences in physiological parameters such as heart rate, respiratory rate, oxygenation and hemodynamic stability (14, 17-19). Repetitive painful interventions can cause atrophy in the brain of babies and lead to neurodevelopmental problems. It also causes complications such as uncontrollable pain, changes in breathing and heart rates, and blood pressure. This can lead to infection, risk of infant death, and increased hospital stay (17).

It is important to reduce the complications seen in negative pressure aspiration, especially in groups that require special care, such as newborns. For this purpose, new methods and procedures are needed to prevent complications. This study aimed to examine the effects of positive pressure aspiration on newborns.

Objective

This study aimed to determine the effects of nasopharyngeal suction with the negative and the positive pressure on the level of pain, respiratory parameters, and mucosal irritation in infants.

Research Hypotheses

H1: The level of pain felt by infants during nasopharyngeal suction with positive pressure is lower than the level of pain they suffer during nasopharyngeal suction with negative pressure.

H2: In infants, nasopharyngeal aspiration with positive pressure has a positive effect on SpO_2 values and respiratory rates.

H3: In infants, the mucosal irritation caused by the nasopharyngeal suction with positive pressure is less than the mucosal irritation caused by the nasopharyngeal suction with negative pressure.

2. METHODS

2.1. Population and Sample

This study was designed as randomized, controlled, and experimental. The population was comprised of term infants who met the inclusion criteria, and were treated and cared for in the Neonatal Intensive Care Unit of a Training and Research Hospital in Istanbul, Turkey, between January 2020 and November 2020. The GPower 3.1 package program was used to calculate the sample size. It was determined that the effect size was 0.65, the type I error was 0.05, and the type II error was 0.8, and the research power was 0.80 (20). Accordingly, the sample size was calculated as 60 infants including 30 in the experimental group and 30 in the control group. The first 60 infants admitted to the neonatal intensive care unit who required nasopharyngeal suction were included in the sample and were randomly assigned to the experimental and control groups. The first hospitalized infant was assigned to the control group, and the second to the experimental group, and the assignment procedure was repeated in this order.

2.2. Implementation of the Study

The nasopharyngeal suction with negative pressure method was employed in the control group in this study. In this method, the nasal secretions were softened with 1-2 ml of physiological saline (PS), and then negative pressure suction was performed using a pine-tipped suction set. In the literature, neonatal aspiration pressure is defined as 60-100 mmHg. In this study, the suction pressure was kept between 60 and 80 mmHg, and no suction lasted for more than 15 seconds (21).

The nasopharyngeal suction with positive pressure method was employed in experimental group. In this method, the infant's head was turned to the side, to the nostril on the other side 1-2 ml of PS was injected with a syringe, and then positive pressure was exerted with the help of the end of the oxygen connection hose from the same nostril, with oxygen or air supply at 5-8 lt/min (if the baby requires oxygen, using an oxygen source) and the nasopharyngeal secretions were

removed from the nostril into which PS has been not injected. The oxygen connection hose was held one centimeter away from the infant's nostril. The researchers prepared a guideline for nasopharyngeal suction with positive pressure based on the literature (22).

In both groups, pain level, respiratory parameters (SpO_2 and respiratory rate), and mucosal irritation (the sign of hemorrhage in the nasal mucosa) were assessed.

The data were recorded in the data collection form created by the researcher. Following the collection of all data, the experimental and control groups were compared in terms of pain level, respiratory parameters, and mucosal irritation. This study was conducted by an NRP-(Newborn Resuscitation Program) certified researcher and the data were recorded by the same researcher.Since the data of the research was collected during the COVID-19 pandemic period, an independent observer could not be used to collect the data.

2.3. Inclusion And Exclusion Criteria

- Inclusion Criteria

- Babies who need suctioning (such as presence of nasal secretion, low SpO_2, wheezing, nose flap breathing, retractions; subcostal, intercostal or supracostal)

- Babies for whom consent can be obtained from their mother/father

- Exclusion Criteria

- Babies who are discharged during the study

- Babies who are intubated

- Infants with neurological and cranial disease were excluded.

2.4. Data Collection Tools

The researcher prepared a form to collect data. The data collection form includes four sections. The assessment periods were set according to the literature (8), and they were completed immediately before the aspiration process (aspiration process will be applied after measurement), 1st minute after the procedure, 5th minute after the procedure, and the 15th minute after the procedure.

- The first section had six questions on demographic data. In this section, data were collected regarding the diagnosis, week of birth, how old he/she was and gender.
- In the second section, the pain level is assessed with the Wong-Baker FACES Pain Rating Scale. There is no attempt to prevent pain during the aspiration process in the unit.
- The third section questioned data on respiratory parameters. The oxygen saturation value (Sp02) and respiratory rate were used to assess respiratory parameters. Since all babies were monitored with a monitor, the Sp02 value was taken from the monitors. Respiratory rate was determined by the researcher performing the aspiration.
- The fourth section evaluated the mucosal irritation as "present" or "absent" by inspecting the hemorrhage development in the nasopharyngeal mucosa. The presence of mucosal irritation was assessed by observation. The presence of mucosal damage was evaluated before the procedure, in 1st minute after the procedure, in 5th minute after the procedure, and the 15th minute after the procedure. It was evaluated as 'present' or 'absent' in terms of the presence of bleeding.

2.5. Ethical Considerations

The ethical approval institution is the Ethics Committee of Göztepe Training and Research Hospital. The approval number is 2020/0072.

The parents of the infants included in the sample gave their consent. It was planned to continue negative pressure suction in infants where nasopharyngeal secretions could not be removed with positive pressure, however, this was not required throughout the study.

The Clinical Trial number was taken as NCT06020638.

2.6. Limitations

The COVID-19 pandemic broke out during the data collection phase of the study. Due to the pandemic, we conducted the study with minimum human resources to reduce the risk of transmission in the neonatal intensive care unit. This inhibited an independent observer from collecting data. The lack of an independent observer is a significant limitation of the study. Due to the pandemic, the length of stay of infants in the neonatal intensive care unit was kept at a minimum level, hence the follow-up interval for mucosal irritation was set to be one day in this study.

2.7. Data Analysis

The data were recorded and analyzed on a computer using SPSS (Statistical Package for Social Sciences) for Windows 22. In the analysis of the data, first the requirements that needed to be met were tested to decide which tests (parametric/ non-parametric tests) to apply. To decide the normality of the distribution, kurtosis and skewness values and histogram graph, which are other events of Kolmogorov-Smirnov, Shapiro-Wilk normal events, were used. Summary values of quantitative (numerical) variables are presented as median (Q1-Q3), and summary values of qualitative (categorical) variables are distributions with frequency and percentage. Man Whitney-U test was used to compare two independent groups, Friedman test was used to compare more than two groups, and Bonferroni corrected pairwise comparisons were used to examine the difference. Chi square test was used to cover categorical variables. In interpreting whether the obtained values were significant or not, 0.05 was used as significance level measurements.

3. RESULTS

When the diagnoses of the babies are examined, it is seen that in the control group, 50% are temporary tachypnea of the newborn, 16.7% are sepsis, 6.7% are asphyxia, 13.3% are vomiting and 13.3% are other diseases. In the experimental group, 46.7% were diagnosed with temporary tachypnea of the newborn, 16.7% with sepsis, 13.3% with asphyxia, 3.3% with vomiting and 20% with other diseases. It was determined that there was no statistically significant difference between the experimental and control groups according to the gestational age (p> .05) and gender, which was considered to affect the study results (p> .05) (Table 1).

Table 1. *Demographic characteristics of the infants*

	Control Group (Negative Pressure Suction) n=30	Experimental Group (Positive Pressure Suction) n=30	Test Statistics	*p*
	Median (*P1-P3)**	**Median (***P1-P3)**		
Gestational Age	40.50 (38.00 – 42.25)	39.00 (37.75 – 41.00)	*Z*=-1.05	*.29
	***n* / %**	***n* / %**		
Female	15 / 48.4	16 / 51.6	X^2=.06	**.79
Male	15 / 51.7	14 / 48.3	X^2=.06	**.79
**Mann Whitney-U Test was run. ** Chi-Square Test was run p<.05 ***P1-P3: Percentile*				

3.1. Pain Level

The pain level mean scores in the 1st minute immediately after the procedure indicate a statistically significant difference in the experimental and control groups (p< .05). The mean scores of pain level at the 5th minute after the procedure indicated a statistically significant difference in the experimental and control groups (p< .05). When the median values were analyzed, it was found that the mean scores of pain level measured in the control group (Median=1) were higher than those in the experimental group (Median=0.33) The mean scores of pain level at the 15th minute after the procedure indicated no statistically significant difference in the experimental and control groups (p> .05) (Table 2).

3.2. Sp02 Values and Respiratuar Rates

A statistically significant difference was found between the SpO2 values that were measured over time in the control group (p< .05) (Table 3). A statistically significant difference was found between the SpO2 values that were measured over time in the experimental group (p< .05). As a result of the Bonferroni-corrected paired comparisons, it was found that the SpO2 values before the procedure were significantly lower than the SpO2 values measured at the 1st minute after the procedure, at the 5th minute after the procedure, and at the 15th minute after the procedure (p< .05) (Table 3).

Table 2. *Findings related to intragroup and intergroup comparison of pain level mean scores*

	Control Group (Negative Pressure Suction) *n*=30	Experimental Group (Positive Pressure Suction) *n*=30	Test Value	*p*
Pain Level After the Procedure	**Median (***P1-P3)**	**Median (***P1-P3)**		
1st minute[a]	5.67(5.33-6.33)	4.33(3.67-4.67)	*Z*=-4.39	**.01
5th minute[b]	1.00(0.67-1.33)	0.33(0.00-0.33)	*Z*=-4.91	**.01
15th minute[c]	0.00(0.00-0.00)	0.00(0.00-0.00)	*Z*=-1.00	*.32
Test Value	X^2=59.05	X^2=55.86		
P	****.01**	****.01**		
Difference	c<a b. b<a	b c<a		
**Mann Whitney-U Test was run. **Friedman Test was run. p<.05 ***P1-P3: Percentile*				

When the findings on the intergroup comparison of the differences between the repeated Spo2 values were examined (Table 3), it was found that the difference between the SpO2 values before the procedure and at the 1st minute after the procedure is higher in the control group than in the experimental group (p< .05), the difference between the SpO2 values at the 1st minute after the procedure and 5th minute after the procedure is higher in the control group than in the experimental group (p< .05), and the difference between SpO2 values at the 5th minute after the procedure and the 15th minute after the procedure is higher in the control group than in the experimental group (p< .05) (Table 3).

A statistically significant difference was found between respiratory rates that were measured over time in the control group (p< .05). As a result of the Bonferroni-corrected paired comparisons made to determine at what time intervals the difference occurred, it was found that the respiratory rate at the 15th minute after the procedure was significantly lower than the respiratory rate values measured before the procedure, at the 1st minute after the procedure and the 5th minute after the procedure. Moreover, the respiratory rate in the 1st minute after the procedure was significantly lower than that before the procedure (p< .05) (Table 3). A statistically significant difference was found between the respiratory rates that were measured over time in the experimental group (p< .05). As a result of the Bonferroni-corrected paired comparisons, it was found that the respiratory rates at the 5th and 15th minutes after the procedure were significantly lower than the respiratory rates measured before the procedure and at the 1st minute after the procedure (p< .05) (Table 3).

When the respiratory rates of the experimental and control groups were compared, it was found that there was no statistically significant difference between the experimental and control groups in terms of the respiratory rates measured before the procedure (p> .05), at the 1st minute after the procedure (p> .05), and the 5th minute after the procedure (p> .05) and 15th minute after the procedure (p> .05) (Table 3).

Table 3. *Findings related to intra-group and inter-group comparison in terms of spo2 and respiratory rate*

Findings	Control Group (Negative Pressure Suction) *n*=30	Experiment Group (Positive Pressure Suction) *n*=30	Test Value	*p*
SpO_2 values	Median (***P1-P3*)	Median (***P1-P3*)		
Before the procedure[(a)]	95.67 (95.33-96.67)	95.83 (94.67-97.08)	*Z*=-0.17	*.86
1st Minute[(b)] after the procedure	92.33 (91.58-93.17)	99.33 (99.25-99.67)	*Z*=-6.03	**.01
5th Minute[(c)] after the procedure	96.00 (95.33-97.00)	98.67 (97.00-99.00)	*Z*=-4.59	**.01
15th Minute[(d)] after the procedure	97.00 (96.00-97.33)	98.33 (97.58-98.67)	*Z*=-4.04	**.01
Test Value	X^2=63.04	X^2=68.64		
p	**.01	**.01		
Difference	b<acd. a<dc. c<d	a<bcd		
Comparison of differences				
Before the procedure[(a)] – 1st Minute[(b)] after the procedure	3.50 (2.66; 4.00)	-3.33 (-4.08; – 2.33)	-6.240	**0.000**
1st Minute[(b)] after the procedure-5th Minute[(c)] after the procedure	-3.66 (-4.33; – 3.00)	1.00 (0.33; – 1.33)	-5.838	**0.000**
5th Minute[(c)] after the procedure-15th Minute[(d)] after the procedure	-0.66 (-1.30; – 0.33)	0.00 (-0.33; 0.33)	-4.149	**0.000**
Respiratory Rates				
Before the procedure[(a)]	51.33(49.33-53.33)	51.00(47.83-53.33)	*Z*=-0.75	*.45
1st Minute[(b)] after the procedure	54.67(51.17-56.00)	53.33(49.83-56.67)	*Z*=-0.68	*.50
5th Minute[(c)] after the procedure	51.33(47.33-52.67)	50.33(45.83-52.17)	*Z*=-0.81	*.42
15th Minute[(d)] after the procedure	50.67(47.33-52.00)	50.00(45.33-52.00)	*Z*=-0.59	*.56
Test Value	X^2=74.22	X^2=69.86		
P	**.01	**.01		
Difference	d<abc. a<b	cd<ab		
*Mann Whitney-U Test was run. **Friedman Test was run. p<.05 ***P1-P3: Percentile				

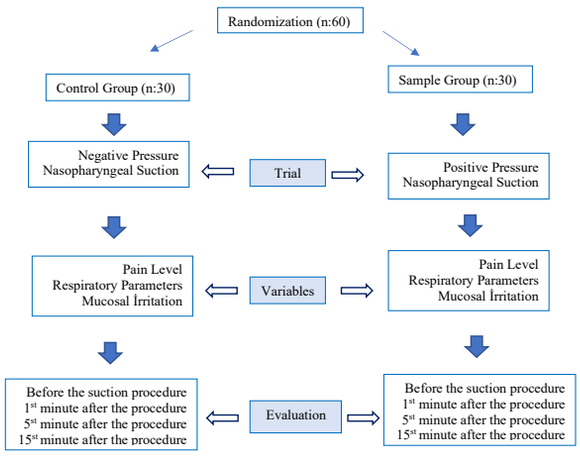

Figure 1. *Flow Chart of the Study*

3.3. Mucosal Irritation

No hemorrhage was observed in the nasal mucosa during the procedure in both groups.

4. DISCUSSION

4.1. Pain Level

Patients suffer pain during the suction procedure and suction is a painful procedure that causes changes in physiological parameters, especially in infants (22,17). The pain suffered by infants in the control group who underwent negative pressure suction alleviated significantly only at the 15th minute after the procedure. On the other hand, the pain levels at the 5th and 15th minute after the procedure were significantly lower than the pain levels immediately after the procedure in infants who underwent positive pressure suction in the experimental group. When these findings were evaluated, it is possible to conclude that the severity of pain decreased within a shorter time in infants who underwent the positive pressure suction procedure.

It was determined that there was a statistically significant difference between the experimental and control groups in terms of the mean score of the pain levels recorded at 1st and 5th minute after the procedure, and the mean score of the pain levels of infants in the control group is significantly higher than the mean score of the pain levels of infants in the experimental group (p< .05). This finding indicated that positive pressure suction caused less pain in infants compared to negative pressure suction. When the mean scores of the pain level measured at the 15th minute after the procedure were analyzed, no statistically significant difference was found between the experimental and control groups (p> .05).

When the literature was reviewed, no study was found that compared the effects of positive pressure nasopharyngeal suction and negative pressure nasopharyngeal suction on pain in infants. It is known, however, that nasopharyngeal suction

causes pain in infants as well as physiological consequences, such as a fluctuation in heart rate, respiratory rate, blood pressure, or a decrease in oxygenation and hemodynamic stability (17, 18). On the other hand, it was underlined that pain caused cognitive impacts in infants proportional to the severity and length of the pain and the severity and length of the pain may affect the infant's future response to pain (17, 18, 23). Positive effects of positive pressure suction on the severity and length of pain were observed in this study when compared to negative pressure suction. Given the impact of pain that may occur during nasopharyngeal suction on the physiological effects and cognitive development of infants, as well as their response to pain throughout their lives, it is believed that it is important to prefer positive pressure nasopharyngeal suction.

4.2. SpO2 Values and Respiratuar Rates

A statistically significant difference was found between the SpO2 values measured over time in the control group ($p< .05$); it is found that the SpO2 values measured at the 1st minute after the procedure were significantly lower than the SpO2 values measured at the 5th and 15th minutes after the procedure, while the SpO2 values measured at the 5th minute after the procedure were significantly lower than the values at the 15th minute after the procedure (Table 3). These findings suggested that negative pressure nasopharyngeal suction lowered the SpO2 value in infants, but the SpO2 value began to rise after the fifth minute. Likewise, a statistically significant difference was found between the SpO2 values measured over time in the experimental group ($p< .05$). However, this difference is found to be in favor of the SpO2 values before the procedure, and there is no significant difference between the SpO2 values measured at the 1st minute after the procedure, the 5th minute after the procedure, and the 15th minute after the procedure (Table 3). These findings indicated that positive pressure suction not only does not lower SpO2 values but increases them by having a positive effect when compared to values before the procedure, hence reducing the risk of hypoxic complications.

When the literature was reviewed, no study was found that compared the positive pressure nasopharyngeal suction and negative pressure nasopharyngeal suction in infants. However, several studies examining the effects of oro/nasopharyngeal suction in infants soon after delivery reported that the SpO2 value was lower in the group with suction was lower than in the group without suction (13, 14). These results suggested that nasopharyngeal suction may lower oxygen saturation in infants. In this study, positive-pressure nasopharyngeal suction had a higher positive effect on oxygen saturation than negative-pressure nasopharyngeal suction, which is considered a significant finding in terms of infant comfort.

A statistically significant difference is found between respiratory rates measured over time in the control group ($p< .05$) (Table 3). Negative pressure suction increased respiratory rate immediately after the procedure but returned to the values before the procedure in the 5th minute after the procedure. Likewise, a statistically significant difference is found between the respiratory rates measured over time in the experimental group ($p< .05$) (Table 3). Positive pressure nasopharyngeal suction increased respiratory rate immediately after the procedure in infants but returned to the values before the procedure at the 5th minute after the procedure, similar to the control group. Invasive treatments have been reported in the literature to raise the respiratory rates of infants (6). When the time-dependent changes in the respiratory rate of the experimental and control groups measured after the procedure were examined, the findings seemed to be compatible with the literature (6). The effects of positive and negative pressure suction methods on respiratory rate were similar in nasopharyngeal suction procedures in infants, and the effect of elevation in respiratory rate immediately after the procedure lasted for a short time in both methods and returned to the values before the procedure at 5th minute after the procedure. When the literature was reviewed, no study was found that compared the effects of positive pressure suction and negative pressure suction on respiratory rates in infants. This finding is found to be significant in terms of contributing to the literature in this regard.

4.3. Mucosal Irritation

No sign of hemorrhage was found in the nasal mucosa observation at the 1st minute after the procedure, at the 5th minute after the procedure, and at the 15th minute after the procedure in the experimental and control groups. It is known that suction of the tracheal, oral, and nasopharyngeal mucosa caused by negative pressure suction may result in hemorrhage and ulceration (16). Unlike the literature, this study revealed no hemorrhage in the nasal mucosa in the repeated observations after the procedure in the infants in the negative pressure suction group. It is believed that suitable practices made by the researcher during the negative pressure suction such as the insertion of a catheter of proper thickness, as well as the adjusting proper pressure by the researcher during the negative pressure suction, may have ensured that the suction did not irritate in the nasal mucosa. Positive pressure suction, on the other hand, did not cause nasal mucosa hemorrhage in infants. This finding was significant for the comfort of the infant after nasopharyngeal suction.

5. CONCLUSION

These findings indicated that the pain level was lower and the SpO2 value was higher in infants who underwent the positive pressure suction, compared to the negative pressure suction method. The effects of both methods on respiratory rate are similar, and in both methods, any mucosal irritation is not detected throughout the observation.

It is recommended that the positive pressure suction method be preferred for nasopharyngeal suction in infants, neonatal

nurses be trained on the effectiveness of the positive pressure suction method, and studies with larger samples and longer follow-up periods be done.

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Design of the study: RK, GKO, NU
Acquisition of data for the study: RK, GKO, NU
Analysis of data for the study: RK, GKO, NU
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Appendix

Application Directive Of Nasopharyngeal Aspiration With Positive Pressure

1.0 Purpose: To ensure adequate ventilation of the lungs by removing secretions and foreign materials from the upper respiratory tract as soon as possible.

2.0 Application:

2-1 Hands are washed according to protocol.

2-2 Aspiration is done in accordance with aseptic technique.

2-3 The aspiration process should not be continued for more than 10-15 seconds.

2-4 Appropriate position is given to the newborn (head is turned sideways).

2-5 During aspiration, the balloon mask system should be kept ready with the newborn.

2-6 The positive pressure to be used for aspiration should be 5-8 lt/min.

2-7 During aspiration, the baby's skin color and vital signs are monitored on the monitor.

2-8 Necessary materials for aspiration should be available with the newborn.

2-9 Aspiration should be done before feeding.

3.0 Conditions Requiring Aspiration

3-1 Wheezing

3-2 Cough

3-3 Presence of secretions

3-4 Presence of foreign substances (milk, food) in the nasopharyngeal region

4.0 Conditions to be Considered in Aspiration Application

4-1 Aspiration should be done when necessary.

4-2 To be more effective, lung physiotherapy should be performed in accordance with the protocol before aspiration.

4-3 Aspiration should be done before feeding.

5.0 Materials

5-1 Positive pressure source (02 – air)

5-2 Glove

5-3 Balloon-mask system

5-4 physiological saline

5-5 Injector

6.0 Pre-Operation Preparation

6-1 The materials are prepared and brought to the patient.

6-2 Positive pressure source is controlled

6-3 The general condition of the baby is evaluated

6-4 Hands are washed according to protocol

6-5 Gloves are worn

6-6 Appropriate position for the baby

7.0 Processing

7-1 In order to soften the secretions, 1-2 milliliters of SF is given to the nostril using an injector.

7-2 Immediately after the administration of SF, positive pressure is given with the end of the oxygen hose through the same nostril.

7-3 The same procedure is applied to both nostrils.

8.0 Post-Processing

8-1 The newborn is followed up for vital signs

8-2 Materials are collected

8-3 Hands are washed according to protocol

8-4 The transaction is recorded

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Clinical and Experimental Health Sciences

The Corpus Callosum in Schizoaffective Disorder: A Shape Analysis Study

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ABSTRACT

Objective: The corpus callosum is the largest white matter structure in the human brain that connects the cortical regions of both hemispheres. Diseases could lead to degenerative alterations in brain structures such as the corpus callosum (CC). Studies have associated CC abnormalities with Schizoaffective Disorder (SAD) symptoms. We predicted that there may be differences in the CC, an important structure connecting the two halves of the brain, in patients with SAD. The present study aims to analyze the CC of patients with statistical shape analysis (SSA) and compare the findings with healthy controls.

Methods: Thirty-nine SAD patients and 39 healthy individuals (11 females and 28 males) of similar age that included subjects participated in the study. CC landmarks were marked on the mid-sagittal images of each participant. The mean 'Procrustes' point was determined, and shape deformations were analyzed with thin plate spline analysis.

Results: Significant differences were observed between the shapes of CC in the two groups, and maximum CC deformation was observed in the posterior regions of SAD patients. There was no significant difference between the CC area of the SAD patients and the controls.

Conclusion: In the present study, the maximum deformation was observed in the posterior region (isthmus and splenium) and the rostrum of the CC. The first CC region, the rostrum (+genu), connects prefrontal and premotor regions, which are associated with cognitive information (landmarks = 1, 7, 8, 9, 13, 15, and 12). The second area, the splenium, connects temporal and occipital cortical areas. These predominantly have auditory, peripheral, and central visual stimulation functions (landmarks = 5, 3, and 4). The current study could assist future studies on the etiology, diagnosis, and treatment of SAD.

Keywords: Corpus callosum, schizoaffective disorder, magnetic resonance imaging, neuroimaging, computer-assisted imaging.

1. INTRODUCTION

Schizoaffective disorder (SAD) is a chronic, potentially disabling psychotic disorder common in clinical settings. SAD has been often used as a diagnostic tool for individuals with an admixture of mood and psychotic symptoms and without a certain diagnosis. Its hallmark is the presence of major mood episode symptoms (either a depressive or manic episode) concurrent with schizophrenia symptoms such as delusions, hallucinations, or disorganized speech (1). When psychotic symptoms are observed exclusively during a mood episode, DSM-5 indicates that the diagnosis should be an adequate mood disorder with psychotic properties; however, when a psychotic condition includes at least two weeks of psychosis without prominent mood symptoms, the diagnosis could be either SAD or schizophrenia. According to the DSM-5, SAD can only be diagnosed if full mood disorder episodes are present during the majority of the total active and residual course of illness, from the psychotic symptom onset until the diagnosis (2).

The corpus callosum is the largest white matter structure in the human brain that connects cortical regions in both hemispheres (3). They contain numerous intra-hemispheric and interhemispheric myelinated axonal projections. Patients who undergo complete or partial corpus callosotomy and callosal lesion intervention have provided further data on their function over the years (3). Corpus callosum shape deformation could reflect a midline neurodevelopmental abnormality (4).

Corpus callosum (CC) size was associated with cognitive and emotional deficits in several neuropsychiatric and mood

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disorders (5). Morphological abnormalities in the corpus callosum were associated with cognitive impairments or abnormal behavior in patients with mental disorders such as schizophrenia and bipolar disorder (6).

Quantitative morphologic analysis of each brain structure commonly entails segmentation observed with volumetric measures in neuroimaging. Volumetric alterations are intuitive markers since they could explain disease-induced atrophies or enlargements. On the other hand, structural alterations such as bending/flattening or changes in a specific section of a structure, for example, thickening of the occipital horn of ventricles, have not been adequately reflected in global volume measurements (7).

Statistical shape analysis, a relatively new method in biological research, compares body forms with specific landmarks determined by anatomical prominence (8). Knowledge of the biological variations of anatomical objects is essential for statistical shape analysis and discrimination between healthy and pathological structures (7).

In addition to schizophrenia and bipolar disorder (6,9,10,11), shape alterations in the corpus callosum (CC) were investigated in various diseases such as Multiple Sclerosis (12), mild cognitive impairment, and Alzheimer's disease (13) , autism subtypes (14), restless leg syndrome (15), and Behçet's Disease (16) with statistical shape analysis (SSA). Our study employed statistical analysis to determine the presence of a correlation between shape changes in the CC in SAD patients.

To our knowledge, this is the first study where statistical analysis was employed to determine whether there was a correlation between shape changes in CC and SAD patients.

2. METHOD

2.1. Participants and study design

The present cross-sectional retrospective study included the data collected from patients, who were diagnosed with SAD based on the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) criteria (17) and admitted to the outpatient or inpatient clinics in a Psychiatric Training and Research Hospital between January 2018 and July 2022. The local ethics committee approved the study (IRB: 07/07/2022–2022.07.177). The images of 39 SAD cases diagnosed based on the DSM-5 criteria, met the participation criteria, and underwent cranial MRI examination were identified in the Hospital Information System (HIS) and examined by a radiologist to determine whether they were suitable for the study. Cases with additional diseases were not included in the study. Participation criteria included age between 18-65, the presence of no other psychiatric diagnosis, mental retardation, a neurological or physiological disease, and no history of alcohol or substance use during the last 6 months based on HIS data and patient statements. The control group included age – and gender-equivalent healthy individuals who met the above-mentioned study criteria, who did not have a psychiatric diagnosis, or who underwent brain MRI examinations for other reasons. In the power analysis performed Post Hoc by comparing it with similar studies (18–20) in the literature, the power was found to be 100 % .

2.2. Data collection and image analysis

All participants were scanned in the same 1.5T Philips Ingenia scanner (Philips Medical System, Best, NL) with an 8-channel array head coil. T2 Turbo Spin Echo MRI images were obtained.

2.3. Determination of the Two-Dimensional Landmarks

Mid-sagittal T2-weighted two-dimensional digital MRI images of each individual that clearly reflected the cerebral aqueduct, CC, and superior colliculus were identified (Figure 1). Corpus callosum was marked (Figure 1) on TpsDig2 version 2.32 software on each image with standardized anatomical landmarks (20–22) for data collection (23).

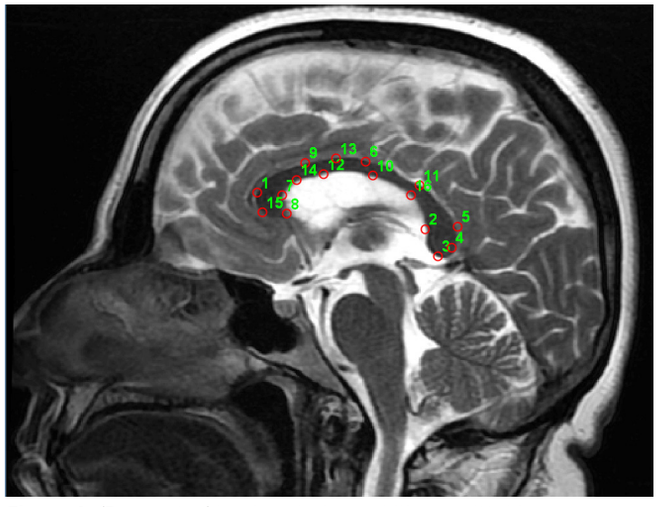

Figure 1. *'Procrustes' landmarks in a midline sagittal image.*

2.4. Statistical Deformation Analysis

The mean 'Procrustes' landmark was calculated and shape deformations were analyzed with thin plate spline (TPS) analysis with Past version 4.10 software (24) Areas with the most significant expansion or contraction were marked with different colors to indicate deformations in this analysis. The homogeneity of the variance-covariance matrices was analyzed with the Box-M test (24, 25) Due to the non-homogeneity of the matrices, the James F_J test based on a resampling procedure was employed to compare the CC shape between the control and SAD groups (25, 26) Furthermore, the root means square of Kendall's Riemann distance rho was compared with the mean to determine the overall shape variation in both groups. The allometric analysis was conducted with multivariate regression analysis of the centroid size (CS, the square root of the sum of the square of the Euclidean distance between each sign and the center) and

tangent coordinate. Model significance was determined with Wilks' lambda test. Model fit was determined based on the mean square error (MSE) and coefficient of determination (R^2) (27, 28).

2.5. Landmark Reliability

A single rater manually defined all landmarks in the present study. Intra-rater reliability was not calculated, since the high reliability of the landmark selection by the same rater was demonstrated in previous studies (28, 15).

2.6. Statistical analysis

Shapes version 1.2.6 software with R version 4.2.0, and PAST version 4.10 were employed for statistical shape analysis (24, 25) Statistical analyses were conducted on SPSS for Windows version 26 (IBM Corporation, Armonk, New York, USA) software. Data are presented in mean±standard deviation. The normal distribution of the data was analyzed with the Kolmogorov-Smirnov test, and adequate tests were employed to compare the groups based on the test results. A p-value of < .05 was considered statistically significant.

3. RESULTS

Eleven out of the 39 patients and 11 out of the 39 controls were female, and the rest were male, and there was no significant difference between the groups based on gender. The mean patient age was 42.08±11.22 and the mean control age was 41.94±12.30, and there was no significant difference between the groups based on age (p= .958).

Since the Box-M test identifies inhomogeneous matrices (F=3.3054, p< .001), the James F_J test was conducted, and it was determined that the CC shapes of SAD patients were significantly different when compared to the controls (T^2=3767.8684, p< .001) (Figure 2). The root means square of Kendall's Riemann distance (rho) to the main shape was 0.06763486 in controls, 0.07620406 in SAD patients, and 0.09275064 for all (Figure 3).

The effect of size-dependent shape changes and mean shape deformations on graphs (shrinkage) was demonstrated and compared for both groups with TPS (Figure 4). Maximum deformation was observed at the landmarks in the rostrum, anterior corpus, and splenium regions of the CC (landmarks 5, 13, 4, 6, 9, 3, 7, 8, 1, 12, 15, 10, 14, 16, 11, and 2 in descending order) (Table 1).

Multivariate regression analysis was conducted to determine the correlation size and shape in allometric analysis, and a statistically significant model (p=4.48E-246, R^2=0.1685, MSE=0.03072, and Wilks' lambda=7.913E-12) was developed.

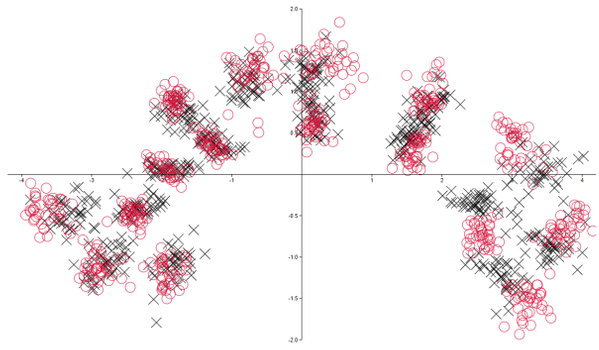

Figure 2. *Landmark scatter plot for controls (O) and schizoaffective disorder patients (X)*

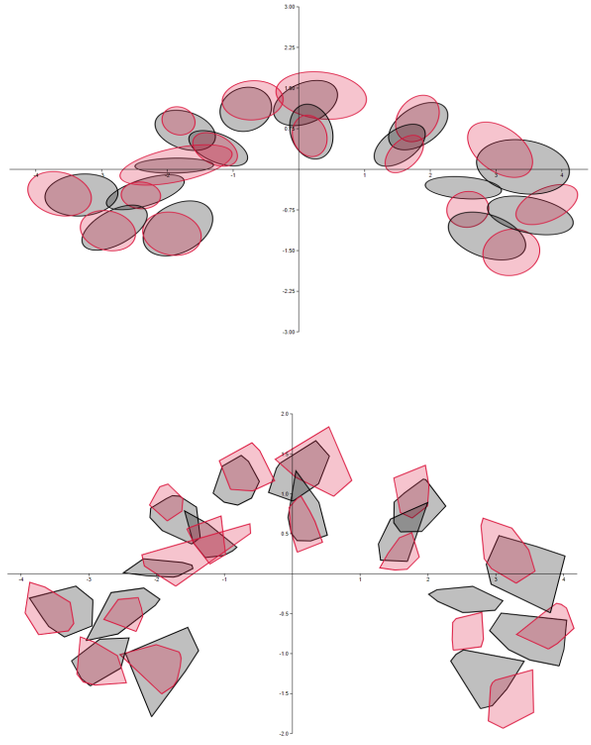

Figure 3. *%95 ellipse/convex hull graph for landmark scatter of controls (grey) and schizoaffective disorder patients (red).*

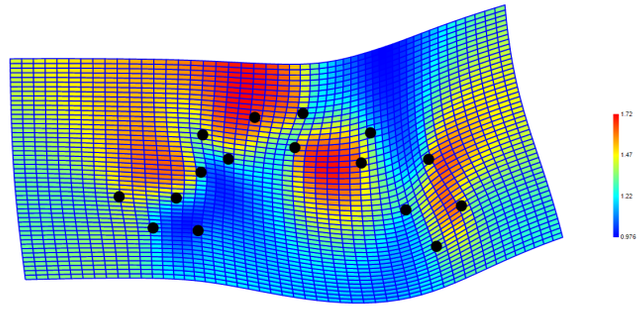

Figure 4. *Thin-plate spline transformation grid with expansion factors of the transformation from the control group to the schizoaffective disorder group.*

Table 1. *Average dissimilarities and contribution rate of the landmarks.*

Landmark	Average dissimilarity	Contribution %	R/p
1	1,9182E+13	2,96	0.5115/< .001
2	2,0122E+12	0,31	
3	3,66E+13	5,66	
4	8,181E+13	12,65	
5	1,5303E+14	23,65	
6	7,069E+13	10,93	
7	3,1194E+13	4,82	
8	2,39E+13	3,69	
9	5,41165E+13	8,37	
10	1,7166E+13	2,65	
11	9,851E+12	1,52	
12	1,8242E+13	2,82	
13	8,3762E+13	12,95	
14	1,61143E+13	2,49	
15	1,8128E+13	2,80	
16	1,1104E+13	1,72	

p-value of < .05

4. DISCUSSION

In the current study, the maximum deformation was identified in the posterior region (isthmus and splenium) and the rostrum of the CC. The first CC region, rostrum (+genu), connects prefrontal and premotor areas, which were associated with cognitive information (landmarks = 1, 7, 8, 9, 13, 15, and 12). The second region, splenium, connects temporal and occipital cortical areas. These areas predominantly have auditory, peripheral, and central visual stimulation (landmarks = 5, 3, and 4) functions (29–32).

Studies on brain structures such as the corpus callosum (CC) are limited in schizoaffective disorder. To our knowledge, there is no study where patient CCs were analyzed with statistical shape analysis (SSA), the results of which were compared with healthy controls. We think that our study will contribute to the literature in this respect.

Morphological abnormalities in the corpus callosum were associated with cognitive impairments or abnormal behavior in patients with mental disorders such as schizophrenia and bipolar disorder(6).

Only one study employed statistical analysis to determine whether there was a correlation between CC shape alterations in schizophrenia patients (SZ) (deficit syndrome (DS) and non-deficit syndrome (NDS)) and healthy controls (HC). The comparison of CC Procrustes shapes between HC and SZ revealed a statistically significant difference. The present study demonstrated callosal shape variations in SZ patients in both the DS and NDS subgroups based on the topographic distribution in CC (9).

In a study where corpus callosum abnormalities were determined based on anatomical signs in autism, no significant difference was reported in the landmark forms between patients and controls; however, the distance between the interior genu and the most posterior section was significantly shorter in the patient group. Thin-plate spline analysis demonstrated significant differences between the landmark configurations of the groups in terms of the diversion from the overall mean. Significant global shape differences were observed in the anterior lower body and posterior bottom, and a local shape difference was determined in the anterior bottom (33).

In a study where corpus callosum was investigated with statistical shape analysis in restless legs syndrome, a statistically significant shape difference was reported between the groups. The highest deformation was determined at the posterior midbody of the corpus callosum. Growth curve analysis demonstrated that an increase in disease duration and severity led to a decrease in the CC size (15).

Morphological analysis of the corpus callosum shape in normal, schizophrenic, and bipolar patients did not reveal significant global shape differences between these mental disorders. The highest differences were observed in the genu-rostrum, posterior body, isthmus, and splenium of schizophrenia and bipolar patients. Sample group comparisons revealed significant differences between all groups and global measurement parameters in various sub-regions. The present study findings suggested that the CC differed significantly in schizophrenia and bipolar disorder when compared to healthy controls, specifically in the anterior body and isthmus in schizophrenia and only in the isthmus in bipolar disorder (6). In our study, the maximum deformation was observed in the posterior region (isthmus and splenium) and rostrum of the CC in SAD patients.

In a previous study, CC area and shape were analyzed in first-episode schizophrenia and affective disorder patients, and the findings were compared with healthy controls with two-dimensional shape analysis. No differences were reported between the corpus callosum areas of the three groups, although differences were observed between the CC shapes of the schizophrenia patients and the controls. Furthermore, as the corpus callosum width narrowed, the angle decreased and led to a more curved shape only in the affective disorder group (4). In our study too, there was no significant difference between the CC area of the SAD patients and the controls.

In a study on CC, subregional volumes and the correlations between these and cognition, psychotic symptoms, and age were investigated in schizophrenia, psychotic bipolar disorder (PBD), SAD patients, their first-degree relatives, and healthy controls, and it was reported that anterior and posterior splenial volumes were significantly reduced in all groups. The schizophrenia and PBD probands exhibited robust and significant reductions, while significant reductions were of intermediate severity in the relatives group. There was a positive but differential correlation between splenial volumes and cognition in the probands and relatives. Proband groups exhibited a significant age-related decrease in the anterior splenium volume when compared to the controls.

The splenial volume was significantly reduced across the psychosis groups (34).

In a study where the morphology of the corpus callosum was compared in various stages of schizophrenia, no significant differences were reported between the total area of the groups. Similarly, in our study, there was no significant difference between the CC area of SAD patients and controls. Reductions in callosal width were observed in the anterior genu region in first-episode disorders. Similar reductions were observed in the anterior genu, as well as the posterior genu and isthmus in the chronic schizophrenia group. Reductions were present in anterior callosal regions that connect the frontal cortex at the onset of schizophrenia, and these were accompanied by changes in other callosum regions that connect cingulate, temporal, and parietal cortices in established illness (35).

In another study, structural-T1 and diffusion magnetic resonance images of SAD patients and healthy controls were obtained to determine surface-based brain morphometry and conduct diffusion tensor imaging analysis. In grey matter, SAD patients exhibited abnormalities in the frontal and temporal lobes, striatum, fusiform, cuneus, precuneus, lingual, and limbic regions. White-matter abnormalities were identified in tracts that connect these areas, including the corpus callosum, superior and inferior longitudinal fasciculi, anterior thalamic radiation, uncinate fasciculus, and cingulum bundle. The spatial overlap of abnormalities across different imaging techniques suggested widespread and consistent brain pathology in SAD. The abnormalities were mainly observed in areas that were commonly reported as abnormal in schizophrenia and, to some extent, in bipolar disorder, which could explain the clinical and etiological similarities in these disorders (36).

According to a systematic review of neuropsychological and neuroimaging studies in schizoaffective disorder, neurocognitive and neuroimaging abnormalities in schizoaffective disorder resembled schizophrenia more when compared to bipolar disorder. This could suggest that schizoaffective disorder could be a subtype of schizophrenia or part of a continuum psychosis spectrum model. schizoaffective disorder patients are more prone to schizophrenia when compared to bipolar disorder (37).

5. CONCLUSION

In the present study, SSA and CC analyses conducted on MRI images revealed significant differences between SAD patients and healthy controls. The maximum deformation was identified in the posterior region (isthmus and splenium) and rostrum of the CC. The study findings emphasized abnormal white matter distribution and subregional variations in the CC of the SAD patients. Future studies that would be conducted with larger samples could contribute to further elucidation of the present study findings.

One limitation of the study was the small sample size. Furthermore, the duration of illness for the patients is not the same. Patients take antipsychotic and/or mood stabilizers and antidepressant medications. One of the limitations of the study is that a clinical scale was not used in the study and whether there was a relationship between anatomical findings and clinical symptoms was not evaluated.

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Peer-review: *Externally peer-reviewed.*
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Research idea: MNN,MB;ÖG
Design of the study: MB,SB
Acquisition of data for the study: ÖG,MB;MNN
Analysis of data for the study: SB,MB
Interpretation of data for the study: SB,MB
Drafting the manuscript:ÖG,MB
Revising it critically for important intellectual content: ÖG,SB,MNN,MB
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Clinical and Experimental Health Sciences

The Effect of Listening to Music, Performing Mathematic Operations and Ball Squeezing in Reducing Pain During Dressing Change in Children

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ABSTRACT

Objective: The aim of this study was to determine the effect of listening to music (LM), ball squeezing (BS), and performing mathematical operations (MO) on the pain and physiological parameters during the first dressing in children aged 8-18 with appendectomy.

Methods: This study was a randomized controlled trial. The sample of the study consisted of 120 children (LM:30, BS:30, MO:30, control:30). Research data were collected using Information Form, Visual Analogue Scale (VAS), mathematical operations form, a softball, a pulse oximeter, and a thermometer. A minute before dressing and during dressing, the children in the LM group listened to music, the children in the SB group squeezed the ball, and the children in the MO group performed mathematical operations.

Results: According to the assessment of the child, parent, and nurse, it was determined that the pain score of the children in the LM and MO groups was lower than that of BS group and control groups ($p< .05$). The mean pulse of the children in the control group was found to be higher during and after dressing than that of LM and MO groups ($p< .05$). In addition, the mean pulse of the children in the BS group during dressing was found to be higher than that of MO group ($p< .05$).

Conclusion: It was determined that listening to music and performing mathematical operations were effective in reducing the pain of children during dressing. It is recommended that nurses use these non-pharmacological methods, which are easy to apply, in reducing children's pain.

Keywords: Appendectomy, children, distraction, dressing, pain.

1. INTRODUCTION

Acute appendicitis is one of the most common surgical causes of acute abdominal pain in pediatric patients (1). Post-operative wound dressing change causes pain, an unpleasant and undesirable feeling associated with tissue injury. It has been reported that the pain of adolescents who have dressing changes is quite high (2,3). Poor pain management leads to discomfort, dissatisfaction, delay in recovery, and prolongation of hospital stay (4,5). If children's pain is not managed effectively after surgery, the severity and duration of the pain increase. Inadequately treated pain after surgery causes children to suffer needlessly and increases the risk of complications (6). Therefore, postoperative pain should be controlled in a timely and effective manner (7).

A clinical guideline for postoperative pain management recommends the use of non-pharmacological methods in combination with various analgesics in children and adults (Recommendation 6-strong recommendation, high-quality evidence) (8). The distraction method is a non-pharmacological pain management technique that is widely used in the reduction of pain. The distraction method aims to draw the child's attention to something engaging and attractive by inhibiting their capacity to participate in painful stimuli, thus reducing pain, distress, and anxiety (9). There are two main techniques of distraction: active and passive (10). Music is a commonly used form of auditory and passive distraction (9,11-17). Practice Guidelines for Music Intervention recommend using music for pediatric patients as a complementary and alternative approach to take advantage of its calming, relaxing, distracting, sedating and pain-relieving effects (18). Ball squeezing is an active distraction method and has been used in a few studies to reduce pain in pediatric patients during intravenous catheterization, blood collection (19-21). No research has been found indicating the effect of ball squeezing in reducing pain during dressing change. The method of having the child perform mathematical operations during painful intervention was used for the first time in this study. It is estimated that the child may have an active distraction effect while trying to perform mathematical operation, as he wants to find the result of the operation correctly. This study aims to determine the effect of listening to music (LM), ball squeezing (BS), and performing mathematical operations (MO) in reducing the pain experienced during the first dressing in children aged 8-18 with appendectomy.

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2. METHODS

2.1. Ethical Considerations

Ethical approval for the study was obtained from the Clinical Research Ethics Committee (Date: 27.06.2018 / Decision no: KAEK/2018.5.07) of the hospital where the study was conducted. Before the study, the children and their parents were informed about the purpose, duration, and plan of the study. In line with the principles of voluntariness and willingness, verbal consent of the child, both verbal and written consent of the parent were obtained.

2.2. Study Design

This study was conducted as a randomized controlled study and carried out with four groups: the listening to music group (LM), the ball squeezing group (BS), the mathematical operations group (MO), and the control group.

2.3. Participants

The population of the study consisted of children who were hospitalized in the pediatric surgery clinic between January 2019-February 2020. The sample was based on a study (22) reporting that distraction with virtual reality application effectively reduced the pain scores of children aged 4 to 16 during dressing change, and the sample size was calculated using G*power 3.1.9.4 program. When the effect size was accepted as 0.8 for the pain score, the alpha error probability was calculated as 0.05 and the power value 0.95, and the sample size was determined to be 28 children in each group. Considering that there may be case losses, the study was carried out with a total of 120 children, who were divided into four groups homogeneously (LM:30, BS:30, MO:30, control:30). The criteria of the sample selection consisted of the child and parent's consents to participate in the study, the child and parent's speaking Turkish at the level of native speaker, the child's being 8-18 years old and undergoing appendectomy, being the body temperature of the child in the normal level (36.5-37.2°C), the child's not having intense nausea and vomiting, the child's not having any physical, intellectual or neurological disability.

2.4. Randomization

Randomization was performed through an online program (URL=https://www.random.org/). Before the sample number was processed into the program, lots was drawn and the 1st set was assigned to the LM group, the 2nd set to the BS group, the 3rd set to the MO group, and the 4th set to the control group. In order to determine which group the children would be in, numbers from 1 to 120 were processed into the program without recurrence of numbers. The children constituting the sample group were randomly divided into 4 groups by the program. The flowchart of the Consolidated Standards of Reporting Trials (CONSORT) for the research was shown in Figure 1 (23).

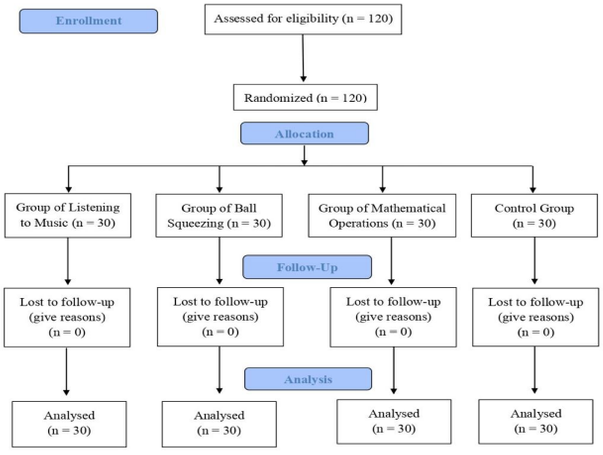

Figure 1. *Consolidated Standards of Reporting Trials (CONSORT) Flowchart*

2.5. Data Collection Tools

Information form, Visual Analogue Scale (VAS), mathematical operations form, ball, and pulse oximeter were used for data collection.

2.5.1. Information Form

In this form, there are 7 questions about the socio-demographic characteristics of the child and parent, appendicitis type, the parent accompanying the child, mobilization time, time of analgesic, antipyretic, anti-inflammatory drugs use, and the child's previous surgery experience. Also, physiological measurement values and pain scores can be written in this form.

2.5.2. Visual Analogue Scale (VAS)

The Visual Analogue Scale (VAS) was used by the child, parent, and nurse to measure the child's pain. The VAS is a valid and reliable tool that is easy for adults and children to use. The VAS consists of a 10 cm horizontal line, where 0 point in this line means 'no pain' and 10 point means 'unbearable pain'. The severity of the pain especially perceived or measured by the person is marked on the line, and the point marked by the person is measured with the help of a ruler and the pain score is determined (24,25).

2.5.3. Mathematical Operations Form

This form was used to have children in the MO group perform mathematical operations. Prepared by the researchers, the form involved mathematical operations such as addition, subtraction, multiplication, and division. The difficulty level of the mathematical operations in the form was determined by age groups of children. Accordingly, mathematical operations were divided into 3 groups primary school, secondary school, and high school levels.

2.5.4. Ball

For the BS group, softball that could fit in the palm and be easily squeezed by the child was used.

2.5.5. Pulse Oximeter

A calibrated Covidien console-type pulse oximeter was used to measure the pulse and oxygen saturation levels of the children.

2.6. Data Collection

The data of the study were collected in 3 stages: pre-dressing, during dressing and post-dressing.

2.6.1. Pre-dressing

Children who met the sample selection criteria and parents were informed about the study. Information about the children and their parents was recorded on the 'Information Form' 5 minutes before the dressing by the researcher. The child and his parents were informed about the VAS. Before the dressing, the child's pain was assessed by the child, his parents and the nurse. The children in the LM group were asked to choose a song they wanted to listen to on their own phones or on their parent's. The children started listening to the music with headphones a minute before the dressing. The children in the BS group started to squeeze the softball a minute before the dressing. The children in the MO group were asked to perform mathematical operations a minute before the dressing. The control group, underwent routine applications, and no distraction method was applied. The pulse and oxygen saturation levels of all groups were measured just before the dressing and recorded in the information form by the nurse.

2.6.2. During Dressing

The first dressing of all children after appendectomy was performed by the doctor in the clinic. No distraction method was applied to the control group during dressing. The LM group were dressed while listening to music. The BS group continued to squeeze the ball during the dressing. The children in the MO group were asked to perform mathematical operations given by the nurse. While the dressing, the pulse and oxygen saturation levels were recorded. At the last stage of dressing, the children were asked to mark the level of pain they experienced during dressing on the VAS, immediately after the sticking plaster was placed on the surgical area. Parents and nurses assessed the pain level of the child during dressing through the VAS.

2.6.3. Post-dressing

When the dressing was over, the application of distraction methods was terminated. The child's pulse and oxygen saturation levels were recorded by the nurse.

2.7. Data Analysis

Data were evaluated using the SPSS package program for Windows (version 21, IBM Corporation, Armonk, NY). Number, percentage, mean and standard deviation were used in descriptive statistics. The normal distribution was evaluated with Skewness and Kurtosis tests.

Pearson chi-square test for categorical variables, one-way analysis of variance in independent groups for numerical variables and Kruskal Wallis test were used in the evaluation of the homogeneity of the descriptive features of the four study groups. T-test in dependent groups was used in the comparison of the mean scores of pain before and after dressing separately by the four study groups (for in-group difference). Analysis of variance in repeated measurements was used in the comparison of the mean pulse and oxygen saturation obtained from three repeated measurements before, during and after dressing (further analysis Bonferroni test). One-way analysis of variance (advanced analysis Tukey HSD) in independent groups was used in the comparison of the difference between the mean scores of pain, pulse and oxygen saturation of the four study groups separately according to the measurement time (for differences between groups). Significance was evaluated at the $p < .05$ level.

3. RESULTS

There was no significant difference between the groups in terms of the age and gender of the children, the parent accompanying them, type of appendicitis, previous surgery, the means of how many hours ago the analgesic/inflammatory drug was taken, and mobilization time ($p>.05$, Table 1).

3.1. Sample Characteristics

It was found that there was no significant difference between the pre-dressing pain scores of the children in the four groups. Post-dressing mean pain score of the children according to the assessment of the child, nurse, and parent was found very significant difference ($p<.001$). In further analysis, it was found that the post-dressing mean pain score of the children in the LM and the MO groups was significantly lower than that of BS and the control groups ($p< .05$). (Table 2).

3.2. Mean Pain Scores

It was found that the post-dressing mean pain score of the LM group was lower than that of pre-dressing, and the difference between the two measurements was very significant according to the assessment of the child ($p<.01$) and highly significant according to the assessment of the parent and nurse ($p<.01$) (Table 2).

The mean pain scores of BS group was examined according to the assessment of the child ($p>.05$), the parent ($p>.05$) and the nurse ($p>.05$), it was found that the post-dressing mean pain score of the children did not change significantly compared to that of pre-dressing (Table 2).

The mean pain score of MO group during dressing was analyzed according to the assessments of the child, parent, and nurse, it was determined that the post-dressing pain score was significantly lower than pre-dressing score ($p<.001$, Table 2).

The post-dressing mean pain score of the control group was found to be higher than pre-dressing and the difference between the two measurements was detected to be significant according to the assessment of parent ($p<.05$), and very significant according to the assessment of child ($p<.01$) and nurse ($p<.01$). ($p< .01$) (Table 2).

The pre – and post-dressing mean pain scores of the children in the four groups were assessed by the child, parent and nurse. There was no significant difference between the pain scores obtained by the three observers ($p> .05$, Table 2).

Table 1. *Comparison of descriptive characteristics*

Characteristics	LM Group (n= 30)		BS Group (n= 30)		MO Group (n= 30)		Control Group (n= 30)		$^{1}x^{2}$	*p*
	n	%	n	%	n	%	n	%		
Gender										
Girl	18	60.0	12	40.0	15	50.0	17	56.7	2.803	.423
Boy	12	40.0	18	60.0	15	50.0	13	43.3	(df: 3)	
Parent accompanying during the procedure										
Mother	22	73.3	25	83.3	26	86.7	25	83.3	2.004	.572
Father/Family member	8	20.7	5	16.7	4	13.3	5	16.7	(df: 3)	
Type of appendicitis										
Perforated appendicitis	26	86.7	22	73.3	24	80.0	25	83.3	1.883	.597
Acute appendicitis	4	13.3	8	26.7	6	20.0	5	16.7	(df: 3)	
Prior surgery status										
Yes	4	13.3	3	10.0	2	6.7	7	23.3	4.038	.257
No	26	86.7	27	90.0	28	93.3	23	76.7	(df: 3)	
	X̄±SD (Min-Max)		X̄±SD (Min-Max)		X̄±SD (Min-Max)		X̄±SD (Min-Max)		^{2}F / ^{3}KW	*p*
Age (years)	11.97±2.62 (8-16)		12.57±2.56 (8-16)		12.17±2.64 (8-16)		12.03±2.55 (8-16)		F: .322	.809
*Medication time (hour)	9.83±1.33 (8-11)		10.75±2.43 (8-16)		9.71±1.38 (8-12)		10.43±1.40 (9-13)		KW: 1.246	.742
**Mobilization (hours)	6.93±2.39 (3-11)		7.34±2.69 (3-12)		7.83±2.56 (3-12)		8.07±2.20 (3-11)		KW: 3.826	.281

LM: Listening to Music, BS: Ball Squeezing (BS), MO: Mathematical Operations, df: Degree of Freedom.1 Pearson Chi-square test.2 One-way analysis of variance in independent groups.3 Kruskal Wallis test. How many hours ago the analgesic/inflammatory drug was taken before the dressing.** How many hours ago the child was mobilized before the dressing. df (degree of freedom): 3 Between-group/in-group/total df (degree of freedom): 3/116/119 (One way ANOVA).*

Table 2*. Comparison of the mean pain scores of the groups pre – and post-dressing*

The person assessing the pain	Time	LM Group[a] (n=30) x̄ ±SD	BS Group[b] (n=30) x̄ ±SD	MO Group[c] (n=30) x̄ ±SD	Control Group[d] (n=30) x̄ ±SD	^{1}F	*p* (difference)
Child	Pre-dressing	2.13±.73	2.10±.76	2.27±.64	2.17±.59	.333	.802
	Post-dressing	1.57±.77	2.20±.96	1.43±.63	2.67±.76	15.888	**.000** (a,c < b,d)
In-group difference	^{2}t	*3.195*	*.571*	*6.113*	*3.340*		
	p	***.003***	*.573*	***.000***	***.002***		
Parent	Pre-dressing	2.17±.70	2.13±.51	2.03±.61	2.07±.78	.255	.857
	Post-dressing	1.33±.71	2.10±1.06	1.47±.68	2.57±.68	15.460	**.000** (a,c < b,d)
In-group difference	^{2}t	*5.473*	*.166*	*4.011*	*2.715*		
	p	***.000***	*.869*	***.000***	***.011***		
Nurse	Pre-dressing	2.23±.68	2.03±.67	2.13±.68	2.10±.84	.399	.754
	Post-dressing	1.50±.63	2.23±1.04	1.33±.61	2.63±.67	19.668	**.000** (a,c < b,d)
In-group difference	^{2}t	*5.430*	*.972*	*5.442*	*3.395*		
	p	***.000***	*.339*	***.000***	***.002***		
Pre-dressing difference between child-parent-nurse	^{3}F	.393	.343	2.016	.230		
	p	.679	.713	.152	.796		
Partial eta squared / Observed power		.03 / .11	.02 / .10	.13 / .38	.02 / .08		
Post-dressing difference between child-parent-nurse	^{3}F	1.130	.556	.649	.389		
	p	.337	.580	.530	.682		

LM: Listening to Music, BS: Ball Squeezing (BS), MO: Mathematical Operations, SD: Standard Deviation. [1] One-way analysis of variance in independent groups, between-group/in-group/total df (degree of freedom): 3/116/119. [2] t test in independent groups, df (degree of freedom): 29. [3] Analysis of variance in repeated measurements, df (degree of freedom): 2.

3.3. Mean Pulse

It was found that there was no difference between the groups before the dressing (p>.05), and there was a very significant difference in during and post-dressing measurements (p<.01). The mean pulse score of the children was determined to be significantly higher in the control group than LM group and MO group (p< .05) during dressing, and the mean pulse score of the children in the BS group was significantly higher than MO group (p< .05). The post-dressing mean pulse the control group was determined to be significantly higher than LM group and MO group (p< .05, Table 3).

It was found that the mean pulse rate of the children in the LM group during dressing was significantly lower than that of pre-dressing (p<.01). It was determined that the pre – and post-dressing mean pulse rate of the children in the BS group was significantly lower than the mean pulse rate during dressing (p<.001). The mean pulse rate of the children in the MO group was found to be significantly lower during and post-dressing than pre-dressing (p<.01). The mean pulse of the children in the control group during and post-dressing was significantly higher than that of pre-dressing (p<.001).

3.4. Mean Oxygen Saturation

No significant difference was found between the pre-, during and post-dressing oxygen saturation mean scores of the children in the four study groups (p> .05, Table 4). When the pre-, during and post-dressing oxygen saturation mean scores of the children was compared within the groups, no significant difference was found in all groups (p> .05, Table 4).

Table 3. *Comparison of the mean pulse of the groups pre – , during and post-dressing*

Time	LM Group[a] (n=30) x̄ ±SD	BS Group[b] (n=30) x̄ ±SD	MO Group[c] (n=30) x̄ ±SD	Control Group[d] (n=30) x̄ ±SD	[1]F	*p* (difference)
Pre-dressing[x]	81.67±8.32	81.00±7.65	81.53±6.60	81.50±7.98	.044	.988
During dressing[y]	80.83±7.17	83.43±6.19	78.63±5.71	85.70±8.61	5.771	**.001** (a, c < d) (b > c)
Post-dressing[z]	78.33±6.08	80.20±5.28	77.77±5.53	83.73±6.65	6.230	**.001** (a, c < d)
In-group difference						
[2]F	7.606	10.977	7.473	13.961		
p (difference)	**.002** (x > z)	**.000** (x, z < y)	**.003** (x > y, z)	**.000** (x < y, z)		

LM: Listening to Music, BS: Ball Squeezing (BS), MO: Mathematical Operations, SD: Standard Deviation.[1] Analysis of variance in independent groups, between-group/in-group/total df (degree of freedom): 3/116/119.[2] Analysis of variance in repeated measurements, df (degree of freedom): 2.

Table 4. *Comparison of the mean oxygen saturation of the groups pre – , during and post-dressing*

Time	LM Group (n=30) x̄ ±SD	BS Group (n=30) x̄ ±SD	MO Group (n=30) x̄ ±SD	Control Group (n=30) x̄ ±SD	[1]F	*p*
Pre-dressing	98.63±.76	98.53±.82	98.63±.72	98.67±.84	.161	.922
During dressing	98.57±.82	98.60±.81	98.77±.77	98.63±.89	.339	.797
Post-dressing	98.60±.72	98.70±.99	98.80±.71	98.57±.86	.485	.693
In-group difference						
[2]F	.059	.265	.486	.103		
p	.943	.769	.620	.902		

LM: Listening to Music, BS: Ball Squeezing (BS), MO: Mathematical Operations, SD: Standard Deviation.

[1] Analysis of variance in independent groups, between-group/in-group/total df (degree of freedom): 3/116/119

[2] Analysis of variance in repeated measurements, df (degree of freedom): 2

4. DISCUSSION

When a distraction technique is performed to relieve pain, the neurocognitive pathways that direct the perception of pain may be disrupted (26). The child's capacity to attend a painful stimuli may be hindered by distracting his attention, enabling him to focus on engaging and attractive things, and therefore his pain, distress, and anxiety can be reduced (9). Active distraction methods require the child's cooperative involvement. Passive methods, are distractions that require such passive interactions as listening to music or watching videos. It has been reported that compared to passive distraction techniques, active distraction methods (distraction cards, playing digital games, virtual reality, etc.) provide a more significant reduction in children's pain and anxiety (22,27-30). However, the use of passive distraction methods has been reported to be more effective in the reduction of pain in painful procedures where no distraction methods were used (13,31,32). It has been reported that the use of active methods such as squeezing balls or playing with electronic devices and the use of passive methods such as watching videos, deep breathing exercises, and listening to music are appropriate in children aged 8-18 during painful interventions (33). In this study, the children in the intervention group carried out activities such as squeezing the ball and performing mathematical operations, among the active methods, and listening to music, a passive method. When the results of the study were examined, it was found that the pain scores of the children who listened to music (passive) and performed mathematical operations (active) were lower than those who squeezed the ball (active) and those in the control group. This result is important as it shows that active methods are not always more effective in reducing pain than passive methods.

Music potentially reduces the need for pharmacotherapy. The distraction effect of music can relieve pain and anxiety (13). In studies examining the effect of music therapy in similar age groups as in our study, the children in the music group were reported to experience less pain (11-13). In the study conducted by Atak and Özyazıcıoğlu (14), three different audio distraction methods were determined to be effective in the reduction of postoperative pain in children aged 7-14. In the study in which the pain related to circumcision was assessed, it was determined that the mean pain scores of the children in the experimental group who listened to music and watched kaleidoscope were lower than the control group (15). In the study in which the effects of distraction cards and listening to music in the reduction of the pain experienced during blood collection were compared, no difference was found between the groups. As in our study, music was compared with the active distraction method in that study and it had a similar effect with the active distraction (16). In the study evaluating the effects of music on pain and anxiety during donor site dressing change in pediatric burn patients aged 6-16, it was found that there was no difference between the groups (17). The reason for a different result between that study and our study in terms of listening to music may be due to the fact that the dressing change performed after appendicitis surgery caused less pain in our study than the donor site dressing change.

Ball squeezing is an easy distraction method that does not require learning and preparation. Sadeghi et al. (19) used a soft ball for squeezing during intravenous catheter insertion and found that it significantly reduced the pain. In another study in which a squishy object was used for squeezing during intravenous catheter insertion, the pain in children over 8 years old was assessed through the VAS, and this distraction method was found to be effective in the reduction of pain (20). In the study conducted by Aydin et al. (34), ball squeezing was found to be effective in reducing pain during blood sampling. Although it was not statistically significant, Aydin et al. (34) compared three different methods of distraction in their study and found that ball squeezing reduced the pain of children compared to the control group. Similarly, it was found in our study that the children in the BS group had lower pain scores than the control group although it was not statistically significant.

According to the gate control theory, pain sensations must pass through various control centers or gates in their pathway in order for pain sensations to be perceived and then be felt as pain. However, each "gate" along the pathway can be opened or closed by other types of stimuli, not just by pain. Thanks to distraction methods, doors can be closed and pain sensations can access fewer pathways (35). It was found that having children perform mathematical operations was effective in reducing pain, and therefore it was a very powerful distraction method. While performing mathematical operations, the child-focused all his attention on the questions, on answering them correctly and on succeeding. No study was found in which children were asked to perform mathematical operations as a distraction method during painful attempts. This method was used for the first time in this study. This study is of great importance in terms of indicating that performing mathematical operations is effective in the reduction of pain during dressing in children with appendectomy. This method, which has no cost, does not require preparation for use and can be easily performed during all kinds of painful interventions, can be used to reduce pain, especially in school-age children.

In our study, it was observed that the pain scores of the LM and MO groups were lower according to the parents' and nurses' assessments of the child's pain. This result was similar to the self-report of children. We consider that the assessments of the parent and nurse are additional evidence to indicate the effect of distraction methods. In many studies, the pain assessment of parents and observers was found to be similar to the self-report of the child (16,21,34,36,37).

It was found that the mean pulse of the MO and LM groups during dressing was lower than the control group. This result indicates that pulse is also affected in parallel with pain. However, no significant difference was found in oxygen saturation levels. Similar to our study, in the study conducted by Hua et al. (22), it was found that the pulse and the pain level of the children to whom the distraction method was applied

during the dressing change decreased, and no difference was detected between the oxygen saturation levels of the groups. In some studies, it has been emphasized that physiological parameters can be affected by various factors such as age, gender, emotions, drugs, and environmental atmosphere and that physiological parameter changes are not reliable indicators in the assessment of the intensity of pain (38,39). In a systematic review study examining the effect of audio-visual distraction on pain and anxiety in children, it was stated that the relationship between pain level and oxygen saturation level was examined in only two studies, and different results were obtained (40).

5. CONCLUSION

In this study, listening to music and performing mathematical operations were found to be effective in reducing pain during the first post-operative dressing in children with appendectomy. It was also determined that ball squeezing reduced the pain, but there was no statistical significance between the control group. The mean pulse of the children who performed mathematical operations and listened to the music was lower during and after dressing than the control group. However, there was no significant difference between the oxygen saturation of the four groups pre-, during, and post-dressing. That listening to music and performing mathematical operations are easy, do not require much preparation, are economical, and are effective in reducing pain makes these them prominent among the non-pharmacological methods used in the research. These methods can be used to relieve pain in children. Since performing mathematical operations does not require any materials, it is recommended to be used in all pediatric clinics during all kinds of painful interventions when the child is conscious.

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Design of the study: BM, ZE, MYA
Acquisition of data for the study: MYA
Analysis of data for the study: BM, ZE, MYA
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Clinical and Experimental Health Sciences

Computational Analysis of Cohesin Complex Genes and their Role in the Pathogenesis of AML

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ABSTRACT

Objective: Anomalies in the cohesion complex contribute to the pathogenesis of myeloid malignancies by affecting the self-renewal capacity of hematopoietic and progenitor stem cells, but the underlying mechanisms of this phenotype are not fully understood. Therefore, this study aims to shed light on the relationship between AML pathogenesis and the cohesion complex by comprehensively determining the mutations and expression profiles in the genes constituting the cohesion complex and investigating the effect of expression on survival using bioinformatics databases and tools.

Methods: A total of 96 different mutations were identified in 13 genes. Out of these 96 mutations, 26 were classified as pathogenic/oncogenic. The expression levels of STAG1, REC8, MAU2, CDCA5, and PDS5B were significantly higher in the patient group compared to the healthy group ($p < .01$). Survival analysis based on low and high gene expression profiles revealed that increased REC8 expression was significantly associated with survival ($p < .05$), which is considered a prognostic marker. In STRING analysis, it was determined that hub proteins interact with acetyltransferases ESCO1 and ESCO2 involved in sister chromatid cohesion, with TERF1, a component of the telomere nucleoprotein complex, and with PDS5A and BRCA2, which are functionally related to genetic stability and genetic recombination, respectively.

Results: An increase in language outcomes, particularly in repetition, was observed following the treatments. It was also found that therapy gains were more robust following bihemispheric stimulation of the posterior temporal sites compared to the inferior frontal targets.

Conclusion: Overall, none of the target genes except the mutated REC8 showed a significant and independent effect on the clinical outcome defined as overall survival. However, we have identified the diversity of genetic alterations in individual cohesin subunits through comprehensive molecular analysis. The results may be beneficial in the development of targeted drug therapies and personalized medicine approaches.

Keywords: Cohesion complex, acute myeloid leukemia, leukemogenesis, mutation, gene expression

1. INTRODUCTION

Acute Myeloid Leukemia (AML) is a hematological malignancy with a poor prognosis. It is believed to originate from functionally complementary genetic abnormalities that cause uncontrolled proliferation and halted maturation of myeloid precursor cells (1,2). Despite advancements in cell biology and comprehensive genomic analyses that have revealed possible leukemogenesis mechanisms, the genetic basis of the disease is not yet fully understood. Recent genome-wide sequencing studies have determined frequent recurring somatic mutations in genes encoding members of the cohesin complex (3-7). Cohesin, a multimeric protein complex, is a large ring-like subunit structure involved in regulating chromosome segregation during cell division (3-9). This structure plays an important role in various cellular processes, including chromatid cohesion, repair of damaged DNA, gene transcription, DNA replication, centrosome biogenesis (6,8-11). The ring-like cohesin complex consists of four proteins: structural maintenance of chromosomes (SMC3 and SMC1A), RAD cohesin complex element (RAD21), and cohesin subunit SA (STAG1/STAG2) (6-12). During the cell cycle, cohesin assists different additional subunits, including NIPBL, MAU2, WAPL, PDS5A, PDS5B, and sororin, in the establishment and dissolution of cohesion (12-15). This complex may also interact with transcriptional suppressor CTCF, promoters, enhancers, RNA polymerase II or transcription factors in their initiating and elongating forms to control chromatin structure and gene transcription (13-15). Undoubtedly, cell division is an important process for every tumor cell, including AML blasts, due to the increased proliferative potential of malignant cells. Mutations and expression abnormalities in genes encoding factors of the cohesin complex can contribute to myeloid malignancies by enabling self-renewal of hematopoietic stem and progenitor cells (4,5,7,8,16). We aimed to reveal a comprehensive genetic

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profile of mutations in genes comprising the cohesin complex, including STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5, and understand how these mutations contribute to leukemogenesis by elucidating their functional consequences behind the phenotype.

2. METHODS

2.1. Formation of the study group

The study group was formed by obtaining the AML dataset (n: 872) from the cBioPortal database. The data was accessed through the cBioPortal. The data was downloaded on July 25, 2022.

2.2. Mutation Profile Analysis

The cBio Cancer Genomics Portal (17) is a tool that provides mutation information, copy number alterations, microarray and RNA sequencing-based mRNA expression levels, methylation values, and protein levels from The Cancer Genome Atlas (TCGA) sourced data. The tumor type of interest was selected from the cBio to comprehensively study the mutations determined in the STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 genes in AML patient samples.

2.3. Oncogenic/ pathogenic impact analysis of identified mutations

The oncogenic/pathogenicity of mutations determined in the *STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2,* and *CDCA5* were founded using the scores given by the PolyPhen-2, SIFT, and OncoKB databases.

PolyPhen-2 (18) is an on-line accessible bioinformatics tool that predicts the potential effects of mutations on the stability and function of proteins by using structural and comparative evolutionary analyses of amino acid positions of potential mutations and SNPs. The program predicts the likelihood of a missense mutation damaging the protein based on a combination of these characteristics and provides the user with a score. The SIFT (19) is a tool that estimates whether an amino acid change impacts protein function based on sequence homology and the physical features of amino acids. OncoKB™ (20) is a precision oncology information base developed at the Memorial Sloan Kettering Cancer Center that provides biological and clinical data about genomic alterations in cancer. OncoKB explains the biological and oncogenic impact, as well as the prognostic and predictive significance of somatic molecular alterations.

2.4. Gene expression and survival analysis

GEPIA (21) is a web server that allows users to perform differential expression analysis at the subtype level. GEPIA is used to analyze gene and isoform expression by comparing TCGA and GTEx projects. Therefore, we used this data provider to determine the differential expression of the STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 genes in AML cohort (n: 173) and healthy tissue samples. Survival analyses of genes according to varying m-RNA expression levels were calculated using GEPIA. Overall survival (OS) and disease free survival (DFS) analyses based on Log-rank test with 95% confidence interval were performed to create survival plots.

2.5. Protein-Protein Interaction Analysis

The STRING database (22) is used estimate protein-protein interactions. The purpose of this database is to create a comprehensive and objective global network, including both physical and functional interactions. The predicted interactions of STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 proteins were performed by STRING database, which identifies *physical* and *functional* relationships between proteins.

2.6. Statistical Analysis

The GEPIA database utilizes the differential analysis method to compare gene expression between tumor and healthy control groups. The one-way ANOVA test is used to calculate differential expression. Overall survival analysis was calcuted using Kaplan-Meier curves. The log-rank test was used to compare the low and high expression groups. $p < .05$ was considered statistically significant in all statistical tests.

3. RESULTS

3.1. Demographic and Clinical Features of the Study Cohort

Detailed demographic and clinical characteristics of the dataset consisting of 872 AML patients were presented in Table 1.

3.2. Results of Mutation Profile of Cohesin Complex Genes in AML

In the AML cohort (n=872), it was determined that 15.7% of patients carried mutations in the study genes. The gene with the highest mutation frequency was STAG2 (3%), while no mutations were identified in PDS5A, SMC1B, and CDC5A genes (Figure 1A). A total of 96 various mutations were founded in 13 genes, including 36 missense, 22 nonsense, 15 splice region, and 23 frame shift mutations. Detailed information on the identified mutations is provided in Table 2**.** Additionally, the top 10 genes with the most frequent mutations in the AML cohort were determined as FLT3, DNMT3A, NPM1, IDH2, TET2, RUNX1, NRAS, SRSF2, WT1, and TP53. Based on the presence or absence of mutations in our target genes, we divided the AML cohort into two different groups. When conducting a graph analysis for the top 10 genes, it can be observed that the frequency of FLT3 mutations is similar in both groups. NPM1 mutations are more prevalent in the group with mutations in the target genes (Figure 1B-C). The localization of mutations founded in the domains of proteins belonging to the study genes in AML cohort is demonstrated in Figure 2.

Table 1. *Demographic, clinical and genetic data of patients with AML*

Characteristic	Patient data n:872 (%)
Gender	
Male/Female/NA	311/251/200
Diagnosis age, years	(1-87)
Chromosomal abnormality	
t(8;21)	11 (1.2)
inv(16)	18 (2.0)
11q23	4 (0.4)
t(15,17)	15 (1.7)
Cytogenetic risk	
Favorable	109 (12.5)
Intermediate	116 (13.3)
Unfavorable	132 (15.1)
NA/other	214 (24.5)
Diagnosis type	
FAB subtype	
M0	10 (1.1)
M1	15 (1.7)
M2	13 (1.5)
M3	11(1.3)
M4	35 (4.0)
M5	32 (3.7)
M7	2(0.7)
NA/other	731 (83.8)
Overall Survival Status	
Living	282 (37.0)
Deceased	423 (55.5)
NA	57 (7.5)
Total Mutation Frequency in AML	**Case (Frequency%)**
STAG1 genetic alteration	0.5
STAG2 genetic alteration	5
RAD21 genetic alteration	3
SMC1A genetic alteration	2.5
SMC3 genetic alteration	2.2
PDS5A genetic alteration	0
WAPL genetic alteration	0.3
NIPBL genetic alteration	0.1
REC8 genetic alteration	0.1
PDS5B genetic alteration	1.9
SMC1B genetic alteration	0
MAU2 genetic alteration	0.1
CDC5A genetic alteration	0

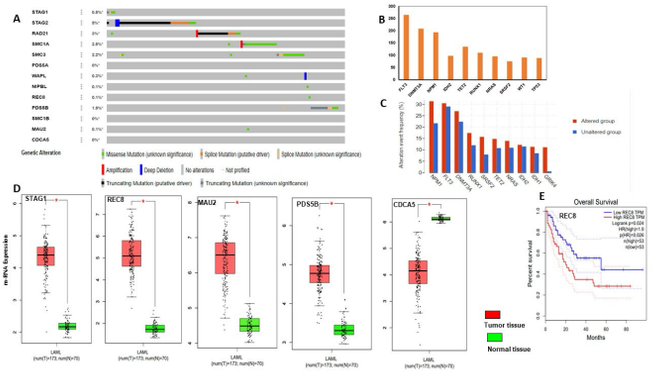

Figure 1. *(A) Distribution of mutations in STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 genes in TCGA AML cohort from cBioPortal. Percentages of overall mutations for each gene are given on the left. (B) Mutation distributions for the first 10 genes in the 872 TCGA AML cohort. (C) Gene Expression Profiling Interactive Analysis (GEPIA) was performed to validate higher expression of seven hub genes (STAG1, REC8, PDS5B, MAU2, and CDCA5) in AML samples compared with normal samples. The red and green boxes represent AML and normal liver tissues respectively. *represented p< .01. (D) Comparison of Kaplan-Meier survival curves of the high and low expression of REC8 in TCGA AML cohort (p< .05). Red line indicates the high expressions of m-RNA; Green line indicates the low expressions of m-RNA.*

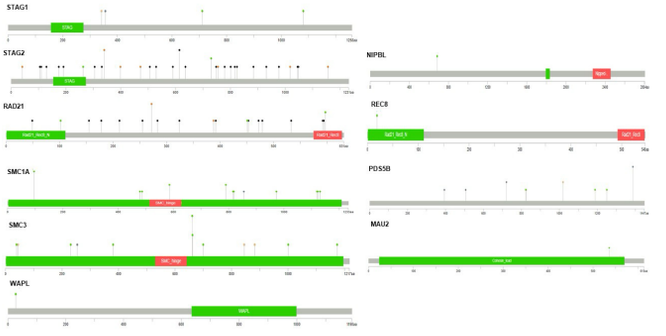

Figure 2. *Schematic representation of domain architecture of the STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 proteins and mutations detected in TCGA AML cohort.*

Table 2. *Characteristics of mutations detected in STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 genes in TCGA AML cohort.*

	Gene	Nt alteration	Rs Number	Alteration Type	Localization	AA position	Type of Cancer and Subtype	Clinical significance		
								Poly-Phen2 (score)	SIFT (score)	OnkoKB (score)
M-1	STAG1	c.3242G>A	NA	Missense mutation	Exon-29	R1081Q	AML	Benign 0.28	Tolerated 0.17	NA
M-2	STAG1	c.2133T>A	NA	Missense mutation	Exon-21	D711E	AML	Benign 0.03	Tolerated 0.68	NA
M-3	STAG1	c.1026+1G>T	NA	Splice region mutation	-	X342_splice	AML	NA	NA	NA
M-4	STAG1	c.1066_1067de	NA	Frame shift deletion	Exon-11	L357Ifs*4	AML-M5	NA	NA	NA
M-5	STAG2	c.123+1G>T	NA	Splice region mutation	-	X41_splice	AML	NA	NA	Likely oncogenic
M-6	STAG2	c.314C>G	NA	Nonsense mutation	Exon-6	S105*	AML	NA	NA	Likely oncogenic
M-7	STAG2	c.328C>T	NA	Nonsense mutation	Exon-6	R110*	AML	NA	NA	Likely oncogenic
M-8	STAG2	c.385_385+1insAA	NA	Frame shift insertion	-	G129Efs*17	AML	NA	NA	Likely oncogenic
M-9	STAG2	c.526dup	NA	Frame shift insertion	Exon-8 STAG domain	C176Lfs*2	AML	NA	NA	Likely oncogenic
M-10	STAG2	c.581_591del	NA	Frame shift deletion	Exon-8 STAG domain	E194Gfs*12	AML	NA	NA	Likely oncogenic
M-11	STAG2	c.913C>T	COSV54354732	Nonsense mutation	Exon-11 SCD domain	R305*	AML-M4	NA	NA	Likely oncogenic
M-12	STAG2	c.992dup	NA	Nonsense mutation	Exon-11 SCD domain	Y331*	AML	NA	NA	Likely oncogenic
M-13	STAG2	c.1018-1_1018del c.1018-1_1018del c.1018-1_1018del	rs205.802.8000	Splice region mutation	Exon-12 SCD domain	X340_splice	AML-M5	NA	NA	Likely oncogenic
M-14	STAG2	c.1197-1G>A	NA	Splice region mutation	-	X399_splice	AML	NA	NA	Likely oncogenic
M-15	STAG2	c.1519dup.1519dup c.1519dup c.1519dup	NA	Frame shift insertion	Exon-16	L507Pfs*2	AML	NA	NA	Likely oncogenic
M-16	STAG2	c.1587_1588dup c.1587_1588dup	NA	Frame shift insertion	Exon-17	R530Ifs*47	AML-M5	NA	NA	Likely oncogenic
M-17	STAG2	c.1768C>T		Nonsense mutation	Exon-19	Q590*	AML	NA	NA	Likely oncogenic
M-18	STAG2	c.1840C>T	COSV54351398	Nonsense mutation	Exon-20	R614*	AML	NA	NA	Likely oncogenic
M-19	STAG2	c.1908C>G c.1908C>G c.1908C>G c.1908C>G c.1908C>G c.1908C>G	NA	Nonsense mutation	Exon-20	Y636*	AML	NA	NA	Likely oncogenic
M-20	STAG2	c.2244_2247dup	NA	Frame shift insertion	Exon-23	E750Nfs*2	AML	NA	NA	Likely oncogenic
M-21	STAG2	c.2336dup	NA	Frame shift insertion	Exon-24	N780Efs*5	AML	NA	NA	Likely oncogenic
M-22	STAG2	c.2450del	NA	Frame shift deletion	Exon-25	P817Lfs*55	AML-M5	NA	NA	Likely oncogenic
M-23	STAG2	c.2470G>T	NA	Nonsense mutation	Exon-25	E824*	AML-M1	NA	NA	Likely oncogenic

M-24	STAG2	c.2653dup	NA	Frame shift insertion	Exon-26 GR domain	I885Nfs*10	AML	NA	NA	Likely oncogenic
M-25	STAG2	c.2793dup	NA	Frame shift insertion	Exon-28 GR domain	Q932Tfs*6	AML	NA	NA	Likely oncogenic
M-26	STAG2	c.2929_2930insTATT	NA	Frame shift insertion	Exon-29 GR domain	G977Vfs*8	AML	NA	NA	Likely oncogenic
M-27	STAG2	c.3054-2A>C	NA	Splice region mutation	Exon-29	X1018_splice	AML	NA	NA	Likely oncogenic
M-28	STAG2	c.3143T>A	NA	Nonsense mutation	Exon-30	L1048*	AML	NA	NA	Likely oncogenic
M-29	STAG2	c.3613C>T	NA	Nonsense mutation	Exon-33	R1242*	AML	NA	NA	Likely oncogenic
M-30	STAG2	c.1840C>T	NA	Nonsense mutation	Exon-20	R614*	AML	NA	NA	Likely oncogenic
M-31	STAG2	c.3133C>T	NA	Nonsense mutation	Exon-30	R1045*	AML	NA	NA	Likely oncogenic
M-32	STAG2	c.2401C>T	NA	Nonsense mutation	Exon-25	Q801*	AML	NA	NA	Likely oncogenic
M-33	STAG2	c.1416+1G>A	NA	Splice region mutation	-	X472_splice	AML	NA	NA	Likely oncogenic
M-34	STAG2	c.2265+2T>C	NA	Splice region mutation	-	X755_splice	AML	NA	NA	Likely oncogenic
M-35	STAG2	c.3467+1G>A	NA	Splice region mutation	-	X1156_splice	AML	NA	NA	Likely oncogenic
M-36	STAG2	c.787A>T	NA	Missense mutation	Exon-9 STAG domain	R263W	AML	Probably damaging (1.00)	Deleterious (0.00)	NA
M-37	STAG2	c.2188G>T	NA	Missense mutation	Exon-23	V730F	AML	Probably damaging (1.00)	Deleterious (0.00)	NA
M-38	RAD21	c.1782_1783insT	NA	Frame shift insertion	Exon-14	A595Cfs*10	AML-M4	NA	NA	Likely oncogenic
M-39	RAD21	c.1774C>T	NA	Nonsense mutation	Exon-14	Q592*	AML	NA	NA	Likely oncogenic
M-40	RAD21	c.1599dup	NA	Frame shift insertion	Exon-12	E534Rfs*3	AML	NA	NA	Likely oncogenic
M-41	RAD21	c.1435A>T	NA	Nonsense mutation	Exon-11	K479*	AML	NA	NA	Likely oncogenic
M-42	RAD21	c.1416dup	NA	Frame shift insertion	Exon-11	P473Tfs*5	AML-M2	NA	NA	Likely oncogenic
M-43	RAD21	c.1175_1176del	NA	Frame shift deletion	Exon-10	C392Sfs*10	AML	NA	NA	Likely oncogenic
M-44	RAD21	c.1162-1G>T	NA	Splice region mutation	-	X388_splice	AML	NA	NA	Likely oncogenic
M-45	RAD21	c.972_973insT	NA	Frame shift insertion	Exon-9	I325Yfs*3	AML-M5	NA	NA	Likely oncogenic
M-46	RAD21	c.815-3_815-2del	NA	Splice region mutation	-	X272_splice	AML	NA	NA	Likely oncogenic
M-47	RAD21	c.532G>T	NA	Nonsense mutation	Exon-6	E178*	AML	NA	NA	Likely oncogenic
M-48	RAD21	c.464_471del	NA	Nonsense mutation	Exon-5	L155*	AML	NA	NA	Likely oncogenic
M-49	RAD21	c.764T>A	COSV52058545	Nonsense mutation	Exon-7	L255*	AML	NA	NA	Likely oncogenic
M-50	RAD21	c.849dup	COSV52059956	Frame shift insertion	Exon-8	V284Rfs*2	AML	NA	NA	Likely oncogenic
M-51	RAD21	c.634G>T	COSV52056385	Nonsense mutation	Exon-6	E212*	AML	NA	NA	Likely oncogenic
M-52	RAD21	c.145dup	COSV52063924	Frame shift insertion	Exon-3	V49Gfs*31	AML	NA	NA	Likely oncogenic

M-53	RAD21	c.1359del	NA	Frame shift deletion	Exon-11	E453Dfs*3	AML	NA	NA	Likely oncogenic
M-54	RAD21	c.1790T>A	NA	Missense mutation	Exon-14	F597L	AML	Probably damaging (00.97)	Deleterious (0.00)	NA
M-55	RAD21	c.1352T>G	rs144953114	Missense mutation	Exon-11	L451R	AML	Probably damaging (0.99)	Deleterious (0.00)	NA
M-56	RAD21	c.305A>G	NA	Missense mutation	Exon-4 SMC3 Domain	E102G	AML	Probably damaging (0.99)	Deleterious (0.00)	NA
M-57	SMC1A	c.3367C>T	NA	Missense mutation	Exon-22	R1123W	AML	Possibly damaging (0.79)	Deleterious (0.02)	NA
M-58	SMC1A	c.2918A>G	NA	Missense mutation	Exon-19	Y973C	AML	Possibly damaging (0.85)	Deleterious (0.01)	NA
M-59	SMC1A	c.2369G>A	COSV59127614	Missense mutation	Exon-15 – Coil-coiled Domain	R790Q-	AML-M2/ M5	Probably damaging (1.00)	Deleterious (0.00)	NA
M-60	SMC1A	c.1756C>T	COSV59128824	Missense mutation	Exon-11	R586W	AML	Probably damaging (1.00)	Deleterious (0.00)	NA
M-61	SMC1A	c.287G>A	COSV59128385	Missense mutation	Exon-2	R96H	AML-M1	Probably damaging (1.00)	Deleterious (0.00)	NA
M-62	SMC1A	c.1757G>A	COSV59127689	Missense mutation	Exon-11	R586Q	AML	Probably damaging (1.00)	Deleterious (0.00)	NA
M-63	SMC1A	c.2563G>T	COSV59129560	Nonsense mutation	Exon-17	E855*	AML	NA	NA	NA
M-64	SMC1A	c.2447G>A c.2447G>A	COSV59131378	Missense mutation	Exon-16	R816H	AML	Probably damaging (0.99)	Deleterious (0.00)	NA
M-65	SMC1A	c.1460C>G	NA	Missense mutation	Exon-9	A487G	AML	Probably damaging (1.00)	Deleterious (0.00)	NA
M-66	SMC1A	c.2456T>A	COSV59127812	Missense mutation	Exon-16	I819N	AML	Benign 0.01	Tolerated 0.17	NA
M-67	SMC1A	c.3358A>G	NA	Missense mutation	Exon-22	K1120E K1120E	AML	Probably damaging (1.00)	Deleterious (0.00)	NA
M-68	SMC1A	c.3391G>A	NA	Missense mutation	Exon-22	G1131R	AML	Probably damaging (1.00)	Deleterious (0.00)	NA
M-69	SMC1A	c.1435C>A	NA	Missense mutation	Exon-9	Q479K	AML	Benign 0.00	Tolerated 0.92	NA
M-70	SMC3	c.117T>A	NA	Missense mutation	Exon-3	S39R	AML-M5	Probably damaging (1.00)	Deleterious (0.00)	NA
M-71	SMC3	c.130+1G>A	NA	Splice region mutation	-	X44_splice	AML	NA	NA	NA
M-72	SMC3	c.691G>C	NA	Missense mutation	Exon-9	E231Q	AML	Probably damaging (0.94)	Deleterious (0.00)	NA
M-73	SMC3	c.1982G>C	NA	Missense mutation	Exon-19	R661P	AML	Probably damaging (0.97)	Deleterious (0.00)	NA
M-74	SMC3	c.1985G>A	NA	Missense mutation	Exon-19	G662D	AML	Probably damaging (1.00)	Deleterious (0.00)	NA

M-75	SMC3	c.2099T>G	NA	Missense mutation	Exon-19	L700R	AML	Benign 0.01	Tolerated 0.07	NA
M-76	SMC3	c.2535+1G>A	NA	Splice region mutation	-	X845_splice	AML	NA	NA	NA
M-77	SMC3	c.1142G>A	NA	Missense mutation	Exon-13	R381Q	AML	Probably damaging (0.97)	Deleterious (0.02)	NA
M-78	SMC3	c.1982G>C	NA	Missense mutation	Exon-19	R661P	AML	Probably damaging (0.97)	Deleterious (0.00)	NA
M-79	SMC3	c.2644+2T>C	NA	Splice region mutation	-	X882_splice	AML	NA	NA	NA
M-80	SMC3	c.760A>T	NA	Nonsense mutation	Exon-10	R254*	AML	NA	NA	NA
M-81	SMC3	c.3001A>G	NA	Missense mutation	Exon-25	I1001V	AML	Benign 0.02	Tolerated 0.06	NA
M-82	SMC3	c.3521C>T	NA	Missense mutation	Exon-28	T1174I	AML	Probably damaging (0.99)	Deleterious (0.00)	NA
M-83	SMC3	c.1984G>T	NA	Missense mutation	Exon-19	G662C	AML	Probably damaging (0.99)	Deleterious (0.00)	NA
M-84	WAPL	c.81A>C	NA	Missense mutation	Exon-2	K27N	AML	Possibly damaging (0.89)	Deleterious (0.00)	NA
M-85	NIPBL	c.2054A>T	NA	Missense mutation	Exon-10	D685V	AML	Benign 0.02	Tolerated 0.06	NA
M-86	REC8	c.57G>T	NA	Missense mutation	Exon-3	W19C	AML	Probably damaging (0.99)	Deleterious (0.00)	NA
M-87	PDS5B	c.1180A>T	rs750827034	Nonsense mutation	Exon-11	R394*	AML	NA	NA	NA
M-88	PDS5B	VCc.4169del c.4169del	NA	Frame shift deletion	Exon-33	N1390Mfs*4	AML-M5	NA	NA	NA
M-89	PDS5B	c.2170dup	NA	Frame shift insertion	Exon-20	R724Pfs*16	AML	NA	NA	NA
M-90	PDS5B	c.1520dup	NA	Frame shift insertion	Exon-14	D508Gfs*4	AML-M1	NA	NA	NA
M-91	PDS5B	c.2469G>T	NA	Missense mutation	Exon-22	M823I	APL with PML-RARA (AML-M3)	Benign 0.02	Tolerated 0.21	NA
M-92	PDS5B	c.2475+1G>T	NA	Splice region mutation	-	X825_splice	AML	NA	NA	NA
M-93	PDS5B	c.3057-1del	rs868311964	Splice region mutation	-	X1019_splice	AML	NA	NA	NA
M-94	PDS5B	c.3748C>T	rs534821517	Missense mutation	Exon-32	R1250W	AML	Probably damaging (1.00)	Deleterious (0.00)	NA
M-95	PDS5B	c.3562A>G	NA	Missense mutation	Exon-31	T1188A	AML	Benign 0.11	Tolerated 0.49	NA
M-96	MAU2	c.1607C>T	NA na	Missense mutation	Exon-17	S536L	AML	Benign 0.02	Tolerated 0.19	NA

Abbreviations: M: Mutation; NA: Not available; ; Nt: Nucleotid; Rs: Register; ; AA: Amino acid; Inv: Inversion: t: translocation

3.3. Cohesin core subunit genes: STAG1/2, RAD21, SMC1A, SMC1B, SMC3, and REC8

Among the six core genes of the complex, namely STAG1/2, RAD21, SMC1A, SMC1B, SMC3, and REC8, four different mutations (2 missense, 1 frame shift, and 1 splice site mutation) were determined in STAG1. The *STAG1* contains the STAG domain, the stromalin protective region (SCD), and the glutamine-rich (GR) domain (23). The identified p.L357Ifs4 and p.X342_splice mutations are located on the sequence encoding the SCD domain. STAG2, which shares 70% homology with STAG1, has 33 different mutations (2 missense, 11 frame shift, 13 nonsense, and 7 splice site mutations). Among the identified mutations, 23 are putative driver mutations. Deep deletion resulting in homozygous allele loss was observed in 2 patients. The somatic mutation frequency is 4.5%. STAG2 shows homology with STAG1 in terms of domains. Frame shift mutations p.C176Lfs2 and p.E194Gfs12 are located on the STAG domain, while 1 missense alteration (p.R263W), 2 nonsense mutations (p.R305 and p.Y331*), and 1 splice region mutation (p.X340_splice) were identified on the SCD domain. Mutations causing frameshift on the GR domain include p.I885Nfs10, p.Q932Tfs6, and p.G977Vfs8. The mutation p.X755_splice is located on the ubiquitination and acetylation region. Additionally, mutations p.N780Efs5, p.P817Lfs55, p.E824, p.I885Nfs10, p.Q932Tfs6, and p.G977Vfs*8 are found in the Ser/Thr phosphorylation region.

RAD21 gene harbors 19 different mutations (8 frame shift, 6 nonsense, 3 missense, 2 splice region mutations). The somatic mutation frequency is 2.5%. Among the identified mutations, 14 are putative driver mutations. Additionally, there is a gene amplification anomaly in RAD21. RAD21 contains the SMC3 domain (1-103aa), STAG1/2 domain (362-403 aa), SMC1A domain (558-628aa), and the LPE motif (255-257 aa) necessary for the specific cleavage of RAD21 by separase (24). The p.C392Sfs*10 mutation, which causes a change in the reading frame, is located in the STAG1/2 domain. The p.X388_splice mutation, located at the exon-intron boundary of the sequences encoding the STAG1/2 domain, is within the splice site that is 100% conserved across species during evolutionary processes, suggesting that this mutation may cause an anomaly in RAD21 m-RNA expression. The same mutation is within RAD21's NLS signaling region, indicating a potential impact on nucleocytoplasmic transport.

SMC1A gene carries 12 missense mutations and 1 nonsense mutation. The somatic mutation frequency is determined as 2.1%. It contains 2 P-loop NTPase domains and 1 SMC hinge domain (514-629 aa) (25) .The p.R586W/Q missense mutation is located within the SMC hinge domain. The p.Y973C missense mutation is in the phosphorylation region, while p.K1120E is in the acetylation region. Among the 18 AML patients carrying mutations in SMC1A, 7 of them carry at least one FLT3 mutation (frame shift insertion, X583_splice region mutation, and D835Y missense mutation). No mutations were detected in SMC1B in the AML cohort. In SMC3, 14 mutations (10 missense, 3 splice region, 1 nonsense mutation) were identified. The somatic mutation frequency is 1.9%. Missense mutations p.R381Q, p.E231Q, and nonsense mutation p.R254* were identified in the N-terminal coiled coil domain. The p.R254* nonsense mutation has a truncated feature that causes early termination of the polypeptide. Patients carrying this truncated mutation also have oncogenic mutations in FLT3, DNMT3A2, and U2AF2 genes. Furthermore, a missense mutation p.R661P was detected in the SMC hinge domain. Missense mutations p.I1001V and p.T1174I are located in the ATPase domain. Splice region mutations p.X845_splice and p.X882_splice are found in the C-terminal coiled coil domain. In REC8, one missense mutation (p.W19C) was identified. The patient with the REC8 mutation has experienced relapse during treatment and carries a total of 636 different mutations, primarily in IDH2, NPM1, and KRAS genes.

3.4. Cohesin loading genes: NIPBL and MAU2

A missense mutation p.D685V was identified in NIPBL in one patient. In MAU2, we identified a somatic missense mutation p.S536L, which is located in the cohesion load domain. The patient with the MAU2 mutation has relapsed during treatment and also carries FLT3 and WT1 mutations.

3.5. Cohesin dissociation genes: PDS5A, PDS5B, WAPL, and CDCA5

No genetic changes were detected in PDS5A. In PDS5B, 9 different mutations (1 nonsense, 3 frame shift, 2 splice region, and 3 missense mutations) were identified. The somatic mutation frequency is determined as 1.7%. Four mutations (p.R394*, p.N1390Mfs4, p.R724Pfs16, and p.D508Gfs*4) have the potential to cause immature termination of the polypeptide and result in truncated protein. One missense mutation (p.K27N) was identified in WAPL, and it is located in the acetylation region. In WAPL, a deep deletion resulting in homozygous allele loss was found in one patient. No mutations were detected in CDCA5 gene.

3.6. Results of In Silico Analysis of Detected Mutations for Pathogenic/Oncogenic Features

According to the analysis results of the Poly-Phen2, SIFT, and OncoKB tools for predicting pathogenic/oncogenic characteristics, out of the 92 mutations detected in our study, 47 were classified as "*Likely oncogenic*" by OncoKB, and 26 were classified as "*disease-causing*" by the Poly-Phen and SIFT programs. Due to the close-to-1 pathogenic scores and the "affected" features of these mutations according to these two programs, it has been determined that they could have pathogenic properties and have been reported to have disease-causing features. The mutations classified as oncogenic/pathogenic are detailed in Table 2.

3.7. Results of gene expression and survival analysis

Survival analysis and m-RNA expression profiles of key genes were analyzed using the AML cohort (n=173) available on GEPIA, along with matched healthy tissues (n=50) for this cancer type. The gene expression profiles of STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL,

REC8, PDS5B, SMC1B, MAU2, and CDCA5 were examined. The expression levels of STAG1, REC8, MAU2, CDCA5, and PDS5B were determined to be upregulated in the patient group compared to the control group ($p < .01$) (Figure 1-D). Survival analysis based on low and high m-RNA expression profiles revealed that the expression level of REC8 significantly impacted the overall survival (OS) of AML patients. Overexpression of REC8 was shown to contribute to poor prognosis and shortened survival in AML, as depicted in Figure 1E.

3.8. Results of Protein-protein interaction analysis

A protein-protein interaction analysis was completed using the STRING analysis to identfy the functional interactions of STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 proteins in cellular processes. According to this analysis, as observed in Figure 2A, the target genes interact with ESCO1 and ESCO2, two enzymes belonging to a conserved acetyltransferase family involved in sister chromatid cohesion (26). The NDC80 protein, which interacts with SMC1A protein, plays a role in organizing and stabilizing microtubule-kinetochore interactions and is crucial for proper chromosome segregation (27). REC8 is shown to interact with PPP2R1A protein, which is involved in the negative regulate of cell growth and division. STAG1 directly interacts with TERF1, a factor of the telomere nucleoprotein complex (28). PDS5A interacts with BRCA2, which plays a role in maintaining genome stability, particularly in the preservation of the homologous recombination pathway for double-stranded DNA repair (29).

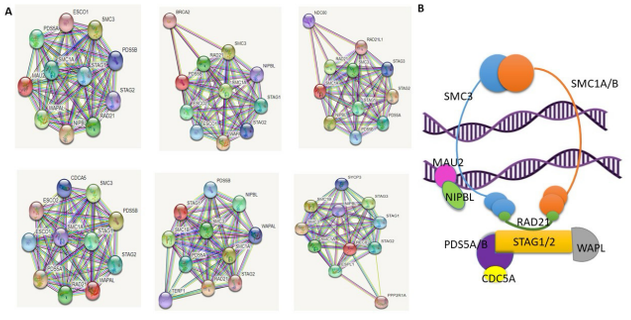

Figure 3. *(A) Schematic representation of known and predicted protein-protein interactions with the STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 genes. Each line has features. [Red line-indicates the presence of fusion evidence; Green line – neighborhood evidence; Blue line – cooccurrence evidence; Purple lineexperimental evidence; Yellow line – textmining evidence; Light blue line—database evidence; Black line—coexpression evidence.]. (B) Depiction of the cohesin complex present in somatic human cells. The core subunits making up the ring-like structure include SMC1A, SMC3, and RAD21. SMC1A and SMC3 both are composed of antiparallel coiled-coil domains joining each other at their hinge domains. RAD21 connects the nucleotide binding domains to close the ring. STAG1/2 joins the complex by associating with RAD21.*

4. DISCUSSION

AML is known to be induced by the collective action of deregulated genes that modify cell proliferation and differentiation. Specifically, chromosomal translocations such as inv(16), t(15;17), t(8:21), t(9;11) are features of AML and play an important role in leukemogenesis. Therefore, approximately 50% of AML cases have a normal karyotype and lack significant chromosomal anomalies (30-32). Recent advancements in next-generation sequencing have allowed us to better understand the genetics of AML and identify numereous mutated genes involved in the pathogenesis of AML. Despite these powerful technological developments, the essence mechanisms underlying leukemogenesis are still not fully elucidated. Therefore, only a few studies have reported that the function of cohesion mutations in the pathogenesis of AML. Increasing evidence suggests that cohesion function deficiency is attributed to cohesion mutations, which may imply a potential tumor suppressor function of cohesion in AML (3-10, 33). In our study, we determined the genetic profiles of genes belonging to the cohesion complex, including STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5, using the genomic and transcriptomic data of 872 patients diagnosed with AML. In addition to our comprehensive mutation profiling, we performed bioinformatics analyses to find the impact of the identified mutations on gene expression profiles and overall survival. Somatic mutations in genes of the cohesion complex are common in different cancer types, including bladder cancer (15-40%), endometrial cancer (19%), glioblastoma (7%), and myeloid leukemias (5-53%) (34). In this study, in parallel with the literature, we identified somatic mutations in the cohesion complex in 15.7% of our AML cohort consisting of 872 patients. Similarly, in a study conducted by TCGA on a cohort of 200 de novo AML patients, one of the most surprising discoveries following whole-genome sequencing was the presence of recurrent somatic mutations in genes encoding the cohesion complex (STAG2, RAD21, SMC3, and SMC1A) in 13% of the patients (31,34). Through genotyping analysis, a total of 96 mutations (36 missense, 22 nonsense, 15 splice region, 23 frameshift mutations) were identified in 13 genes. In our cohort, STAG2 and RAD21 were the most commonly mutated genes in the cohesion complex, while no mutations were found in PDS5A, SMC1B, and CDCA5 genes. The co-occurrence of mutations in SMC1A with WAPL and MAU2 was determined to be statistically significant.

RAD21 is a crucial component of the cohesion complex and forms a trimeric ring with SMC1A and SMC3 (Figure 3B). RAD21 has three binding domains that interact with corresponding proteins: SMC3 (1–103 aa), STAG1/2 (362–403 aa), and SMC1A (558–628 aa); an LPE motif (255-257 aa) (24, 35). Out of the identified mutations in RAD21, 14 are characterized as driver mutations. Driver mutations are known to be the mutations that contribute to the transformation of a normal cell into cancer. In our study group, the L155* mutation located on the LPE binding motif and the Q592* nonsense mutations in the SMC1A protein-binding region have the potential to create a stop codon and result in the formation of

truncated proteins. When we specified the RAD21 mutation analyzes according to AML subtypes, frameshift mutations with oncogenic character were detected in the AML-M2, M4 and M5 subgroups (Table 2). PDS5 plays crucial roles in the establishment, maintenance of sister chromatid cohesion (14,36,37). The regulatory complex of cohesion is regulated positively or negatively with chromosomes depending on which protein binds to the region in PDS5 (14). The missense mutations p.T1188A and p.R1250W found in the study cohort are located in the regulatory region where the NLS signal is present. The p.R394* nonsense mutation in the binding sequence of RAD21 protein in the N-terminal HEAT repeats region has the potential to create a stop codon, leading to truncated protein and loss of the RAD21 protein-binding domain. Mutations in the HEAT repeat regions, namely the 22nd exon/23rd intron boundary region (p.x825_splice) and the 28th exon/29th intron boundary region (p.x1019_splice), can create alternative branch sites in the spliceosome complex, resulting in intron retention, exon skipping, and the generation of non-functional transcripts with intronic extensions. Splice region mutations are one of the main driver mutations in AML and have been reported in different myeloid neoplasms such as MDS, AML, and myeloproliferative neoplasms (MPNs) (38). During the folding stage of the PDS5 protein, multiple HEAT repeats form extended superhelical structures and serve as a scaffold to facilitate the assembly and disassembly of other cohesion complex components (36,37). Mutations in this region can negatively affect the binding and dissociation of other components within the complex. It is known structurally that inositol hexakisphosphate (IP6) binds to the base of the PDS5 clamp (39,40). Other high inositol polyphosphates such as IP5 and IP4, including IP6, are abundant lipid-derived metabolites in eukaryotic cells (39,40). In particular, it is known that IP6 governs protein-protein interactions, thus regulating the interaction between proteins involved in the cohesion complex, such as RAD21 and WAPL, with PDS5 (14,40). In our study, we identified frame shift mutation p.R724Pfs*16 and p.X825_splice mutations in this region, which can have a negative impact on binding. The frameshift mutations p.N1390Mfs*4 and p.D508Gfs*4 have been identified in patients with subtypes M5 and M1, respectively, but the pathogenic properties of these mutations in AML are unknown. In addition, in our study, the benign p.M823I missense mutation was detected in a patient with APL with PML-RARA subtype (AML-M3).I n our study group, it is observed that individuals with high mRNA expression of PDS5A in the AML cohort are statistically significant. PDS5A is known to interact with BRCA2, which plays a role in maintaining genomic stability, particularly in the preservation of the homologous recombination pathway for double-stranded DNA repair. WAPL is an important negative regulator of cohesion and forms a complex with PDS5 to facilitate the release of cohesion from chromatin. There is a binding site for WAPL on PDS5 (40,41). No mutations were detected in these regions in our study. However, the p.K27N missense mutation is located in the acetylation site of WAPL and has the potential to affect the regulation of gene activity. REC8 is the essential protein for the cohesion complex, holding the lateral elements necessary for synaptonemal complex formation and homologous recombination (42). In the AML cohort, there is a p.W19C missense mutation, and it is observed that individuals with high mRNA expression of REC8 in the AML cohort are statistically significant and have shorter overall survival. This finding implies that REC8 expression may serve as a poor prognostic marker.

The SMC1A protein consists of five different domains: two coiled-coil domains, a hinge domain, and N – and C-terminal domains. The N-terminal region contains a nucleotide triphosphate (NTP) binding motif and is responsible for ATP binding (43). The ATP-related domain contains a pathogenic missense mutation p.R96H. This mutation can affect ATP processes such as SMC1A/SMC3 main domain dimerization. In our study group, pathogenic missense and nonsense mutations were identified in the coiled-coil domain, which we present in detail in Table 2. Since SMC proteins form heterodimers, the coiled-coil interactions are reported to be crucial for proper folding of an SMC monomer (43,44). Additionally, it is suggested that the coiled-coil domains may directly interact with DNA and/or be necessary for protein-protein interactions (43-45). Hence, mutations detected in the coiled-coil domain in our study group may impact hinge activity or disrupt interactions with other cohesion subunits. On this domain, the oncogenic p.R790Q missense mutation was detected in patients with AML-M2 and M5 subtypes.

STAG1/2 are subunits of the cohesion complex that are essential for sister chromatid cohesion, chromosome segregation, DNA repair, genome organization, and m-RNA expression (46). Specifically, STAG2 is the cohesion component that harbors the most likely pathogenic mutations. STAG1 protein carries an AT hook to bind telomeric sequences, including a Stromalin Conserved Domain (SCD), in its N-terminal region (46,47). The p.x342_splice mutation may create an alternative branch site in the spliceosome complex, resulting in intron retention, exon skipping, and the generation of non-functional transcripts with intronic extensions. In a patient (AML-M5) carrying the oncogenic/driver mutation NPM1, a frame-shift mutation p.L357Ifs4 was identified in the 11th exon of the STAG1 gene, which can lead to premature termination of the STAG1 polypeptide early in its 34th exon. The *STAG2* is located on the X chromosome and is identified as the most frequently mutated subunit of the cohesion complex. The 11 frame shift mutations identified in the STAG2 gene occur throughout the gene body due to a change in the reading frame and are likely to result in loss of protein expression, possibly due to mRNA decay. Among the identified mutations in the STAG2 gene, 24 are characterized as driver mutations. Specifically, p.X755_splice mutations are located in the acetylation and methylation regions, indicating their oncogenic nature, which may disrupt gene activity. In addition, the p.x340_splice mutation can create an alternative branching site in the spliceosome complex, leading to intron retention, exon skipping and the generation of non-functional transcripts with intronic expansions. This oncogenic mutation was

identified in a patient (AML-M5) carrying the oncogenic/driver mutation NPM1. It has been reported in the literature that alterations in cohesin genes interact with other genetic events in driver genes such as NPM1, potentially promoting malignant transformation (48). The frame shift mutation p.C176Lfs2, which is also oncogenic, is located in the phosphorylation region and can lead to loss of the phosphorylation region, thereby impairing gene activity. The STAG1/2 subunits of the cohesion complex also possess the ability to bind RNA localized in the nucleus (49-51). In cancer cells with STAG2 mutations, STAG inhibition has been reported to cause chromosome segregation defects and homozygous mutations leading to embryonic lethality (50,51). In the STRING protein-protein interaction analysis, our core (hub) proteins interact with two enzymes belonging to a conserved acetyltransferase family involved in sister chromatid cohesion, ESCO1, and ESCO2. Specifically, it has been observed that PDS5A interacts with BRCA2, a protein involved in DNA repair and particularly important for the preservation of homologous recombination pathway in double-stranded DNA repair.

Although we conducted comprehensive molecular profiling analysis of genes responsible for cohesion complex anomalies, we are aware of certain limitations in our study. Current study was conducted with a limited experimental design using bioinformatics tools. Hence, to clarify the impact of STAG1/2, RAD21, SMC1A, SMC3, PDS5A, WAPL, NIPBL, REC8, PDS5B, SMC1B, MAU2, and CDCA5 on the pathogenesis of the complex, further wet lab studies with a larger sample size are required. Understanding the functional consequences of cohesion mutations in a lineage-specific and signal-dependent manner will help identify new pathophysiological mechanisms of the disease and inform the development of new therapeutic targets. These findings indicate that mutations in the cohesion complex may contribute to leukemogenesis.

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Authorship contribution

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

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Clinical and Experimental Health Sciences

The Effect of Breathing Exercise on Self-Care Management in Chronic Obstructive Pulmonary Patients

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ABSTRACT

Objectives: The aim of this study is to investigate the effect of breathing exercise on self-care management in chronic obstructive pulmonary patients.

Methods: The study was conducted in a cross-sectional descriptive type with 415 COPD patients between 04.01-04.02.2023. COPD patient identification form and self-care management scale in chronic diseases were used to collect research data. The research data were evaluated with the statistical package program.

Results: The rate of application of breathing exercises by the participants was found to be 47.7%. The mean self-care management of patients with COPD was found to be 93.53±35.32. It was determined that the mean of self-care management of the COPD patients who participated in the study who applied breathing exercise was 100.97±29.86, the mean of self-care management of the participants who did not apply respiratory exercise was 86.73±38.48, and the difference between the averages was statistically significant ($p < .001$).

Conclusion: As a result of this study, it was determined that the level of application of breathing exercises in COPD patients was below fifty percent, and the level of self-care management was below the middle. It was determined that the practice of breathing exercise was effective on self-care management.

Keywords: Breathing exercise, COPD, self-care management

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic, irreversible disease that progresses with a lung infection and respiratory symptoms. COPD is one of the leading causes of mortality and morbidity in the whole world and in our country (1). COPD is responsible for 6% of all deaths, and it was reported that more than three million people died due to COPD in 2012 (2). While 35331 people died from respiratory system-related diseases in Turkey in 2013, this number increased to 52568 in 2018, showing an increase of 48.79% (3). COPD is a very important health problem due to the fact that it is a disease that negatively affects the patient physiologically, psychologically, socially, and economically, which occurs at this frequently. The inflammation that occurs in the lungs in the disease causes irreversible damage to the bronchi and bronchioles, causing air to be trapped in the alveoli. This condition leads to the patient experiencing symptoms related to the respiratory system (4). In addition to medical treatment, pulmonary rehabilitation practices are included in the treatment of COPD disease. Breathing exercises, on the other hand, are an important part of pulmonary rehabilitation (5). It has been reported that breathing exercises have a positive effect on dyspnea, exercise capacity, and quality of life in individuals with COPD (6). Nurses have important duties in teaching breathing exercises and patient education. In this sense, nurses participate in increasing patient motivation, teaching, and applying these exercises to the patient and managing the disease (7). On the other hand, the practice of breathing exercises by individuals with COPD is thought to be effective in self-care management.

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Self-care is the satisfaction of the individual's needs in social, emotional, and psychological terms, which he practices in order to prevent diseases and accidents and to live in a healthy way. Self-care management, on the other hand, is the individual making all kinds of decisions about his/her health and acting toward it. If it is desired to define self-care management in individuals with chronic illness, it is that the individual makes a decision about his/her illness, which negatively affects his/her health, and exhibits behavior towards it (10). There is a decrease in self-care management in individuals with chronic diseases. This situation is valid in COPD disease. In individuals with COPD, there is a decrease in self-care management due to the symptoms and problems caused by the disease. However, in COPD disease, it is particularly important that the individual has a high level of self-management of the disease (11).

A high level of self-management requires awareness of the disease and high compliance with treatment (10). In this sense, it is thought that regular practice of breathing exercises is also related to self-management, as it will benefit from keeping the disease under control. There have not been any studies in the national and international literature investigating the effect of the use of breathing exercises on self-care management in chronic obstructive pulmonary patients. Therefore, in this study, it was aimed to investigate the effect of breathing exercise practice on self-care management in chronic obstructive pulmonary patients. For this purpose, answers to the following research questions are being sought.

Research Questions

1. What is the percentage of breathing exercise application in COPD patients?
2. What is the level of self-care management score of COPD patients?
3. Is there a statistical significance between the state of application breathing exercises in COPD patients and the self-care management scale and its sub-dimensions decisively?
4. Are the defining characteristics of COPD patients effective in breathing exercise application status?
5. Are the defining characteristics of COPD patients effective in self-care management and self-care management sub-dimensions?

2. METHODS

2.1. Ethical Considerations

Before the study, the ethics committee of the university dated 04.01.2023 and E-67796128.000.2300000489 the numbered ethics committee permission was obtained. Consent was obtained from the participants participating in the study. The research was conducted according to the principles in the Helsinki Declaration.

2.2. Study Design and Sample Selection

The research was conducted in descriptive cross-sectional type between the dates of January 4, 2023, and February 4, 2023. The population of the study consisted of patients diagnosed with COPD living in Turkey. The sample size of the research was determined through a program (12). Accordingly, it was determined that the minimum sample size to be included in the study was 237 with a 5% error, 95% power, and 19.1% COPD prevalence (13). A total of 451 patients were accessed, and 34 patients who did not meet the research criteria were excluded from the scope of the study. The sample of the study consisted of 415 patients. Criteria for inclusion in the study; being diagnosed with COPD by a doctor, having been receiving COPD treatment for at least one year, being over 40 years of age, using an Android phone, being able to use the social media account and voluntarily agreeing to participate in the research. Patients who were under the age of 40, who could not use an Android phone, who had vision problems, and who had been receiving COPD treatment for less than a year were excluded from the study.

Data Collection: Research data was collected online via WhatsApp, Facebook and Instagram. The participants were reached through snowball sampling. Snowball sampling is a known and applicable method for recruiting study participants who are not easily accessible or unknown to researchers (14-16). It is a widely used sampling method in which individuals are included in the research by contacting others who connect them to the research participants in cases where researchers cannot reach the participants directly (14,15,17). Researchers proved that COPD patients had to rely on social media to reflect the participants through snowball sampling and in general in Turkey, as providing transportation outside the hospital environment or an institution is especially important in terms of respiratory exercise and self-care management (14). The exacerbation status of the patients was not questioned.

2.3. Data Collection Forms

In the collection of research data, the COPD patient form and the self-care management scale for chronic diseases were used.

COPD Patient Form: It is a form consisting of 14 questions created by scanning the literature in order to determine the descriptive characteristics of the participants and their characteristics related to the disease (8,9,18). The questions in the form of patients age, gender, marital status, children status, place of residence, economic status, family type, non-smoking status, year of diagnosis, COPD, difficulty breathing can interfere with the condition, the condition to be able to do breathing exercises, breathing exercises finding useful and investigates the situation of the affected level. The level of exposure to the disease consists of a content that allows the patient to assess the level of exposure to COPD in the range

from 1 to 10. With this, it is aimed to evaluate the patient's own perception. '1' indicates the lowest level of exposure, while '10" indicates the highest level of exposure.

Self-Care Management Scale in Chronic Diseases: Hancerlioglu and Senuzun Aykar made the validity and reliability of the scale developed by Jones and Pruett to evaluate self-care management in chronic diseases in Turkish in 2018 (18). The scale, which consists of a total of 35 items and is scored in Likert type, has two sub-dimensions as self-protection and social protection. Some items of the scale such as 3., 15., 19., and 28. are scored in reverse. The lowest score that can be taken from the scale is 35, and the highest score is 175. As the score obtained from the scale increases, self-care management increases. In the validity and reliability study of the scale, Cronbach alpha was used in the scale of .75, in the self-protection sub-dimension .78, and in the social protection sub-dimension, it is .78 (18). The Cronbach alpha coefficient numbers in this study are, respectively, .96, and .95, and it was determined as .90.

2.4. Statistical Analysis

The research data were evaluated with Statistical Package for Social Sciences (SPSS) 26 program. Number, percentage, mean, and standard deviation were given for descriptive data. Skewness and Kurtosis for compliance of data with normal distribution – 2-+2 the values were taken as a basis (19). It was seen that the data were in this range and were in accordance with the normal distribution. Independent samples T-test was applied for the difference between the self-care management and subscale averages according to the participants' breathing exercise status. In order to investigate the differentiation of breathing exercise according to descriptive features, independent samples t-test for binary variables and One Way ANOVA test for more than two variables were applied. Bonferroni test was used as post hoc analysis. Multiple regression analysis was performed for the level of influence of self-care management and its sub-dimensions on descriptive data. Statistical significance was accepted as $p < .05$.

3. RESULTS

3.1. Findings of the Descriptive Characteristics of the Patients

Table 1 shows the descriptive characteristics of COPD patients. Accordingly, it was found that the average age of the patients participating in the study was 64.46±9.41, the year of diagnosis of COPD was 13.56±7.81, and the level of exposure to the disease was 6.79±2.04. It was proved that 60% of the participants are male, 38.6% are in the 60-69 age group, 78.6% are married, 47.7% are literate, 78.6% have children, 68.7% live in the province, 73% have moderate economic status, 68% are in a large family type, 55.7% have quit smoking, and 50.6% are actively working (Table 1).

Table 1. *Descriptive features of COPD patients*

Variables		Mean ± SD	
Age		64.46±9.41	
COPD diagnosis year		13.56±7.81	
The level of being affected by the disease		6.79±2.04	
Categories		**n**	**%**
Gender	Female	166	40.0
	Male	249	60.0
Age groups	40-49 age	51	12.3
	50-59 age	59	14.2
	60-69 age	160	38.6
	70 years and over	145	34.9
Marital status	The married	326	78.6
	Single	55	13.3
	Widowed/divorced	34	8.2
Educational status	Literate	198	47.7
	Secondary education	163	39.3
	High school and above	54	13.0
Do you have children?	Yes	326	78.6
	No	89	21.4
Where you live?	Province	285	68.7
	District	78	18.8
	Village	52	12.5
Economical status	Good	89	21.4
	Middle	303	73.0
	Bad	23	5.5
Family type	Nuclear family	118	28.4
	Extended family	282	68.0
	Broken family	15	3.6
Smoking status	I use	97	23.4
	I quit	231	55.7
	I dont use	87	21.0
Working status	I am working	210	50.6
	I am not working	205	49.4

47.7% (n=198) of the COPD patients participating in the study reported that they performed breathing exercises, while 52.3% (n=217) reported that they did not perform any breathing exercises (Figure 1).

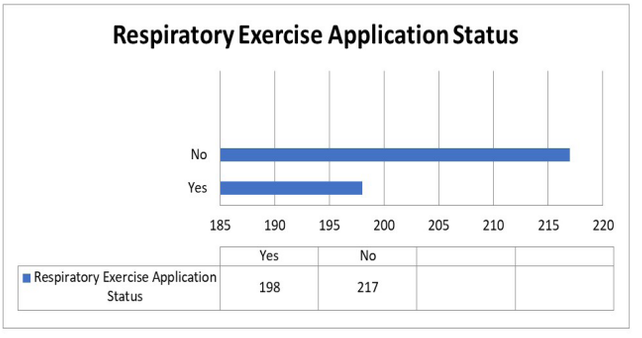

Figure 1. *Respiratory Exercise Application Status*

When the status of finding this exercise useful in COPD patients who performed respiratory exercise was examined, it was found that 95.5% of the patients found the respiratory exercise useful (n=189) (Figure 2).

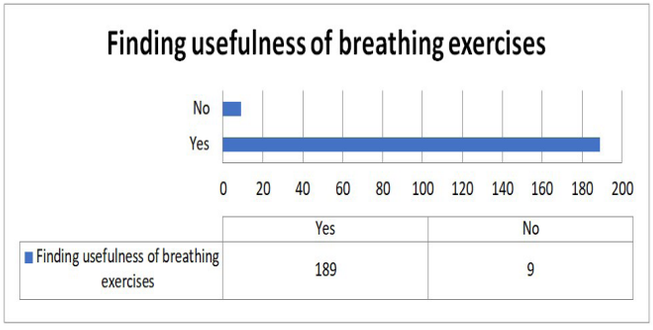

Figure 2. *Finding Usefulness of bBreathing Exercises*

According to the state of attendees making the breathing exercises, self-care management scores compared to self-protection ($p < .01$), social protection ($p < .01$), and self-care management, general ($p < .01$) scores of making the breathing exercises were seen to differ according to the state at a statistically significant level (Table 2).

Table 2. *Distribution of self-care management scores according to breathing exercise application status*

	Breathing exercise application status		Statistical test/p
	Yes	No	
Self protection	58.24±16.15	50.08±21.23	t=3.036, p = **.003**
Social protection	42.73±14.04	36.64±17.58	t=4.039, p = **.001***
Self-care management general	100.97±29.86	86.73±38.48	t=3.489, p = **.001**

**p < .001*

When the participants were asked 'How are you struggling with your breathing difficulties?' it was seen that 27.7% of the participants answered with medications, 23.6% by resting, and 17.6% by doing breathing exercises (Figure 3).

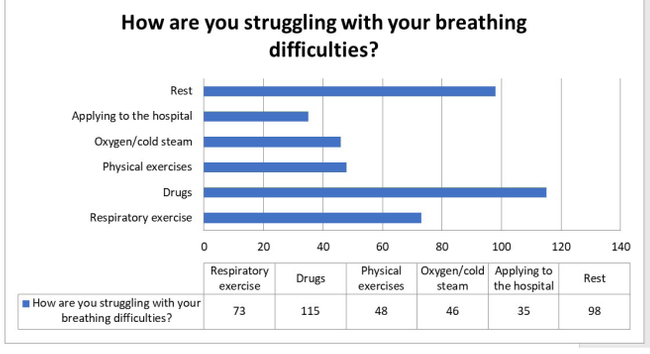

Figure 3. *Ways to Cope with Breathing Difficulties*

3.2. Findings of the Factors Affecting the Patients' Breathing Exercise Practice Status

Table 3 shows a comparison of the breathing exercise application status according to descriptive data. According to this, it was found that the individuals' breathing exercise status differed statistically significantly according to age groups ($p < .001$), having children ($p < .001$), economic status ($p < .001$), smoking status ($p < .01$), and working status ($p < .001$).

Table 3. *Comparison of breathing exercise application status with descriptive data*

Categories		Breathing exercise application status	
		X̄±SD	Signifcance between groups/p
Gender	Female Male	1.54±0.50 1.51±0.50	t=0.641 p = .522
Age groups	40-49 age[a] 50-59 age[b] 60-69 age[c] 70 years and over[d]	1.06±0.23 1.25±0.43 1.48±0.50 1.85±0.36	F=58.447 p = **.001**** a>c, a>d, b>c, b>d*
Marital status	The married Single Widowed/ divorced	1.51±0.50 1.56±0.50 1.62±0.49	F=0.976 p = .378
Educational status	Literate Secondary education High school and above	1.55±0.49 1.51±0.50 1.46±0.50	F=0.750 p = .473
Do you have children?	Yes No	1.47±0.50 1.73±0.44	t=-4.518 p < **.001****
Where you live?	Province District Village	1.49±0.50 1.64±0.48 1.54±0.50	F=2.934 p = .05
Economical status	Good[a] Middle[b] Bad[c]	1.20±0.40 1.61±0.48 1.61±0.49	F=26.115 p < **.001**** a>b, a>c*
Family type	Nuclear family Extended family Broken family	1.55±0.50 1.51±0.50 1.60±0.51	F=0.502 p = .606
Smoking status	I use[a] I quit[b] I dont use[c]	1.46±0.50 1.59±0.49 1.41±0.49	F=4.836 p = **.008** b>c*
Working status	I am working I am not working	1.30±0.46 1.75±0.43	t=-10.014 p < **.001****

**Bonferroni test, **p < .001*

3.3. Findings of the Factors Affecting the Patients' Self-Care Management Levels

Table 4 shows the results of the regression analysis conducted for the situation of self-care management and its sub-dimensions being affected by descriptive data. Accordingly, it was found that the regression model established between the self-care management scale and decipherment data was significant ($p < .001$). It was learned that the explanatory power of the created model was 19.5%. It was found that age ($p < .01$), child-bearing status ($p < .001$), family type ($p < .01$), and year of COPD diagnosis ($p < .001$) affected self-care management at a statistically significant level (Table 4).

It was found that the regression model established between the self-protection sub-dimension, and decipherment data from the sub-dimensions of the self-care management scale was significant (p < .001). It was found that the explanatory power of the model was 21.5%. It was found that age (p < .01), child-bearing status (p < .001), family type (p < .01), and year of COPD diagnosis (p < .001) were significantly affected by the self-protection sub-dimension at a statistically significant level (Table 4).

Table 4. *Results of regression analysis between self-care management of individuals with COPD and descriptive data*

Dependent variable	Independent variable	*B*	*SD*	*β*	*t*	*p*
Self-care management general Overall	(Constant)	164.846	16.474		10.006	**.001***
	Age	-0.510	0.185	-0.136	-2.759	**.006**
	Gender	-6.449	3.635	-0.090	-1.774	.077
	Marital status	4.223	2.930	0.073	1.441	.150
	Status of having	-16.673	4.297	-0.194	-3.880	**.001***
	children	4.223	2.401	0.085	1.767	.078
	Living place	-4.815	3.444	-0.067	-1.398	.163
	Economical	9.783	3.339	0.141	2.930	**.004**
	status	-4.141	2.458	-0.078	-1.685	.093
	Family type	4.122	3.480	0.058	1.184	.237
	Smoking status	-1.895	0.226	-0.419	-8.381	**.001***
	Working status					
	COPD diagnosis					
	year					
R= 0.464, R^2=0.215, Adjusted R^2= 0.195, F=11.060, Durbin Watson= 0.337						
Self protection Overall	(Constant)	94.950	8.933		10.629	**.001***
	Age	-0.305	0.100	-0.148	-3.036	**.003**
	Gender	-3.634	1.971	-0.092	-1.844	.066
	Marital status	2.320	1.589	0.073	1.461	.145
	Status of having	-8.238	2.330	-0.175	-3.536	**.001****
	children	2.193	1.302	0.080	1.684	.093
	Living place	-2.904	1.868	-0.074	-1.555	.121
	Economical	5.169	1.810	0.136	2.855	**.005**
	status	-1.816	1.333	-0.062	-1.362	.174
	Family type	2.248	1.887	0.058	1.192	.234
	Smoking status	-1.119	0.123	-0.451	-9.125	**.001***
	Working status					
	COPD diagnosis					
	year					
R=0.484, R^2=0.234, Adjusted R^2=0.215, F=12.363, Durbin Watson=0.355						
Social protection Overall	(Constant)	69.895	7.711		9.065	**.001***
	Age	-0.206	0.087	-0.119	-2.377	**.018**
	Gender	-2.815	1.701	-0.085	-1.655	.099
	Marital status	1.903	1.371	0.071	1.388	.166
	Status of	-8.434	2.011	-0.213	-4.194	**.001***
	having	2.051	1.124	0.089	1.825	.069
	children	-1.911	1.612	-0.058	-1.185	.237
	Living place	4.614	1.563	0.145	2.952	**.003**
	Economical	-2.325	1.150	-0.095	-2.021	**.044**
	status	1.874	1.629	0.058	1.150	.251
	Family type	-0.776	0.106	-0.373	-7.334	**.001***
	Smoking status					
	Working status					
	COPD					
	diagnosis year					
R=0.434, R^2=0.189, Adjusted R^2=0.169, F= 9.398, Durbin Watson=0.364						

**p < .001*

It was found that the model established between the social protection sub-dimension and decipherment data from the sub-dimensions of the self-care management scale was statistically significant (p <.001). It was also found that the explanatory power of the model was 16.9%. It was found that age (p < .05), child-bearing status (p < .001), family type (p < .01), smoking status (p < .05), and year of COPD diagnosis (p < .001) were significantly affected by the social protection sub-dimension (Table 4).

4. DISCUSSION

Breathing exercises in COPD provide strengthening inspiratory muscles, increase exercise capacity (20,21), increase pulmonary functions, improving health-related quality of life (21,22), reduction of COPD exacerbations, increase lung capacity, and reducing symptoms (23). For this reason, the implementation of breathing exercises is especially important in COPD patients. In this study, it is observed that the rate of COPD patients' breathing exercise practice remains below fifty percent. When the patients were questioned about their status of considering breathing exercises useful, it was found that 95.5% of them found breathing exercises useful. Although patients find breathing exercises so useful, it is believed that the reason why more than half of them do not practice is due to their low level of education (47.7% of them are literate). In contrast to our study, in a study conducted with 182 individuals with COPD in Nepal, 36.3% of the participants reported that breathing exercises were necessary, and the low visual acuity required by breathing exercises was also associated with a low level of education (24).

In our research, it was seen that the patient's breathing exercise practice status differed statistically significantly according to age, child ownership, economic status, smoking status, and working status. Breathing exercises and the results of a systematic review of physical activity done by treating it as when we examined the barriers to physical activity of COPD health status of individuals with personal problems, lack of support, external factors, smoking status has been reported (25). When a study examining the obstacles in front of doing pulmonary exercise was examined, it was found that age, gender, and smoking were effective (26). It is seen that the results of this study coincide with our research.

COPD is a disease that makes it difficult for individuals to perform self-care due to problems caused by many symptoms and symptoms that occur. In this sense, when the literature is examined in general, it is seen that the self-care power of individuals with COPD is low (9,27). In our research, it was seen that the general, self-protection sub-dimension and social protection sub-dimensions of the self-care management scale of COPD patients were below the average level. In the Turkish validity and reliability study of the self-care management scale for chronic diseases, in contrast to our study, it was found that the general average of the self-care management scale was high, the self-protection sub-dimension was at a moderate level, and the social protection

was below the moderate level, similar to our study (18). In contrast to our study, in other studies, it has been reported that the overall score of the self-care management scale is high and its sub-dimensions are at a good level (28,29). It is believed that the differences between the studies are due to the fact that the studies were conducted with individuals of different sample sizes and characteristics.

In our research, it was found that the practice of breathing exercises affects self-care management at a statistically significant level (p <**.001**). It was seen that the state of doing breathing exercises was affected by the self-care management self-protection sub-dimension, age, gender, child-bearing status, economic status, working status, year of COPD diagnosis, the state of finding exercise useful, and the state of being affected by the disease (p <.**05**). There has not been a study in the literature on the effects of doing breathing exercises.

In our research, it was seen that the self-care management scale of COPD patients was affected by age, child-bearing, family type, and year of COPD diagnosis (p < .**05**). It was found that gender, marital status, place of residence, economic status, smoking status, and working status did not affect self-care management (p >.05). In the studies conducted, it was seen the economic situation did not affect self-care, similar to our study (28,30). In contrast to our study, there is also a study that found that self-care management is affected by income level (29).

In a study conducted with individuals with COPD, in contrast to our study, married individuals' self-care power was found to be higher (31). According to the results of regression analysis in a study conducted with COPD patients in China, it was reported that self-management behaviors are influenced by age, marital status, and place of residence (32). While the situation of age affecting self-care management coincides with our study, marital status and place of residence were not affected by self-care management according to our study. It is thought that this situation may be due to the fact that the proportion of married people (79.2% of participants are married) and the rate of living in rural areas (64.4%) are higher compared to our study in a study conducted in China, without receiving more social support. The social support received from the family and the environment contributes to self-care management (33).

In our research, there was no relationship between education level and self-care management. On the contrary, it has been found that there is a linear relationship between educational level and self-care behaviors in the studies conducted (31, 34). In our study, child ownership and family type are affected by self-care management. This situation can be explained by the fact that high social support provides better self-care management (35).

In our research, it was found that the self-protection sub-dimension was affected by age, having a child, family type, and year of COPD diagnosis (p < .**05**). It was figured out that social protection was affected by age, child-bearing status, family type, smoking status, and year of COPD diagnosis. According to the results of regression analysis conducted in a study with COPD patients, it was reported self-protection is affected by age and family type, social protection is affected by smoking (15). This ultimately coincides with the results of our study.

Our research has some limitations. The research was conducted with COPD patients living in Turkey, aged 40 and over, and using smartphones. This situation limits the generalization of the results to all age groups. The data in the study were collected online by snowball sampling, which may cause the participants to focus on a specific social media network usage and environment, which may be inadequate in terms of representing the general population. Another limitation of our study is that treatment compliance, which is a factor affecting self-care, was not evaluated.

5. CONCLUSION

The results of the study, which examined the effect of breathing exercise application on self-care management in COPD patients, are as follows. It was found that the level of exposure of COPD patients to the disease is high, the rate of performing respiratory exercises is 47.7% and the level of self-care management is below average. It was detected that the difference between the breathing exercise practice status, the self-care management, and the sub-dimension averages was statistically significant decisively.

Based on the results of this study, it is seen that the practice of breathing exercises from COPD patients is an effective determinant in self-care management. In this sense, it is recommended to conduct randomized controlled trials with a high level of experimental-based evidence with larger samples, taking into account the stages of COPD. In addition, it is proposed to raise public awareness through congresses and conferences where large audiences are included in respiratory exercise, self-care management training, and interventional studies for this purpose.

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Clinical and Experimental Health Sciences

Does Root Canal Shaping Effect the Accuracy of Electronic Apex Locators in Curved and Straight Root Canals?

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ABSTRACT

Objective: To assess the influence of root canal preparation (RCP) on the precision of electronic apex locators (EALs) in both straight and curved root canals.

Methods: The experimental setup included forty-two extracted human mandibular molars featuring both straight distal canals (0-10 degrees) and curved mesial root canals (30-50 degrees). Initial working length (AWL1) measurements were conducted under a stereomicroscope at X15 magnification prior to root canal preparation (RCP). Subsequently, electronic working length (EWL1) was determined using two different electronic apex locators (EALs) – Propex-Pixi and EndoRadar. RCP was carried out with ProTaper Ultimate F1, F2 files in mesial canals, and F1, F2, F3 files in distal straight root canals. The RCP procedure involved the implementation of a standard irrigation protocol along with the use of a sonic device for activation. Following RCP, measurements were repeated under a stereomicroscope and documented as AWL2. EWL measurements were also repeated and recorded as EWL2 after the preparation. The variation was calculated by subtracting AWL from EWL for each tooth (EWL1-AWL1 and EWL2-AWL2). Statistical analysis employed Paired Sample t-test and Wilcoxon tests.

Results: There was no statistically significant difference observed in the accuracy of Propex-Pixi and EndoRadar Pro before and after root canal preparation for both straight and curved canals.

Conclusion: Root canal preparation did not alter the accuracy of the electronic apex locators (EALs) used in this study, whether applied to curved or straight root canals.

Keywords: Working length, root canal preparation, curvature, straight canal

1. INTRODUCTION

A successful endodontic treatment depends on various factors, but canal preparation is one of the most important steps (1). The distance between the reference point located at coronal part and the minor apical foramen where the root canal treatment (RCT) should terminate is defined as working length (WL) (2). Many studies (3-5) have shown that determination of reliable WL significantly affects the success of RCT. The ideal WL should terminate at the cementodentinal junction serves as a histological marker that establishes the boundary between pulp tissue and periodontal tissue (3). Reduced WL may result in a recontamination of the root canal space, and over-instrumentation may damage periapical healing (6).

Despite efforts to maintain accurate WL, many studies (2,7,8) have shown a reduction in WL during RCT due to canal anatomy variations and this reduction is most commonly observed in curved canals. This phenomenon arises due to the predisposition of the file to become straighter within the root canal, concluding in the excessive removal of dentin from both the inner wall of the curve and the outer wall of the apical region of the root canal (9,10). Consequently, this action leads to the straightening of the root canal and potentially shortening the WL.

In clinical practice, various methods are employed to locate the canal terminus and measure the WL, including electronic apex locators (EALs), radiographic techniques and tactile perception (11). The traditional radiographic techniques have been the most reliable and popular approach for WL determination. This method provides direct observation of the root canal anatomy. However, there are some disadvantages that render this method unsuitable for every situation; for instance, the radiographic apex and anatomic apex may not always coincide, and there are concerns regarding radiation dosages and superpositions of anatomies (12,13).

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EALs have been a major innovation in RCT. They offer several advantages compared to the radiographic method, including continuous monitoring of WL and a reduction in radiographic exposures (14,15). Once the WL is obtained, regardless of the method used, it is crucial to maintain that length throughout the treatment. However, the accuracy of EAL can be affected by the presence of different electrolytes, residual pulp tissue, excessive bleeding in the root canal (16).

The aim of the present study was to assess the impact of RCP on the establishing of WL using Propex-Pixi (Dentsply Maillefer, Ballaigues, Switzerland) and EndoRadar Pro (Woodpecker, Guilin, China) in teeth with curved and straight root canals. The null hypothesis stated that there would be no difference in the accuracy of EALs for before and after RCP.

2. METHODS

The methodology of the study underwent review and took an approval from the The Clinical Research Ethics Committee of Akdeniz University Faculty of Medicine, with decision number of KAEK-398. The size of sample was calculated using G*Power 3.1.9.7. according to results of the pilot study *(α=.05, power=.95, effect size=0.4*). Fourty-two human mandibular molars in similar length were included in this study.

The study excluded the extracted teeth that showed cracks, fracture lines, or had previous RCT after the examination under the operating stereomicroscope (Zeiss Stemi, Carl Zeiss, Germany). Periapical radiographs were taken in the bucco-lingual direction for mandibular molars. Then, the curvature angle of the canal was established using method of Schneider (17). The study exclusively included teeth with straight distal root canals and curved mesiobuccal root canals exhibiting curvature angles falling within the range of 30 to 50 degrees.

With a scaler, residues of hard and soft tissues on the teeth were eliminated. The teeth were immersed in a saline solution to prevent drying out during the study. In all teeth, endodontic access cavities were created. After the apical patency was verified using a number 8 K-file, the teeth in which the number 10 K-file became stuck in apical foramen were included in this study. To ensure a fixed and uniform reference point at coronal area, the cusps of the selected teeth were smoothed down.

2.1. Determination of WL Before Preparation

Each tooth was decoronated to achieve a standardized WL of 15 mm, and patency of apical foramen was checked using a number 10 K-file. Utilizing a stereomicroscope (Zeiss Stemi) with a magnification of X15, a #10 K-type file was meticulously advanced until it became visible at the apical end. Once the file was observed apically, a rubber stop was affixed to the incisal edge. The distance between this rubber stop and the tip of the file was precisely gauged using a digital caliper, and this measurement was documented as the actual length (AL1).

Subsequently, alginate was prepared following the instructions of the manufacturer. The lip clip of the EAL was positioned within the mold containing freshly prepared alginate, and the teeth were submerged into the mold up to the cement enamel-margin. Following this, the electronic length was assessed employing the Propex-Pixi and EndoRadar Pro devices. A number 10 K-file was cautiously advanced into the canal until both EALs (Propex-Pixi and EndoRadar Pro) displayed '0.0' indications on their respective screens.

Once these indications stayed steady for 5 sec on the EALs' screens, a rubber stop was positioned at that specific point to denote the location. Using a digital caliper, the measurement of the distance between the file's tip and the rubber stop was conducted, and this specific value was recorded as Electronic Length 1 (EL1).

2.2. Preparation of Teeth

For the mesial curved canals, ProTaper Ultimate F1 and F2 files (Dentsply Ballaigues, Switzerland) were used for preparation, while the straight distal canals were prepared with F1, F2, and F3 files. Throughout the preparation process, canals were irrigated after each file with sodium hypochlorite (NaOCl, 2 mL of 2.5%) and 5 mL sterile saline solution.

2.3. Determination of WL After Preparation

The measurements were replicated in the same manner prior to the root canal preparation (RCP) phase, and the updated measurements were denoted as AL2 and EL2. To ensure precision and mitigate the potential for operator discrepancies, this complete process was reiterated thrice for each tooth. All the measurements were made by one single operator and another operator read and recorded them.

The extent of variation was established by deducting EL1 from AL1 and EL2 from AL2 for each tooth under consideration. Negative values (-) represented instances where the response was shorter than the recorded value, while positive values (+) indicated instances where the response exceeded the recorded value.

2.4. Statistical Analysis

In this study, statistical analyses were made using SPSS (25.0; IBM, Newyork, USA). The significance level of 5% was set for all tests. The Shapiro-Wilk test was used to evaluate the normality. Wilcoxon Signed Rank Test and Paired T-test was used to compare mean and standard deviation values of WL change for each EAL (Table 1). The Chi-square test was used to compare the success rate of EALs (Table 2).

Table 1. *Mean and standard deviation values of WL change (mm) in root canals for before and after RCP.*

EAL	Mesial canal			Distal canal		
	Mean ± SD		p	Mean ± SD		p
	Before (EL1-AL1)	After (EL2-AL2)		Before (EL1-AL1)	After (EL2-AL2)	
Propex-Pixi	-.140±.96	-.088±.84	**.797**	-.131±63	-.231±68	**.506**
EndoRadar	-.120±1.20	-.065±.78	**.392**	.008±.84	-.029±.55	**.664**

**WL: working length, RCP: root canal preparation, SD: Standart deviation, EL: Electronic length, AL: Actual length.*

Table 2. *Success rate of Propex-Pixi and EndoRadar at ±0.5 mm tolerance range on mesial and distal canals.*

EAL	Success rate of EAL in mesial canals				Success rate of EAL in distal canals			
	Before RCP		After RCP		Before RCP		After RCP	
	n	%	*n*	%	*n*	%	*n*	%
Propex – Pixi	20[a]	47.6%	22[a]	52.4%	18[a]	42.9%	23[a]	54.8%
EndoRadar	21[a]	50.0%	23[a]	54.8%	25[a]	59.5%	24[a]	57.1%

3. RESULTS

A total of 42 mandibular molars were included in this study, encompassing teeth with both straight distal canals and teeth with curved mesial canals. Mean and standard deviation values of WL change for before and after RCP were shown in Table 1. It was observed that no significant difference was found between the pre and post-preparation measurements in the distal and mesial root canals when using Propex-Pixi and EndoRadar Pro. With a tolerance limit for accuracy set at ± 0.5 mm, the achievement of the EALs were displayed in Table 2. The analysis demonstrated that there existed no notable distinction between the success rates of measurements taken before and after the RCP for both the mesial and distal canals, as observed in both the Propex-Pixi and EndoRadar Pro devices. Thus, it can be concluded that both EALs were not affected by the RCP.

4. DISCUSSION

In a curved and narrow root canal, the principal objective of RCP is to transform it into a consistently conical and tapering structure, wherein the smallest area is achieved at the apical extent of RCP. This tapered form allows for better disinfection, and obturation of the root canal, promoting successful RCT outcomes. The cleaning and shaping procedures invariably result in the removal of dentin from the canal walls, irrespective of the chosen preparation technique (18). However, it is essential to maintain a balance during this process to avoid excessive dentin removal, which can lead to complications including thinning of the canal walls, perforation or transportation. ProTaper Ultimate file which was preferred in this this study for RCP, has distinct crystallographic arrangements, which play a key role in ensuring a well-balanced combination of flexibility and strength (19). The system prioritizes proper shaping in the apical third for effective RCT. This also ensures a conservative approach in the coronal two-thirds, minimizing unnecessary removal of tooth structure and preserving as much of the natural tooth as possible (20).

The straightening of canals during the instrumentation process is more likely to occur in cases of curved canals (21). As files navigate through the curvatures, they tend to straighten, resulting in the removal of additional dentin from both the outer wall of the apical section and the inner wall of the curvature of the root canal. This alteration can lead to a modification in the original canal shape, which might influence the WL and the final outcome of the RCT. It has been observed that with an increase in the curve of the apical area of the canal, the likelihood of obtaining inaccurate radiographic WL measurements also rises (22). On the other hand, the determination of the WL in the mesial root canals of mandibular molars has exhibited a higher degree of accuracy and remained relatively consistent with their actual lengths, even when varying degrees of canal curvature were present (23). These observations align with the outcomes of our study. In contrast, studies by Sadeghi et al. (24) and Santhosh et al. (25) have highlighted notable differences between Electronic Length (EL) and Actual Length (AL) in curved canals. However, it's important to note that these studies didn't incorporate canal preparation stages, thereby not assessing the impact of root canal preparation on the precision of EALs in curved canals.

In the context of embedding extracted human teeth for *in vitro* EAL studies, it is important to use materials that closely mimic the properties of the periodontal ligament, such as having similar electroconductive and colloidal consistency. Many studies have utilized materials like agar-agar, gelatin, alginate, or saline solution for this purpose (3,12,15). Among these options, alginate molds have been favored due to their firm consistency, which prevents intrusion of materials, as well as their good electroconductive properties (24). In this study, an alginate mold was selected to closely replicate the properties of the periodontal tissue, aiming to achieve accurate and reliable results in the evaluation of the EALs.

Utilizing a saline solution has been demonstrated to yield dependable measurement data. Kaufman et al. (26) stated that saline and EDTA solutions are considered reliable solutions for electronical measurements. Goldenberg et al. (27) chose saline solution for protocols because it possesses similar properties with alveolar bone. Therefore, sterile solution was preferred in this study during electronic measurement.

After advancements in endodontic treatments, the number of single-visit endodontic therapies has increased (28). Modern endodontic techniques, advancements in canal shaping, irrigation methods, and intra-canal materials have led to shorter treatment times while enhancing the success and reliability of the treatment. However, it is important to note that single-visit treatment may not be suitable for every case. In such cases, to avoid experiencing a loss of WL between visits, it is necessary to perform a re-measurement. By re-measuring, the risk of over – or under-preparation of

the canal can be minimized, leading to a more successful endodontic outcome. Thus, it is important for the EALs to measure the WL accurately after the RCP.

In the context of RCP, a major challenge lies in precisely determining the WL of the canal. Some variables have been established to impact the accuracy of EALs, including the presence of electroconductive fluids, periapical pathosis, the dimension of the major apical foramen, the shape and size of the file, and the operator's skill level (29,30). Among many studies exploring the effectiveness of EALs in defining the apical foramen, it has been noted that wider apical diameters can indeed influence the precision of EAL measurements (31,32). Taking into account Herrera et al.'s (32) investigation, which demonstrated that EAL measurements were notably more precise when the majör foramen diameter was 0.25 mm compared to measurements within the range of 0.45 to 0.70 mm, the current study incorporated this finding into its approach. In alignment with this, the present study observed an enhanced success rate of the EALs after preparation, except for the EndoRadar Pro in post-preparation measurements of the distal lateral canal, which is consistent with previous research. To ensure consistency, the study selected teeth with a smaller foramen diameter, allowing for the passage of a 10/15 file. This selection strategy aimed to optimize the accuracy of EAL measurements by focusing on teeth with an appropriate foramen size that is more conducive to obtaining reliable readings.

This study exclusively focused on the mesiobuccal canals of mandibular molars for inclusion. This deliberate selection aimed to mitigate potential significant discrepancies in apical termination sizes that might arise from different types of canals. It's worth noting that the dimension of the canal at its apical termination point can impact the precision of EL determination (33).

Olivera et al. (34) conducted an efficacy study of five apex locators and found that Propex Pixi demonstrated a success rate of 67% when the measurement was 1 mm short of apical foramen. In the study on 30 single-rooted extracted teeth conducted by Serna Pena et al. (35) it was found that the accuracy of Propex-Pixi was 73% when the measurement was in ±1 mm tolerance range. De-Deus et al. (36) investigated the precision of EALs using µCT and disclosed that the accuracy of Propex-Pixi was determined to be 52.1% within a ±0.5 mm tolerance range. In another study conducted on curved mesial canals (37), the accuracy rate of Propex-Pixi was found to be 54.3% within a ± 0.5 mm range. In this study, the success rates of Propex-Pixi and EndoRadar Pro at ±0.5 mm tolerance range on curved canals were 52.4% and 54.8% respectively. The variation in the success rate of accuracy can be due to the tolerance range, variation in the solution used during measurements, and root canal morphology. In addition, a previous study (38) stated that the performance of Propex-Pixi and Root ZX II were not affected from the different root canal sealers. Another study (39) investigated the accuracy of different apex locators including Propex-Pixi in determining the minimum perforation diameter and showed that all devices were successful in identifying the root perforations. However, EndoRadar Pro is a new EAL, and as far as our knowledge, no literature is available to compare its accuracy.

There are some studies reported changes in WL following due to root canal Preparation (8,36,37). However, any previous study was evaluated the effect of RCP on the accuracy of EALs during determination of WL according to our knowledge. The null hypothesis that root canal preparation has no affect on the accuracy of EALs usen in this study was accepted based on the results of this study.

This study has certain limitations, including the lack of periodontal ligament and intraoral conductive fluids due to its in vitro nature. Although occlusion reduction was performed to establish a reference point, achieving consistent WLs within each tooth is complicated because of the inherent variations in canal anatomy for mesial canals of mandibular molars. While the present study offers valuable insights into the impact of RCP on WL measurements using Propex-Pixi and EndoRadar Pro, researchers and readers should consider these limitations when interpreting the results and applying them to clinical scenarios. Future studies with more representative in vivo conditions may help further validate and refine these findings.

5. CONCLUSION

In the scope of this study, the extent of root canal curvature was found to lack any substantial influence on the precision of the EALs. Likewise, the process of root canal preparation did not exhibit any discernible effect on the accuracy of the EALs. Consequently, the findings propose that neither the curvature of root canals nor the RCP significantly affect the accuracy of the EALs within the context of this specific investigation.

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Clinical and Experimental Health Sciences

Effects of Contrast Limited Adaptive Histogram Equalization (CLAHE) on Manual and Automated Tracing of Lateral Cephalometric Radiographs

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ABSTRACT

Objective: The aim of this study is to compare the difference between original lateral cephalometric radiographs (LCRs) and Contrast Limited Adaptive Histogram Equalization (CLAHE) LCRs in two examiners and WebCeph.

Methods: A total of 200 LCRs were selected, and CLAHE (tile size: 20*20) was applied to the original LCRs. 27 LCR landmarks were manually determined by two examiners and, the selected LCR's determined automatically using the WebCeph program. Absolute differences between the original LCRs and CLAHE-LCRs were calculated in the x-y axes and Euclidean distance. The Kruskal Wallis test was used for comparisons between the examiners and WebCeph. The Wilcoxon Signed Rank Test was used to evaluate the x and y axes within each group.

Results: The best accuracy values were seen in examiner 1 along the x-y axes and Euclidean distance, while the worst accuracy values were seen in WebCeph. The mean differences according to the methods were higher along the y-axis than along the x-axis for both examiners (except PNS, Me') and WebCeph (except Po, Co). The mean Euclidean distances were above 2 mm only in Co, PNS at Examiner 1, PNS, Po, Ba, Co, Go, Pog, U1RT, Me', Pog at Examiner 2, and WebCeph in all measurements. However, the differences in Euclidean distances were less than 4 mm for both examiners and WebCeph.

Conclusion: CLAHE-LCRs require more adjustments for landmark determination in WebCeph than the in the manual system.

Keywords: Orthodontics; radiography; cephalometry; image enhancement; artificial intelligence; diagnostic imaging

1. INTRODUCTION

Lateral cephalometric radiographs (LCRs) are an essential evaluation tool in orthodontics, providing clinicians with information about dental and facial morphology. Changes during and post-treatment, tooth movements, facial growth, the relationship between the maxilla and mandible with the cranium, and the soft tissue profile are evaluated with LCR (1). The LCR landmarks are identifiable points that signify anatomical structures of hard or soft tissue. The structures are used as LCR landmarks for the determination of different cephalometric angles and measures (2).

Artificial intelligence (AI) is a machine's ability to mimic rational human behavior, including complex tasks (3). AI is becoming more widespread and reducing human performance requirements. Dentistry's applications have also significantly advanced. Dentists use AI algorithms to evaluate medical imaging and plan treatments (4,5). A significant development in orthodontics is the automatic identification of LCR landmarks to aid in diagnosing and treating dental and skeletal discrepancies (2). Several authors have used AI algorithms designed for a specific study and web-based software on search engines and mobile apps to study the accuracy of crucial landmark detection in cephalometric analysis. The studies compared AI algorithms' accuracy in localizing cephalometric landmarks on LCRs to manual tracing and examined algorithm differences. CephX, Ceppro, AudaxCeph, WebCeph, CephNinja, CS imaging V8, and CephNet are Commercial Software/Applications (6).

To ensure the accuracy of measurements as a diagnostic tool, it is essential to have high-quality LCRs and accurately marked anatomical landmarks. However, because of the intricate nature of the craniofacial region, there are challenges in identifying these landmarks, such as superimposition on two-dimensional images and variations in dentofacial morphology (7). Identification errors arise from the ability

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to identify anatomical landmarks in the correct localization. Improving the image quality of LCRs can significantly reduce these errors and improve the accuracy of identifying landmark localization (8,9). Therefore, it is crucial to strive for high-quality LCRs and accurate identification of anatomical landmarks to ensure reliable diagnostic information in orthodontic evaluations (10).

Image enhancement is a widely used technique in medical imaging systems to improve the quality of medical images. The use of image enhancement techniques can improve the quality of radiological images, allowing for more accurate and effective diagnosis (11). Histogram-based algorithms, such as Contrast-Limited Adaptive Histogram Equalization (CLAHE), are popular techniques for improving image contrast, brightness, and details (12). These contrast enhancement techniques not only aid in the assessment of radiographs by dentists but also function as a preliminary step for sophisticated automatic identification schemes based on deep learning methodologies (13). Most of the studies in the literature studied the point localization of experts as ground truth and compared it with AI-based programs how efficient they are (7,14). On the contrary, the main goal of our study was to compare the response of both the examiners and the AI-based program in differences between original and CLAHE LCRs, rather than ground truth-based evaluation.

2. METHODS

This retrospective study was approved by the research ethics committee of Recep Tayyip Erdogan University (number: 2021/168) and utilized LCRs obtained from patients referred for orthodontic treatment at the University's Department of Orthodontics in the Faculty of Dentistry. The LCRs were acquired using a Planmeca Promax 2D S2 device (Planmeca Oy; Helsinki, Finland) with exposure parameters set at 66 kVp, 10 mA, and 10.5 s. LCRs were obtained by the same technician.

Informed written consent forms were obtained from all patients at the beginning of their treatment, allowing their records to be used in scientific studies. Patients with the following features were selected from the orthodontic archive: a) in permanent dentition, b) no radiographs with projection errors, c) no individuals with craniofacial deformity, d) no individuals with impacted teeth or hypodontia, e) no presence of foreign body causing image artifact, f) no orthodontic attachments in the mouth and g) LCRs in which soft tissue was visible by both examiners.

Two orthodontists, one with ten and one with five years of clinical experience, analyzed the LCRs of 553 patients. A total of 353 LCRs that did not meet the inclusion criteria were excluded. In total, 200 LCRs were assessed in this study.

2.1. CLAHE Method

CLAHE is an image processing technique that enhances the image contrast while mitigating the amplification of noise and artifacts (15). Unlike traditional histogram equalization, which can produce unnatural results, CLAHE achieves this by dividing the image into smaller tiles and equalizing the histogram of each tile individually. A contrast limit was imposed on each tile to prevent over-amplification (12).

The optimal tile size depends on image characteristics, such as resolution and contrast properties. Smaller tiles enhance local contrast but may introduce graininess, whereas larger tiles smooth out noise but may reduce local contrast and detail. The optimal tile size depends on image characteristics, such as resolution and contrast properties. Images with low spatial frequencies generally benefit from larger tile sizes, whereas images with high spatial frequencies may require smaller ones. Dental tissues have small sizes and varied densities, requiring a delicate balance between contrast, detail, and artifact reduction (16). The visibility of soft tissues, such as organs or muscles, must be improved while retaining the contrast and reducing noise. When enhancing different tissues, tradeoffs are frequently encountered. CLAHE is a technique for achieving an acceptable balance by increasing local contrast (17). One method for addressing tradeoffs is selecting different enhancing techniques based on the region of interest. Adaptive approaches, such as CLAHE, can improve skeletal and dental tissues while maintaining the natural appearance of soft tissues and optimize overall picture quality while ensuring tissue specificity (15).

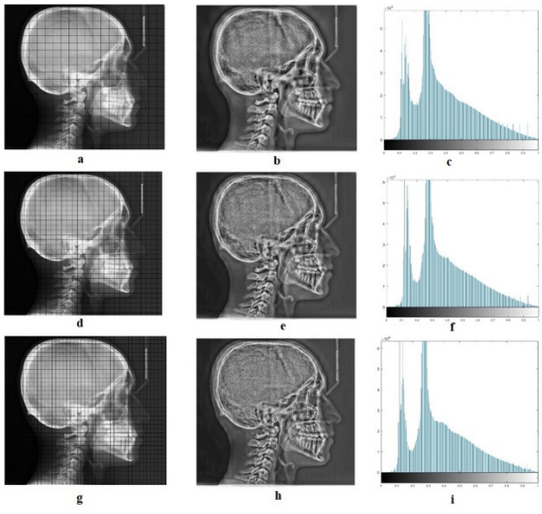

Figure 1. *The demonstration of the effects of different tile sizes in Contrast to Limited Adaptive Histogram Equalization (CLAHE) on Lateral cephalometric radiographs and histograms representing the distribution of pixel intensities.* ***a.*** *Original lateral cephalometric radiographs processed with a 20x20 tile size.* ***b.*** *The output image shows the modified contrast and clarity due to the 20x20 tile size.* ***c.*** *The image's histogram shows how the 20x20 tile size has affected the pixel intensity distribution in CLAHE.* ***d.*** *Original lateral cephalometric radiographs processed with a 30x30 tile size.* ***e.*** *The output image shows the modified contrast and clarity due to the 30x30 tile size.* ***f.*** *The image's histogram shows how the 30x30 tile size has affected the pixel intensity distribution in CLAHE.* ***g.*** *Original lateral cephalometric radiographs processed with a 40x40 tile size.* ***h.*** *The output image shows the modified contrast and clarity due to the 40x40 tile size.* ***i.*** *The image's histogram shows how the 40x40 tile size has affected the pixel intensity distribution in CLAHE.*

Figure 1 illustrates the original X-ray images processed with three different CLAHE tile sizes—20x20, 30x30, and 40x40—and their corresponding histograms. This figure succinctly demonstrates how different CLAHE tile sizes can influence the final image quality and contrast distribution. In our study, we selected a clip limit of 0.01, and the number of tiles was 20x20 for these tradeoffs using Matlab Version: R2020a 64bit (Figure 2).

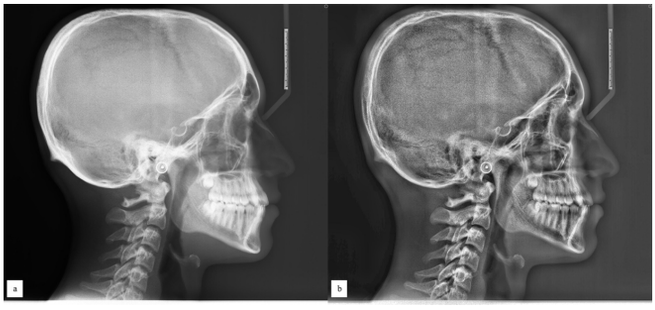

Figure 2. a: *Original lateral cephalometric radiograph,* ***b:*** *CLAHE20*20 applied lateral cephalometric radiograph*

2.2. Study Design

A fiduciary point was created in the upper-left corner of the radiograph to determine the difference between the locations of the LCR points in the original LCR and CLAHE-LCR. A macro was written using ImageJ v1.52 software (National Institutes of Health, Bethesda, MD, USA) so that the fiduciary point could be placed in a standard region in LCRs, and the location of the fiduciary point was standardized on all radiographs. Vertical and parallel planes were created from this fiduciary point, and the distances of the points at the LCRs to these planes were calculated.

CLAHE was applied to the original LCRs. The original LCR and CLAHE-LCR are shown in Figure 1 a-b, respectively. Two examiners decided on the LCRs used in this study—the soft tissue boundaries needed to be visible to both examiners without any adjustments. The brightness and contrast settings were not changed during the determination of the LCR landmark manually, so that the evaluation of the visual enhancement could be made clear. The definitions of the LCR points used in this study are listed in Table 1.

Table 1. *The definitions of the cephalometric points*

Skeletal cephalometric points	A: The deepest point of the curve of the maxilla, between the anterior nasal spine (ANS) and the dental alveolus.
	ANS (Anterior nasal spine): The tip of the anterior nasal spine.
	Ar (Articular): The intersection between posterior contour of mandible and cranial base.
	B: The deepest point of the curvature between the pogonion point and the alveolus of the mandibular incisor
	Ba (Basion): Midpoint of the anterior border of foramen magnum
	Co (Condylon): The most superior point of the mandibular condyle
	Go (Gonion): The intersection point of the lower edge of the corpus mandibularis and the posterior edge of the ramus mandibularis.
	Gn (Gnathion): Most anterior and lowest point on the mandibular symphysis
	Me (Menton): The most inferior point in the mental symphysis
	Na (Nasion): The most anterior point on frontonasal suture
	Or (Orbita): The lowest point in the inferior border of bony orbit
	PNS (Posterior nasal spine): The tip of the posterior nasal spine.
	Po (Porion): The most superior point of the external auditory meatus.
	Pog (Pogonion): The most anterior point on the chin.
Dental cephalometric points	U1IT (U1 Incisal Tip): Tip of the most prominent maxillary central incisor.
	U1RT (U1R Root Tip): Apex of the most prominent maxillary central incisor.
	L1IT (L1 Incisal Tip): Tip of the most prominent mandibular central incisor.
	L1RT (L1 Root Tip): Apex of the most prominent mandibular central incisor.
Soft tissue cephalometric points	A' (Soft tissue A): Most concave point between subnasale and the anterior point of the upper lip
	B' (Soft tissue B): Most concave point between the lower lip and the soft-tissue chin
	Gn' (Soft tissue Gnathion): Midpoint of the chin soft tissue outline between the soft tissue pogonion and soft tissue menton
	Me' (Soft tissue menton): The point where the lowest point of the soft tissue chin tip intersects with the neck plane
	Pog' (Soft Tissue pogonion): Point on the anterior curve of the soft-tissue chin
	Prn' (Pronasale): The most anterior point of nose tip
	Subnasale': Point where the nose connects to the center of the upper lip
	UpperLip': Most anterior point on the curve of the upper lip
	LowerLip': Most anterior point on the curve of the lower lip

In this study, cephalometric points were manually identified by two examiners (10 years of experience and 5 years of experience) using the AudaxCeph Advantage Cephalometric X-Ray Analysis Software Ver 4.2.0.3101 (Ljubljana, Slovenia). In addition, the same points were determined automatically using the WebCeph program, and the landmark table module was used. The working principle of the program module creates parallel and vertical planes by using the Sella point as a reference and calculating the distance of other LCR points to these planes. Thus, distance calculations were standardized by moving the sella point to the fiduciary point. Therefore, changes in the sella were not evaluated in this study.

The localization of the 27 LCR landmarks was determined by two examiners and WebCeph in both the original LCRs and the CLAHE-LCRs.

The absolute differences between the original LCRs and the CLAHE-LCRs were calculated millimeters along the x and y axes. The Euclidean distance was evaluated along the x – and y-axes by taking the square root of the sum of the squares of the changes. According to the two examiners and WebCeph, absolute differences were compared.

2.3. Measurement Error

The same orthodontist with ten years of experience repeated all measurements one month after the initial evaluation. Intraclass correlation coefficients (ICC) were utilized to assess the intra-rater agreement of landmarks in both the original and CLAHE-LCRs along the x-y axes.

2.4. Statistical Analysis

The G*Power 3.1 software (Heinrich-Heine University of Dusseldorf, Germany) was used to calculate the sample size. Post-hoc power analysis was also performed using one-way ANOVA with a 95% confidence level (1-α), d = 0.25 (medium effect size according to Cohen), three groups, and a sample size of 200. The test strength (1-β) was calculated to be 89% (18). Statistical analysis was conducted using IBM SPSS (version 21.0) software (SPSS, Chicago, IL, USA), and descriptive statistics were presented as minimum, maximum, median, mean, and standard deviation (SD). The normality of the data was assessed using histograms, normality curves, and the Kolmogorov-Smirnov test. The homogeneity of variance was checked using Levene's test.

To compare the absolute differences along the x and y axes and the Euclidean distances between the original LCRs and CLAHE-LCRs according to the examiners and WebCeph, the Kruskal Wallis test was used. Bonferroni corrections were carried out on all pairwise comparisons using Kruskal–Wallis tests. Absolute differences in the x – and y-axes within each group were evaluated using the Wilcoxon signed-rank test. In all cases, p-values of <.05 were accepted as statistically significant.

3. RESULTS

A second evaluation one month later of the same observer with ten years of experience was used to estimate the intra-class correlation coefficient (ICC). ICC greater than 0.9 indicates excellent reliability; between 0.75 and 0.9 indicates good reliability; between 0.5 and 0.75 indicates moderate reliability; and below 0.5 indicates poor reliability (19). For all cephalometric points in both the original and CLAHE-LCRs along the x-y axes, the intra-rater agreement was excellent, with an ICC value of at least 0.987.

When the accuracy values on the x-axis of the 27 landmarks were evaluated, no statistically significant difference was found among the examiners and WebCeph in A, ANS, PNS, and U1RT (p>.05). The best accuracy values along the x-axis were seen by examiner 1 in 23 of 27 landmarks, in both examiners 1 and 2 in 6 landmarks (Or, Ar, Po, Ba, Co, Go), by both examiner 1 and WebCeph in 8 landmarks (Gn, L1RT, Subnasale, Upper Lip, Lower Lip, Gn', Me', Pog') (Tables 2, 3, 4). The mean differences for the x-axis between the original LCR and CLAHE-LCR are demonstrated in Figure 3.

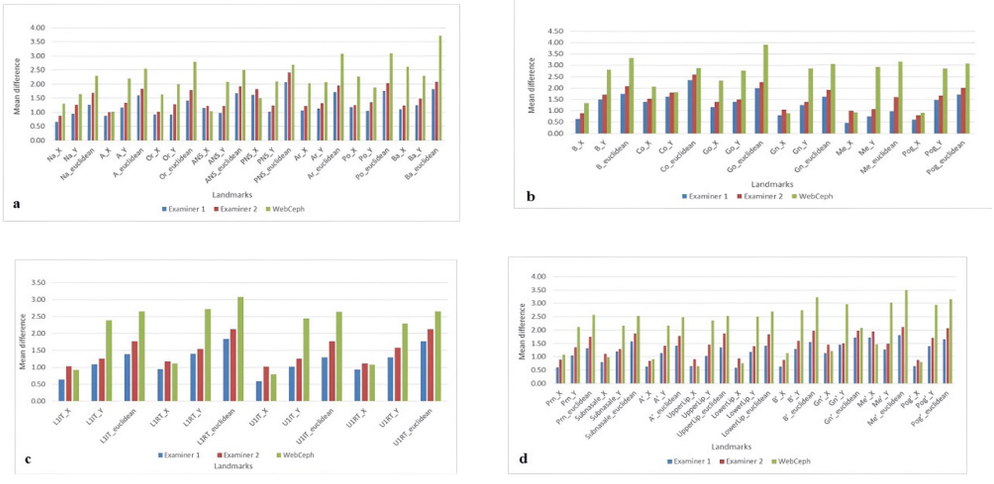

Figure 3. a-b: *Absolute difference of means along the x-y axes and Euclidean distance in skeletal landmarks,* ***c:*** *Absolute difference of means along the x-y axes and Euclidean distance in dental landmarks,* ***d:*** *Absolute difference of means along the x-y axes and Euclidean distance in soft tissue landmarks*

Table 2. *Comparison of the effects of skeletal absolute differences between the original LCR and CLAHE-LCR along the x and y axes by examiners and WebCeph*

		Examiner 1					Examiner 2					WebCeph						Post hoc comparison (mean diff.)			More accurate		
LCR Points	axis	Med.	Min.	Max.	Mean	SD	Med.	Min.	Max.	Mean	SD	Med.	Min.	Max.	Mean	SD	P	E1-E2	E1-W	E2-W	E1	E2	W
Na	X	0.54	0	2.83	0.66	0.53	0.71	0.06	3.06	0.87	0.68	1.15	0	6.95	1.3	1.19	**<.001**	**.011 (-0.21)**	**<.001 (-0.64)**	**.005 (-0.43)**	✓		
Na	Y	0.76	0.01	6.46	0.95	0.92	1.06	0	6.72	1.26	1.01	1.3	0	12.69	1.65	1.48	**<.001**	**.001 (-0.31)**	**<.001 (-0.7)**	.077 (-0.39)	✓		
A	X	0.76	0.02	4.39	0.88	0.68	0.81	0.01	4.22	1.01	0.76	0.85	0	4.53	1.01	0.79	.215	(-0.13)	(-0.13)	(0)			
A	Y	0.94	0.01	4.86	1.17	0.91	1.19	0.01	4.97	1.34	1.04	1.99	0	9.14	2.19	1.7	**<.001**	.493 (-0.17)	**<.001 (-1.02)**	**<.001 (-0.85)**	✓	✓	
Or	X	0.7	0.01	4.15	0.92	0.77	0.8	0.02	5.02	1.02	0.89	1.39	0.01	6.76	1.62	1.29	**<.001**	1 (-0.1)	**<.001 (-0.7)**	**<.001 (-0.6)**	✓	✓	
Or	Y	0.69	0	6.47	0.92	0.82	1.08	0	4.99	1.28	0.94	1.82	0.01	7.68	1.99	1.58	**<.001**	**<.001 (-0.36)**	**<.001 (-1.07)**	**<.001 (-0.71)**	✓		
ANS	X	1	0	5	1.14	0.95	1.02	0	4.55	1.22	0.99	0.79	0	5.27	1.04	0.91	.109	(-0.08)	(0.1)	(0.18)			
ANS	Y	0.82	0.01	3.53	0.98	0.78	1	0.01	4.81	1.22	0.92	1.6	0	9.5	2.07	1.77	**<.001**	**.046 (-0.24)**	**<.001 (-1.09)**	**<.001 (-0.85)**	✓		
PNS	X	1.42	0.01	6.73	1.62	1.34	1.55	0.01	6.94	1.82	1.37	1.27	0.01	7.95	1.5	1.17	.075	(-0.2)	(0.12)	(0.32)			
PNS	Y	0.9	0.01	3.78	1.03	0.76	1.07	0.01	4.64	1.24	0.93	1.8	0.01	9.48	2.09	1.62	**<.001**	.133 (-0.21)	**<.001 (-1.06)**	**<.001 (-0.85)**	✓	✓	
Ar	X	0.98	0.01	4.72	1.06	0.81	1.1	0.02	4.88	1.22	0.83	1.77	0	10.48	2.04	1.63	**<.001**	.222 (-0.16)	**<.001 (-0.98)**	**<.001 (-0.82)**	✓	✓	
Ar	Y	1.02	0.02	3.73	1.14	0.78	1.16	0	4.2	1.33	0.95	1.73	0.03	8.39	2.06	1.62	**<.001**	.349 (-0.19)	**<.001 (-0.92)**	**<.001 (-0.73)**	✓	✓	
Po	X	0.94	0	6.46	1.18	0.93	1.06	0.01	5.42	1.25	1.04	1.97	0	9.5	2.27	1.63	**<.001**	1 (-0.07)	**<.001 (-1.09)**	**<.001 (-1.02)**	✓	✓	
Po	Y	0.82	0.01	5.05	1.05	0.89	1.2	0.02	5.17	1.36	1.03	1.5	0.01	9.54	1.87	1.51	**<.001**	**.008 (-0.31)**	**<.001 (-0.82)**	**.007 (-0.51)**	✓		
Ba	X	0.87	0.01	4.37	1.1	0.89	1.04	0.02	5.25	1.23	0.9	2.28	0.02	9.62	2.62	1.99	**<.001**	.374 (-0.13)	**<.001 (-1.52)**	**<.001 (-1.39)**	✓	✓	
Ba	Y	1.04	0.01	5.24	1.25	1.02	1.27	0.02	5.99	1.48	1.11	1.86	0	9.22	2.3	1.82	**<.001**	.113 (-0.23)	**<.001 (-1.05)**	**<.001 (-0.82)**	✓	✓	
B	X	0.57	0	2.13	0.64	0.47	0.72	0	3.49	0.89	0.69	1.07	0.01	5.46	1.33	1.09	**<.001**	**.007 (-0.25)**	**<.001 (-0.69)**	**<.001 (-0.44)**	✓		
B	Y	1.28	0.05	6.22	1.49	1.13	1.51	0	6.78	1.7	1.25	2.36	0.02	11.61	2.81	2.05	**<.001**	.405 (-0.21)	**<.001 (-1.32)**	**<.001 (-1.11)**	✓	✓	
Co	X	1.08	0.04	5.43	1.38	1.07	1.35	0.04	5.98	1.52	1.15	1.78	0.03	9.66	2.05	1.55	**<.001**	.712 (-0.14)	**<.001 (-0.67)**	**.003 (-0.53)**	✓	✓	
Co	Y	1.27	0.01	6.87	1.62	1.35	1.47	0.01	7.86	1.79	1.45	1.51	0	7.42	1.82	1.46	.326	(-0.17)	(-0.2)	(-0.03)			
Go	X	0.96	0.01	4.22	1.16	0.86	1.2	0.01	4.39	1.39	0.98	1.92	0	8.94	2.32	1.8	**<.001**	.103 (-0.23)	**<.001 (-1.16)**	**<.001 (-0.93)**	✓	✓	
Go	Y	1.21	0.02	4.99	1.39	1.06	1.25	0.01	5.37	1.5	1.1	2.39	0.01	10.36	2.77	2.15	**<.001**	1 (-0.11)	**<.001 (-1.38)**	**<.001 (-1.27)**	✓	✓	
Gn	X	0.67	0.01	4.91	0.81	0.68	0.91	0	3.97	1.05	0.75	0.75	0	4.78	0.89	0.74	**.002**	**.002 (-0.24)**	.896 (-0.08)	**.048 (0.16)**	✓		✓
Gn	Y	1.01	0	5.23	1.24	1	1.12	0.01	5.09	1.38	1.14	2.55	0	12.78	2.86	2.16	**<.001**	1 (-0.14)	**<.001 (-1.62)**	**<.001 (-1.48)**	✓	✓	
Me	X	0.35	0	2.67	0.47	0.43	0.84	0.01	3.36	1	0.67	0.78	0.01	4.98	0.93	0.75	**<.001**	**<.001 (-0.53)**	**<.001 (-0.46)**	.178 (0.07)	✓		
Me	Y	0.51	0	5.11	0.75	0.86	0.83	0.01	5.41	1.07	0.95	2.59	0	12.63	2.93	2.23	**<.001**	**<.001 (-0.32)**	**<.001 (-2.18)**	**<.001 (-1.86)**	✓		
Pog	X	0.46	0.02	2.59	0.6	0.5	0.68	0	2.53	0.81	0.59	0.78	0.01	4.45	0.91	0.72	**<.001**	**.001 (-0.21)**	**<.001 (-0.31)**	.581 (-0.1)	✓		
Pog	Y	1.14	0.01	5.65	1.47	1.16	1.41	0.01	5.5	1.67	1.27	2.49	0.02	12.79	2.85	2.12	**<.001**	.488 (-0.2)	**<.001 (-1.38)**	**<.001 (-1.18)**	✓	✓	

[*] *Significant results according to Kruskal Wallis test, Bonferroni corrections were carried out on all pair-wise comparisons of Kruskal–Wallis tests. (p<.05)*

LCR; lateral cephalometric radiograph, Med; median, Min; minimum, Max; maximum, SD; standard deviation, E; Examiner, W;WebCeph, Clahe;Contrast-Limited Adaptive Histogram Equalization

Table 3. *Comparison of the effects of dental absolute differences between the original LCR and CLAHE-LCR along the x and y axes by examiners and WebCeph*

		Examiner 1					Examiner 2					WebCeph						Post hoc comparison (mean diff.)			More accurate		
LCR Points	axis	Med.	Min.	Max.	Mean	SD	Med.	Min.	Max.	Mean	SD	Med.	Min.	Max.	Mean	SD	P	E1-E2	E1-W	E2-W	E1	E2	W
L1IT	X	0.54	0.01	2.1	0.64	0.49	0.88	0.01	3.51	1.03	0.66	0.85	0.01	3.91	0.93	0.7	**<.001**	**<.001 (-0.39)**	**<.001 (-0.29)**	.127 (0.1)	✓		
L1IT	Y	0.88	0	4.18	1.09	0.87	1.11	0.03	4.25	1.26	0.92	2.07	0	9.74	2.39	1.79	**<.001**	.246 (-0.17)	**<.001 (-1.3)**	**<.001 (-1.13)**	✓	✓	
L1RT	X	0.74	0.01	3.77	0.95	0.75	1.06	0.01	5	1.17	0.86	0.94	0.01	5.16	1.11	0.85	**.018**	**.019 (-0.22)**	.124 (-0.16)	1 (0.06)	✓		✓
L1RT	Y	1.13	0.01	5.33	1.4	1.08	1.45	0.01	6.12	1.54	1.12	2.32	0.04	11.99	2.73	2.03	**<.001**	.588 (-0.14)	**<.001 (-1.33)**	**<.001 (-1.19)**	✓	✓	
U1IT	X	0.52	0	2.69	0.59	0.49	0.88	0	3.57	1.02	0.73	0.7	0.01	3.74	0.79	0.62	**<.001**	**<.001 (-0.43)**	**.005 (-0.2)**	**.005 (0.23)**	✓		
U1IT	Y	0.81	0	4.04	1.03	0.82	1.05	0	4.37	1.26	0.95	2.22	0.01	10.33	2.45	1.84	**<.001**	.092 (-0.23)	**<.001 (-1.42)**	**<.001 (-1.19)**	✓	✓	
U1RT	X	0.77	0	3.46	0.94	0.76	0.97	0	4.99	1.12	0.86	0.86	0	5.28	1.07	0.91	.111	(-0.18)	(-0.13)	(0.05)			
U1RT	Y	1.09	0	4.16	1.3	0.99	1.26	0.01	5.6	1.58	1.24	2.07	0.02	7.99	2.29	1.66	**<.001**	.214 (-0.28)	**<.001 (-0.99)**	**<.001 (-0.71)**	✓	✓	

* *Significant results according to Kruskal Wallis test, Bonferroni corrections were carried out on all pair-wise comparisons of Kruskal–Wallis tests. (p<.05)*

LCR; lateral cephalometric radiograph, Med; median, Min; minimum, Max; maximum, SD; standard deviation, E; Examiner, W;WebCeph, Clahe;Contrast-Limited Adaptive Histogram Equalization

Table 4. *Comparison of the effects of soft tissue absolute differences between the original LCR and the CLAHE-LCR along the x and y axes by examiners and WebCeph*

		Examiner 1					Examiner 2					WebCeph						Post hoc comparison (mean diff.)			More accurate		
LCR Points	axis	Med.	Min.	Max.	Mean	SD	Med.	Min.	Max.	Mean	SD	Med.	Min.	Max.	Mean	SD	P	E1-E2	E1-W	E2-W	E1	E2	W
Prn	X	0.46	0	2.44	0.6	0.52	0.69	0	2.93	0.88	0.65	0.93	0	6.2	1.07	0.92	**<.001**	**<.001 (-0.28)**	**<.001 (-0.47)**	.535 (-0.19)	✓		
Prn	Y	0.76	0	4.45	1.04	0.91	1.16	0	5.29	1.34	1.03	1.7	0.01	8.53	2.11	1.65	**<.001**	**.007 (-0.3)**	**<.001 (-1.07)**	**<.001 (-0.77)**	✓		
Subnasale	X	0.69	0.01	2.74	0.8	0.6	0.93	0.01	4.47	1.11	0.84	0.89	0	3.47	0.98	0.71	**.001**	**.001 (-0.31)**	.52 (-0.18)	.645 (0.13)	✓		✓
Subnasale	Y	0.98	0.02	5.39	1.2	0.97	1.08	0	5.63	1.29	1.04	1.84	0	9.6	2.16	1.68	**<.001**	1 (-0.1)	**<.001 (-0.96)**	**<.001 (-0.87)**	✓	✓	
A’	X	0.55	0	2.05	0.63	0.46	0.76	0.01	2.75	0.85	0.61	0.82	0.01	3.06	0.9	0.67	**<.001**	**.002 (-0.22)**	**<.001 (-0.27)**	1 (-0.05)	✓		
A’	Y	0.87	0.01	4.96	1.14	0.98	1.25	0.01	4.91	1.41	1.08	2.03	0	9.57	2.16	1.66	**<.001**	**.042 (-0.27)**	**<.001 (-1.02)**	**<.001 (-0.75)**	✓		
UpperLip	X	0.49	0	3.32	0.65	0.59	0.85	0	3.47	0.91	0.62	0.51	0	4.41	0.64	0.6	**<.001**	**<.001 (-0.26)**	1 (0.01)	**<.001 (0.27)**	✓		✓
UpperLip	Y	0.7	0	4.82	1.03	0.92	1.25	0	5.31	1.45	1.16	2.05	0	10.14	2.35	1.81	**<.001**	**.001 (-0.42)**	**<.001 (-1.32)**	**<.001 (-0.9)**	✓		
LowerLip	X	0.52	0	2.22	0.59	0.46	0.81	0	3.16	0.94	0.7	0.57	0	3.43	0.75	0.66	**<.001**	**<.001 (-0.35)**	.087 (-0.16)	**<.001 (0.19)**	✓		✓
LowerLip	Y	0.94	0.01	4.83	1.18	0.93	1.23	0.01	4.46	1.39	1	2.1	0	11.09	2.5	1.96	**<.001**	.144 (-0.21)	**<.001 (-1.32)**	**<.001 (-1.11)**	✓		
B’	X	0.54	0	2.22	0.63	0.46	0.75	0	3.03	0.87	0.63	0.85	0	11.27	1.13	1.17	**<.001**	**.001 (-0.24)**	**<.001 (-0.5)**	.389 (-0.26)	✓		
B’	Y	0.97	0.01	6.21	1.29	1.11	1.39	0	6.12	1.59	1.21	2.34	0	12.81	2.74	2.07	**<.001**	.055 (-0.3)	**<.001 (-1.45)**	**<.001 (-1.15)**	✓	✓	
Gn’	X	0.99	0.01	5.1	1.14	0.93	1.26	0.01	6.47	1.46	1.11	1.01	0	9.95	1.2	1.07	**.006**	**.007 (-0.32)**	1 (-0.06)	**.044 (0.26)**	✓		✓
Gn’	Y	1.17	0.02	6.5	1.45	1.14	1.19	0.01	6.46	1.5	1.27	2.52	0.03	13.55	2.96	2.31	**<.001**	1 (-0.05)	**<.001 (-1.51)**	**<.001 (-1.46)**	✓	✓	
Me’	X	1.38	0	6.51	1.72	1.38	1.68	0.01	8.34	1.95	1.43	1.27	0.01	6.54	1.47	1.17	**.002**	.193 (-0.23)	.304 (0.25)	**.001 (0.48)**	✓		✓
Me’	Y	0.93	0.02	4.77	1.28	1.05	1.25	0.02	5.18	1.48	1.1	2.65	0	12.74	3.03	2.3	**<.001**	.192 (-0.2)	**<.001 (-1.75)**	**<.001 (-1.55)**	✓	✓	
Pog’	X	0.54	0	3.22	0.64	0.52	0.78	0	3.18	0.87	0.64	0.66	0.01	10.22	0.79	0.9	**.001**	**<.001 (-0.23)**	.23 (-0.15)	.131 (0.08)	✓		✓
Pog’	Y	1.14	0	5.07	1.4	1.09	1.52	0	5.49	1.7	1.21	2.5	0.01	13.73	2.94	2.2	**<.001**	.074 (-0.3)	**<.001 (-1.54)**	**<.001 (-1.24)**	✓	✓	

* *Significant results according to Kruskal Wallis test, Bonferroni corrections were carried out on all pair-wise comparisons of Kruskal–Wallis tests. (p<.05)*

LCR; lateral cephalometric radiograph, Med; median, Min; minimum, Max; maximum, SD; standard deviation, E; Examiner, W;WebCeph, Clahe;Contrast-Limited Adaptive Histogram Equalization

*Kruskal Wallis test, Bonferroni corrections were carried out on all pair-wise comparisons of Kruskal–Wallis tests. *significance on p < .05 scale*

Table 5. *Comparison of absolute differences between original LCRs and CLAHE LCRs along the x and y axes within examiners and WebCeph themselves*

LCR Points	Examiner 1		Examiner 2		WebCeph	
	mean diff. (x-y)	p	mean diff. (x-y)	p	mean diff. (x-y)	p
Na	-0.29	**<.001**	-0.39	**<.001**	-0.35	**.001**
A	-0.29	**<.001**	-0.33	.1	-1.18	**<.001**
Or	0	.972	-0.26	**.003**	-0.37	**.004**
ANS	0.16	.151	0	.817	-1.03	**<.001**
PNS	0.59	**<.001**	0.58	**<.001**	-0.59	**<.001**
Ar	-0.08	.212	-0.11	.109	-0.02	.464
Po	0.13	.119	-0.11	.188	0.4	**<.001**
Ba	-0.15	.77	-0.25	**.006**	0.32	.25
B	-0.85	**<.001**	-0.81	**<.001**	-1.48	**<.001**
Co	-0.24	.54	-0.27	**.045**	0.23	**.008**
Go	-0.23	**.014**	-0.11	.395	-0.45	**.007**
Gn	-0.43	**<.001**	-0.33	**.001**	-1.97	**<.001**
Me	-0.28	**<.001**	-0.07	.541	-2	**<.001**
Pog	-0.87	**<.001**	-0.86	**<.001**	-1.94	**<.001**
L1IT	-0.45	**<.001**	-0.23	**.009**	-1.46	**<.001**
L1RT	-0.45	**<.001**	-0.37	**.001**	-1.62	**<.001**
U1IT	-0.44	**<.001**	-0.24	**.003**	-1.66	**<.001**
U1RT	-0.36	**<.001**	-0.46	**<.001**	-1.22	**<.001**
Prn	-0.44	**<.001**	-0.46	**<.001**	-1.04	**<.001**
Subnasale	-0.4	**<.001**	-0.18	**.038**	-1.18	**<.001**
A’	-0.51	**<.001**	-0.56	**<.001**	-1.26	**<.001**
UpperLip	-0.38	**<.001**	-0.54	**<.001**	-1.71	**<.001**
LowerLip	-0.59	**<.001**	-0.45	**<.001**	-1.75	**<.001**
B’	-0.66	**<.001**	-0.72	**<.001**	-1.61	**<.001**
Gn’	-0.31	**.002**	-0.04	.928	-1.76	**<.001**
Me’	0.44	**<.001**	0.47	**.001**	-1.56	**<.001**
Pog’	-0.76	**<.001**	-0.83	**<.001**	-2.15	**<.001**

** Significant results according to Wilcoxon Signed Rank Test (p<.05)*
LCR; lateral cephalometric radiograph, E; Examiner, W;WebcephX, Clahe;Contrast-Limited Adaptive Histogram Equalization
*Wilcoxon Signed Rank Test. *significance on p < .05 scale*

When the accuracy values in the y-axis of 27 landmarks were evaluated, no statistically significant difference was found between the examiners and WebCeph in Co (p>.05). The best accuracy values along the y-axis were seen by Examiner 1 in 26 of the 27 landmarks, while in both examiners 1 and 2 in 17 landmarks. The worst accuracy values were obtained for all landmarks in WebCeph (Tables 2-4). The mean differences for the y-axis between the original LCR and CLAHE-LCR are demonstrated in Figure 3.

When the differences in the x and y axes were compared with each other, there was no statistically significant difference in Or, ANS, Ar, Po, Ba, and Co by Examiner 1, in A, ANS, Ar, Po, Go, Me by Examiner 2, and Ba in WebCeph (p>.05). Statistically, differences between examiners and WebCeph were generally more pronounced on the y-axis (p<.05). The exceptions were PNS and Me’ for examiners and Po and Co for WebCeph (p<.05) (Table 5). The mean differences in the x – and y – axes according to image methods by examiners and WebCeph are demonstrated in Figure 4.

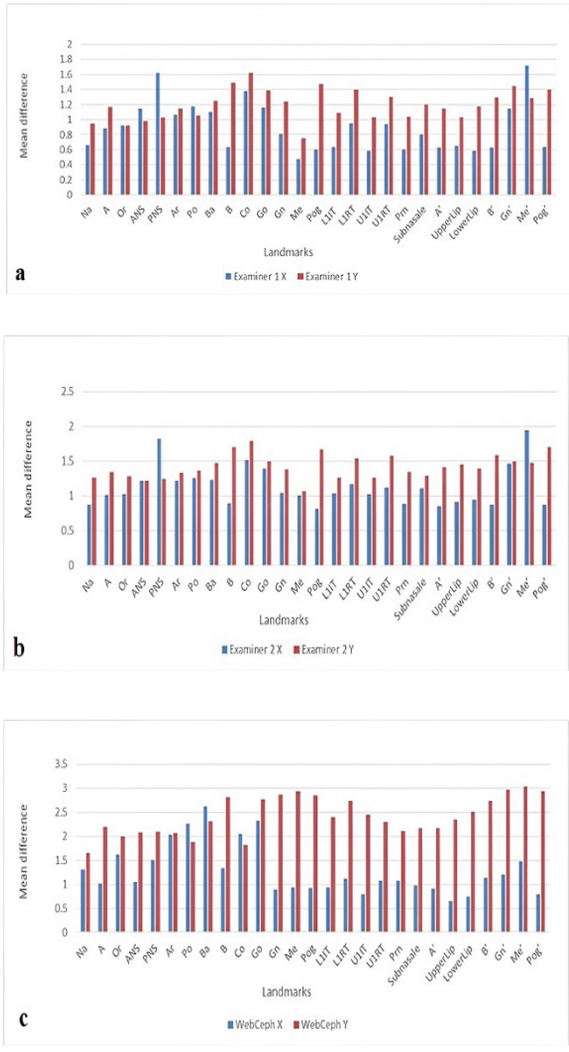

Figure 4. *a: Absolute difference of means along the x-y axes by Examiner 1, b: Absolute difference of means along the x-y axes by Examiner 2, c: Absolute difference of means along the x-y axes in WebCeph*

When the accuracy values in the Euclidean distance of 27 landmarks were evaluated, significant differences were found between the examiners and WebCeph in all measurements. The best accuracy values were seen by Examiner 1 for all landmarks, and by both examiners 1 and 2 for five landmarks. The worst accuracy values were obtained for all landmarks using WebCeph (Table 6). The mean differences in the Euclidean distance between the original LCR and CLAHE-LCR are shown in Figure 3.

The differences between the original LCR and the CLAHE-LCR are shown in supplementary material for both two examiners and WebCeph.

Table 6. *Comparison of the effects of euclidean differences between the original LCR and the CLAHE-LCR by examiners and WebCeph*

		Examiner 1					Examiner 2					WebCeph						Post hoc comparison		More accurate		
LCR Points		Med.	Min.	Max.	Mean	SD	Med.	Min.	Max.	Mean	SD	Med.	Min.	Max.	Mean	SD	P	E1-E2	E1-W	E2-W	E1	E2	W
Na	euclidean	1.07	0.04	6.54	1.27	0.92	1.5	0.26	6.84	1.69	0.99	1.95	0.08	12.71	2.29	1.66	**<.001**	**<.001 (-0.42)**	**<.001 (-1.02)**	**.002 (-0.6)**	✓		
A	euclidean	1.39	0.16	5.04	1.6	0.94	1.63	0.14	5.02	1.83	1.04	2.22	0.16	9.67	2.54	1.7	**<.001**	.63 (-0.23)	**<.001 (-0.94)**	**<.001 (-0.71)**	✓	✓	
Or	euclidean	1.18	0.09	7.69	1.42	0.98	1.59	0.08	6.92	1.79	1.07	2.5	0.15	8.3	2.79	1.72	**<.001**	**.001 (-0.37)**	**<.001 (-1.37)**	**<.001 (-1)**	✓		
ANS	euclidean	1.55	0.07	5.12	1.67	0.99	1.73	0.08	5.35	1.91	1.07	2.16	0.12	9.5	2.5	1.76	**<.001**	.66 (-0.24)	**<.001 (-0.83)**	**.017 (-0.59)**	✓	✓	
PNS	euclidean	1.9	0.12	6.78	2.07	1.32	2.27	0.14	7.01	2.41	1.34	2.42	0.21	12.37	2.69	1.83	**.001**	**.014 (-0.34)**	**.001 (-0.62)**	1.00 (-0.28)	✓		
Ar	euclidean	1.61	0.14	4.73	1.71	0.88	1.85	0.17	4.88	1.95	1.02	2.82	0.11	11.7	3.07	2.05	**<.001**	.111 (-0.24)	**<.001 (-1.36)**	**<.001 (-1.12)**	✓	✓	
Po	euclidean	1.58	0.12	6.49	1.76	1.02	1.78	0.09	5.72	2.03	1.19	2.6	0.25	11.75	3.09	2.01	**<.001**	.114 (-0.27)	**<.001 (-1.33)**	**<.001 (-1.06)**	✓	✓	
Ba	euclidean	1.57	0.04	5.38	1.82	1.14	1.81	0.31	6.36	2.08	1.19	3.49	0.14	13.32	3.72	2.36	**<.001**	.14 (-0.26)	**<.001 (-1.9)**	**<.001 (-1.64)**	✓	✓	
B	euclidean	1.56	0.15	6.24	1.73	1.06	1.91	0.09	6.79	2.08	1.17	3.01	0.3	12.03	3.31	2.02	**<.001**	**.011 (-0.35)**	**<.001 (-1.58)**	**<.001 (-1.23)**	✓		
Co	euclidean	2.1	0.2	7.63	2.34	1.43	2.45	0.15	8.1	2.6	1.47	2.39	0.09	9.97	2.87	1.95	**.036**	.164 (-0.26)	**.044 (-0.53)**	1 (-0.27)	✓	✓	
Go	euclidean	1.81	0.13	5.07	1.98	1.09	2.17	0.2	5.45	2.25	1.13	3.44	0.23	11.8	3.9	2.38	**<.001**	**.08 (-0.27)**	**<.001 (-1.92)**	**<.001 (-1.65)**	✓		
Gn	euclidean	1.4	0.13	5.73	1.61	1.03	1.67	0.12	5.48	1.91	1.11	2.76	0.06	13.64	3.05	2.21	**<.001**	**.028 (-0.3)**	**<.001 (-1.44)**	**<.001 (-1.14)**	✓		
Me	euclidean	0.79	0.01	5.17	0.98	0.85	1.4	0.11	5.56	1.6	0.96	2.76	0.11	13.58	3.16	2.25	**<.001**	**<.001 (-0.62)**	**<.001 (-2.18)**	**<.001 (-1.56)**	✓		
Pog	euclidean	1.5	0.06	5.71	1.71	1.09	1.79	0.28	5.92	2.01	1.16	2.61	0.08	13.54	3.08	2.13	**<.001**	**.03 (-0.3)**	**<.001 (-1.37)**	**<.001 (-1.07)**	✓		
L1IT	euclidean	1.25	0.05	4.3	1.38	0.82	1.7	0.03	4.57	1.77	0.88	2.31	0.07	10.5	2.65	1.8	**<.001**	**<.001 (-0.39)**	**<.001 (-1.27)**	**<.001 (-0.88)**	✓		
L1RT	euclidean	1.55	0.19	5.48	1.84	1.09	2	0.06	6.79	2.12	1.11	2.68	0.28	13.05	3.08	2	**<.001**	**.042 (-0.28)**	**<.001 (-1.24)**	**<.001 (-0.96)**	✓		
U1IT	euclidean	1.17	0.02	4.13	1.3	0.79	1.68	0.02	4.79	1.76	0.96	2.3	0.05	10.99	2.64	1.85	**<.001**	**<.001 (-0.46)**	**<.001 (-1.34)**	**<.001 (-0.88)**	✓		
U1RT	euclidean	1.66	0.08	5.18	1.77	1	1.98	0.12	6.39	2.12	1.22	2.26	0.25	8.82	2.65	1.72	**<.001**	**.016 (-0.35)**	**<.001 (-0.88)**	**.03 (-0.53)**	✓		
Prn	euclidean	1.08	0.07	5.03	1.32	0.9	1.57	0.1	5.77	1.74	1.02	2.22	0.13	9.56	2.57	1.6	**<.001**	**<.001 (-0.42)**	**<.001 (-1.25)**	**<.001 (-0.83)**	✓		
Subnasale	euclidean	1.43	0.25	5.41	1.58	0.94	1.76	0.07	5.82	1.87	1.08	2.26	0.2	10	2.52	1.6	**<.001**	**.022 (-0.29)**	**<.001 (-0.94)**	**<.001 (-0.65)**	✓		
A’	euclidean	1.23	0.13	5.33	1.4	0.94	1.68	0.09	5.01	1.78	1.03	2.36	0.14	10.01	2.48	1.59	**<.001**	**<.001 (-0.38)**	**<.001 (-1.08)**	**<.001 (-0.7)**	✓		
UpperLip	euclidean	1.16	0.07	4.85	1.36	0.91	1.69	0.1	5.38	1.86	1.09	2.16	0.1	10.71	2.52	1.81	**<.001**	**<.001 (-0.5)**	**<.001 (-1.16)**	**.019 (-0.66)**	✓		
LowerLip	euclidean	1.29	0.03	4.91	1.42	0.9	1.83	0.06	4.47	1.84	0.96	2.21	0.12	11.61	2.7	1.96	**<.001**	**<.001 (-0.42)**	**<.001 (-1.28)**	**.004 (-0.86)**	✓		
B’	euclidean	1.26	0.15	6.47	1.55	1.04	1.78	0.06	6.14	1.97	1.12	2.88	0.48	13.1	3.22	2.02	**<.001**	**<.001 (-0.42)**	**<.001 (-1.67)**	**<.001 (-1.25)**	✓		
Gn’	euclidean	1.63	0.14	5.34	1.72	0.92	1.86	0.2	6.77	1.97	1.09	1.78	0.15	10.12	2.09	1.38	**.024**	.064 (-0.25)	**.046 (-0.37)**	1 (-0.12)	✓	✓	
Me’	euclidean	1.66	0.1	5.37	1.8	1.08	1.94	0.28	5.95	2.11	1.14	3.24	0	14.84	3.49	2.42	**<.001**	**.033 (-0.31)**	**<.001 (-1.69)**	**<.001 (-1.38)**	✓		
Pog’	euclidean	1.37	0.13	5.13	1.66	1.04	1.85	0.08	5.5	2.06	1.12	2.64	0.1	14.24	3.15	2.24	**<.001**	**.002 (-0.4)**	**<.001 (-1.49)**	**<.001 (-1.09)**	✓		

** Significant results according to Kruskal Wallis test, Bonferroni corrections were carried out on all pair-wise comparisons of Kruskal–Wallis tests. (p<.05)*

LCR; lateral cephalometric radiograph, Med; median, Min; minimum, Max; maximum, SD; standard deviation, E; Examiner, W;WebCeph, Clahe;Contrast-Limited Adaptive Histogram Equalization

4. DISCUSSION

"Accuracy" throughout the manuscript refers to evaluating a cephalometric landmark by comparison of the differences (x, y-axes, and Euclidean distances) between the original LCRs and CLAHE LCRs. When evaluating original LCRs and CLAHE LCRs from the same individual, consistent results in LCR point determination, regardless of visual characteristics, mean high accuracy.

The majority of the research in the literature compared the efficiency of AI-based algorithms to expert point localization as ground truth (7,14). Rather than ground truth-based evaluation, the primary purpose of our study was to compare the responses of both examiners and the AI-based software in inconsistencies between original and CLAHE LCRs.

To our knowledge, no previous study has investigated the efficacy of CLAHE in identifying cephalometric landmarks in WebCeph and human examiners. The efficacy of CLAHE was tested on 27 LCR landmarks. The first null hypothesis, which stated that there would be no difference in landmark identification accuracy between WebCeph and human examiners based on the method used, was not rejected. The second null hypothesis, which stated that there would be no difference in accuracy in identifying LCR landmarks between the x – and y – axes based on comparisons made by both examiners and WebCeph itself, was also not rejected.

Interpreting radiographic images requires radiological information, pattern recognition, and image quality. Improving landmark determination accuracy improves image quality (20,21). Computerized digital radiography systems can adjust image contrast and brightness. Edge enhancement is one of the most popular methods since it selectively increases image edges, making identifying anatomical landmarks easier. Based on the particular requirements of the radiographic examination, these approaches can improve image quality in darker or lighter areas or the overall radiographic image. These image enhancement methods can improve landmark identification accuracy and consistency, contributing to more accurate diagnoses (22).

Studies in the literature have evaluated the effects of image enhancement on landmark detection errors based on manual tracing in standard LCRs and digital LCRs along the x-y axes (23-25). In digital radiography, sharpness and contrast changes can enhance the image. Thus, anatomical landmarks can be determined more easily (10,26). The differences between the examiners were less than 1 mm in all measurements of differences between the original LCR and the CLAHE-LCR. Although there were statistically significant differences in some landmarks between the examiners, it may not be a clinically important difference. From a clinical point of view, 1 mm is an acceptable error limit for identifying cephalometric points regarding the examiners (27).

Eppley and Sadove (10) reported that standard LCRs and digitally enhanced LCRs showed comparable accuracy in identifying bone landmarks, but digital enhancement was superior in identifying soft tissue. Nikneshan et al. (28) reported that the original LCRs provided better reliability at Co, Or, and Pog, while the emboss image enhancement LCRs provided better reliability at A, ANS, B, Ba, Me', L1IT. It was stated that the effect of the embossing method on the reliability of landmarks was greater in the x plane than in the y plane.

In this study, the CLAHE method changed the y-axis more than the x-axis in two examiners and WebCeph. The x-axis results were more consistent for examiners (except PNS, Me') and WebCeph (except Po, Co).

The literature mentions acceptable mean errors for automatic landmark detection in orthodontic practice and research. For the x coordinate, 0.59 mm of mean error is acceptable, and for the y coordinate, 0.56 mm. Although the Euclidean mean error value of ±0.81 mm is recommended, it is not commonly used. A successful difference in automatic landmark detection is generally considered to have a difference of ≤2 mm from a human operator and an acceptable difference of ≤4 mm. (29-34). In this study, the CLAHE caused over 0.59 mm error on the x-axis and 0.56 mm on the y-axis in all measurements for both examiners and AI. The mean Euclidean distances were greater than 2 mm in Co, PNS by Examiner 1, Po, Ba, Co, Go, Pog, U1RT, Me', Pog by Examiner 2, and in all measurements by WebCeph. Both examiners and WebCeph had Euclidean distance differences of less than 4 mm. This study showed that WebCeph had higher Euclidean distance differences than examiners. This indicated that WebCeph performed less accurately than trained orthodontists manually.

Hwang et al. (33) reported that YOLOv3 performed better than human examiners in repeated measurements in original LCRs. Our study compared the differences between the original LCR and CLAHE-LCR. For almost all LCR landmarks, WebCeph's differences were greater than the differences of examiners.

CLAHE has been used in dentistry to detect caries in radiographs, identify gingivitis in photos, pulp capping treatment, endodontic therapy, and improve radiographic image quality (16,35-39). In orthodontics, Nishimoto et al. (40) found no significant difference between manual and AI-assisted LCR point determination in angular and linear measurements using CLAHE. The gonion had the highest landmark error in Nishimoto's and our study (Examiner 2 and WebCeph). However, angular or linear measurements were not examined in our study.

The highest Euclidean errors in skeletal landmarks were Co and PNS in examiners and Go and Ba in WebCeph. Anatomical superpositions are common in the posterior LCR region, where these landmarks are. The Co and Ba landmarks overlap complex anatomical structures, making identification difficult. The auricular structures of X-ray machines may cover the area, making identification challenging (23,41). Ba was also the second-least accurate landmark in WebCeph. Blurring the image led by the CLAHE method and overlapping adjacent or bilateral structures have made it challenging to identify Ba.

The PNS, the posterior limit of the hard palate, can be difficult to locate on radiographic images because, as the hard palate extends towards the back, it becomes less visible on the image because of the more transparent soft palate (23). The CLAHE technique reduced image contrast and sharpness, making it difficult for examiners to find the PNS border horizontally rather than vertically.

Go is on a curved structure connecting the corpus and mandibular ramus. Mandibular shape or head position variations overlap structures, making locating difficult (23,41,42). Identifying the gonion in CLAHE-LCRs is more challenging because the image-processing technique could grey out surrounding areas, especially in double-contoured regions.

WebCeph had higher dental Euclidean mean errors than the examiners, and Examiner 1 had the lowest. The highest errors by the examiners belonged to U1RT and L1RT, while the highest for WebCeph belonged to L1RT. On WebCeph, however, the Euclidean errors of the other dental landmarks (except L1RT) were close. The reason for this can be seen in L1RT rather than U1RT may be that the bone in the maxilla has a porous structure, while the mandible has a compact structure, and this situation is more pronounced in the mandible due to the decreased sharpness and increased greyness in CLAHE.

The CLAHE method enhanced the visibility of soft tissue. WebCeph had higher Euclidean mean soft tissue landmark differences than examiners. Examiner 1 had the lowest soft tissue landmark error. The highest soft tissue landmark errors were Me' and Gn' for examiner 1, Me' and Pog' for examiner 2, and Me' and Pog' for WebCeph. Gn' and Pog' changed significantly on the y-axis for both examiners and WebCeph, while Me' changed significantly on the x-axis.

Ha et al. (43) used a YOLOv3-based convolutional neural network model to diagnose mesiodens and found that CLAHE images had lower accuracy, sensitivity, and specificity internally and externally. Image enhancement may not improve AI-based application accuracy.

Menezes et al. (44) reported that their AI-based program is excellent for cephalometric analysis. However, low brightness and strong contrast affect program landmark reproducibility and require human supervision to be clinically reliable. They suggested extra machine learning rounds for accurate landmark localization in images with variable brightness and contrast, especially for bilateral landmarks like Or and Po. Our findings confirmed the need for manual adjustment, similar to them. The differences between the original LCR and CLAHE-LCR were lower in examiners than in WebCeph.

The main limitation of this study is that only two investigators evaluated reliability, and it was a single-center study. Another limitation is that the study sample was selected only from individuals with permanent dentition. No evaluation was performed in individuals with mixed dentition or orthodontic appliances in the mouth.

5. CONCLUSION

The use of CLAHE in WebCeph requires more adjustments to identify landmarks accurately compared to the manual system. Therefore, further research is required to enhance the performance of the CLAHE method in automated systems. In most measurements, the mean differences between the original LCR and CLAHE-LCR were more significant along the y-axis than the x-axis. For this reason, the y-axis should be evaluated more carefully when evaluating the landmark position in CLAHE-LCR.

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Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: MG
Design of the study: MG, ÇST
Acquisition of data for the study: MG, ŞG
Analysis of data for the study: MG, ÇST
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Clinical and Experimental Health Sciences

Effect of Cementation and Thermomechanical Aging on the Marginal Adaptation of Veneered and Monolithic Zirconia

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ABSTRACT

Objective: The objective of current study was to evaluate the marginal discrepancy of a manually veneered CAD/CAM zirconia and two different monolithic CAD/CAM zirconia all-ceramic crowns; before and after cementation; before and after artificial aging by using a chewing simulator.

Methods: A total of 36 specimens were divided into 3 groups (n=12) and crown restorations were fabricated by using 3 different type of zirconia materials for each group: Group MZ1: monolithic zirconia crowns (GC initial), Group MZ2: monolithic zirconia crowns (InCoris TZI), Group BZ: bilayered zirconia crowns; zirconia core (InCoris ZI) veneered with a low-fusing glass-ceramic. The specimens were duplicated using epoxy resin before and after cementation and after thermo-mechanical fatigue. The marginal adaptations of replicated specimens were evaluated at six points. The margins were evaluated under a scanning electron microscope. Statistical analysis was performed using SPSS. Tukey HSD test were used to investigate the differences between the three types of zirconia crown restorations. Statistically significant difference was determined at ($p < .05$).

Results: There is a statistically significant difference between specimens before and after cementation, and as well after thermos-mechanical fatigue for the three zirconia materials.

Conclusion: The cementation process showed a significant effect on the marginal gap size in all groups. Additionally, thermo-mechanical fatigue significantly increased the marginal gap in all groups.

Keywords: Marginal fit, monolithic zirconia crown, thermomechanical fatigue.

1. INTRODUCTION

Due to increasing demands for aesthetics, metal-free ceramic restorations have been widely used in the last few years (1,2). The use of tetragonal zirconia polycrystalline which is stabilized by yttria (Y-TZP) for the manufacture of all ceramic frameworks using CAD/CAM technology is common nowadays owing to its exceptional biocompatibility, minimal plaque accumulation, and superior mechanical properties (3,4). Since zirconia has an opaque nature, the desired aesthetics can be achieved by veneering it with more translucent feldspathic porcelain (5).

Previous research has indicated that porcelain veneering and firing may have an adverse effect on the marginal integrity of zirconia-based restorations. (4,6,7).

The fitting accuracy of a full restoration may be influenced by the veneering process due to thermal distortions of the core (8). Castellani et al (9) observed significant defects in the marginal area due to the veneering process in single crowns produced using various all-ceramic systems. The all-ceramic crowns that were investigated showed higher sensitivity to repeated porcelain firing cycles compared to metal ceramic restorations. The veneering process may result in discrepancies in the marginal area, which could contribute to a gap between the restorations and the prepared teeth. (10).

The improvement of zirconia's translucency has been achieved through a reduction in the quantity of light scattering

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sources, such as alumina particles, while maintaining its mechanical characteristics at the same level. Therefore, monolithic zirconia restorations, which are anatomically shaped, were subsequently introduced (11).

With the development of high-translucency zirconia, the production of monolithic zirconia crowns has become possible without the necessity of porcelain veneering. This eliminates potential complications such as porcelain chipping and discrepancies in marginal gaps. Furthermore, monolithic zirconia systems exhibit improved mechanical and aesthetic properties (12,13).

Long term clinical success of dental restorations is affected by fracture resistance, esthetic quality and marginal fit (14). The achievement of optimal marginal fit is an essential factor that significantly influences the success and longevity of dental restorations. Inadequate marginal fit of dental restorations has been found to play a role in the accumulation of plaque and an increased risk of microleakage. These unfavorable conditions might eventually lead to the development of secondary dental caries, pulpal lesions, periodontal disease, and bone loss (15,16).

The marginal gap between 100 and 120 µm has been used as a clinically acceptable range according to the study by McLean and von Fraunhofer (17). The main factors that influence marginal fit are: tooth preparation design, cement space parameters and veneering process (18). The precision of fit may be influenced by variables among different CAD-CAM applications of production, such as: the level of scanning accuracy, the effectiveness of CAD software, the state of zirconia during milling, and the grinding protocol employed by the CAD-CAM system. Furthermore, it has been demonstrated that the post-milling hand adjustments performed by the dental technicians, make significant contributions towards improving the fit of CAD-CAM restorations (14,19,20).

Cementation may also increase the marginal discrepancies of fixed restorations according to the cement space distance. To obtain the precision of fit, the old studies recommended a cement space between 30-50 µm for resin luting cement (21). However, evidence suggests that the cement space of CAD-CAM zirconia crowns should be set at no smaller than 60 µm for better seating on the abutment with minimal need of manual adaptation (19,20).

When subjected to the conditions in oral cavity, zirconia restorations are directly in contact with moisture and exposed to pH changes, mechanical loads and variations in temperature. All of these factors may cause instability in the tetragonal phase that may lead to aging (18,22). Therefore, the evaluation of the marginal fit accuracy is performed after artificial aging as well, that maintains more accurate results of the long-term stability and outcome of the restorations (7).

The objective of the current study was to evaluate the marginal discrepancy of a bilayered CAD/CAM zirconia and two different monolithic CAD/CAM zirconia all-ceramic crowns; before and after cementation; before and after thermomechanical aging by using a chewing simulator. The null hypothesis was that the marginal discrepancy of bilayered would be affected significantly more than monolithic ones by both the cementation and thermomechanical aging.

2. METHODS

This study was approved by the Clinical Research Ethics Committee of Marmara University Faculty of Dentistry (Date-Number:27.12.2018-2018/253). Thirty-six recently extracted human mandibular molars were selected (n=36) according to the criteria including: being intact, non-carious and having similar dimensions bucco-lingually and mesio-distally. The exclusion criteria were that the teeth had: caries, restorations, anatomical defects and visible fracture lines. All teeth were cleaned by using an ultrasonic scaler and stored in 0.1% thymol solution at room temperature until tooth reduction process. The teeth were mounted in a self-curing acrylic resin (Imicryl SC; Imicryl Dental Materials, Inc, Konya, Turkey) by pouring in a cylindrical PVC mold to make cylindrical acrylic blocks. The teeth were positioned perpendicular to upper surface of acrylic blocks and 1 mm below the cement-enamel junction by using a dental surveyor (Kavo EWL; Kavo Elekrotechnisches Werk GmbH, Leutkirch im Allgau, Germany). Following a standardized tooth preparation protocol, the tooth reduction was made using a diamond rotary instrument with a convergence angle of 60 (Meisinger 880G, Hager & Meisinger, Neuss, Germany) after fixing the high-speed handpiece on the same dental surveyor (Kavo EWL, Germany). The high-speed handpiece was positioned to obtain the diamond bur parallel to the axis of the tooth to obtain approximately 60 convergence angle after reduction (Figure 1). A 1.5 mm circumferential reduction at axial and 1.5-2 mm reduction at occlusal surfaces were performed with a chamfer finish line. All sharp edges and margins were rounded. Impressions of the prepared tooth samples were made by using a putty-wash technique in dental plastic cups. After light-body silicone impression material (Elite HD+; Zhermack Spa, Badia Polesine, Italy) was syringed around the prepared teeth and putty silicone in the plastic cups, the prepared teeth were inserted in the cup by handling from the acrylic blocks. Five hours after removing the impression from the tooth samples, a type IV dental stone (Fujirock EP, GC Europe, Leuven, Belgium) was poured in the impressions. After a setting time of 1 hour, casts were removed from the impressions.

A total of 36 specimens were divided into 3 groups (n=12) and crown restorations were produced by using 3 different type of zirconia materials for each group:

- Group MZ1: monolithic zirconia crowns (GC initial; GC America, Alsip, IL, USA) (n=12)
- Group MZ2: monolithic zirconia crowns (InCoris TZI; Dentsply Sirona, Charlotte, USA) (n=12)
- Group BZ: bilayered zirconia crowns; zirconia core (InCoris ZI; Dentsply Sirona, Charlotte, USA) veneered with a low-fusing glass-ceramic (IPS Emax Ceram; Ivoclar Vivadent AG, Schaan, Liechtenstein) (n=12)

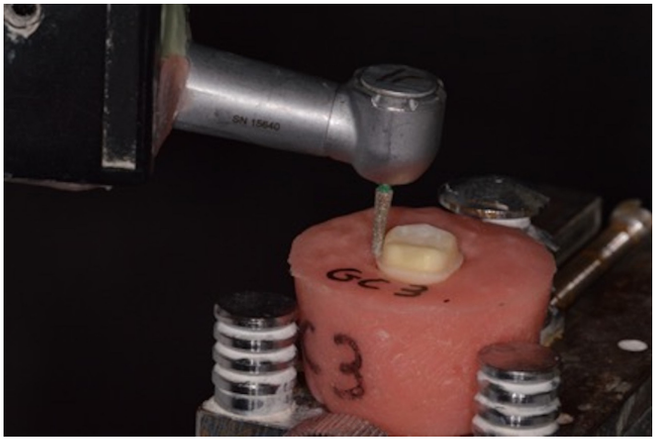

Figure 1. *The position of the high-speed handpiece fixed on the dental surveyor to obtain the reduction with a 6⁰convergence angle.*

The casts were scanned and digitized (CEREC inLab; Sirona Dental Systems GmbH, Bensheim, Germany) to fabricate the zirconia crown restorations. The crowns of the first 2 groups (Group MZ1, Group MZ2) and the frameworks of Group BZ were designed and milled by a CAD/CAM unit (CEREC inLab; Sirona Dental Systems). The crowns of Group MZ1 and Group MZ2 were milled from different pre-sintered zirconia discs (respectively; GC Initial; GC America, Alsip, IL, USA; InCoris TZI; Dentsply Sirona, Charlotte, USA) in the milling unit (CEREC inLab; Sirona Dental Systems GmbH) and sintered in a furnace (InFire HTC speed; Sirona Dental Systems). The zirconia frameworks of Group BZ (thickness 0.7 mm) were steam cleaned and veneered with layering porcelain (IPS e.max Ceram; Ivoclar Vivadent AG). The ceramic material performed the firing process in a furnace (Programat S1; Ivoclar Vivadent AG). The manufacture of monolithic restorations (GC Initial; InCoris TZI) were left without ceramic layering and only a glaze layer was applied (Ceram Glaze; Ivoclar Vivadent AG, Schaan, Liechtenstein). A cement space of 60 µm was selected for all crowns during the designing procedure.

The crowns were seated on their relevant abutments and an impression was made for each sample using polyvinyl siloxane impression material (Elite HD+ silicone impression material; Zhermach, Badia Polesine, Italy). Then, the models were obtained by pouring epoxy resin material (Morasin; Moravia, İstanbul, Türkiye) in the impressions. Thus, the replicas of crowns before cementation were prepared for measuring the marginal gaps. To measure the marginal gaps of crown after cementation, all crowns were cemented on their relevant abutments using a self-adhesive resin cement (G-CEM LinkAce; GC America, Alsip, IL, USA). After the resin cement was applied in the crowns, the crowns were cemented with finger pressure and light cured for 20 seconds to all surfaces and margins. The impression was made for cemented crowns and, the epoxy models for crowns after cementation were made as mentioned above. Thus, the replicas of crowns after cementation were prepared for measuring the marginal gaps.

All specimens were artificially aged in a computer-controlled chewing simulator CS-4 (SD Mechatronik). The cemented crowns were submitted to an aging procedure: 2 400 000 cycles, 80 N, at 37°C under artificial saliva bath. The load was applied vertically to the central occlusal fossa of the crowns using a 1.7 Hz steel antagonist ball with a diameter of 6 mm. The test chambers were subjected to a thermal cycling process involving the flooding of deionized water at a temperature of 5°C for a duration of 30 seconds and subsequent flooding with water at a temperature of 55°C for another 30 seconds to result a total of 3000 thermal cycle. After the simulation in the chewing machine the impression made on artificially aged crowns and the epoxy models for crowns were made as mentioned above. Thus, the replicas of crowns after artificial aging were prepared for measuring the marginal gaps.

The replicated specimens were subjected to evaluation of the marginal adaptation for six points: two buccal, two lingual, one mesial and one distal points. The margins were evaluated in entirety under a standard 200x magnification scanning electron microscope (Zeiss EVO LS100 Carl Zeiss AG, Germany) to distinguish the marginal gap between the three groups materials before cementation, after cementation and after thermomechanical fatigue test. The marginal gap interfaces were identified by an expert technician using SEM images (Figure 2).

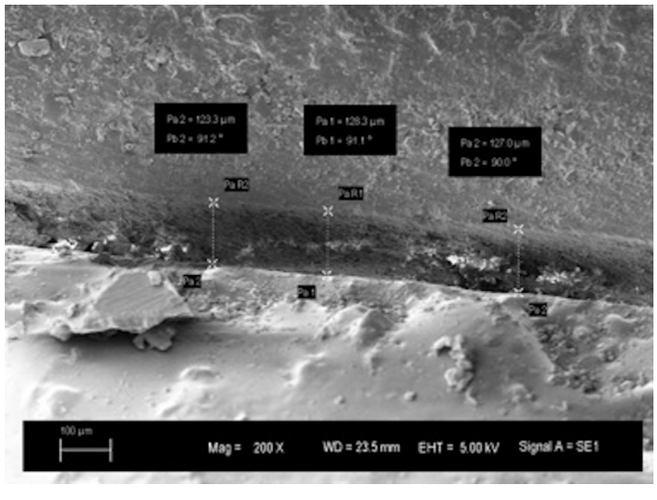

Figure 2. *The measurement of the marginal gaps on 200x magnification SEM images.*

Data was entered and analyzed using SPSS (SPSS 23.00, SPSS Inc., Chicago, IL, USA), descriptive statistics and inferential statistic techniques were used for data analysis. One-way ANOVA test was used to compare between the groups. Repeated measures ANOVA was used to analyze the effect of cementation and aging. Tukey HSD test was used to analyze the differences between the three types of Zirconia. Statistically significant difference was determined at $p< .05$.

3. RESULTS

The mean values and standard deviations (SDs) of the measured vertical marginal gaps at the six measuring points for the three groups are shown in table 1. The lowest marginal gap was recorded in MZ1 group before cementation while the highest marginal gap was recorded in the BZ group after aging. One-way ANOVA test showed no significant difference in the mean marginal gap between the three groups before cementation, after cementation and after aging ($p < .05$). Repeated measures ANOVA test showed that statistically significant difference found between before cementation, after cementation and after aging in each one of the three groups ($p < .001$).

Tukey results showed that all groups showed statistically similar marginal gaps before cementation and after cementation while there was statistically significant difference after aging between MZ2 and BZ groups (Table 2).

Table 1. *Mean and standard deviation values of marginal gap before cementation, after cementation and after aging for MZ1, MZ2 and BZ (µm*

Group	Before Cementation	After Cementation	After Aging	p*
MZ1	64.48(13.92)	104.25(8.75)	123.84(10.07)	.000
MZ2	64.71(10.98)	101.71(7.75)	120.68(9.35)	.000
BZ	62.52(10.42)	103.58(9.03)	126.05(11.62)	.000
#p	.660	.602	.306	

**Repeated measures ANOVA test, p= .001*
 # One-way ANOVA Test, p= .05

Table 2. *Paired comparisons between groups*

Groups	Before cementation		After Cementation		After aging	
MZ1	MZ2	BZ	MZ2	BZ	MZ2	BZ
	.993	.582	.177	.884	.165	.410
MZ2	MZ1	BZ	MZ1	BZ	MZ1	BZ
	.993	.511	.177	.390	.165	.006*
BZ	MZ1	MZ2	MZ1	MZ2	MZ1	MZ2
	.582	.511	.884	.390	.410	.006*

According to Tukey HSD test
**The mean difference is significant at the p= .05 level.*

4. DISCUSSION

This study evaluated the marginal fit of 3 different zirconia all-ceramic crowns before cementation, after cementation and after thermomechanical fatigue loading in a masticatory simulator. The null hypothesis, that the marginal discrepancy of bilayered zirconia would be affected significantly more than monolithic ones by both the cementation and thermomechanical aging was accepted.

CAD/CAM zirconia is fabricated mainly by 2 different methods such as: directly digitalization intraorally and indirectly digitalization of the cast produced by an analog impression. It was reported that, although direct digitalization method indicates significantly smaller values for marginal fit in addition to time and material consuming disadvantages of indirect digitalization, the marginal fit for both methods were within the range of clinical acceptance. In current study, indirect digitalization method was used for all group samples' fabrication (20).

In current study, to evaluate the marginal gaps at different fabrication stages between different groups of materials, replica technique was used. This technology takes less time to create specimens and the original abutment tooth can be conserved (7).

Scanning Electron Microscopy (SEM) technique was also used to determine the gap marginal adaptation of crowns fabricated with 3 different zirconia CAD/CAM materials. This method is considered reliable and accurate method for the evaluation of accuracy of dental restorations (23). Some studies in CAD/CAM restorations measure the marginal gap using SEM with different techniques; either directly or from epoxy replicas (24-26). On the other hand, Groten et al (27) reported that the potential of error in SEM is around 10%, making it unsuitable for the assessment of marginal gap. This is mostly due to variances in electron beam strengths in SEM, which result in disparities between black and white graphic regions on the scanned materials. The thermomechanical fatigue and cementation that was applied to the ceramics, proved deterioration in marginal gap, in the current study, thus it could be argued that the SEM is a good predictor of the marginal gaps (28,29).

According to Ferrini et al (30) measurements were taken at the buccal, palatal, mesial, and distal parts of the abutment-coping interface, as well as at intermediate levels of these points for a total of eight readings, which is close to current study. In the current study marginal gap was measured two points from buccal, two points from palatal, one point from mesial and one point from distal surfaces in total 6 readings.

In all experimental groups the mean marginal gap before cementation was between 62.5 µm and 64.4 µm, and after cementation it ranged between 101.7 µm and 104.2 µm. This is considered as a clinically acceptable gap. Guess et al (31) found a mean marginal gap of 30-35 µm before cementation and 49–63 µm after cementation. Other studies also reported that the marginal fit before cementation ranged between 50 to 60 µm (32,33). The marginal fit usually increases significantly after cementation due to numerous factors such as viscosity of the luting agent, filler particle size and preparation design (34). In this study, the finger of the researcher was used to press on the crowns during the cementation procedure. This step was used to simulate the clinical procedure. Att et al (7) also used finger pressure to simulate the clinical procedure but stated that it is considered as a limitation of the study because the pressure of the finger is variable and couldn't be standardized.

The main objective of the study was to determine the difference between before and after cementation and after thermo-mechanical fatigue for each zirconia material. The

results presented show a statistically significant difference in nearly all the variables, this accepts the null hypothesis. In all zirconia materials, there was a statistical difference between before and after cementation, a difference between before cementation and after thermo-mechanical fatigue, as well there was a difference between after cementation and after thermo-mechanical fatigue.

The results of this study were consistent with Martinez-Rus et al (35) who evaluated the marginal adaptation between computer-aided design/computer-assisted manufacture lithium disilicate, pressed lithium disilicate and CAD yttrium-stabilized tetragonal zirconia polycrystalline on implant abutments before and after cementation and concluded that marginal discrepancies increased significantly after cementation for all abutment – crown combinations. In addition, Kale et al (13) compared the marginal fit of monolithic zirconia crowns before and after cementation and found that the cementation significantly affected the marginal gaps of CAD-CAM monolithic zirconia crowns.

On the other hand, Gonzalo et al (21) evaluated the marginal fit of 3-unit FPDs manufactured from Lava All-Ceramic System, Procera Bridge Zirconia, VITA In-Ceram before and after cementation and concluded that the marginal gap was increased slightly after cementation in all the groups but the difference was not significant and explained this result that the luting space of 50 µm is enough to obtain adequate space for the cement.

Regarding the thermomechanical aging effect on the marginal gap, several study results were consistent with the results of this study (36-38).

Thermal and mechanical load cycles may generate significant stresses at the interface of the restorations, resulting in the failure of the cement interface. The marginal adaptation might undergo additional degradation due to the varying thermal expansion between the cement and the tooth or restoration, and also because of the repeated application of mastication forces (36,39,40). On the contrary, Del Pinal et al (18) conducted a study comparing the marginal fit before and after thermomechanical fatigue of veneered zirconia and monolithic zirconia crowns and stated that the aging process did not alter the marginal fit of zirconia crowns.

Regarding the differences of the marginal gap between the groups, there was no significant differences between any of the groups before cementation or after cementation. After thermomechanical fatigue, BZ group showed significantly higher mean marginal gap compared to MZ2 group.

Similar to this result, Rayyan (12) compared the marginal fit between porcelain veneered zirconia crowns and high-transluceny monolithic zirconia crowns and concluded that monolithic zirconia crowns showed superior marginal accuracy than porcelain-veneered zirconia crowns. The larger marginal space of veneered copings may be attributed to a number of factors, including the firing shrinkage of veneering porcelain. The porcelain shrinkage produces a compressive force on the frameworks and causes enlargement of the gap (41).

Conversely, Saraswathi et al (42) compared between monolithic zirconia and multilayer zirconia crowns in terms of marginal gaps and found no significant differences. They suggested that the resistance to porcelain firing shrinkage of zirconia copings was a result of their superior strength.

Several limitations were identified in the present study. The use of extracted natural teeth might alter the optimal standardized conditions. Additionally, finger pressure was used during cementation to press on the crowns until the cement was set. These are considered limitations but were performed to simulate the clinical conditions.

5. CONCLUSION

With regard to the limitations of this study, the subsequent results may be derived: All evaluated materials before cementation and after cementation showed clinically accepted mean marginal fit (less than 120). After aging process, all evaluated materials showed mean marginal gap higher than the accepted range. The cementation process showed a significant effect on the marginal gap size in all groups. Additionally, thermo-mechanical fatigue significantly increased the marginal gap in all groups.

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Clinical and Experimental Health Sciences

The Relationship of Trace Element Levels with Obesity and Cardiovascular Health

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ABSTRACT

Objective: One of the most important factors affecting cardiovascular health is obesity. Trace elements, which play a role in every stage of metabolism, are also related to our cardiovascular health. The aim of this study was to determine serum trace element levels in individuals and to examine the relationship between trace elements and cardiovascular risk.

Methods: This cross-sectional study was conducted with individuals who applied to the outpatient clinic between 01.03.2022-31.04.2022. Sociodemographic characteristics, BMI, and Framingham risk score values were recorded. Serum iron, zinc, copper and selenium levels were analyzed in the laboratory.

Results: A total of 180 individuals were included in the study. Of the individuals, 33.3% were overweight and 32.8% were obese. The mean Framingham Risk Score (FRS) was 9.31±7.99 (1-37). FRS values of normal weight individuals (7.84±3.05) were lower than the mean FRS values of overweight (8.80±3.39) and obese (13.39 ± 6.24) groups (p<.001). There was a weak positive correlation between serum copper levels and BMI (r=.176 p=.018). No significant correlation was found between serum iron, zinc and selenium levels and BMI and FRS.

Conclusion: There was a weak positive correlation between copper levels and BMI values, however Se, Zn and Fe levels were not associated with BMI and FRS values. Randomized controlled trials are needed to introduce serum trace element determination in to practice in the diagnosis and follow-up of obesity and cardiovascular disease.

Keywords: Serum trace element, body mass index, Framingham risk score.

1. INTRODUCTION

Obesity and cardiovascular diseases (CVD), which are increasing in frequency in the general population, are two diseases that are closely related to each other in terms of both etiology and prevalence in the society (1). According to studies conducted in recent years, reactive oxygen derivatives formed as a result of oxidative stress and immune response triggered by cell damage come to the fore in the etiology of both diseases (2). Elements whose amount in the human body is less than 100 mg/kg are called trace elements. This group includes elements such as iron, selenium, chromium, copper, zinc, and iodine (3). A link has been established between deficiency or excess of these trace elements and many diseases (e.g. iron deficiency – anaemia or zinc deficiency – dermatitis) (4). Some trace elements such as zinc, copper, and selenium are closely associated with inflammation and peroxidation and may be responsible for the pathogenesis of cardiovascular disease and obesity (5,6).

Zinc (Zn) is essential for the maintenance of normal cellular structure and functions. The extracellular and intracellular levels of Zn are also related to cardiovascular health (7). According to Little et al., plasma Zn levels decrease with age and have a strong association with increasing CVD (8). Moreover, recent advances in cardiac biology and pathophysiology have highlighted the critical contribution of perturbations in Zn homeostasis to myocardial ischemia/reperfusion injury and

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the role of Zn signaling in cardioprotection against ischemia/reperfusion injury (9). Zn has a regulatory role in oxidative stress, inflammation, apoptosis and insulin secretion. In addition, Zn has roles in carbohydrate-lipid metabolism and cell division-regeneration (10). Many of these metabolic pathways are found to be impaired in obese patients (11). Considering these conditions, it may be thought that Zn deficiency or excess plays a role in the etiology of obesity. Obesity, oxidative stress and inflammation are factors known to predispose to cardiovascular diseases (12).

Iron (Fe) is an important mineral needed by almost all living organisms (13). The maintenance of iron homeostasis is essential for proper cardiac function. A growing body of evidence suggests that Fe imbalance is the common denominator in many subtypes of cardiovascular disease (14). Fe is a trace element that can affect the clinical course of many chronic diseases such as obesity, type 2 diabetes and atherosclerosis (15). In many studies, it has been associated with weight gain, cardiovascular disease and metabolic syndrome development, insulin receptor resistance and Type-2 diabetes due to the lack or excess of Fe and the inflammation caused by these conditions (16-18).

Copper (Cu) deficiency plays an important role in the unspecific manifestations of hematologic, neurologic, immunologic, dermatologic, cardiovascular, and skeletal defects. Cu in the body is one of the cofactors of enzymes involved in mitochondrial function, inflammatory response and anti-oxidative functions (19). Additionally, copper is involved in vascular changes in the heart. Kunutsor et al. showed that a higher serum Cu level increases the risk of atherosclerotic heart disease by regulating lipid metabolism, low-density lipoprotein oxidation, and inflammatory response (20).

Selenium (Se) plays an important role in the proper functioning of the entire human body. It is involved in the processes of reproduction, carcinogenesis, mechanisms of the immune response and in regulating the work of cardiovascular system (21). Recent studies have demonstrated the role of Se in the modulation of important molecular in adipose tissue (22,23). Therefore, both selenium deficiency and excess selenium may contribute to adipose tissue dysfunction and metabolic alterations that are particularly important in the context of obesity and cardiovascular disease.

It is hypothesized that excessive adiposity alters the absorption, distribution, metabolism, and excretion of micronutrients, thereby compromising their bioavailability and limiting their function in the body (24-26).

Few studies have examined the relationship between cardiovascular risk, obesity and trace elements together. In this context, the aim of the study is to determine the Framingham Risk Score and serum trace elements (copper, zinc, iron and selenium) and examine the relationship with obesity.

2. METHODS

2.1. Study Design

The cross-sectional study consisted of individuals who applied to the Family Medicine Polyclinic for periodic health examination between 01.03.2022 and 31.04.2022. Volunteers who applied to the family medicine outpatient clinic after the ethics committee approval and met the inclusion criteria were divided into 3 groups according to BMI. Individuals who applied from each group were included in the study by selecting consecutive odd numbers (1-3-5-7...) in order, randomisation was ensured. During the selection, an attempt was made to keep the age distribution, gender and smoking similar between group. Since the number of individuals in the population was unknown, the sample size was calculated using the appropriate formula. According to this calculation, taking into account a 10% margin of error, the study was completed with 180 people.

2.2. Exclusion Criteria

Those under 18 years of age, those with a psychiatric diagnosis that would impair cooperation, those who could not communicate, those with a diagnosis of syndromic obesity, those with known cardiovascular disease, those with a diagnosis of cancer, use of mineral supplements, and those with a diagnosis of gastrointestinal malabsorption were excluded.

2.3. Ethical Authorisation of the Study

Prior to the study, Necmettin Erbakan University Faculty of Medicine ethics committee approval was obtained (Number: 2021/3431 Date: 01.10.2021). After ethical approval, Scientific Research Project (BAP) support was obtained from Necmettin Erbakan University (Project number 221518001). Individuals were briefly informed about the purpose of the study and their verbal and written informed consent was obtained according to the principles of the Declaration of Helsinki.

2.4. Collection of Data

The sociodemographic introductory questionnaire form for the individuals included in the study was prepared by examining the literature on the subject. The form consisted of questions about sociodemographic characteristics such as marital status, age, employment status, presence of chronic diseases, medication use, education level and smoking status. Those who had a hemogram, total cholesterol, HDL and LDL cholesterol level, insulin level, fasting blood level and thyroid stimulating hormone (TSH) level planned in the last month or in the current examination, and those who also met the criteria were included in the study after their consent was obtained. The results of these individuals were obtained from the application files and recorded in the form. Blood pressures were measured with a suitable cuff in a

rested and sitting position. Shoes were removed and height was measured with a standard meter on a hard surface and weight was measured with a standard weighing device after removing excess clothing. Body mass index (BMI) of the participants was calculated in kg/m2.Those with BMI<25 kg/m2 were considered normal weight, those with BMI=25-30 kg/m2 were considered overweight and those with BMI≥30 kg/m2 were considered obese. Participants' waist circumference (WC) was measured above the umbilicus.

2.4.1. Framingham Risk Score

It is one of a number of scoring systems used to determine a person's ten-year risk of developing cardiovascular disease. There are two separate scoring for women and men. In the study, after calculating the parameters of age, smoking, systolic blood pressure, being on antihypertensive treatment, total cholesterol, HDL-cholesterol level, the ten-year cardiovascular disease risk of the participants was calculated via the website https://www.mdcalc.com/framingham-risk-score-hard-coronary-heart-disease. FRS classification was made according to the following ranges "Low risk (10-year risk of coronary heart disease < 10%)"; "Moderate risk (10-year risk of coronary heart disease 10-20%)"; "High risk (10-year risk of coronary heart disease > 20%)" (27).

2.4.2. Laboratory Analysis

Collection of Blood Samples

Human venous blood samples were collected in gel tubes containing clot activator. The blood samples were centrifuged in a Hettich Rotina 46R (Hettich Zentrifugen, Tuttlingen, Germany) refrigerated centrifuge at 40°C, 1000 g for 10 minutes and serum samples were separated.

Measurement of Serum Copper Levels

Cu levels in serum samples were measured by spectrophotometry using a Beckman Coulter AU 480 autoanalyzer (Beckman Coulter, Brea, California, USA) and kits, calibrators and controls from Archem (Archem Diagnostic Industry, Istanbul, Türkiye). Serum copper levels were calculated as µg/dL.

Measurement of Serum Zinc Levels

Zn levels in serum samples were measured by spectrophotometry on a Beckman Coulter AU 480 autoanalyzer (Beckman Coulter, Brea, USA) using a kit, calibrator and controls from Archem (Archem DiagnosticIndustry, Istanbul, Turkey). Serum zinc levels were calculated as µg/dL.

Measurement of Serum Iron Levels

Fe levels in serum samples were measured by spectrophotometry on a Roche Cobas c702 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany) using kits, calibrators and controls from Roche (Roche Diagnostics GmbH, Mannheim, Germany). Serum Fe levels were calculated as µg/dL.

Measurement of Serum Selenium Levels

Se levels in serum samples were measured by inductively coupled plasma mass spectrometry (ICP-MS) on a NexION 350X ICP-MS analyzer (Perkin Elmer Inc., Shelton, Connecticut, USA) using kits, calibrators and controls from Perkin Elmer (Perkin Elmer Inc., Shelton, Connecticut, USA). Serum selenium levels were calculated as µg/L.

2.5. Statistical Analysis

Statistical analyses were performed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) for Windows 22.0. Mean and standard deviation values of numerical data were calculated. Compliance with normal distribution was evaluated with the Kolmogorov-Smirnov test, and categorical and numerical data were compared with appropriate statistical analyzes such as chi-square, student-t test, and one-way ANOVA. Correlation analysis was used to determine the relationship between variables. In cases where there was a difference between groups, significance was evaluated with the Post-Hoc Tukey test. Significance value was accepted as $p< .05$.

3. RESULTS

The study was completed with 180 individuals. 50.0% of the individuals included in the study were male (n = 90). Of the participants, 76.12% (n = 137) were married and 47.22% (n = 85) had a university degree. Sociodemographic data of the participants are given in Table-1.

The mean age of the participants was 43.36±8.58 (30-65) years, mean systolic blood pressure was 117.96±12.46 mmHg and mean diastolic blood pressure was 76.17±9.35 mmHg. Mean FRS was 9.31±7.99 (1-37), and mean BMI was 27.58±5.70 (18-60.55) kg/m2. Individuals were 33.9% (n=61) normal weight, 33.3% (n=60) overweight and 32.8% (n=59) obese. Men were 34.4% normal weight, 33.33% overweight and 32.2% obese. For females, there was an equal distribution of 33.3% in each BMI group ($p > .05$). The mean FRS of the individuals was 7.84±3.05 in the normal weight group, 8.80±3.39 in the overweight group and 13.39±6.24 in the obese group. The mean FRS of the normal weight group were significantly lower than those of the obese and overweight groups ($p < .001$ and $p < .001$, respectively).

Mean trace element levels were measured as serum Fe 81.09±39.22 µg/dL, serum Zn 112.48±38.14 µg/dL, serum Cu 101.29±35.44 µg/dL and serum Se 83.03 ± 9.11 µg/L (Table 2). The mean trace element levels of the individuals who participated in the study were compared in groups formed according to BMI values. No statistically significant difference was found between the groups in terms of mean Fe, Zn, Cu and Se levels ($p > 0.5$). Comparison of mean trace element levels of individuals according to BMI and statistical analysis values are given in Table 3.

Table 1. *Distribution of the Sociodemographic Characteristics of the Participants*

	n	%
Sex		
Male	90	50.00
Female	90	50.00
Marital Status		
Married	137	76.12
Single	43	23.88
Educational status		
Primary school	44	24.44
Middle school	17	9.44
High school	34	18.89
University	85	47.22
Working status		
Working	127	70.56
Not-working	53	29.44
Chronic disease condition		
Yes	72	40.00
No	108	60.00
Use of medication		
Yes	63	35.00
No	117	65.00
Smoking		
Yes	89	49.44
No	91	50.56
Total	**180**	**100**

Table 2. *Demographic and clinical characteristics of the participants (n=180)*

Parameters	Mean ± SD	Min	Max
Age (Year)	43.36 ± 8.58	30	65
Systolic Blood Pressure (mmHg)	117.96 ± 12.46	90	167
Diastolic Blood Pressure (mmHg)	76.17 ± 9.35	50	101
Framingham Risk Score (FRS)	9.31 ± 7.99	1	37
Waist Circumference (cm)	95.63 ± 15.08	61	132
Body Mass Index (BMI)	27.58 ± 5.70	18.0	60.55
Serum Iron (µg/dL)	81.09 ± 39.22	12.20	237.50
Serum Zinc (µg/dL)	112.48 ± 38.14	36.0	336.0
Serum Copper (µg/dL)	101.29 ± 35.44	8.0	225.0
Serum Selenium (µg/L)	83.03 ± 9.11	52.30	99.60

Mean Cu levels were 103.70±31.66 µg/dl in the low FRS group, 85.21±33.84 µg/dl in the intermediate risk group and 111.80±44.23 µg/dl in the high risk group. Mean Cu values were statistically significantly lower in the intermediate risk group compared to the low and high-risk groups ($p < .01$; $p < .005$, respectively). There were no significant differences between the risk groups in terms of other trace element levels. Comparison of mean trace element levels of individuals according to FRS is given in Table 4.

Table 3. *Comparison of mean trace element levels of individuals according to BMI*

BMI*	Normal weight (n = 61)	Overweight (n = 60)	Obese (n = 59)		
Parameters	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	***F***	***p*****
Serum Iron (µg/dL)	88.04 ± 48.47	73.18 ± 32.74	81.94 ± 33.34	2.222	.112
Serum Zinc (µg/dL)	107.59 ± 40.47	110.86 ± 29.75	119.18 ± 42.66	1.475	.232
Serum Copper (µg/dL)	106.24 ± 40.77	97.41 ± 29.28	100.12 ± 35.24	.986	.375
Serum Selenium (µg/L)	83.45 ± 8.62	83.11 ± 9.26	82.51 ± 9.58	.161	.851

**BMI: Body Mass Index **One Way ANOVA*

Table 4. *Comparison of mean trace element levels of individuals according to Framingham Risk Score*

FRS	Low risk (n = 112)	Moderate risk (n = 37)	High risk (n = 31)		
Parameters	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	***F****	***p****
Serum Iron (µg/dL)	85.30 ± 38.09	77.90 ± 46.08	69.67 ± 32.41	2.100	.125
Serum Zinc (µg/dL)	114.30 ± 36.44	106.29 ± 30.60	113.29 ± 50.80	.618	.540
Serum Copper (µg/dL)	103.70 ± 31.66	85.21 ± 33.84	111.80 ± 44.23	5.714	**.005**
Serum Selenium (µg/L)	83.22 ± 9.43	83.54 ± 7.88	81.74 ± 9.49	.390	.677

**:One Way ANOVA*

4. DISCUSSION

Non-communicable diseases such as cardiovascular diseases (CVD), obesity, cancer, chronic respiratory diseases and diabetes are leading causes of morbidity and mortality in both developed and developing countries. Lifestyle changes such as healthy nutrition, regular physical activity and smoking cessation are necessary to prevent CVD (1). The oxidant or antioxidant functions of metals such as Fe, Zn, Cu and Se may also have effects on cardiovascular health (28). However, there is no consistent evidence that these supplements may affect CVD in healthy individuals without known nutritional deficiencies (29). Despite the known role of trace elements in maintaining general health, there is controversy regarding their specific effects on CVD risk. There are few studies in the literature examining the relationship between obesity, cardiovascular mortality risk and trace elements in Türkiye. This study is one of the few studies that simultaneously investigate Zn, Fe, Se and Cu elements along with obesity and CVD in individuals. the presented study, There was no evidence that trace element levels affect cardiovascular risk, although a correlation was found between serum trace element levels and lipid levels.

Obesity directly contributes to the development of cardiovascular risk factors such as dyslipidemia, type 2 diabetes, hypertension and sleep disorders (1). Recent data emphasize that abdominal obesity, as measured by waist circumference, is a marker of cardiovascular disease risk independent of body mass index (30). Thus, even in normal weight individuals, higher waist circumference (WC) may indicate higher CVD risk because WC is an indicator of abdominal body fat, which is associated with cardiometabolic disease and CVD and predicts mortality (31). The present study has demonstrated that those with a higher 10-year risk of coronary heart disease exhibit a higher waist circumference. In a large-scale study by Dhaliwal et al., it was demonstrated that central obesity is associated with a high FRS score (32). Given the close relationship between obesity and cardiovascular disease, it can be posited that this is also valid for central obesity (33).

Trace element levels in the human body have a great importance in the tissues of the cardiovascular system as well as in all tissues of the human body (15,21,34). Cu, a trace element, is essential for enzyme function and has an important role as both a pro-oxidant and an antioxidant. It acts as a catalytic cofactor of enzymes such as Cu/Zn superoxide dismutase, ceruloplasmin, and lysyl oxidase, which has a central role in the strength and integrity of the heart and blood vessels (35). Cu is also essential to mitochondrial respiration and Fe absorption. Elevated Cu levels may increase the production of reactive oxygen species (ROS) and consequently oxidative stress (36), resulting in the oxidation of lipids, proteins, DNA, homocysteine and other particles (37). Cu deficiency, on the other hand, can cause peroxidative damage (38). Both Cu deficiency and overload play key roles in atherogenesis. As an essential trace element, Cu has been considered to play an important role in lipid metabolism. High serum Cu was associated with elevated serum concentrations of TC and HDL cholesterol (39). The zinc-copper-blood pressure relationship is important because of the biochemical relationship between Zn and Cu and because both elements play important roles in blood pressure regulation (40). In this study, no correlation was found between Cu levels and BMI. However, Cu levels were higher in those with a higher 10-year atherosclerotic heart disease (ASCVD) risk.

Cheng et al (2022) observed negative associations of chromium (Cr) and Se with 10-year ASCVD risks in their study, in which they aimed to evaluate the factors that they thought could predict the relationship of trace elements with the 10-year risk of ASCVD in 607 elderly adults living in China. In addition, a positive relationship between Cr and HDL cholesterol and a negative relationship between Se and systolic blood pressure were found in both linear regression (41). In this study, a positive correlation was found between total cholesterol levels and Se levels. As the key component of glutathione peroxidase with unique antioxidant properties, Se has been considered to play an important part on lipid metabolism (22). Ju revealed that selenium concentrations were positively correlated with TC, HDL-c, TG and LDL-c (42). In the present study, no significant correlation was found between serum trace element levels and age, systolic and diastolic blood pressure, and TG levels.

Analysis of factors relevant to CHD identified associations between Fe levels and risk factors including inflammation, obesity, proatherogenic as well as antiatherogenic and antioxidant components. Such associations reveal the pathophysiologic mechanisms of the relationship between Fe and CHD. Fe trapped in the macrophages within the arterial wall serves as an oxidative stress mediator and has been identified as a novel risk factor for vascular disease progression (28). Cebi et al. investigated the relationship between iron and coronary heart disease and found that serum iron levels were lower in individuals with coronary heart disease (38). Iron deficiency (ID) is also an important predictor of cardiovascular events and all-cause mortality (43). Fe can also stimulate the formation of reactive oxygen species (ROC) and thus cause lipid peroxidation and atherosclerosis (44). However, several observational studies and meta-analyses have not supported the adverse effect of Fe status on CHD risk (27). This evidence challenged the hypothetical cardiotoxic effect of Fe and its role in the development of cardiovascular events. In the present study, no association was found between serum iron levels and 10-year ASCVD risk. In the NHANES-3 study, individuals were followed closely for 6 years in terms of cardiovascular diseases and low serum Se levels were found to be associated with high cardiovascular disease (45).

In a study conducted in postmenopausal women, it was reported that the daily intake of all minerals evaluated was not dependent on BMI (46). Another study found that high Se levels were associated with an increased risk of obesity, while high Cr levels were associated with a decreased risk of obesity (47). In a study evaluating the association of trace elements with obesity and hypertension in adult women, Fe and Cu were elevated in obese patients, Cu and Se content was higher in patients with hypertension. Zn levels were significantly lower in obese women with and without hypertension compared to healthy controls and normal weight women with hypertension (48). In the present study, no correlation was found between BMI and serum trace element levels.

A review of the literature revealed a paucity of studies investigating the relationship between 10-year ASCVD risk and serum trace element levels. The study's strength lies in its evaluation of trace elements in conjunction with BMI and 10-year ASCVD risk.

Limitations

This study has some limitations. First of all, we did not include the effect of daily consumed food and supplementary foods/pills in our study parameters. Second, the study was conducted on a limited number of participants. It cannot be generalized. It can be considered as a preliminary study for a large-scale study to be conducted in the future with more

samples from different regions of Türkiye. We think that more comprehensive studies are needed in which individuals' chronic diseases, nutritional status and geography are questioned, and factors that will affect trace element levels in the blood are minimized.

In conclusion, the results of study provide some evidence for the association of serum trace element levels with obesity and the risk of developing cardiovascular disease. There was no evidence that trace element levels affect cardiovascular risk, although a correlation was found between serum trace element levels and lipid levels. Considering the prevalence of cardiovascular diseases in the population, weight control may prevent death or disability due to cardiac causes. In this context, dietary interventions and appropriate physical activity for weight control in obese people need to pay more attention to both the quantity and quality of food. As family physicians, we are able to predict the risks that may occur as we constantly follow the individual from birth to death. The breadth of the facilities of the clinician in charge of diagnosis and follow-up in people with obesity and cardiovascular disease is an important issue. More well-designed, large-scale studies are needed to clarify the relationship between trace elements and cardiovascular health or evaluate the possibility of using these elements as biomarkers in clinical practice.

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Clinical and Experimental Health Sciences

Evaluating the Role of Nrf-2/HO-1 Pathway in Glioblastoma Treatment Efficacy: A Co-Culture Study

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ABSTRACT

Objective: Glioblastoma (GB) is a highly lethal form of brain tumor. Although standard therapy appears to be effective, the survival time is quite short, and the recurrence rate is high. Bortezomib (BTZ), is a proteasome inhibitor, used in GB therapies and resulted in serious off-target effects. Carfilzomib (CFZ), is an alternative for BTZ, has known with nonserious off-target effects. This study aimed to examine the potential off-target effects caused by BTZ and CFZ in terms of the therapy related activation of antioxidant mechanisms regarding to Nuclear factor (erythroid-derived 2)-like 2 (Nrf-2)/Heme Oxygenase-1 (HO-1)-dependent response.

Methods: GB cells were co-cultured with heathy astrocyte (HA) cells to mimic the tumor microenvironment in some extent. Cell viability was determined following ionizing radiation (IR), temozolomide (TMZ), BTZ and CFZ alone and in combination. Nrf-2 and HO-1 protein expressions were analyzed by western blotting assay.

Results: Co-culture results showed that the GB cells in the BTZ-treated groups expressed higher levels of Nrf-2 and HO-1 than in the CFZ-treated groups. In the HAs, the group treated with CFZ showed higher Nrf-2 expression than the group treated with BTZ alone, while the same groups in combination with TMZ&IR showed exactly opposite results. HO-1 expression was also not seen in any of the HA groups.

Conclusion: The significant increase in Nrf-2 levels in the CFZ-treated group in the HAs could also be interpreted as CFZ promoting the defence of healthy cells against therapy-induced stress conditions. Although further studies are needed, these preliminary results show that the evaluation of CFZ as a second-line therapy could be a milestone for the treatment of GB.

Keywords: Glioblastoma, proteasome inhibitors, bortezomib, carfilzomib, nuclear factor (erythroid-derived 2)-like 2, heme oxygenase-1

1. INTRODUCTION

Glioblastoma (GB) is the most common and most aggressive grade IV astrocytoma in adults. As seen in clinical studies, GB is a disease with a poor prognosis with a 2-year survival rate of 26-33% and a 5-year survival rate of only 4-5% (1–4). The standard treatment includes the combination of temozolomide (TMZ) and ionizing radiation (IR) after surgical resection of the tumor (if possible) (3,5).

TMZ is an alkylating chemotherapy agent that causes DNA damage in tumor cells, including DNA double strand breaks, which trigger apoptosis and cytotoxicity (6,7). However, development of high rate of resistance to IR and TMZ in tumor cells, results in the recurrence of the disease. Since the exact mechanism and related pathways that underlying the recurrence and resistance of disease is not clear, the presence of GB stem cells (GSCs) is known as leading cause as for now (8,9). As shown in a recently published study (10), one of the struggles about GSCs that must be solved is their higher levels of proteasome activity which causes resistance to therapy. Another cause of therapy resistance is the reversal of the TMZ activity in tumor cells by the help of O-methylguanine-DNA-methyltransferase. It is seen that the proteasome inhibitor, bortezomib, is able to both inhibit proteasome and methyltransferase enzyme and helps to overcome this resistance with its chemo-sensitizing effect (11,12). In the light of these and related other studies, the need of proteasome inhibitors in GB treatment is confirmed.

The ubiquitin proteasome system which is responsible for the degradation of damaged proteins, regulation of signal

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pathways and immune response, is the major protein degradation mechanism in eukaryotic cells to balance their cellular homeostasis. Since cancer cells maintain their higher proliferation rates mostly depend on this system, proteasome inhibitors have been tried for the treatment of diverse types of cancer including GB, for more than 15 years (13,14). Bortezomib (BTZ), one of the FDA-approved first-generation proteasome inhibitors, is frequently used in combination in the clinic in hematological cancers and increases survival at a high rate (15). BTZ is a proteasome inhibitor that reversibly inhibits both the caspase-like and chymotrypsin-like activities of proteasome with its boronic acid pharmacophore (15–17). The off-target interactions of BTZ causes significant side effects like peripheral neuropathy and limits its use in solid tumor cancers (16,18,19). The extremely aggressive nature of chemotherapy agents used in solid tumor treatment, combined with the effect of BTZ, makes it exceedingly difficult for the patient to overcome all these side effects. Not all patients respond equally to BTZ-centered treatments, and it appears that cancer may recur in some patients who initially respond. Especially solid tumors may develop resistance to BTZ in some cases. Hence, second generation proteasome inhibitors have been studied and new proteasome inhibitors have been developed, like Carfilzomib (CFZ) (18,20,21). CFZ is an irreversible and highly selective proteasome inhibitor, differing from BTZ by its structure and mechanism of action (22). It binds covalently and irreversibly to the 20S proteasome and composes morpholino adducts with its epoxyketone structure, which is not active as boron moiety of BTZ, and inhibits the chymotrypsin-like activity of proteasome (19). Both the minor off-target and superior on-target interactions of CFZ provide it more potential against BTZ (16,19). By causing less nervous system damage and peripheral neuropathy than BTZ, CFZ is a promising proteasome inhibitor to combine in standard therapies (16,21–23).

However, triggering of Nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) dependent antioxidant mechanisms limit the efficiency of proteasome inhibitors, during the treatment (14). Nrf-2 is a key transcription factor involved in the regulation of a wide variety of cytoprotective genes such as inflammation proteins, antioxidant, and detoxification enzymes (8,24,25). Nrf-2 activation is tightly regulated by Kelch-like ECH associated protein (KEAP1), an adapter for Cullin 3 (Cul3)-based ubiquitin E3 ligase. KEAP1 binds to Nrf-2 and enables the ubiquitination process to occur. KEAP1-Nrf-2 system, one of the ubiquitin-proteasome-based regulatory systems, is unique in that it can sense oxidative and electrophilic stresses through reactive cysteine residues in KEAP1 and mediate the expression of cytoprotective enzyme genes through Nrf-2 activity. When cells are exposed to reactive oxygen species (ROS) or electrophilic toxicants, the reactive cysteine residues in KEAP1 are covalently modified by ROS or electrophiles, which stops ubiquitination of Nrf-2. As a result, the amount of Nrf-2 begins to accumulate in the cell. This accumulation induces the transactivation of cytoprotective genes. Nrf-2 regulates the expression of genes encoding detoxifying and antioxidative enzymes like Heme Oxygenase-1 (HO-1). In contrast, Nrf-2 also down-regulates genes encoding proinflammatory factors such as interleukin-6 (IL-6) and interleukin-1β (IL-1β) (26).

Through the proteasomal activation, Nrf-2 levels are kept restricted within the cell under normal physiological conditions and rises very rapidly when cells are exposed to a variety of environmental stresses (24,27). In healthy cells, the overexpression of Nrf-2 is correlated with suppression of tumor formation. However, higher Nrf-2 levels cause dysregulation of autophagy, tumor progression, and chemoresistance in tumor cells along with poor prognosis (8,24,25). In various studies, Nrf-2 has been found to be overexpressed in GSCs, increases their proliferation; and causes tumor recurrence and aggression (25,28). It was also observed that increased Nrf-2 expression in GB cells decreased apoptosis and increased HO-1 expression (29). Nrf-2 and HO-1 upregulation is correlated with the progressed, vascularized, aggressive and therapy resistant GB cells (9,14,24).

Herewith, we aimed to elucidate the mechanisms of second-generation proteasome inhibitor CFZ against first-generation proteasome inhibitor BTZ mediated alterations on the Nrf-2/HO-1 dependent antioxidative response in term of both cancer and healthy cells in GB and healthy astrocyte (HA) cells co-cultivation.

2. METHODS

2.1. Cell Culture

In this study, GB and HA cells were used. GB cells were purchased from ATCC (American Type Culture Collection) with the code DBTRG (ATCC® CRL2020™, Manassas, VA United States). HAs were purchased from Innoprot (P10251, Derio, Bizkaia, Spain). The flasks and petri dishes that were used in astrocytes culture, were coated with Poly-L-lysine to provide cell adhesion. Poly-L-lysine (stored at +4 °C) was mixed with dH_2O to obtain 30 µg/mL concentration and then the surfaces of culture dishes were coated with 1 mg/mL Poly-L-lysine. The covers of the dishes were wrapped in parafilm to be isolated, and they were kept at +4 °C for 3 days. The excess Poly-L-lysine was aspirated before use, the dishes were washed once with 1X DPBS (Dulbecco's phosphate-buffered saline) and then appropriate medium was added on the dishes and cells were cultured. As recommended by the ATCC, GB cells were cultured in high DMEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum), 1% penicillin/streptomycin, and 0.1% amphotericin; and astrocytes were cultured in DMEM-F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) containing 2% FBS, 1% penicillin/streptomycin, 0.1% amphotericin, and 1% astrocyte growth supplement in Poly-L-lysine coated flasks in 5% CO_2 and 37 °C incubator conditions.

2.2. Co-cultivation of glioblastoma cells with astrocytes

For co-cultivation of GB cells with HAs, inserts were used in 24-well plates. The used inserts were obtained as being appropriate for 6/24 well plates, having 0.4 µm pore size (PICM01250, Merck Millipore Millicell, Burlington, MA United States). The structures of pores were small enough to prevent cell escape and large enough to permit the medium's ingredients and signal molecules. To perform co-cultivation, the wells were coated with the 450 µL Poly-L-lysin and $15x10^3$ HA cells were cultured onto coated wells in 600 µL medium. Following, inserts were placed into the Poly-L-lysin coated wells and $10x10^3$ GB cells were cultured in inner surface of inserts in 400 µL medium. The cells were incubated in 5% CO_2 and 37 °C conditions for 24h prior to drug exposures.

2.3. Drug and ionizing radiation treatments

The next day following the co-cultivation, TMZ (ALX – 420-044-M025, Enzo Life Sciences, Lausen, Switzerland), BTZ (PS341, Selleck Chemicals, Houston, TX United States) and CFZ (PR-171, Selleck Chemicals, Houston, TX United States) administrations were performed. Stock drug solutions were prepared with DMSO (Dimethyl sulfoxide) in 10 µM concentrations. Formerly obtained IC_{50} drug doses of TMZ, BTZ and CFZ by our group were administered: 300 nM for BTZ, 500 nM for CFZ and 50 µM for TMZ (in 0.1% DMSO) for 48h. Before the IR exposures, medium of cells were refreshed with phenol red free medium due to the detrimental effects of IR to structure of phenol red. Cells were exposed to single dose 2 Gy ionized radiation by using 6 MV photon energy. Following the IR exposure, the mediums of cells were replenished, and drug administrations were performed. The treatment groups were classified as; control (CTRL), TMZ treated, BTZ treated, CFZ treated, IR treated, TMZ&IR treated, TMZ&IR&BTZ treated and TMZ&IR&CFZ treated groups. The IR, TMZ&IR, TMZ&IR&BTZ and TMZ&IR&CFZ groups were IR exposed groups. The mediums of IR-exposed groups were Phenol red-free. Following the IR, cells were incubated 5% CO_2 and 37 °C conditions for 1 hour. After incubation, drug administrations were performed for all groups for 48h.

2.4. Cell viability assay

Following the 48h drug exposures, cell viability was assessed with MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, Sigma-Aldrich, St. Louis, MO United States) according to manufacturers' protocol. Briefly, 5 mg/mL MTT solution was added into each well and cells were incubated for 3 h at 37 °C. Then, all the media were discarded from wells and 300 µL DMSO was added onto each well to dissolve the formed tetrazolium salts. After the incubation of plate for 10 minutes at 37 °C, the absorbance value was measured at 590 nm using an EnSpire multimode plate reader (PerkinElmer, Waltham, MA United States) to detect cell viability. Experiments were designed as triplicated.

2.5. Western blotting

Isolation of total proteins from cells was performed via Cell Lysis Buffer (Cell Signaling Technology, CST, Danvers, MA United States) containing protease inhibitor cocktail and PMSF (phenylmethylsulphonyl fluoride). Determination of the protein concentrations of the samples were performed with Pierce™ BCA (Bicinchoninic acid) Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA United States) and 20 µg of protein sample was loaded into gels per group. The protein samples were separated with 10–12% SDS-PAGE (Sodium dodecyl-sulphate polyacrylamide gel electrophoresis) and were blotted onto nitrocellulose membranes (Thermo Fisher Scientific, Waltham, MA United States). The membranes then were blocked with 5% non-fat dry milk-TBST (Tris-buffered saline with 0.1% Tween 20 detergent) and incubated with primary antibodies at 4 °C overnight. Following incubation with HRP (Horseradish peroxidase) conjugated secondary antibody for 2 h at room temperature, blots were developed using ECL (enhanced chemiluminescence) reagent (Thermo Fisher Scientific, Waltham, MA United States). The bands were visualized using ChemiDoc™ MP System (Bio-Rad Laboratories, Hercules, CA United States) and band densities were quantified using Image Lab software. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as the loading control.

Antibodies

The following antibodies were used: Nrf-2 (Cell Signaling, 12721S, 1:1000), HO-1 (Cell Signaling, 5061S, 1:1000), GAPDH (Novus, NB300-221, 1:1000), HRP conjugated anti-rabbit (Cell Signaling, 7074P2, 1:10000).

2.6. Statistical analysis

Statistical analyses were performed with GraphPad Prism 9 (GraphPad Prism version 9.0.0 for Windows, GraphPad Software, Boston, MA USA). The data expressed as mean ± SD of three independent experiments and were analysed by one-way ANOVA followed by Tukey's post-tests between groups. A p-value <.05 was selected as statistically significant, and p value < .0001 was considered extremely significant.

3. RESULTS

3.1. BTZ and CFZ significantly decrease the cell viabilities

Cell viability of GB and HA cells decreased significantly after 48 hours of exposures to 50 µM TMZ, 300 nM BTZ, 500 nM CFZ and their combinations along with the IR exposures. The values did not differ between the groups treated with BTZ and CFZ in GB cells. However, the CFZ-treated groups showed higher cell viability than the BTZ-treated groups in HA cells (Fig. 1).

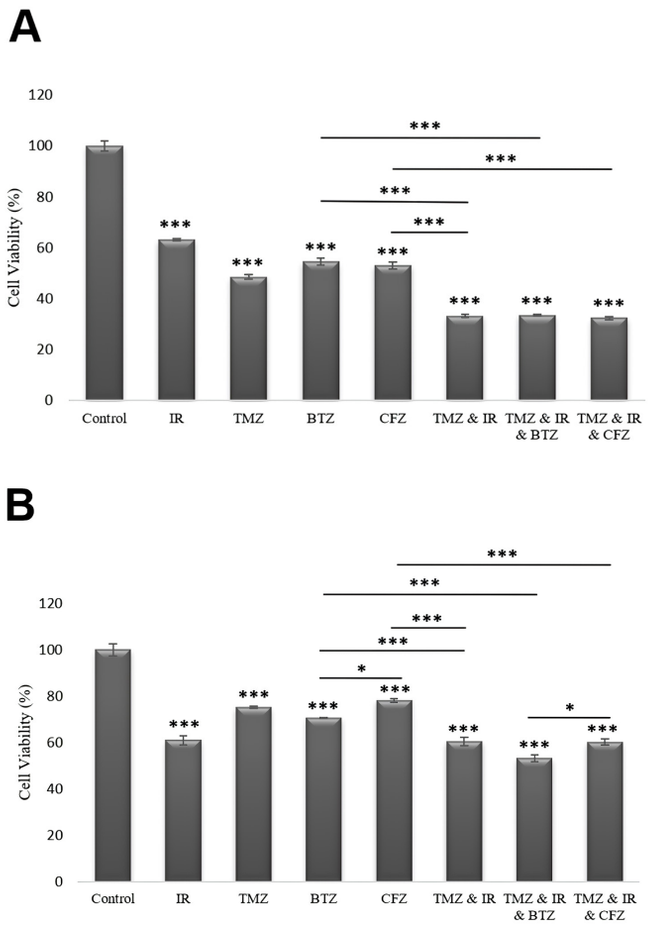

Figure 1. *Percentage viability of* **(A)** *glioblastoma cells and* **(B)** *human astrocytes after IR, 48 h of BTZ, CFZ, TMZ and their combinations treatments with MTT-assay. All the data are presented as means ± SD (n=3). *P < .05, ***P < .001 The P value is < .0001, considered extremely significant for both cell viabilities.*

3.2. BTZ and CFZ treatments effects the Nrf-2 and HO-1 expression levels of cells

According to the western blotting analysis, Nrf-2 and HO-1 protein levels in GB cells showed variable results in response to different therapy combinations (Fig. 2A). The Nrf-2 protein levels of BTZ-treated group was significantly higher compared to the control, CFZ and TMZ & IR & BTZ-treated groups. CFZ treatment alone decreased the Nrf-2 levels compared to both BTZ treatment alone and TMZ & IR & CFZ treated groups (Fig. 2B). HO-1 protein concentrations in the BTZ, CFZ, TMZ & IR & BTZ and TMZ & IR & CFZ treated groups were significantly higher than in the control group, in which HO-1 protein concentrations were strikingly low. BTZ treatment alone increased HO-1 levels compared to the CFZ treatment group (Fig. 2C).

On the other hand, the Nrf-2 levels in the HAs varied in the different treatment groups (Fig. 3A). Compared to the control group, significant increases were observed in the groups treated with BTZ, CFZ and TMZ & IR & BTZ. Nrf-2 levels were lower in the BTZ-treated group than in the CFZ-treated group, the exact opposite of the GB cells. At the same time, Nrf-2 levels were higher in the CFZ-treated group than in the TMZ & IR & CFZ-treated group (Fig. 3B). However, the expression of HO-1 in HAs was not observed as there was no induction factor for this expression.

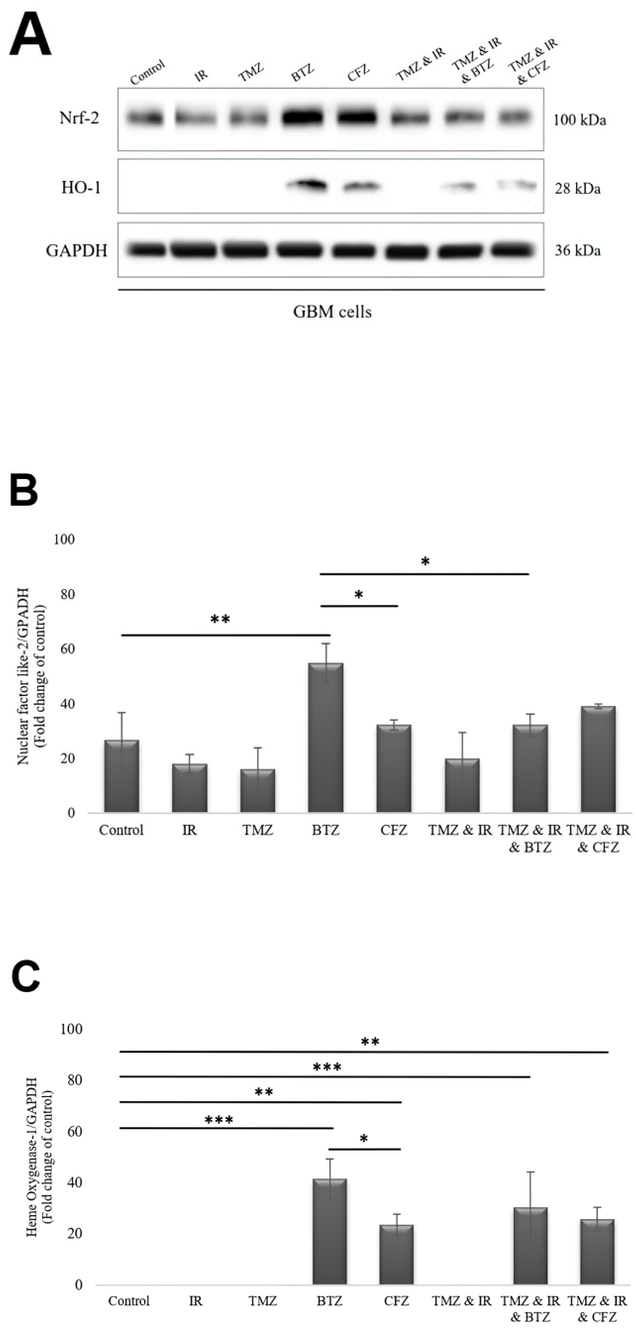

Figure 2. *IR, TMZ, BTZ, CFZ and their combination treatments affect the Nrf-2 and HO-1 levels in glioblastoma cells A Western blotting analysis of protein levels of GB cells treated with IR, 50 µM TMZ, 300 nM BTZ, 500 nM CFZ and their combinations with indicated antibodies. B – C Quantification of Nrf-2 and HO-1 protein levels after IR, TMZ, BTZ, CFZ and their combination treatments for 48 h in GB cells. All the data are presented as means ± SD (n=3). *P < .05, **P < .001, ***P < .001 The P value is < .0001, considered extremely significant for protein expressions.*

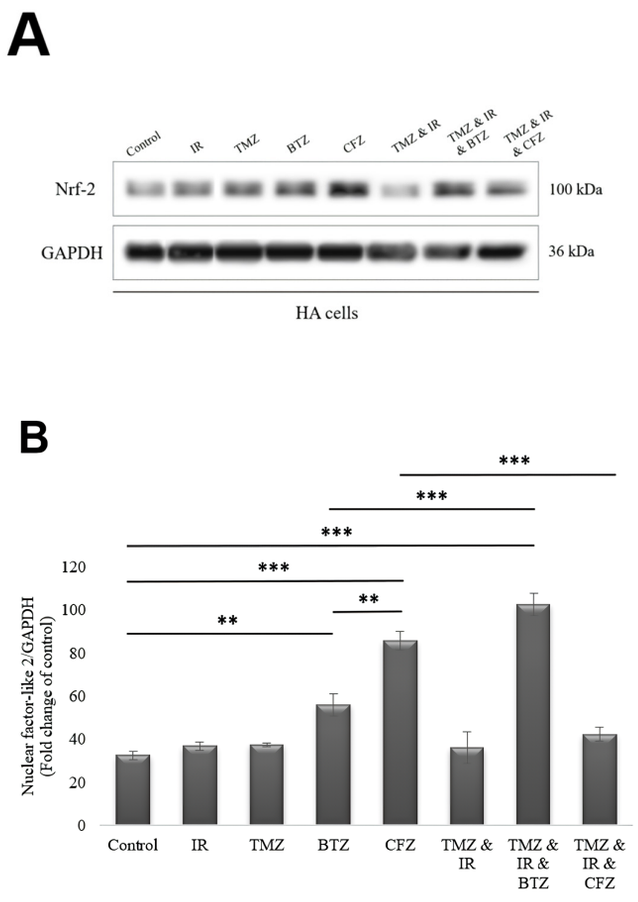

Figure 3. *IR, TMZ, BTZ, CFZ and their combination treatments affect the Nrf-2 levels in human astrocytes* **A** *Western blotting analysis of protein levels of HA cells treated with IR, 50 µM TMZ, 300 nM BTZ, 500 nM CFZ and their combinations with indicated antibody.* **B** *Quantification of Nrf-2 protein levels after IR, TMZ, BTZ, CFZ and their combination treatments for 48 h in HA cells. All the data are presented as means ± SD (n=3). **P < .01, ***P < .001 The P value is < .0001, considered extremely significant.*

4. DISCUSSION

In this study, we tested proteasome inhibitors BTZ and CFZ in co-cultivation of GB-HA cells. Since diverse types of antioxidant mechanisms get activated as a response to applied treatments, we aimed to observe the given Nrf-2/HO-1 dependent antioxidant response in combined BTZ and CFZ treatments from GB and HA cells by co-culturing and analyzing the relevant protein expressions of the cells.

The aim of the therapies is to eradicate the malignant tumor without affecting the surrounding healthy cells when it comes to overcoming tumors. However, the healthy cells responsible for maintaining the microenvironment and the development of the tumor will most likely be affected by potential off-target effects of the administered chemotherapies (30). Herein, we focused on the off-target effects of applied GB therapies on both GB and HA cells in terms of unintentionally activated antioxidant signalling pathways.

Nrf-2 is an essential transcription factor that regulates the mechanisms of cellular protection against a wide variety of stress conditions (13). Their levels are kept low within the cells in the absence of stress conditions by way of KEAP-1 proteins. KEAP-1 regulates Nrf-2 levels by inclining the ubiquitination of Nrf-2 proteins and hence causing degradation in proteasomal systems (13,31,32). When we considered the GB cells underwent the cellular stress by applied treatments and through the exposed proteasome inhibitors, the degradation of Nrf-2 proteins through proteasomal pathways halted and consequently, Nrf-2 levels and accordingly HO-1 levels increased within the cells. Not surprisingly, the GB cells are more prone to be affected by proteasomal inhibition when compared to HA cells. Together with the remarkable decrement in proteasomal activity, Nrf-2 and HO-1 levels reached higher levels in proteasome inhibitor treated groups, especially with BTZ treatment.

The following groups of GB cells showed increased Nrf-2 expressions; BTZ, CFZ, TMZ & IR & BTZ and TMZ & IR & CFZ; and in parallel with this finding, HO-1 levels in these groups showed significant increases. Thus, in GB cells, with the increased stress conditions by applied treatments, cells resisted to survive by increasing cytoprotective Nrf-2 and HO-1 proteins to prevent apoptosis which is not intended by an efficient treatment.

On the other hand, while BTZ, CFZ and TMZ & IR & BTZ treated groups showed increased Nrf-2 levels in HA cells, we did not observe HO-1 expression in HA cells with western blot analysis even with increased level of protein concentrations loaded to gel. HO-1 is a downstream target of Nrf-2 and serves as an essential indirect antioxidant enzyme within the cell (33). Unlike the tumor cells, the induction of HO-1 expression by Nrf-2 with the drug exposures did not occur. HO-1 as being the downstream protein of Nrf-2, is expressed in highly activated intracellular antioxidant response. Since the drug exposures were not sufficient to generate excessive oxidative stress and there were any known inducers, like heavy metals, UV light, hydrogen peroxide, and lipopolysaccharide, to induce HO-1 expression in healthy cells (31,34), HO-1 expression was not seen in HA cells. The elevation of Nrf-2 levels in HA cells indicates the attempt of the cells to defense themselves both to applied treatments and culminated side effects.

The proteasomal activity increment is one of the upregulation mechanisms of cancer cells to enhance their survival rates. Thus, proteasomal system inhibitors are highly promising approaches to overcome cancer cell survival that results from proteasomal system activity. However, as seen in this study, inhibiting proteasomal activity may result in the activation of other cytoprotective mechanisms within the cells, like increased Nrf-2 and HO-1 levels. Accordingly, as an alternative promising therapy approach beyond the proteasomal inhibitors may be targeting the downregulation of the Nrf-2 levels within the cells (24).

For GB cells, both the Nrf-2 and HO-1 levels of BTZ treated group are over the levels of CFZ treatment except for combination groups and vice versa for HA cells. As

hypothesized, the increment in antioxidant protein levels in tumor cells and the decline in healthy cells may be the reason for the inefficaciousness and off-target outcomes of the treatment like neuropathy. Most of the side effects of BTZ eventuates through inflammation and oxidative stress because of its multiple intracellular actions caused by its boron moiety (19). In this regard, the lower Nrf-2 and HO-1 levels of CFZ present intended therapy results as having morpholino adducts with its epoxyketone structure, which is not active as boron moiety of BTZ and ends up with lower off-target results. In another study with healthy mouse NSC cells (21), the fact that stress proteins like stress-70 protein, superoxide dismutase [Mn], Protein disulfide-isomerase A3, heat shock protein (HSP) 90-alpha, protein disulfide-isomerase A6, catalase, HSP32, HSP47 and HSP70 expressions that can induce neuropathy were lower in the CFZ group compared to BTZ or were expressed at the same levels as the control group confirms the results of our current study.

The groups with combined treatment have the opposite results to the groups treated with BTZ and CFZ alone. While the combined treatment with BTZ leads to a higher induction of the antioxidant signalling pathways in HA cells, the treatment with BTZ alone shows a lower induction, but in GB cells these results are exactly opposite and not consistent with the fate of cell survival. Furthermore, the combined treatment with CFZ is shown to lead to a decrease in the relevant proteins, while treatment with CFZ alone increases the levels in HA cells as intended, whereas the combined treatment groups maintain relatively higher levels in GB cells. However, the effects of the combined treatment on antioxidant metabolic pathways are not maintained by cellular viabilities. It can be concluded that as the complexity of the applied therapy increases, the number of affected metabolic pathways increases and it becomes more difficult to control all metabolic pathways.

Besides, as Fig.2 shows TMZ&IR treated group has lower Nrf-2 and HO-1 levels when compared to BTZ and CFZ groups. However, this reduction does not state an increment in the cellular viabilities of same groups. In Fig.1, it is clearly seen that BTZ and CFZ treated groups show higher levels than TMZ&IR treated group.

If we consider all these results together, we concluded that neither the treatment with TMZ&IR nor the combination treatment with BTZ and CFZ guarantees effective treatment. On the other hand, CFZ treatment alone outperforms BTZ treatment alone and outperforms all treatment groups.

In summary, we present that Nrf-2 levels increased within the cells in response to proteasomal inhibition. Besides, in GB cells, increased Nrf-2 levels induced the elevation of HO-1 expression levels. Through the activation of cytoprotective mechanisms, cancer cells take advantage of Nrf-2 to withstand to undergoing apoptosis with all its strength. This effect especially is seen in the administration of BTZ rather than CFZ. While the activation of Nrf-2 causes tumor progression and resistance to therapy, silencing of Nrf-2 in tumor cells shows diminishment of cell migration and metastasis (14,24). Targeting the Nrf-2 may seem to be an efficient way to overcome the challenges resulting from its overexpression. However, since the physiological roles of Nrf-2 is highly vital for healthy cells, on-target therapy may result in deleterious side effects in patients (35). Alternatively, targeting indirectly by the way of related cellular pathways may be more prone to get constructive results. In this regard, prioritizing the second-generation proteasome inhibitor CFZ as a replaced therapy, may provide the intended Nrf-2 levels which are lower for GB cells and higher for HA cells. Further studies that will replace the treatments with CFZ may provide an outstanding insight for the GB treatment.

5. CONCLUSION

Besides the known roles of Nrf-2 and HO-1 in cancer development and progress, there are still lots of mysteries. In consideration of our results, it may be hypothesized that the inefficacy of current treatments that includes BTZ, may be the result of increased levels of devastating Nrf-2 and HO-1 in GB cells. Replacing the standard treatments with CFZ and investigating the Nrf-2 originated tumor progression and resistance in GB may likely focus on directly the basis of tumor bulk and give more consistent results in future.

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Author Contributions:
Research idea: BY, SAY, MFB
Design of the study: BY, AŞ, BMA, OK
Acquisition of data for the study: ZG, SAY, MFB
Analysis of data for the study: ZG, SAY, AŞ
Interpretation of data for the study: ZG, AŞ, BY
Drafting the manuscript: ZG, AŞ, BY
Revising it critically for important intellectual content: ZG, AŞ, BY
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Clinical and Experimental Health Sciences

Nursing Principles Practice Basic Competencies Scale – Turkish Adaptation Study

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ABSTRACT

Objective: The main objectives of clinical education in nursing education are to develop the student's competences in cognitive, affective and psychomotor areas. Reliable measurement tools are needed for the evaluation of these competences that begin to be gained with the first clinical practice. This methodological study investigated the cultural and Turkish validity of the Core Competence in Basic Nursing Practice Scale (CCFNPS) for undergraduate nursing students.

Methods: A methodological research has been carried out on the cultural validation and adaptation of the scale. The construct's validity was examined using both exploratory and confirmatory factor analysis in a sample of 466 students. The scale's internal consistency reliability was evaluated using Cronbach's alpha. Test-retest reliability was assessed using a dependent sample t-test and intraclass correlation with a sample of 30 students.

Results: The scale's content validity index was 0.98. The results of exploratory factor analysis revealed a five-factor structure similar to the original scale, the variance explained was 69.62%, but four items were removed from the scale due to low factor loading. Confirmatory factor analysis results (fit indices values) applied to the twenty – one items and five sub-dimensional structure were found to be acceptable. Scale Cronbach's alpha values were above 0.70.

Conclusion: The 21-item Turkish version of CCFNPS was found to be a valid and reliable scale for measuring the competencies acquired by students after their first clinical training.
Keywords: Clinical competence, clinical practice, self-assessment, nursing students, reliability.

1. INTRODUCTION

Competent nurses provide safe and effective care in health care delivery. For this reason, nursing competencies have been identified and nursing competencies that must be acquired during the nursing education have been defined (1). Field practices in nursing education are critical in the acquisition of these competencies (2). During these practices, nursing students apply their theoretical knowledge to practical experience, develop the necessary technical skills, learn interpersonal skills, make clinical judgements, ensure professional socialization, develop professional values and learn to provide systematic care through the nursing process (3).

The Fundamentals of Nursing Course (FNC) offers nursing students their first clinical field experiences. The aim of the FNC, which forms the basis of vocational courses and practices, is to build knowledge, skills and attitudes related to the main principles, basic skills and understanding of the nursing process of the profession. This course consists of theoretical learning, professional skills, laboratory studies and clinical education. The most essential objectives of clinical education in the nursing education are to improve the student's competencies in cognitive, affective and psychomotor areas; to instill habits for lifelong learning; and and to cultivate critical thinking and problem-solving abilities (4,5). As the process of assessment of student nurses' competence in clinical practical (6), clinical evaluation determines the extent to which these skills are mastered. Literature reviews indicate that the evaluation of nursing students in clinics is difficult, may be far from objectivity and there are unresolved problems (3,4,7-11). Two systematic reviews indicate that clinical assessment is a complex task for educators, emphasize the necessity of developing reliable

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and valid assessment tools, as well as consistent assessment approaches. (4,11).

Clinical competence assessment usually involves educators and clinical mentor nurses. Multidimensional evaluation is considered valuable in evaluation of clinical proficiency and students' self-evaluation is considered an integral part of this (8,4). However, students may overestimate their competencies while self-assessing and may need support to make an objective self-assessment (10). The reliability of students' self-assessments is still unclear (4,7). Students should clearly understand the desired level of nursing competence and determine their weaknesses and strengths according to these standards (4,11).

The literature states that scales assessing biomedical knowledge, basic clinical skills, critical reasoning and judgement, care and life-long learning evaluate the competence of final year nursing students in clinic practical (5,12,13). Chang et al. developed the "Core Competence in Fundamental Nursing Practicum Scale-CCFNPS" measurement tool because they found the existing tools unsuitable for evaluating the nursing students' self-efficacy entering clinical practice. To provide a new measurement tool, they determined a reliable scale to validly measure nursing students' perceptions of core competence during their first clinical practice. This measurement tool can be used by educators to define basic competencies that students perceive as weak and to improve these areas.

Nursing education in Turkey begins in the first or second year with the FNC, which is the basic professional course. In the third and fourth years, students are given other professional courses such as Internal Medicine and Surgery Nursing. The expectations of the students who follow these courses in clinical practice vary according to the objectives of the courses and the criteria for assessment become more demanding. As the FNC covers basic nursing knowledge and skills, the assessment criteria for clinical practice should be at a basic level consistent with the objectives of the course. In Turkey, there no scale has been developed to evaluate students' self-assessment of their efficacy in clinical practice within the scope of the FNC. For this reason, we planned to conduct a Turkish reliability and validity study to use Chang et al.'s scale in our country.

2. METHODS

2.1. Aim

This methodological research investigated the cultural and Turkish validity of the CCFNPS for undergraduate nursing students.

2.2. Design

This research was carried out in two stages: cultural adaptation, and translation and validation of CCFNPS in Turkish. International guidelines (14,15) were used for the translation, validation and adaptation phase of the scale. Permission from the author of the original CCFNPS was obtained to adapt it into Turkish. This study was approved by İstinye University Human Research Ethics Committee (Number /date: 22-101/July 21,2022). Participation in this research was voluntary.

The CCFNPS was translated into Turkish by two native Turkish-speaking linguists who also teach English as a second language. The scale was then back-translated into English by two native Turkish-speaking translators. The translators and members of the research group checked the translation and back-translation against the initial version in order to clarify concepts and reach consensus. Meanwhile, in two of the scale items (items 23 and 24), there was no semantic consensus in translation and retranslation. The original scale author was consulted to clarify the meaning and these two items were reformulated in the back translation and Turkish version.

This research interviewed 30 first-year nursing students for pilot testing. Because the students stated that the scale statements and the instructions for completion were understandable, no changes were made to the scale. Next, ten experts were consulted to ensure the scale's conceptual and content equivalence; 6-10 experts is considered a sufficient number for this (14,15). For the expert panel, the study selected faculty experienced in scale development and adaptation studies who teach the FNC in university nursing departments. The experts were requested to evaluate every statement in scale and to convey their opinions by choosing one of the responses: "not appropriate (1)", "the item should be adapted (2)", "appropriate, but minor changes are required (3)" or "very appropriate (4)". The content validity ratio of the instrument items and the content validity index (CVI) of the scale were calculated according to the responses of the experts. For this, the Davis technique was used (16). The mean Item-CVI of the scale items was 0.86 (Min=0.80 – Max=1.0) and the sum CVI of the scale was 0.98. Since the CVI scores of the scale items and the sum of the instrument were 0.80 and above, design reliability and validity analyses of the instrument items were initiated (14,15).

2.3. Setting and Sample

This research was conducted in the nursing schools of one state and two private universities in a city in western Turkey. The FNC, which includes theory and practical courses, is given in the spring of the first year in two of the nursing programme where the research was conducted and in the spring of the second year in the remaining programme. In the three nursing schools where the research was conducted, 563 students were taking the FNC in the 2021-2022 academic year. The CCFNPS evaluates the competencies gained by students after their first professional laboratory and clinical practical training (13). The study included 471 students who had successfully completed the first iteration of the FNC course and voluntarily participated in the research. Students who had attended the course and

clinical practice for a second time and students whose first language was different from Turkish were excluded from the study. The study sample comprised 466 students who completed the questionnaires. A minimum of 30 pairs of data should be included in the retest (17). In this study, the retest was carried out with 30 students from the third school. Data were collected from these students twice at an interval of 30 days (18).

2.4. Instrument

Data collection used a Demographics Form and a form entitled CCFNPS. The demographics form consisted of 6 questions surrounding age, gender, grade point average, high school graduated, place of residence and willingness to enter the profession. CCFNPS is a 5-point Likert-type scale consisting of five factors and a total of 25 items. The first factor is "Communication (COM)" (3 items), the second is "Application of nursing processes (ANP)" (6 items), the third is "Basic biomedical science (BBS)" (4 items), the fourth is "Nursing skills and ability to perform a care process (NSAPCP)" (7 items) and the fifth is "Professional attitude (PA)" (5 items). Each item was rated on a 5-point Likert scale (1-not satisfied at all, 5-very satisfied). Total scale and each factor points were analysed by the average of the total item scores. A high score indicates more competence (better performance) during the application of nursing principles. Cronbach's alpha for CCFNPS subscales ranged from 0.83 to 0.92, and for the total scale it was 0.94 (13).

2.5. Data Collection

Following the FNC clinical practice at two universities, three researchers collected data for construct validity and internal consistency analyses and completed the examination and grade evaluations. The students were explaine about the research and the questionnaires were handed to them. The students who ticked the box "I want to participate in the research" in the questionnaire were given 15 minutes to complete the questionnaire. For the test-retest, same questionnaire was administered to the students of the third university. For this, the students were asked to put a pseudonym on the questionnaire and the questionnaire was re-administered one month after.

2.6. Analysis of Data

The data analysis was conducted using SPSS 26 and AMOS 22. Student demographic characteristics (number, percentage, mean and standard deviation) and scale scores (Skewness, Kurtosis) were analyzed by descriptive statistics. Pearson Correlation Analysis was used to analyze the relationship between the scores of the sub-dimensions of the total scale.

The scale's construct validity was evaluated through exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) using AMOS 22. Factor analysis identifies clusters of relevant variable dimensions underlying a broad construct (19). For the purpose of construct validity, the total sample size was divided into two parts: the EFA was carried out with data from 233 nursing students, followed by the CFA with data from the same number of students. Prior to conducting the EFA test, we evaluated the data's suitability for factor analysis by performing the Kaiser-Meyer-Olkin test (KMO, range 0-1) and Bartlett's test ($p < .05$) (20). Validity analyses provided us with values for composite reliability (CR), average variance extracted (AVE), maximum reliability (MaxR), and maximum shared variance (MSV). To assess internal consistency, we calculated Cronbach's alpha scores. The scale's invariance was tested using dependent group t-tests and intraclass correlation (ICC) analyses.

3. RESULTS

Of the nursing students, 80.5% were female, 85.8% were between the ages of 18-22 years, and 66.3% lived with their families, 76.6% chose the profession willingly and the mean age of all subjects was 20.29 ± 1.92 years.

3.1. Construct Validity

An exploratory factor analysis was conducted using principle components analysis and varimax rotation to identify the items included in the different dimensions of the CCFNPS. The KMO value was 0.843 (≥.70 are desired) and Bartlett's test result was meaningful (Chi-Square:2125.349; Bartlett's Test Sphericity (df): 210; $p<.01$) indicating a sufficient sample size.(20) EFA determined that the scale items showed a five-factor structure. Due to low factor loadings, items 5, 7, 15, and 21 were eliminated from the scale (20,21). The factors including the remaining items in the scale were the same as the original scale and the factor loadings ranged between 0.41 and 0.86 (Table 1). As a result of EFA, the total explained variance was 62.69%.

Table 1 presents the item-total score correlation coefficients of the scale items and Cronbach's alpha coefficients after the items were removed. Item-total score correlation coefficients were found to be between 0.42 and 0.63, a value above the desired value (>0.30 is desired) (21). When the item was removed, the Cronbach's alpha coefficient was 0.89.

Confirmatory factor analysis was applied to verify EFA structure determined in the scale, which was resized with the Varimax method. Model fit index values (22) were taken into consideration in interpreting the CFA analysis. Figure 1 presents the CFA sub-dimensions visualizations resulting from EFA analysis. Table 2 presents model fit index values. It was seen that χ^2/sd, goodness of fit index (GFI), normed fit index (NFI), incremental fit index (IFI), comparative fit index (CFI) and root mean square error of approximation (RMSEA) were within the "acceptable fit value ranges". Table 3 presents validity statistics for the model shown in Figure 1. CR > 0.7, AVE > 0.5 and MaxR > 0.7, and MSV < AVE (23)

Table 1: *Varimax rotated structures matrix (n=233)*

	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted	NSAPCP	ANP	BBS	PA	COM
Q17.Ability to complete required homework or report assignments.	.607	.887	.738				
Q18.Ability to operate instruments correctly when performing nursing skills.	.634	.886	.700				
Q19.Ability to properly perform nursing skills to patients.	.631	.886	.687				
Q20.Ability to properly prepare tools or materials for conducting nursing skills.	.594	.887	.612				
Q16.Ability to search for information.	.525	.889	.512				
Q14.Ability to organize data when writing a report.	.521	.889	.411				
Q6.Ability to execute care plans developed for patients.	.445	.891		.693			
Q9.Ability to correctly perform physical assessments of patients.	.498	.890		.636			
Q8.Ability to evaluate changes to patients after the implementation of care plans.	.526	.889		.537			
Q4.Ability to make appropriate nursing diagnoses for patients.	.447	.891		.456			
Q11.Ability to understand reasons for prescribing specific medications for patients.	.427	.892			.857		
Q10.Ability to understand the side effects of medications used by patients.	.419	.892			.622		
Q13.Ability to understand mechanisms of medication used by patients.	.580	.887			.619		
Q12.Ability to instruct patients on medication use.	.529	.889			.538		
Q24.Ability to perform self-reflections**.	.516	.889				.667	
Q25.Ability for proper time management after the practicum.	.428	.892				.656	
Q22.Possession of a positive learning attitude.	.507	.889				.593	
Q23.Ability to perform emotional self-regulation.*	.403	.893				.533	
Q2.Ability to use communication skills to communicate with caregivers.	.542	.889					.814
Q1.Ability to use communication skills to communicate with patients.	.525	.889					.766
Q3.Ability to observe the nonverbal needs of patients..	.420	.892					.468

Note: *It should be noted that in order to utilise this scale, permission must be obtained from the author who developed the original scale.*

ANP: *Application of Nursing Processes, BBS: Basic Biomedical Science, COM: Communication, NSAPCP:Nursing Skills and Ability to Perform a Care Process, PA: Professional Attitude*

p<.001***Emotion regulation skills*** *are the skills that persons can identify, control and express their emotions, and transfer them to their environment. Emotion regulation involves controlling not only positive emotions but also negative emotions of the persons and not allowing these negative emotions to affect their lives.*

***** Reflection*** *is concentrating one's all thoughts on a problem, thinking through and examining a subject in detail.*

Table 2. *Model fit ındex values (n=233)*

Fit Indexes	Good Fit	Acceptable Fit	Model Fit Index
χ^2/df (CMIN/DF)	$0 \leq \chi^2/sd \leq 2$	$2 < \chi^2/sd \leq 5$	1.799
GFI	0.95≤ GFI< 1.00	0.90 ≤ GFI<0.95	.951
AGFI	0.95≤ GFI< 1.00	0.90 ≤ GFI<0.95	.916
NFI	0.95≤ NFI< 1.0	0.90≤NFI<0.95	.951
IFI	0.95 ≤ IFI< 1.00	0.90≤ IFI<0.95	.963
TLI	0.95≤ TLI< 1.00	0.90 ≤ TLI<0.95	.934
CFI	0.95≤CFI<1.00	0.90≤CFI<0.95	.962
RMSEA	0 ≤ RMSEA ≤ .05	.05 ≤ RMSEA ≤ .08	.058
RMR	0 ≤ RMR ≤ .05	.05 ≤ RMR ≤ .08	.034

AGFI: Adjustment Goodness of Fit Index; CFI: Comparative Fit Index; GFI: Goodness of Fit Index; IFI: Incremental Fit Index; NFI: Normed Fit Index; RMR: Root Mean Square Residual RMSEA: Root Mean Square Error of Approximation; TLI: Tucker Lewis Index χ^2: Chi-square; df: Degrees of Freedom

Table 3. *Validity analysis results (n=233)*

	CR	AVE	MSV	MaxR (H)	COM	NSAPCP	ANP	BBS	PA
COM	0.815	0.602	0.316	0.857	**0.776**				
NSAPCP	0.858	0.518	0.406	0.872	0.505	**0.711**			
ANP	0.738	0.541	0.319	0.745	0.562	0.565	**0.644**		
BBS	0.801	0.513	0.289	0.811	0.385	0.526	0.538	**0.710**	
PA	0.742	0.529	0.406	0.747	0.481	0.637	0.543	0.408	**0.647**

ANP: Application of Nursing Processes, BBS: Basic Biomedical Science, COM: Communication NSAPCP: Nursing Skills and Ability to Perform a Care Process, PA: Professional Attitude AVE: Average Variance Extracted, CR: Composite Reliability, MSV: Maximum Shared Variance, MaxR(H): Maximal Reliability.

3.2. Internal Consistency and Correlation Analysis

The mean scores, Cronbach's alpha values and correlation values of the sub-dimensions of the scale are presented in

Table 4. The Cronbach's alpha value for the total scale was found to be 0.92, with sub-dimension scores ranging from 0.73 to 0.85. Correlation values between the subscale scores ranged from 0.31 to 0.53, indicating a positive and statistically significant relationship between the sub-dimensions (p<.01).

Table 4. *Scale sub-dimensions mean scores and correlation analysis results (n=233)*

	Min-Max	Mean±SD		NSAPCP	ANP	BBS	PA	COM
NSAPCP	1-5	4.06±0.55	r	1	.481	.451	.530	.437
			p		.000*	.000*	.000*	.000*
ANP	1-5	3.87±0.55	r		1	.416	.332	.493
			p			.000*	.000*	.000*
BBS	1-5	3.62±0.70	r			1	.306	.325
			p				.000*	.000*
PA	1-5	3.96±0.63	r				1	.306
			p					.000*
COM	1-5	4.04±0.64	r					1
			p					
Cronbach Alfa		Overall – 0.92		0.85	0.73	0.80	0.76	0.79

*ANP: Application of Nursing Processes, BBS: Basic Biomedical Science, COM: Communication, NSAPCP:Nursing Skills and Ability to Perform a Care Process, PA: Professional Attitude r: Pearson Product-moment Correlation Test, *p<.001*

Table 5. *CCFNPS total and sub-dimensional test-retest and confidence coefficients (n=30)*

CCFNPS	Application	Mean	SD	t p	r p	ICC p
Factor 1: COM	1. Application	4.16	.51	t= .560	r=.961	ICC=.997
	2. Application	4.14	.49	p=.580	p=.000*	p=.000*
Factor 2: ANP	1. Application	4.35	.52	t= – 1.608	r=.908	ICC=.950
	2. Application	4.29	.49	p=.119	p=.000*	p=.000*
Factor 3: BBS	1. Application	3.61	.53	t= – 1.901	r=.897	ICC=.946
	2.Application	3.69	.52	p= .067	p=.000*	p=.000*
Factor 4: NSAPCP	1.Application	3.92	.25	t= – .369	r=.708	ICC=.828
	2.Application	3.93	.27	p=.715	p=.000*	p=.000*
Factor 5: PA	1.Application	3.84	.54	t= – .565	r=.821	ICC=.901
	2.Application	3.87	.52	p=.576	p=.000*	p=.000*
Overall CCFNPS	1.Application	4.16	.40	t=-1.794	r=.994	ICC=.997
	2.Application	4.14	.40	p=.083	p=.000*	p=.000*

ANP: Application of Nursing Processes, BBS: Basic Biomedical Science, COM: Communication, NSAPCP:Nursing Skills And Ability To Perform A Care Process, PA: Professional Attitude

*ICC: Intraclass Correlation Coefficient, r: Pearson Product-moment Correlation Test, SD: Standard Deviation, t: t Test in Dependent Samples, *p<.001*

3.3. Test-Retest Reliability

Table 5 shows the scores of the dependent groups t-test and intraclass correlation values applied to determine the difference in scores of the two measurements applied one month apart to thirty students. The test-retest reliability analyses of CCFNPS resulted in a first measurement mean score of 4.16±0.40 and a second measurement mean score of 4.14±0.40. Mean scale scores between the first and second administrations were not statistically significantly different (t: – 1.794, p> .05). Correlation analysis comparing the scale scores of the two measurements identified a statistically significant, positive correlation (r=0.99, p< .001). The ICC between the scale total scores of the two interventions performed one month apart was found to be statistically significant (ICC: 0.99, p< .001).

4. DISCUSSION

This research adapted Chang et al.'s CCFNPS (13) to Turkish in order to obtain a tool for evaluating the basic competencies of nursing students at the end of their first clinical practice.

The language and content of the instrument were examined for validity. The psychometric properties of the Turkish version of the instrument were evaluated using internal consistencies, item reliability, construct validity and test-retest methods.

Before using a measurement tool in a different culture, language and cultural adaptation is necessary, then psychometric testing to determine whether it is valid and reliable for that society (24,25). In the adaptation phase, translation, expert panel evaluation, back translation and piloting took place, followed by necessary corrections to two items. The scale was re-translated into English and sent to the authors, who developed meaning-concept equivalence. Following positive feedback, it was decided that language equivalence had been reached.

Content validity evaluated the scale items' measurement of the concept in question (26); for this, the research obtained expert opinions from 10 faculty members and evaluated them accordance with the Davis method. The scale items CVI value was between 0.90 and 1.00. Davis accepted the value of 0.80 as a benchmark for CVI. According to this reference value, it was seen that the Turkish form of the scale met the desired criteria for content validity (16).

This research tested the structural validity of the instrument with CFA and EFA. KMO (0.843) and Bartlett's value (p< .001) evaluated the suitability of the measurement tool for factor analysis and showed that the sampling sizes were adequate (20).

According to EFA results, the scale items showed a five-factor structure as in the original scale. However, the factor loadings of four items were below 0.40. Since the factor loading should be above 0.40 (20,21,26), "Item 5, 7, 15 and 21" were removed from the Turkish version of CCFNPS.

In factor analysis models are re-tested after item removals due to factor loadings and item-total score correlation examinations (27). After 4 items were excluded from the instrument, the 21-item and five-factor model was re-examined with CFA. The items' factor loadings ranged from 0.41 to 0.86. As they were above 0.40, path coefficients showing the relationship between the items and the sub-dimension met the validity criterion. The 21-item 5-factor structure explained 62.69% of the total variance, a ratio deemed sufficient; the criteria that the explained

variance ratio should be 40-60% and each factor should have at least three met items (28,29). Confirmatory factor analysis confirmed the factor structure of the items in the scale, formed as a result of EFA. A series of descriptive and inferential fit indices of CFA help to evaluate the goodness of fit of the overall CFA model. It was seen that χ^2/sd, GFI, NFI, IFI, CFI, RMSEA and RMR, which are among the CFA model goodness of fit index values, were within the "good fit value ranges" and AGFI and TLI values were within the "acceptable fit value ranges"(30). These results indicate that the scale items are interrelated and that the data is consistent with the model.

Tests for internal consistency, time invariance and equivalence are recommended for the evaluation of reliability (19). The test-retest method was used to determine the time invariance of the scale; individuals perform the first test and then repeat the test for ten days to one month later to identify the coefficient of correlation between the two measurements. The closer the correlation coefficient to 1, the more reliable the test (18). This research conducted the retest after 30 days. The test-retest correlation results of the instrument were 0.96 for communication, 0.90 for application of nursing processes, 0.89 for basic biomedical science, 0.70 for nursing skills and ability to perform a care process and 0.82 for professional attitude, and 0.99 for the total instrument. The results indicate that the instrument has high reliability and consistency over time, as shown by the test-retest results.

Cronbach's alpha value in internal consistency analysis is acceptable at a value > 0.7 (31). The Cronbach's alpha values of the original 25-item CCFNPS are 0.94, with sub-dimension values between 0.83 and 0.92. In our study, the Cronbach's alpha values of the 21-item CCFNPS were 0.92, with sub-dimension values between 0.73 and 0.85, showing the scale adapted into Turkish is reliable according to accepted criteria.

It is possible that the data may be biased as the students who participated in the research were studying at nursing schools where the researchers were employed. In order to guarantee generalisation, it is recommended that the validity and reliability of the scale be validated with the participation of students nationally.

5. CONCLUSIONS

The research goal to determine the reliability and validity of the Turkish version of CCFNPS. The findings indicate that the scale is a reliable and valid instrument for evaluating the basic competencies acquired by nursing students after their first clinical practice. The Likert-type scale's 21 items include five sub-dimensions. The Turkish form of CCFNPS can be used to determine the basic competences that students gain after the first clinical practice. Determining the areas where students are least competent will provide important information for the development of educational strategies that will ensure the development of the student. Future research conducted with this scale will contribute significantly to recognition of the scale's measurement power. Still, it is recommended that studies be carried out to verify the reliability and validity of the instrument in other populations.

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Clinical and Experimental Health Sciences

Reflection of Violence in Health to Physicians, Its Effect on Anxiety and Depression Levels

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ABSTRACT

Objective: The aim of this study is to examine the impact of violence on health and the effect of violence on anxiety and depression levels in physicians.

Methods: 442 physicians working in internal medicine and surgery departments were included in this cross-sectional study. The collected data were analysed using IBM SPSS Statistics, version 27.0.

Results: It was found that 63.1% of the physicians were exposed to violence from patients/patient relatives. Anxiety and depression scores were significantly higher among those exposed to violence and those who reported that health-related violence negatively affected their approach to patients.

Conclusions: All forms of violence experienced by research assistants, who play an important role in the provision of health care, in the course of their work have a negative impact on their professional and social lives.

Keywords: Residency, violence in health, depression, anxiety, hospital anxiety and depression scale

1. INTRODUCTION

Violence is a major public health problem that affects all aspects of human life and is increasing worldwide (1). According to the World Health Organization (WHO), violence has emerged throughout human history and is defined as the intentional use of force against oneself, another person, or a group that may cause or contribute to injury, death, or psychological harm (2). Although violence can be found in all areas today, it is particularly prevalent in the workplace and is becoming a serious problem in all professions. In particular, health care institutions are among the high-risk workplaces in terms of exposure to workplace violence. Violence perpetrated in these institutions "comes from the patient, the patient's relatives or any other person and poses a risk to the health care worker; threatening behaviour, verbal threats, physical assault and sexual assault (3,4). Despite all the precautions and proposals, violence in healthcare has increased over the years. Because of this increase, it is stated that physicians and health care workers are at risk of being exposed to violence ranging from physical to psychological violence (3,5).

WHO emphasizes that 8-38% of those working in health institutions have been exposed to physical violence at any point in their working life (6). More attention and reporting of physical violence in health institutions causes psychological and verbal violence to be ignored. For this reason, it is estimated that the frequency of violence in health institutions is higher than the stated rates (7). It is stated that especially psychological violence can be more dangerous than physical violence for physicians and health workers in terms of leaving permanent psychological and psychosomatic effects (8).

The main causes of violence experienced in health institutions are factors such as the 24-hour uninterrupted service of the institutions, the presence of stressed patient relatives, the long wait for service, the inability to benefit from health services, the workload, the inadequacy of the number of employees, the work in a crowded environment, the lack of knowledge of the employee about coping with violence, the absence of adequate security personnel, and the inadequacy of laws limiting violence (9,10).

Violence in health institutions is an important issue not only with its causes but also with its consequences. Violence or the threat of violence perpetrated in this area can cause

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both dissatisfaction of the employees and negative effects on the structure of the institutions by affecting the service quality. Employees exposed to violence; It is stated that psychological effects of violence such as confusion, anger, helplessness, fear, burnout, loss of confidence, anxiety and depression occur, and post-traumatic stress disorder may develop especially in employees who have been subjected to physical violence (11,12).

The aim of this study is to investigate the reflection of violence in health and the effect of violence on anxiety and depression levels among physicians working as research assistants in the internal and surgical departments of Necmettin Erbakan University Medical Faculty Hospital.

2. METHODS

The study was designed as a cross-sectional study. Written permission to conduct the study was obtained from the Non-Pharmaceutical and Medical Device Ethics Committee of Necmettin Erbakan University Medical Faculty (Decision No. 2023/4125) and the Chief Physician of Necmettin Erbakan University Medical Faculty Hospital.

The population of the research consists of 567 research assistants working in the internal and surgical departments of Necmettin Erbakan University Medical Faculty Hospital. The sample size of the study; Based on the chi-squared test in the G Power program, the type 1 error was calculated as 0.05, the power was calculated as 95%, the effect size was small-medium (0.2), and the maximum degree of freedom was 3, with a minimum of 390. Data from the study sample were collected using stratified simple random sampling. First, doctors were stratified according to their specialty, and the number of residents to be reached from each specialty was determined by weighting according to the number of residents in each specialty. Residents in each branch were then contacted using simple random sampling. The inclusion criteria for the study were defined as working as a research assistant in the medical and surgical departments of the hospital, volunteering to participate in the study, and answering all questions on the data collection form. Six participants were found to have deficiencies in the data collection form and were excluded from the study. The research was completed with 442 doctors working as research assistants in internal medicine and surgery who gave verbal consent to participate in the study between 1 and 20 January 2023.

After the literature review for the research, a data collection form consisting of 36 questions and 4 parts was prepared. The first part of the form, consisting of 6 questions, asks about the socio-demographic characteristics of the physicians, the second part, consisting of 4 questions, asks about the characteristics of the working life, and the third part, consisting of 12 questions, asks about the physicians' views on violence in health care. The fourth part of the data collection form is the Hospital Anxiety and Depression Scale (HADS). The HADS is a self-administered scale. It was developed by Zigmond and Snalth and a Turkish validity and reliability study was conducted by Aydemir et al. (13,14). There are 14 questions in the scale, 7 of which measure anxiety and 7 of which measure depression and are presented in a 4-point Likert structure. For Turkish individuals, the cut-off score for anxiety was 10 and the cut-off score for depression was 7. The scores that can be obtained from both the anxiety and depression subscales of the scale are a minimum of 0 and a maximum of 21 points. The Cronbach's alpha coefficient calculated in the validity and reliability study of the scale was 0.918 (14). In this study, the Cronbach's alpha coefficient of the scale was calculated to be 0.860. Data collection forms were administered to volunteer participants under observation. The forms took approximately 15 minutes to complete.

2.1. Statistical analysis

Statistical analysis of the data was performed using IBM SPSS, version 27.0 (IBM Corp, Armonk, N.Y. USA). Visual (histograms and probability plots) and analytical (Kolmogrorov-Smirnov) methods were used to test the conformity of the data with the normal distribution. Numerical data were evaluated using arithmetic mean±standard deviation, median (1st and 3rd quarter); frequency distributions and percentages were used to summarise categorical data. Anxiety and depression scores and data; evaluated using Mann-Whitney U and Kruskal-Wallis H tests. Post-hoc Mann-Whitney U test with Bonferroni correction was performed for pairwise comparisons between groups with significant Kruskal-Wallis H test results. Categorical data were compared with the chi-square test. Statistically, cases with p less than .05 were considered significant.

3. RESULTS

51.4% (n=227) of the study group consisting of 442 physicians with a mean age of 27.71±2.39 years were female and 58.1% (n=257) were single. The sociodemographic characteristics of the participants are shown in Table 1.

Table 1. *Sociodemographic characteristics of participants*

Characteristic		n (%)
Gender	Female	227 (51.4)
	Male	215 (48.6)
Marital status	Married	185 (41.9)
	Single	257 (58.1)
Living with	Living alone	177 (40.0)
	With mother and/or father	61 (13.8)
	With spouse and/or child	180 (40.7)
	With a friend	24 (5.4)
Child present	No	259 (81.2)
	Yes	83 (18.8)
Presence of chronic illness	No	412 (93.2)
	Yes	30 (6.8)
Presence of hobby	No	281 (63.6)
	Yes	161 (36.4)

72.2% (n=319) of the physicians included in the study worked as research assistants in internal medicine and 27.8% (n=123) in surgery. The median length of practice was 2.50 (1.50-4.00) years. It was found that 86.4% (n=352) of doctors worked 7.00 (5.00-8.00) shifts per month.

It was found that 63.1% (n=279) of physicians were exposed to violence from patients/patient relatives, mostly verbal violence. It was found that 63.9% of female doctors and 62.3% of male doctors were exposed to violence. The rates of exposure to violence were similar between male and female doctors (p = .405). Physicians who were exposed to violence were mostly exposed to violence in the polyclinic (66.3%) and by patients' relatives (89.2%) (Table 2).

Table 2. *Physicians' exposure to violence*

Characteristic		n (%)
Exposure to violence	No	163 (36.9)
	Yes	279 (63.1)
Type of violence (n=279)*	Verbal violence	269 (96.4)
	Physical violence	55 (19.7)
	Sexual violence	13 (4.7)
Place of violence (n=279)*	Policlinic	185 (66.3)
	Service	152 (54.5)
	Other (emergency, intensive care)	44 (15.8)
Violent (n=279)*	Patient	119 (42.7)
	Patient's relatives	249 (89.2)
	Hospital staff	21 (7.5)

**Participants ticked more than one option.*

According to physicians, the three most important causes of violence in health care were impatience of patients and/or their relatives with 74.7% (n=330), loss of prestige of physicians and health care professionals with 73.5% (n=325) and insufficient criminal sanctions with 73.5% (n=325). The most important reason for female physicians was the decrease in respect towards physicians and healthcare professionals with 81.5% (n=185), and for male physicians, the impatience of patients and their relatives with 71.6% (n=154) (Table 3).

71.9% (n=318) of the doctors surveyed said that violence in health care had a negative impact on their approach to patients. Of the 318 physicians who reported a negative impact, 61.9% (n=197) reported that they felt the need to take precautions against the possibility of violence, 51.6% (n=164) reported that they had difficulty tolerating patients' demands, and 50.6% (n=161) reported that they were afraid to be physically close to the patient. When male and female doctors were assessed separately, the most distressing situation for both groups was "feeling the need to take precautions against the possibility of violence" (Table 4).

If confronted with violence in the workplace, 63.3% (n=280) of doctors said they would give a white code, 22.2% (n=98) would react/defend themselves, 12.2% (n=54) would try to calm the perpetrators and the remaining 2.3% (n=10) would not react.

Table 3. *Main causes of health-related violence according to doctors*

The main causes of violence in healthcare*	All participants (n=442)	Female physicians (n=227)	Male physicians (n=215)
	n (%)	n (%)	n (%)
Patient and family impatience	330 (74.7)	176 (77.5)	154 (71.6)
Lack of respect for doctors and health professionals	325 (73.5)	185 (81.5)	140 (65.1)
Insufficient criminal sanctions	325 (73.5)	174 (76.7)	151 (70.2)
Health policies	304 (68.8)	159 (70.0)	145 (67.4)
Excessive workload for doctors and health professionals	194 (66.5)	165 (72.7)	129 (60.0)
Misdirection of my society by news and series in the media	267 (60.4)	144 (63.4)	123 (57.2)
Insufficient safety measures in health centres	250 (56.6)	136 (59.9)	114 (53.0)
Insufficient time for patients and their families	245 (55.4)	122 (53.7)	123 (57.2)
Inadequate communication between patient and doctor	175 (39.6)	76 (33.5)	99 (46.0)

**Participants ticked more than one option.*

Table 4. *Affected type of physician's approach to patients*

Influence on approach to patients*	All participants (n=318)	Female physicians (n=173)	Male physicians (n=145)
	n (%)	n (%)	n (%)
Feeling the need to take precautions against the possibility of violence	197 (61.9)	107 (61.8)	90 (62.1)
Difficulty tolerating patient demands	164 (51.6)	95 (54.9)	69 (47.6)
Avoiding being physically close to the patient	161 (50.6)	97 (56.1)	64 (44.1)
Avoidance of out-of-hours medical responsibilities	148 (46.5)	79 (45.7)	69 (47.6)
Reduced verbal communication with patient	137 (43.1)	66 (38.2)	71 (49.0)
Feeling the need to consult other departments more	137 (43.1)	76 (43.9)	61 (42.1)
Decreased ability to explain to patients and their families	101 (31.8)	50 (28.9)	51 (35.2)
Shortening of history and examination process	89 (28.0)	44 (25.4)	45 (31.0)
Not wanting to be in hospital	141 (44.3)	89 (51.4)	52 (35.9)
Don't want to give up medicine	73 (23.0)	47 (27.2)	26 (17.9)

**Participants ticked more than one option.*

It was found that 80.1% (n=354) of doctors were concerned about being exposed to violence in the course of their work. In the hospital where they work, 30.3% (n=134) of the doctors reported feeling safe, 20.1% (n=89) were undecided, and 49.6% (n=219) did not feel safe. 66.1% (n=292) of doctors reported that the violence they experienced and/or witnessed had a negative impact on their social life.

Table 5. *Comparison of socio-demographic characteristics of physicians with their HADS scores*

Characteristic	Anxiety Score		Depression Score	
	Median (Quarter 1-3)	*p value*	Median (Quarter 1-3)	*p value*
Gender				
Female	9.00 (6.00-11.00)	**.003***	8.00 (5.00-10.00)	.840*
Male	8.00 (6.00-10.00)		7.00 (5.00-10.00)	
Marital status				
Married	8.00 (6.00-11.00)	.367*	7.00 (4.00-10.00)	.459*
Single	8.00 (6.00-11.00)		8.00 (5.00-10.00)	
Living with				
Living alone	8.00 (6.00-11.00)	.789**	8.00 (5.00-10.00)	.359**
With mother and/or father	8.00 (5.50-11.00)		7.00 (3.50-9.00)	
With spouse and/or child	8.00 (6.00-11.00)		7.00 (4.00-10.00)	
With a friend	8.50 (6.00-10.00)		8.00 (6.25-11.00)	
Child present				
No	8.00 (6.00-11.00)	.476*	8.00 (5.00-10.00)	.079*
Yes	8.00 (6.00-11.00)		7.00 (4.00-9.00)	
Presence of chronic illness				
No	8.00 (6.00-11.00)	**.005***	7.00 (5.00-10.00)	.523*
Yes	10.50 (6.75-13.25)		7.00 (4.75-12.25)	
Presence of hobby				
No	8.00 (6.00-11.00)	.178*	8.00 (5.00-10.00)	.118*
Yes	8.00 (5.00-10.00)		7.00 (4.00-10.00)	
Branch				
Internal Sciences	8.00 (6.00-11.00)	.054*	7.00 (5.00-10.00)	.262*
Surgical Sciences	9.00 (6.00-11.00)		8.00 (5.00-10.00)	

**Mann-Whitney U test*
***Kruskal-Wallis H test*

The median HADS anxiety score for physicians was 8.00 (6.00-11.00) and the median depression score was 7.00 (5.00-10.00). While 36.2% (n=160) of physicians had an anxiety score of 10 and above, 60.2% (n=266) had a depression score of 7 and above. The comparison of socio-demographic characteristics of physicians with anxiety and depression scores is shown in Table 5. The anxiety scores of female doctors were significantly higher than those of male doctors (p= .003). Anxiety scores were significantly higher in patients with chronic diseases than in those without (p= .005).

Anxiety and depression scores were found to be significantly higher among those who were exposed to violence, those who reported that violence in health care had a negative impact on their approach to patients, those who worried about being exposed to violence while performing their duties, and those who reported that violence in health care had a negative impact on their social life. The anxiety and depression scores of doctors who reported feeling unsafe at work were significantly higher than those who were undecided and those who reported feeling safe (p< .001) (Table 6).

Table 6. *Comparison of physicians' responses to the questions on health-related violence with the scores they received from HADS*

Characteristic	Anxiety Score		Depression Score	
	Median (Quarter 1-3)	*p value*	Median (Quarter 1-3)	*p value*
Exposure to violence				
No	7.00 (5.00-10.00)	**.006***	6.00 (3.00-9.00)	**<.001***
Yes	8.00 (6.00-11.00)		8.00 (5.00-10.00)	
Affected approach to the patient				
No	7.00 (4.00-9.00)	**<.001***	6.00 (3.00-9.00)	**<.001***
Yes	9.00 (6.00-11.00)		8.00 (5.00-10.00)	
Living the Worry				
No	7.00 (5.00-10.00)	**.004***	6.00 (3.25-9.75)	**.020***
Yes	8.00 (6.00-11.00)		8.00 (5.00-10.00)	
Negative impact on social life				
No	6.00 (4.00-9.00)	**<.001***	5.00 (2.00-8.00)	**<.001***
Yes	9.00 (7.00-12.00)		8.00 (6.00-10.00)	
Feeling safe at work				
Feels safe	7.00 (4.75-10.00)	**<.001****	5.00 (2.00-9.00)	**<.001****
Indecisive	8.00 (5.00-10.00)		7.00 (5.00-10.00)	
Doesn't feel secure	9.00 (7.00-12.00)		9.00 (6.00-10.00)	

**Mann-Whitney U test*
***Kruskal-Wallis H test*

4. DISCUSSION

The aim of this study was to examine the impact of violence in health on the work and social life of physicians working as research assistants and to determine the effect of this impact on the level of anxiety and depression in physicians. The study

found that more than half of the research assistants were exposed to violence. In the studies conducted, it was found that the prevalence of violence in health care was 25.0% in the USA, while this rate was 19.0% in Iran (15,16). A meta-analysis study conducted in China reported that the overall prevalence of healthcare workers experiencing violence was 62.4% (17). In studies conducted in our country, these rates varied from 49.3-68.2% (18-21). These differences in the prevalence of violence have been attributed to socio-cultural differences between the provinces or countries in which the studies were conducted, the fact that the participants were selected from different health care units, working conditions such as the number of patients cared for daily, the number of shifts, and personal characteristics such as age and year of work.

This study found that almost all of the doctors who reported being exposed to violence were exposed to verbal violence and about one fifth were exposed to physical violence. Similar studies of national and international health professionals have found rates of exposure to verbal and physical violence similar to the findings of this study (19,22).

In this study, the frequency of violence was similar between male and female doctors. This finding differs from studies showing that women are exposed to more violence (19,27).

4.7% of the doctors included in the study reported that they had been exposed to sexual violence. It has been reported in the literature that rates of sexual violence against health care workers vary from 2.2 to 8.6% (19,22,27). Sexual violence ranges from shouting and gestures to rape. Some acts of sexual violence may be considered as physical or verbal violence. This can be interpreted as a reason for the differences in rates.

The study found that physicians were most exposed to violence in the polyclinic and by patients' relatives. Some studies found that the most common places of violence were emergency services and inpatient services (23,24). A study using white code data also reported that violent events were more frequent in the emergency department (25). In contrast to the literature, this study found a higher proportion of people who reported experiencing violence in outpatient settings. The reason for this difference may be related to the patient load of the hospital where the study was conducted, the waiting time in the polyclinic and the socio-cultural level of the society in which the study was conducted. Violence, as a social phenomenon, has a dynamic structure. In this context, the differences in the place and time of the research and the people who applied to the hospital may have caused the differences in the places where violence was experienced. Similar to the literature, the study found that the majority of violence was perpetrated by the patients' relatives (19,26). Impatient and unsympathetic behaviour of patients' relatives, insufficient information about the diagnosis and treatment of their patients, and ineffective communication with doctors can be considered as reasons for this finding.

In this study, impatience of patients and/or their relatives, reduced respect for doctors and health professionals and inadequate criminal sanctions were found to be the three most common causes of violence in health care. In a similar study conducted by Uskun in 2022, the most common causes were: illiteracy, low socio-economic status, psychological and social problems, health policies, inadequate legal regulations and safety measures, and the media (27). In a study conducted in Pakistan, lack of patient education was found to be the most common reason (28). Although the ways in which the reasons are expressed in the studies vary, it can be interpreted that the reason for violence is related to the level of education of individuals in the society and the cultural and sociological structure of the society. For this reason, it can be concluded that violence in health is multidimensional and it is an issue that should be evaluated from this perspective.

For female doctors, the most important reason for violence against health workers was the lack of respect for health workers, while for male doctors it was the impatience of patients and their relatives. A male-dominated social structure, gender perceptions and power imbalances between the sexes make it difficult for women to be respected in both their professional and social lives. This may have paved the way for the inability of patients and their relatives to respect female doctors and why violence in health care is the most important reason for female doctors.

The study found that almost three-quarters of doctors had experienced a negative impact on their approach to patients as a result of witnessing or experiencing violence. The doctors who reported being most negatively affected were found to feel the need to take precautions against the possibility of violence, to have difficulty tolerating the demands of patients, and to be afraid of being physically close to the patient. In a similar study, it was found that health workers who were exposed to violence displayed a more cautious and self-protective attitude towards patients and did not enjoy their work as much as before (27). Another study showed that the doctor-patient relationship, which is based on trust in the health care system, can deteriorate because of health care violence (29). Mistrust, negative emotions and self-protective attitudes between doctor and patient can disrupt patient-doctor communication and increase the risk of violence. This highlights the importance of improvements in the area of violence in health care, both to improve the quality of health care and to protect the doctor-patient relationship, which should be based on trust.

The study found that about a third of doctors screened with the HADS had anxiety and more than half had depression. In some international studies, the general prevalence of anxiety among physicians ranged from 20.0-74.1%, and the prevalence of depression from 21.3-62.0% (30-33). The rates of anxiety and depressive symptoms found in this study are consistent with international studies, but the figures may vary depending on the country, setting and screening tool used. In

addition, similar to the literature, female doctors were found to have higher levels of anxiety than males (32,33).

In this study, those who were exposed to violence had higher levels of anxiety and depression than those who were not, and those who reported that violence in health care had a negative impact on their approach to patients. It has been supported by studies that various psychological problems such as post-traumatic stress disorder, anxiety, depression and burnout can occur in health care workers exposed to violence (11,15,19,26,30). In addition, it has been noted that friends and family members of victims of violence may also experience psychological distress, and even the occupational and psychological status of those who witness violence may be adversely affected (34).

In addition, the anxiety and depression scores of doctors who reported that they did not feel safe at work were higher than those who were undecided and those who reported that they did feel safe. In a study conducted by Yılmaz in 2020, 68.0% of healthcare workers who were exposed to violence reported that they did not receive support from their managers (35). The fact that doctors and health workers do not receive enough support from managers after the violence they experience, the lack of a system that protects them when they are right, the inadequacy of criminal sanctions and the inability to provide quick and adequate solutions as a result of complaints lead to a decrease in the trust of health workers towards the system they are in and the hospital.

5. CONCLUSIONS

As a result of this study, which was conducted on 442 physicians working as research assistants in the Department of Internal and Surgical Sciences at Necmettin Erbakan Faculty of Medicine Hospital, it was found that more than half of the physicians were exposed to at least one type of violence. The study found that the prevalence of anxiety was 36.2% and the prevalence of depression was 60.2% among physicians screened with the HADS. Anxiety and depression scores were higher among those who had been exposed to violence, those who reported that violent incidents had affected their approach to patients, those who did not feel safe while working in the hospital, those who reported that the violent incidents they experienced and/or witnessed had a negative impact on their social life, and those who reported that they were worried about encountering violence while performing their duties.

All forms of violence experienced by research assistants, who play an important role in the provision of health care, in the course of their work have a negative impact on their professional and social lives. It prevents physicians exposed to violence from performing their work in a prejudiced way, reduces their tolerance towards patients and prevents them from performing their work in a healthy way. It is necessary to solve the problems of violence against health professionals and to create a safe environment for doctors to practice their profession. It is also extremely important to identify the factors that lead to violence and to recommend solutions to prevent it. Before doctors can effectively care for their patients, they must feel safe. Managers should also fulfil their obligations to ensure a safe environment. Physicians should be informed about code white practices and legal recourse available after violence, and should be supported in implementing these steps after violence. Staff exposed to violence should be provided with immediate and appropriate security support. If necessary, psychological support should be provided.

Our study had a number of limitations. Due to the cross-sectional design of the study, causal relationships between different factors associated with anxiety and depression could not be evaluated for a long time. In addition, only research assistants working at the Faculty of Medicine were included in the study. It is an important limitation that those who work as health professionals in different institutions and in different areas are not included in the study. Finally, the HADS used to assess the prevalence of anxiety and depression is a self-administered screening tool and is not used for diagnostic purposes. Therefore, studies using diagnostic tools such as the Structured and Clinical Interview for DSM or the Mini International Neuropsychiatric Interview are recommended to confirm our findings. Despite the above limitations, this study highlights the negative impact on the mental health of people exposed to health violence and contributes to the literature on the importance of this issue.

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Clinical and Experimental Health Sciences

Novel Thiazole-Hydrazide Derivatives and Their Anticancer Properties

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ABSTRACT

Objective: Cancer is described as uncontrolled cell division, and it is a major problem in Türkiye, as well as around the world. Current treatment options are insufficient in some cases, particularly the treatment rate for lung cancer cases, which is very low. Meanwhile, current pharmaceuticals have several side effects, such as drug-drug interactions, and cognitive disorders. Additionally, developing drug resistance is a major problem for current and future management of the disease. Accordingly, the search for new molecules or alternative treatment options is actively achieved.

Methods: In this study, eight novel thiazole-hydrazide analogs were designed and synthesized, and their structural elucidation was performed via HRMS, 1H-NMR, and 13C-NMR. Their biological activity profile was investigated on A549 lung carcinoma and MCF7 breast adenocarcinoma cells. To determine the selective cytotoxicity on cancer cells, they were also tested against NIH/3T3 healthy cell line. Besides that, an *in silico* study was performed to understand the binding modes of the compounds.

Results: The results showed that in the serial 4f and 4g, the most bulky analogues, showed no inhibition against any cell type, even at the highest concentration tested. On the other hand, 4a, 4b, 4d, 4e, and 4h showed less cytotoxicity on healthy cells than A549 cells, so they exhibited significant cytotoxicity and a selective profile against A549 cancer cells. While they also inhibited MCF7 cells. The major point is that para-chlorophenyl analogs at the fourth position on thiazole (4a and 4d) displayed a better anticancer profile than ortho-chlorophenyl analogs. These two compounds were also investigated for their apoptotic effects using in silico studies. Both experimental and *in silico* studies revealed that the combination of thiazole and hydrazinoacetyl has a significant impact against cancer cells, and *in silico* study also suggested that tri-substitute thiazole ring has anticancer potential that induced cancer cell death via apoptosis.

Conclusion: Results of this study was presented that compound 4a was the most potent compound against lung cancer cells (A549) and 4d was the most potent compound against breast cancer cells (MCF-7). Furthermore, analyzing the molecular docking study for promising compounds (4a and 4d) suggested that interactions with the loop region residues have a pivotal role in inducing caspase-3 enzyme activity. It was concluded that hybridization of thiazole and hydrazinoacetyl moieties is responsible for the anticancer activity.

Keywords: Thiazole; hydrazide; anticancer; A549 lung cancer; MCF7 breast cancer

1. INTRODUCTION

Cancer is a public health problem with high incidence and mortality worldwide, including Türkiye (1). According to the WHO 2020 report (2), there were approximately 37070 lung and 7161 breast cancer deaths in Türkiye with an annual incidence of close to 41264 and 24175 cases, respectively. Chemotherapy is one of the treatment options, however, there are a lot of challenges, such as drug resistance (3, 4), and side effects (5, 6). Therefore, it is obvious that humanity needs the development of new therapeutic agents. For this purpose, pharmaceutical chemists have a great responsibility in designing and finding new molecules. The cytotoxic effects of molecules containing the thiazole ring system in their structure on various cells, from cancer (7, 8) to invasive microbial organisms (9, 10), have been investigated to date. Some studies specifically reported that the effect of thiazole-containing compounds on A549 and MCF7 cell lines is significant because of its lower cytotoxicity against healthy cell lines (11-14). Moreover, this ring system has also been

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revealed to overcome developing drug resistance issues (15, 16). Molecular mechanistic studies have indicated that the effectiveness of thiazole derivatives in cancer treatment may be achieved through many mechanisms (15, 17, 18) such as aromatase inhibition (19, 20), EGFR inhibition (21), and MMP-9 (22) inhibition. This ring system has also reported as apoptosis inducer (23-25). All this information points to the anticancer potential of thiazole-derived compounds. On the other hand, previous published papers have revealed that the parts substituted to the thiazole ring system are decisive for the inhibitory strength of the molecule, its mechanism of action or some side effect profiles. Although the substituted parts augment the cytotoxic activity of the molecule mainly by ensuring and/or increasing the stability of the ligand-protein complex, they can also be responsible for serious problems such as drug-drug interactions (26, 27), high cytotoxicity on healthy cells (28, 29), and cognitive dysfunctions (30, 31). For example, substitution of the thiazole ring with hydroxamate has been shown to reduce oral bioavailability, metabolism and selectivity and cause in vivo instability problems in many drugs. For this reason, hydroxamate has been reported as an undesirable group in designing drug molecules. Therefore, it is of great importance to realize substitutions intelligently and evaluate the designed molecules in terms of possible negative effects during the drug design process (32-34). It is known that some groups such as amides, esters and hydrazides are frequently used in drug design studies. Studies have shown that acetamide moiety substitution increases the anticancer effect of thiazoles (35, 36). Moreover, some other studies have reported that hydrazide has a significant effect on the anticancer activity of the molecule because it can form H-bonds (37, 38). In this study, we aimed to design and synthesize effective, safe, stabile, and economic (39-41) anticancer molecules by bringing these parts together on the same molecule. Moreover, *in silico* studies have attempted to elucidate whether the caspase-3 enzyme is a possible molecular target of the tested thiazole-hydrazide analogues.

2. METHODS

2.1. Chemistry

All chemicals used in the syntheses were purchased either from Merck Chemicals (Merck KGaA, Darmstadt, Germany) or Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA). The reactions and the purities of the compounds were observed by thin-layer chromatography (TLC) on silica gel 60 F_{254} aluminum sheets obtained from Merck (Darmstadt, Germany). The melting points of the synthesized compounds were recorded by the MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and presented as uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded by a Bruker 300 MHz NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) using DMSO-d_6 as a solvent for the samples. In the NMR spectra, splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants (*J*) were reported as Hertz. High-resolution mass spectrometric (HRMS) studies were performed using an LC/MS-IT-TOF system (Shimadzu, Kyoto, Japan).

2.1.1. General Synthesis of Ethyl 2-(2-chlorophenoxy) acetate (1)

2-chlorophenol (0.05 mol, 6.4 g), and ethyl 2-chloroacetate (0.06 mol, 6.42 mL) were added into a flask and then potassium carbonate (0.075 mol, 10.35 g) was put in this mixture. This mixture was refluxed in 150 mL of acetone for 20 h. The completion of the reaction was controlled with TLC analysis. The solvent was evaporated, and the raw material was filtered and washed with water before recrystallization with ethanol.

2.1.2. General Synthesis of 2-(2-chlorophenoxy) acetohydrazide (2)

Ethyl 2-(2-chlorophenoxy)acetate (**1**) (0.025 mol, 5.36 g), and hydrazine hydrate (0.1 mol of 85 %) were stirred at room temperature in ethanol (100 mL). The completion of the reaction was controlled using TLC. Stirring was stopped and the mixture was awaited till a precipitate was formed. The precipitated material was filtered. After drying, the product was recrystallized with ethanol.

2.1.3. General Synthesis of 2-(2-(2-chlorophenoxy)acetyl)-N-phenylhydrazinecarbothioamide Derivatives (3)

2-(2-Chlorophenoxy)acetohydrazide (**2**) (0.017 mol, 3.4g) and phenylisothiocyanate derivatives (0.02 mol) were refluxed in 100 mL of ethanol for 3h. TLC was used to observe the reaction. The solvent was evaporated, and the scraped material was removed. The raw product was recrystallized from ethanol.

2.1.4. General Synthesis of 2-(2-chlorophenoxy)-N'-(3,4-diphenylthiazol-2(3H)-ylidene) acetohydrazide Derivatives (4a–4h)

The synthesized intermediates 2-[2-(2-chlorophenoxy)acetyl]-*N*-phenyl hydrazinecarbo-thioamide derivatives (**3**) (0.001 mol) were refluxed with phenacyl bromide derivatives (0.001 mol) for 2h in ethanol. After overnight standing in a cool place, crystals of the final thiazole compounds were filtered off.

2.2. Antiproliferative Activity

The cytotoxic activities of the compounds were performed against NIH/3T3, MCF-7, and A549 cell lines according to the previously reported method (39), using doxorubicin as the standard drug. The inhibitory concentrations were determined for each compound against the tested cell lines, and the results are presented in µg/mL.

2.3. ADME Parameters

Some physicochemical properties of the compounds were predicted with Swiss ADME software (40, 41).

2.4. In silico study on caspase-3 enyzme

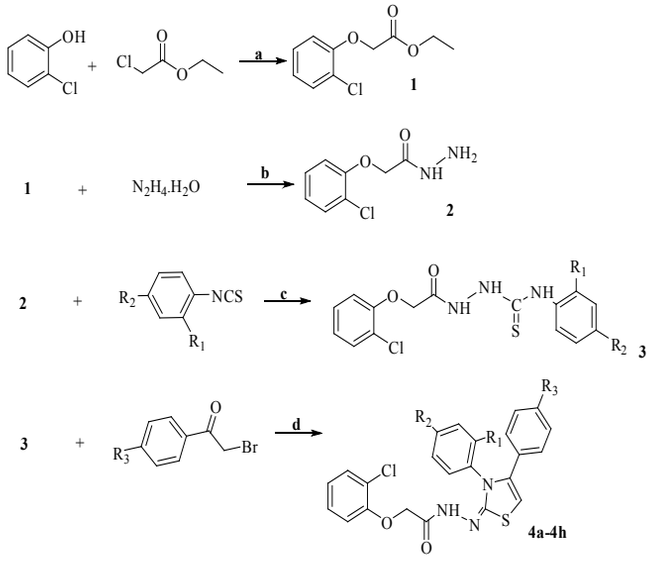
The *in silico* study was run using the Schrodinger Maestro Drug Discovery program (42). The most active compounds (4a and 4d) were prepared using the LigPrep module for the docking study. The X-RAY crystal structure of the caspase-3 enzyme was retrieved from the Protein Data Bank web server (PDBID: 4QTX), this structure was prepared using the Protein Wizard module in the default setting. The area of residues in the allosteric binding cleft was mapped by the Grid Generation module, then the docking procedure was applied similarly to our previous studies (43).

3. RESULTS

3.1. Chemistry

The synthesis diagram of the final molecules 4a-4h was displayed in Figure 1. At first, the starting material, ethyl 2-(2-chlorophenoxy)acetate (1), was obtained from the reaction of 2-chlorophenol and ethyl 2-chloroacetate. Then, compound 1 was treated with hydrazine monohydrate to gain 2-(2-chlorophenoxy)acetohydrazide (2) (44). The resulting molecule (2) was reacted with phenylisothiocyanate derivatives to synthesize 2-[2-(2-chlorophenoxy)acetyl]-N-phenylhydrazinecarbothioamide derivatives (3) (44), and those products were also refluxed with phenacyl bromide derivatives to gain 2-(2-chlorophenoxy)-N'-(3,4-diphenylthiazol-2(3H)-ylidene)acetohydrazide derivatives (4a-4h). The structures of all synthesized compounds (4a-4h) were confirmed by high-resolution mass spectroscopy (HRMS), 1H-NMR, and 13C-NMR. All analysis results were shared with the spectra in the supplementary file.

In both NMR (1H-NMR and 13C-NMR) spectra, the peaks of the aromatic and aliphatic regions were observed at estimated areas. The mass spectra of the compounds' [M+1] peaks were observed in agreement with their predicted molecular formula (4a-4h).



Compound	R^1	R^2	R^3	Compound	R^1	R^2	R^3
4a	-H	-Cl	-H	4e	-Cl	-H	-H
4b	-H	-Cl	-Cl	4f	-Cl	-H	-Cl
4c	-H	-Cl	$-NO_2$	4g	-Cl	-H	$-NO_2$
4d	-H	-Cl	-OMe	4h	-Cl	-H	-OMe

Figure 1. *The synthesis scheme of the compounds* ***4a-4h.*** *Reagents and conditions:* ***(a)*** *K_2CO_3, Acetone, ref., 20 h;* ***(b)*** *EtOH, 0-5 °C, then r.t 3 h;* ***(c)*** *Acetone, Potassium carbonate, r.t.,* ***(d)*** *EtOH, reflux, 2h.*

3.2. ADME Parameters

The predictable physicochemical properties which are number of H-bond acceptor (HBA), H-bond donor (HBD), topologic polar surface area (TPSA, Å²), partition coefficient (Log Po/w), water solubility (Log S), gastrointestinal absorption level (GIA), skin permeation (Log Kp, cm/s), violation number of drug-likeness rules and synthetic accessibility (SA) of the final compounds and standard drug, was determined and they are shown in Table 1. Accordingly, it was determined that the compounds had an HBA number of 3-5, an HBD number of 1 and a TPSA of 83.86-129.68 Å². Log P values of the compounds were determined in between 4.56-5.89 whereas they have Log S values of – 8.72 – -7.93. Except for compounds 4c and 4g, the synthesized compounds were predicted to have high gastrointestinal absorption. The synthetic accessibility indicates that the compounds can be synthesized easily., that SA values were calculated at a range of 3.55-3.73 (1 is for very easy and 10 is for very difficult.)

Table 1. *Physicochemical, pharmacokinetic, and medicinal chemistry properties of the final compounds (by SwissAdme) (4a-4h).*

	HBA	HBD	TPSA	Log $P_{o/w}$	Log S	GIA	RoF (V)	SA
4a	3	1	83.86	5.36	-7.93	High	Yes (1)	3.55
4b	3	1	83.86	5.89	-8.59	High	No (2)	3.56
4c	5	1	129.68	4.56	-8.72	Low	Yes (1)	3.66
4d	4	1	93.09	5.29	-8.10	High	No (2)	3.70
4e	3	1	83.86	5.35	-7.93	High	Yes (1)	3.59
4f	3	1	83.86	5.86	-8.59	High	No (2)	3.60
4g	5	1	129.68	4.57	-8.72	Low	Yes (1)	3.69
4h	4	1	93.09	5.26	-8.10	High	No (2)	3.73
Ref. D.	12	6	206.07	0.52	-3.91	Low	Yes (1)	5.81

HBA: *H-bond acceptor,* ***HBD:*** *H-bond donor,* ***TPSA:*** *Topologic polar surface area (Å²)* ***Log $P_{o/w}$:*** *Consensus Log $P_{o/w}$ (Average of all five predictions),* ***Log S:*** *Water Solubility,* ***GIA:*** *Gastrointestinal absorption,* ***Log K_p:*** *skin permeation (cm/s)* ***RoF (V):*** *Rule of Five (violation number),* ***SA****: Synthetic accessibility from 1 (very easy) to 10 (very difficult).* ***Reference drug:*** *Doxorubicin*

3.3. Cytotoxic Activity

The antiproliferative activities of the final synthesized compounds (4a-4h) were tested on healthy mouse embryoblast cells NIH/3T3, lung cancer cell line A549, and breast cancer cell line MCF-7 cells. The results in micromolar are presented in Table 2. The reference compound Doxorubicin found to be non-toxic against healthy cells, and its IC50 values against A549 and MCF-7 were calculated as 10.76 µM and 5.51 µM, respectively.

Table 2. *IC_{50} values of the compounds 4a-4h against NIH/3T3, A549, and MCF-7 cell lines (μM)*

Compound	NIH/3T3	A549	MCF-7	Selectivity index A547/ MCF7
4a	>500	26.53±0.08	64.41±0.02	>18.85/>32.21
4b	319.31±0.95	31.59±0.12	246.81±0.09	10.11/1.29
4c	280.94±0.69	429.14±0.57	195.67±0.61	0.66/1.44
4d	>500	37.82±0.06	40.18±0.05	>13.22/>12.44
4e	>500	45.86±0.17	108.62±0.52	>10.90/>4.60
4f	>500	>500	>500	n.c./n.c.
4g	>500	>500	>500	n.c./n.c.
4h	461.46±0.41	69.46±0.33	127.33±0.16	6.64/3.62
Doxorubicin	>500	10.76±0.04	5.51±0.03	n.c.

n.c.: *not calculated*

Compounds 4f and 4g did not show inhibition against all three cells even at the highest concentration tested. The lowest limit of cytotoxicity of all compounds against healthy cells was observed to be an IC50 value of 280 μM. Compound 4a was determined to be the most potent compound among the compounds, with an IC50 value of 26.53 μM. This is approximately half the potency of the standard drug against A549 cells. Significant cytotoxicity potency against A549 was observed in the following order 4a, 4b, 4d, 4e, and 4h, where 4a is the most potent and 4h is the least potent compound. However, none of the compounds reached the potency of doxorubicin. The IC50 value of doxorubicin against MCF-7 was 5.51 μM, while that of compound 4d was 40.18 μM which is considered the most potent of the synthesized compounds followed by compound 4a with an IC50 value of 64.41 μM. The potencies of compounds 4e and 4h followed these compounds. On the other hand, the selectivity index calculations for the compounds' selectivity against A549 cancer cells were determined in the following order 4a> 4d>4e>4b>4h>4c while against MCF7 cancer cells were as 4a>4d>4e>4h>4c>4b.

3.4. In silico results

Possible molecular interactions of the test compounds with caspase-3, a cysteine-protease group enzyme that plays an important role during apoptosis (45, 46), were investigated using in silico methods. The best poses collected from molecular docking studies performed for both compounds are shown in Figure 2. Obtained findings indicated that both compounds docked to enzyme cavity and interacted commonly with Glu123 (aromatic H-bond), Cys163 (H-bond/ Halogen bond), Tyr204 (π-π stacking and π-cation contact/ Ar H-bond), Ser205 (H-bond), Trp206 (π-π stacking), Arg207 (Ar H-bonds), and Phe256 (π-π stacking and π-cation contact) residues. On the other hand, compound 4a interacted specifically with Gly122 (Halogen bond) while 4d formed halogen bond with Gly165 at the same cavity, in addition to that, oxygen of 4-methoxyphenyl group of 4d also formed H-bond with Ser251 residue. The interactions were summarized in Table 3. As seen in the table, hydrazide moiety was localized between loop regions (Cys163 and Arg207 residues) which are identified for allosteric stimulation.

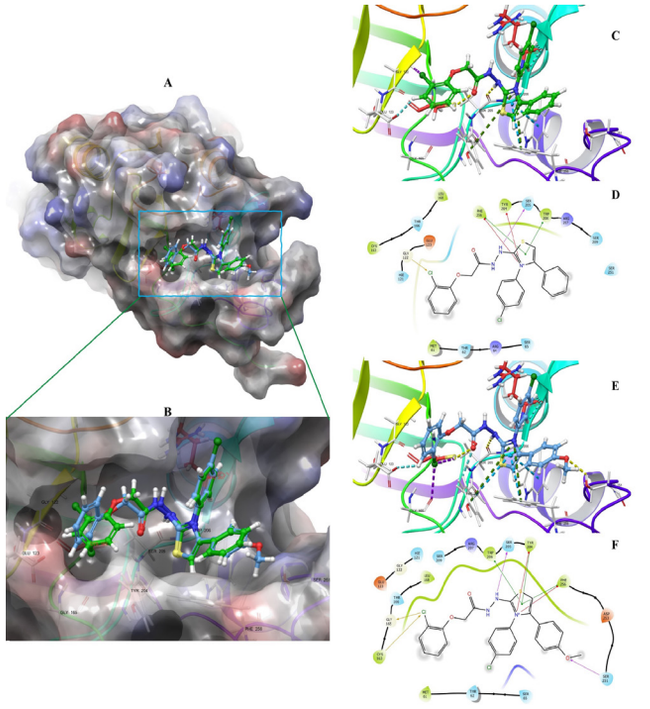


Figure 2. *Poses obtained from Molecular docking study.* ***A and B:*** *Superimposed of* ***4a*** *(green carbons) and* ***4d*** *(blue carbons) at the allosteric cavity of caspase-3 (PDBID: 4QTX);* ***C*** *and* ***D****: 3D and 2D interaction diagrams between* ***4a*** *and enzyme binding cavity;* ***E*** *and* ***F****: 3D and 2D interaction diagrams between* ***4d*** *and enzyme binding cavity.*

Table 3. *Interaction sites of the caspase-3 with the active compounds*

Compound	Moiety	Interaction type
4a	Chlorine of 2-chlorophenoxy	Gly122 (Halogen bond)
	$Hydrogen_3$ of 2-chlorophenoxy	Glu123 (Aromatic H-bond)
	Oxygen of hydrazide	Cys163 (H-bond)
	$Nitrogen_2$ of hydrazide	Ser205 (H-bond)
	Nitrogen of thiazole	Trp206 (π-cation)
		Phe256 (π-cation)
	$Hydrogen_3$ of 4-chlorophenyl	Arg207 (Ar H-bond)
	$Hydrogen_2$ of 4-chlorophenyl	Arg207 (Ar H-bond)
	Thiazole ring	Trp206 (π-π stacking)
		Phe256 (π-π stacking)
4d	$Hydrogen_3$ of 2-chlorophenoxy	Glu123 (Aromatic H-bond)
	Chlorine of 2-chlorophenoxy	Cys163 (Halogen bond)
		Gly165 (Halogen bond)
	Oxygen of hydrazide	Tyr204 (Ar H-bond)
	Nitrogen of thiazole	Tyr204 (π-cation)
		Phe256 (π-cation)
	$Nitrogen_2$ of hydrazide	Ser205 (H-bond)
	$Hydrogen_3$ of 4-chlorophenyl	Arg207 (Ar H-bond)
	$Hydrogen_2$ of 4-chlorophenyl	Arg207 (Ar H-bond)
	Thiazole ring	Tyr204 (π-π stacking)
		Trp206 (π-π stacking
		Phe256 (π-π stacking)
	Methoxy of 4-methoxyphenyl	Ser251 (H-bond)

4. DISCUSSION

Eight new thiazole-hydrazide analogs (4a-4h) were designed and synthesized in this work. The identity and purity of the final molecules were confirmed by using analytical and spectral methods. The 1H-NMR spectra of the compounds indicated that the signals at δ 3.82-4.80 ppm were observed as singlet peaks for Ph-OCH3 protons. O-CH2 protons peaked as a singlet at δ 4.68-4.86 ppm. The propanehydrazide N–H proton was observed as a broad singlet peak at δ 11.42-11.88 ppm. The appearance of a pair of singlet, doublets, triplets and/or multiplets at δ 6.57–8.25 ppm indicated that the aromatic protons. Meanwhile, the 13C-NMR spectra of final molecules peaked at δ 55.77–55.80 ppm for Ph-OCH3 carbon, at δ 66.68-66.94 ppm for O-CH2 carbon, at δ 96.67-160.79 ppm for aromatic carbon and at δ 167.48-167.99 for the carbonyl carbon (C=O). M±1 peaks in HRMS spectra agreed with the calculated molecular weight of the final compounds (4a-4h).

To determine their antitumor potential, they were evaluated on A549 lung carcinoma and MCF7 breast adenocarcinoma cells. They were also tested against the NIH/3T3 healthy cell line to ascertain the selective cytotoxicity on cancer cells. Results showed that even at the highest dose tested, 4f and 4g, were unable to demonstrate inhibition against all three cells. On the other hand, 4a, 4b, 4d, 4e, and 4h exhibited significant cytotoxicity and a selective profile against A549 while they also inhibited MCF7 cells, except 4b because of its low selectivity on cancer cells.

When molecular structures are examined, the serial compounds were divided into two parts according to whether they contained N-(2-chlorophenyl)thiazole or N-(4-chlorophenyl)thiazole substitution. Both series were derived by substituting the phenyl ring at the 4th position of the thiazole ring with the same groups (H, 4-Cl, 4-NO2, 4-OCH3). At the end, compounds 4c and 4g were the lowest cytotoxicity profile against both cancer cells in the series. These molecules are containing nitro group and chlorine atom together.

It was determined that derivative containing non-substituted phenyl ring (4a) were more active than the derivatives containing other substituents (Cl, NO2 and OCH3) against A549 cancer cells. Similar to previous results (10, 27), the results indicated that the small groups more attractive in contributing to the anti-non-small cell lung carcinoma (NSCLC) activity than bulky groups. In another previous study (39), it was declared that the non-substituted thiazole ring is more effective than the 4-substituted thiazole analogs because of its ability to form interactions via nitrogen and aromatic ring system.

The major point is that para-chlorophenyl analogs at the fourth position on thiazole displayed a better anticancer profile than ortho-chrlorophenyl analogs. Among them, 4a against for A549 cells and 4d against for MCF7 were reported as more promising compounds than others.

The results of in vitro studies revealed that compounds 4a and 4d may be effective agents for both NSCLC and breast cancer. Within the scope of this study, the effects of these two compounds, which were comparable to the standard drug, and their possible relationship with apoptosis were also investigated. Based on the knowledge that the caspase-3 enzyme plays a role in intrinsic and extrinsic pathways in both types of cancer (48, 49), possible allosteric activation of the caspase-3 enzyme was investigated by molecular docking studies. As seen clearly in Figure 2-B, the superimposed pose indicated that the variable groups have an impact directly on the localization of the 2-chlorophenoxy as an invariable moiety of the final molecules while the 4-chlorophenyl group was stabilized and was not affected by the variable groups. These findings revealed that hydrazinoacetyl moiety enabled the rotation of the ligands, thus, its flexibility was found important for the activity since the ligands can take a position against loop amino acids, that's why these interactions between ligand and loop residues stabilized the ligand-protein complex. The results of in silico studies suggest that molecules carrying thiazole and hydrazinoacetyl pharmacophores in their structures may lead to the death of cancer cells through caspase-3 activation, and the in vitro anticancer activities of these compounds revealed in this study may be mediated by the activation of apoptotic mechanisms.

In the ADME estimation studies, obtained results showed that there was no more than one violation of Ro5 (Lipinski's rule of five) (47). These results of basic computational chemistry suggested that the final compounds might have good pharmacokinetic profiles. Therefore, it can be suggested that the hit molecules (4a, 4c, 4e, and 4g) might be good candidate anticancer agent(s).

5. CONCLUSION

In the scope of this study, new thiazole-hydrazide structured compounds that may play a role in the treatment of non-small cell lung cancer and breast cancer were designed and structural analyses were performed after the synthesis with the high yield. Then, the activity profiles were evaluated on A549 lung carcinoma and MCF7 breast adenocarcinoma cell lines. They were also tested against the NIH/3T3 healthy cell line to ascertain the selective cytotoxicity on cancer cells. Results showed that 4a, 4c, 4e, and 4g appear to have good druggable profiles. However, even at the highest dose tested, 4f and 4g, the bulkiest analogs in the series, were unable to demonstrate inhibition against all three cells. On the other hand, 4a, 4b, 4d, 4e, and 4h exhibited significant cytotoxicity and a selective profile against A549 while they also inhibited MCF7 cells, except 4b because of its low selectivity on cancer cells. The major point is that para-chlorophenyl analogs at the fourth position on thiazole displayed a better anticancer profile than ortho-chlorophenyl analogs. Among them, 4d and then 4a were reported as more promising compounds than others. Furthermore, analyzing the molecular docking study for promising compounds (4a and 4d) suggested that

interactions with the loop region residues have a pivotal role in inducing caspase-3 enzyme activity. Briefly, the 3-substituted thiazole-hydrazide combination is marked as a promising pharmacophore structure against non-small cancer cells.

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Clinical and Experimental Health Sciences

The Effects of Home-Based Walking on Cancer-Related Fatigue in Patients with Breast Cancer: A Systematic Review

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ABSTRACT

Objective: Home-based walking programs can be a good option for breast cancer patients with fatigue. However, studies on the effectiveness of home-based walking exercise programs have not fully defined proper exercise prescriptions that may be safe and beneficial for breast cancer patients, and their effects on fatigue are still controversial. This systematic review aimed to evaluate the effect of home-based walking on fatigue management in patients with breast cancer.

Method: In this study, electronic databases such as Scopus, Pubmed, Web of Science, Medline, Science Direct, and Ebsco Ultimate were searched. The randomized controlled studies published between January 2002 and February 2022 were included in the study. The data were summarized narratively.

Results: Patients in the studies consisted of young individuals. The majority of their cancer stages ranged from I to III. A significant part of them received chemotherapy. Home-based walking was found to have positive effects on cancer-related fatigue in women with breast cancer. Home-based walking prescriptions were provided to patients through face-to-face or telephone counseling or printed learning materials. It was determined that the exercise frequency, duration and intensity of home-based walking programs varied in the studies.

Conclusion: Home-based walking was found to have positive effects on cancer-related fatigue in women with breast cancer. Therefore, home-based walking can be a simple, cost-effective and safe approach to women with breast cancer. The heterogeneity in reporting procedures suggests that further high-quality and uniform studies should be conducted to reach a stronger consensus on the effects of home-based walking program on fatigue.
Keywords: Breast cancer, walking, fatigue, exercise, physical activity

1. INTRODUCTION

Breast cancer is one of the prominent malignancies that causes significant morbidity in women and places a serious burden on health systems globally (1). It is the most common type of cancer in women worldwide and is increasing day by day (2). According to Globocan 2022 data, approximately one in four women in Türkiye (n=25 249; 23.5%) are newly diagnosed individuals (3). However, mortality pattern has a slowing acceleration for breast cancer. Most of these women are expected to have a good prognosis following treatment. Cancer treatments have many side effects that negatively affect the quality of life. Fatigue is a common symptom and negatively affects activities of daily living. Moreover, persistent fatigue may adversely affect recurrence and survival in patients with breast cancer (4-6). Cancer-related fatigue is particularly high in breast cancer patients compared to other cancer cases, due to extensive anticancer therapy (7).

Despite of the increased number of pharmacological and non-pharmacological approaches recommended in the management of cancer-related fatigue, no “gold standard” treatment is yet available (8). However, exercise-based interventions are the most promising and recommended first-line therapy for cancer-related fatigue (8). These exercise interventions can be performed by under the guaidance of personal trainers or at home settings. However, supervised exercises may not provide time flexibility for patients. Moreover, it is very difficult for tired patients to attend these exercise sessions (9). At this point, home-based exercise programs may be a good option for breast cancer patients (10, 11). Since these exercise programs minimize the frequency of commuting hospital for patients, they also reduce commuting time and energy required for patients with fatigue. Thus, home-based walking programs offer cancer patients control and guidance beyond simple walking recommendations. They also help cancer patients overcome barriers to hospital commuting and attendant accessibility. Moreover, home-based walking

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programs are reliable, simple, convenient and inexpensive, requiring no special skills (9, 10, 12)

Although there are studies suggesting that home-based walking can be used in the management of fatigue in patients with cancer, its effects are still controversial (13). Many issues such as the effect(s), type, frequency and intensity of home-based walking still remain unclear (14). Therefore, this study aimed to evaluate the effect of home-based walking on fatigue management in patients with breast cancer.

2. METHODS

2.1. Research Strategy and Selection Criteria

The protocol for this systematic review was recorded in the PROSPERO registry (CRD420.223.35068). The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) was used in this review. The PRISMA flowchart was created. In the study, the participant, intervention, comparator, and outcome framework were used to develop eligibility criteria as follows. The participants included adult patients with breast cancer, those diagnosed with breast cancer or breast cancer survivors (aged ≥18 years) receiving anticancer therapy, regardless of cancer stage and current treatment (P); intervention: only home-based walking exercise or home-based walking exercise combined with other exercises (I) those in the control group received only usual care or other types of exercise (C) and the outcome was to evaluate the effect of home-based walking on fatigue, using fatigue as a primary or secondary outcome included randomized controlled trials (O). However, studies with supervised exercise processes and group training for those in the intervention group were excluded from this study. Also, studies involving home-based walking exercises for those in the control group were also excluded to avoid contamination of the results.

A systematic literature review was conducted using the databases of Scopus, Pubmed, Web of Science, Medline, Science Direct and Ebsco Ultimate and the keywords of ((“breast neopl*” [MesH] OR tumor [MesH] OR “breast cancer” [MesH]) AND (step OR “home-based walking” [title/abstract] OR “home based walking” [title/abstract] OR pedometer* [title/abstract] OR accelerometer* [title/abstract] OR treadmill [title/abstract]) AND fatigue [MesH]). Due to word limit, a combination of the most mentioned keywords related to the subject such as [(“breast cancer”) AND (“home-based walking” OR “home based walking” OR pedometer) AND fatigue] was used for the Science Direct database. The review was conducted independently by two authors and their disagreements were resolved by a third independent researcher. The database searches were restricted to studies published in English between January 01, 2002 and February 21, 2022.

2.2. Selection Criteria

Table 1 summarizes the selection criteria.

Table 1. *Inclusion and exclusion criteria*

Inclusion Criteria	Exclusion Criteria
• Studies involving adult patients with breast cancer, those diagnosed with breast cancer and receiving anticancer treatment regardless of cancer stage and current treatment, or breast cancer survivors (≥18 years old) • Studies comparing any type of home-based walking with usual care/other types of exercise • Studies with interventions that included only home-based walking training or home walking training combined with other exercises. However, supervised exercises and group training were excluded. • Studies involving home-based walking exercises for those in the control group were excluded to avoid contamination of the results. • Studies using fatigue as a primary or secondary outcome • Randomized controlled studies • Studies published in English • Studies published between 2002-2022	• Studies involving cancer types other than breast cancer • Male patients • Gray Literature • Expert opinions • Qualitative studies • Animal experiments • Study protocols • Presentations • Studies whose full text could not be reached • Systematic reviews, quick review, meta-analysis • Studies not published in English • Studies with poor quality

2.3. Study Selection

Existing studies were screened by the authors for systematic review. Eligible or potentially eligible studies were independently retrieved by the authors for abstract and full-text review. Disagreements between them during the screening process were resolved through discussion and consensus. After the abstract review, studies that met the inclusion criteria were recorded and their full texts were accessed. This process was reported in the PRISMA flowchart (Figure 1).

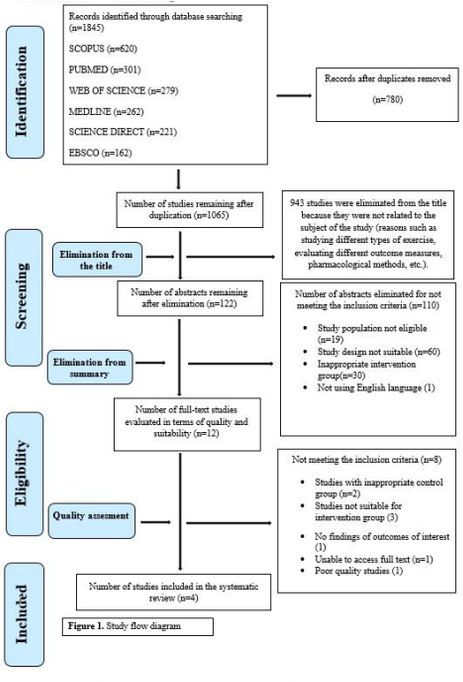

Figure 1. *Study flow diagram*

2.4. Data Extraction

The population, intervention, comparison and outcome (PICO) approach was used in the study. The authors independently extracted the data. Any disagreements between them were resolved through discussions or by an independent researcher outside the study. Data extraction included author and year of publication, country, purpose of study, cancer stage, available treatment, intervention and control groups, data collection tool, sample size, age/sex of participants, and results.

2.5. Study Evaluation and Risk of Bias Assessment

Quality assessments for each selected study were made by two independent researchers. Two researchers independently assigned an overall risk of bias to each trial using the Checklist for Randomized Controlled Trials, and in case of their disagreement, another researcher (H.CA.) was called to resolve the discrepancy. It was adapted into Turkish by Hür et al (15). Items in the tool generally aim to assess four types of bias in studies: performance, selection, detection, and attrition bias. After reading items in the tool and making a methodological evaluation of each item, their relation to the study is marked as "yes", "no", "uncertain" and " inappropriate". The answers of "inappropriate" and "uncertain/ inappropriate" are evaluated as 0 points. The JBI critical assessment probable score is between 0-13.

The JBI Critical Appraisal Checklist for Randomized Controlled Trials was used in the review. In the quality evaluation by two independent researchers, all studies received a low score in the category of "blindly assigning participants into groups". Studies that received a score of 7 or more in the evaluation made by ignoring this category were included in the systematic review. In the quality evaluation, there was a statistically significant and very high agreement between the researchers ($p<.05$). Figure 2 shows the distribution of quality assessment scores of the four studies included in the systematic review. Table 2 presents the consensus table showing the quality scores assigned to the studies.

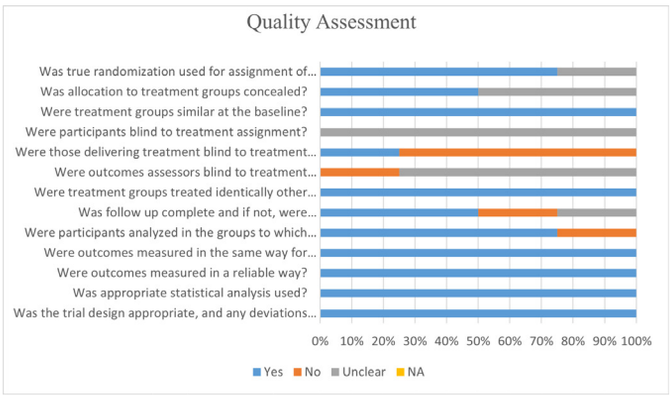

Figure 2. *Graph showing the distribution of scores from each domain according to the JBI Quality Assessment Tool for Randomized Controlled Studies for all studies evaluated as quality.*

Table 2. *Quality evaluation scores of independent evaluators*

	Methodological Quality Assessment			
Authors/Year/ Country	1. Evaluator	2. Evaluator	3. Evaluator*	The Evaluators' Decision
Payne et al (2008) ABD (16)	4/13	4/13	4/13	4/13
Baruth et al. (2015) Colombia (17)	6/13	7/13	7/13	7/13
Gokal et al. (2016) United Kingdom (18)	8/13	9/13	9/13	9/13
Yee et al. (2019) Australia (19)	7/13	8/13	8/13	8/13
Huang et al. (2019) Taiwan (20)	11/13	11/13	11/13	11/13

Quality assessment was carried out by two independent researchers. In order to resolve discrepancies, another researcher was consulted and a final decision was made. Studies with a score of 7 and above in the quality assessment were included.

2.6. Data Synthesis and Analysis

A narrative synthesis of the results of the studies included in our study was structured considering their study design, country of study, sample characteristics, cancer stage, available treatment, treatment/control group characteristics, and scales. The statistical analysis was performed using Microsoft Excel (Microsoft Corporation, 2016). Categorical variables were presented as number of participants (N) and percentage (%).

2.7. Ethical Considerations

An ethics committee approval was not required for the study. No funding source was used in this study.

3. RESULTS

A total of 1845 studies were obtained by searching six databases and using keywords. Of the studies, 780 were eliminated due to duplication. Then, an additional 110 studies, including those with irrelevant subject (n=943), those with unsuitable research design (n=60), those with unsuitable study population/sample (n=19), those with unsuitable intervention (n=30), and those published in languages other than English (n=1) were also eliminated. Full texts of the remaining 12 studies were reviewed for relevance and quality by two independent researchers. As a result, a total of seven studies were eliminated, including one without full text (n=1), one without proper results (n=1), one with unsuitable control group (n=2), and three with unsuitable intervention group (n=3). The remaining five studies were evaluated using the JBI Critical Appraisal Checklist for Randomized Controlled Trials and cone of them was eliminated as it received poor grade (n=1). Finally, four studies (n=4) with a score of seven or higher according to the quality assessment were included in this systematic review. Figure 1 shows the flowchart for selecting studies.

Table 3. *Characteristics of the randomized controlled studies examined*

Author/Year/ Country	Study Purpose	Sample Size (HBW/CG)	Age (mean±SD)/ Sex	Cancer Stage	Treatment	Treatment Group	Control Group	Scales	Results
Baruth et al. (2015) Colombia (15)	To examine the effects of a 12-week home-based walking program on quality of life and fatigue in survivors of early-stage breast cancer. The secondary aim is to examine whether changes in these outcomes are associated with the changes in walking behavior.	20/12	HBW 57.4±6.1 CG 54.9±6.5 Female	I-III	None Completed adjuvant therapy within the last 12 months (Surgery/ Chemotherapy/ radiotherapy/ hormone therapy)	In week 1, participants were instructed to walk at a moderate intensity (RPE 10-11) for 20 minutes, 3 days a week (3 times/wk). If participants could do more than their initial fitness level, they were encouraged to do so. By week 8, participants were instructed to walk at a moderate to vigorous intensity (RPE 12-15) for 30 to 40 minutes 5 days a week and maintain this level of walking for the remainder of the study. A total of 12 weeks of intervention	Participants were asked to maintain their usual level of physical activity, 12 weeks	FACT-Fatigue	Most participants in the walking intervention group had improved fatigue and quality of life outcomes. In contrast, fatigue and quality of life generally remained unchanged or worsened over time, with a few minor improvements for those in the control group. Participants in the walking intervention group experienced greater reductions in fatigue than those in the control group, and this effect was small to moderate. Changes in fatigue/QOL outcomes have generally been associated with changes in walking behavior, ranging from small to moderate effects. **FACT-Fatigue Score Change Baseline Week 12** HBW 14.5 10.0 CG 15.3 14.1
Gokal et al. (2016) United Kingdom (16)	To evaluate the effectiveness of a self-administered home-based moderate-intensity walking intervention on psychosocial health outcomes among breast cancer patients undergoing chemotherapy.	25/25	HBW 52.08± 11.7 CG 52.36±8.9 Female	I-III	Chemotherapy	Home-based, self-managed, moderate intensity walking, 5 times/wk, 30 minute,	Usual care,12 wk	FACT-F	Self-administered home-based intervention was found to have positive effects on fatigue. **FACT-F Score Change Pretest Posttest** **HBW** 32.16± 8.42 26.04±3.80 **CG** 34.24±9.48 33.60±7.29

** Calculated considering participants who are currently undergoing treatment. * HBW: Home Based Walking*

*** Calculated considering all participants. *CG: Control Group*

Table 3. *Characteristics of the randomized controlled studies examined (continued)*

Author/Year/ Country	Study Purpose	Sample Size (HBW/CG)	Age (mean±SD)/ Sex	Cancer Stage	Treatment	Treatment Group	Control Group	Scales	Results
Yee et al. (2019) Australia (17)	To determine the safety and feasibility of a physical activity program for patients with metastatic breast cancer and to investigate the effectiveness of this program.	8/6	HBW 60.1±12.7 CG 65.0±6.9 Female	IV	Hormone therapy/ chemotherapy/no current treatment	Unsupervised walking program, 10-15 minutes of brisk walking followed by 30-40 minutes of resistance training, 2 times a week, 8 weeks	Maintaining their usual level of physical activity, 8 weeks	FACIT-F ECOG Performance Scale (Scale scores of 0-2 were included in the study)	Compared to the mean change scores from baseline to post-intervention, positive trends were observed in favor of those in the exercise group compared to those in the control group. **FACIT-F** **Week 8 Week 16** HBW 5.60±3.20 6.10±3.60 CG – 1.80±3.90 0.8±5.70
Huang et al. (2019) Taiwan (18)	To examine the short – and long-term effects of a personalized, home-based brisk walking program in breast cancer patients undergoing chemotherapy.	81/78	HBW 48.32±7.90 CG 48.31±8.65 Female	I-III	Chemotherapy/ Radiotherapy/ hormone therapy/ Target therapy	Our tailored, home-based brisk walking program (30%-70%), 3 to 5 times a week, gradually increasing from 15–25 minutes/ session to 35–40 minutes/session, 12 weeks	Maintaining spontaneous exercise behaviors, 12 weeks	Brief Fatigue Inventory Karnofsky Performance Status scale (Average Karnofsky Performance Status of participants was 89.91 (SD = 5.16)	Fatigue levels increased over time in both the intervention and control groups after completing treatment. At the end of the 12-week exercise program, those in the exercise program experienced less fatigue than those in the control group, and this group difference was maintained throughout the entire study period. At the end of the exercise program, those who spent more time exercising before diagnosis experienced less fatigue than those who exercised less. In addition, patients' fatigue levels measured at various time points fluctuated, along with their physical performance, sleep disturbances, and depression. Overall fatigue levels increased significantly over time (P = 0.027), but this increase was more significant for those in the control group than those in the exercise group. The attenuating effect of exercise on time slope coefficient was not significant (P = 0.157).
Total		N:255 HBW:134 CG:121	%100 Female	**Stage I-III:%94.5 (n:241)	*Chemotherapy: %85..5 (n:189) **Being treated/ untreated: %13.3 (n:34)				

** Calculated considering participants who are currently undergoing treatment. * HBW: Home Based Walking*

*** Calculated considering all participants. *CG: Control Group*

3.1. Properties of Studies

Table 3 shows the properties of the four studies included in this study (properties of the study, properties of the participants, walking intervention, control groups, scales, and results).

3.2. Participants

Four studies included a total of 255 patients who met the inclusion criteria (intervention: 134; control: 121). The studies were conducted in the United Kingdom, Taiwan, Australia and Colombia. The mean age of the participants in the studies was 48 ~ 65 years. The majority of their cancer stages ranged from I to III. Most of them received chemotherapy. There were also patients who received some other treatments such as radiotherapy, target therapy, hormone therapy.

3.3. Interventions

The researchers/trainers provided participants with home-based walking prescriptions through face-to-face training/counseling, telephone counseling, and printed learning materials. Face-to-face counseling included several topics such as the concept and benefits of brisk walking, correct walking techniques and preparation process, goal setting and exercise safety. Telephone counseling was conducted for various reasons such as motivating participants and increasing their commitment to walking program during the intervention, monitoring participant safety and solving some possible difficulties and inconveniences that may occur during the exercise. Participants in the intervention group were given pedometers and exercise diaries/physical activity records to measure their daily step count, walking time, and perceived exertion, allow them to provide immediate feedback, encourage their self-monitoring, and motivate them. In three studies, the patients' heart rates were monitored during physical activity (18-20). In one study, patients optionally recorded their heart rate during activity in their exercise diaries (17). In only one study, patients did not use a physical activity monitoring tool, but followed up their perceived exertion using Borg's Rating of Perceived Exertion (18).

The interventions included only home-based walking or other exercises such as home-based walking and strength/resistance training. The intensity of home-based walking was mostly moderate. The arrangement of walking programs differed by the studies. Participants were advised to increase their walking time consistently in one study (18), and were asked to increase their steps at certain intensity (RPE 11-13) by 10% each week in another study (19). In addition, the frequency, duration and intensity of exercise were gradually increased together in the studies (17, 20). The home-based walking exercises were lasted between 10-40 minutes and performed 2-5 times a week for 8 to 12 weeks.

3.4. Control Groups

In two studies, participants in the control group were asked to maintain their usual level of physical activity and were not given any advice on exercise or physical activity (17, 19). In addition, those in the control group received only usual care (medical care only) in one study (21), and weekly phone calls to offset the therapist-contact effect in another study (20). During the phone calls, the nurse talked to patients about the management of chemotherapy-related side effects, but gave them no advice about physical activities (20).

3.5. Scales

In the studies evaluated the fatigue level using FACT-F, Brief Fatigue Inventory, FACIT-F, and FACT-Fatigue measurement tools.

4. DISCUSSION

This systematic review examined studies on the effect of home-based walking on fatigue management in breast cancer patients. A total of four articles written in English were included in the review. The studies evaluated the home-based walking programs' safety and feasibility, short – and long-term effects, effectiveness on psychosocial health outcomes, and effects on quality of life and fatigue among breast cancer patients (17-20).

Fatigue is one of the most widespread symptoms during and after cancer treatment and is a subjective experience for cancer patients (22). It is perceived as an inevitable consequence of the disease and its treatment that patients have to tolerate (23). Therefore, although it is considered a common problem, it is often not noticed and treated by healthcare professionals (21). While 17% of prostate cancer patients report fatigue, the prevalence of fatigue is approximately 33% in colorectal cancer survivors, reaching 40% in breast cancer patients (24).

Despite promising evidence of the efficacy of different forms of exercise in many studies, patients are often reluctant to participate in exercise programs because they believe that treatment-related side effects will worsen by exercises (25,26). Misguided perceptions of risk and safety concerns are commonly reported by patients as barriers to physical activity. These barriers can be attributed to a lack of inconsistent, vague or reliable information on physical activity provided by health professionals (25, 27). However, Huang et al. reported that home-based brisk walking program had a positive effect on reducing fatigue during chemotherapy (20). This result shows that exercise interventions are possible during cancer treatment and can be initiated early to help patients establish long-term healthy behaviors in breast cancer survivor (20). In addition, the COVID-19 pandemic has adversely affected the lifestyle behaviors of breast cancer patients and created a sedentary environment in which it has become difficult to comply with physical activity guidelines (28). Home-based exercise prescriptions, perhaps with remote support, can positively affect cancer-related fatigue in breast cancer patients (29, 30). The main results of our systematic review suggest that home-based walking programs have positive effects on cancer-related fatigue (17-20).

It is very important to know the demographic, disease and treatment properties that make breast cancer patients more likely to develop severe fatigue during and after treatment (31). However, results regarding their demographic variables and fatigue levels are controversial in the literature (32, 33). Studies have confirmed the high prevalence of fatigue in breast cancer patients in all individuals, young and old (31, 34). But several studies report that younger women experience more fatigue (35, 36). Young women may be more vulnerable to the disease and may have fewer resources to manage a diagnosis of breast cancer that is extremely complex and threatening in many ways (physical, survival, future etc.) (37). This explains why the participants were selected from this target group in studies about the effect of home-based walking on fatigue. In this context, age may be a determining sociodemographic factor. Therefore, health professionals should prepare young people for the effects of breast cancer in the best way possible.

A meta-analysis revealed that one in four cancer patients suffers from severe fatigue, and that higher disease stage and type of treatment received are important risk factors (38). Breast cancer patients with stage II or III who are undergoing chemotherapy and those who have had surgery, radiotherapy, chemotherapy and/or hormone therapy are at higher risk. Hormone and targeted therapy are not significant risk factors (38). The fact that a significant part of the population selected in the studies included in our review received chemotherapy compared to other treatment modalities supports these data.

Studies have shown that women with breast cancer undergo various exercise programs to reduce unpleasant symptoms and improve cardiopulmonary function and body composition. These studies included patients receiving different types of treatment at various stages of breast cancer (39, 40). Types of the exercise interventions (home-centered/supervised) and approaches used to measure exercise outcomes differed in these studies. Their results are not particularly helpful for healthcare professionals in providing clear recommendations on exercise to patients (26). New studies are needed in this area.

Previous studies have not fully identified the safe and beneficial exercise prescriptions for women with breast cancer (41, 42). Therefore, it is very important to determine a safe and feasible home-based walking program and evaluate its effectiveness in this population. Our systematic review shows that there are differences in exercise prescriptions across four studies (17-20). Gokal et al. have recommended that participants begin the 12-week program by completing a 10-minute walk, then steadily increase the walking time to 30 minutes five times a week. In this process, the perceived effort in the intervention group was monitored using Borg's Rating of Perceived Exertion (18). In another study, the exercise intensity was set to moderate in the walking program, and the heart rate reserve was gradually increased from 30% to 70% over a 12-week period. In this process, the exercise intensity was monitored using a sports pulse ring. In the same period, the exercise frequency (3-5 times a week) and duration (15-25 minutes/session to 35-40 minutes/session) were gradually increased (20). Yee et al., followed the eight-week walking program using a pedometer and Borg's Rating of Perceived Exertion, and targeted the exercise intensity as RPE 11-13 (19). In another study (2015), the participants were requested to maintain 20-minute walk with moderate-intensity (RPE 10-11) three days a week in the first week and 30-40 minute walk with moderate-to-vigorous intensity (RPE 12-15) five days a week until the 8th week (17). It is noteworthy that the studies used objective (heart rate, etc.) or self-reported (Borg's Rating of Perceived Exertion) measurements to monitor exercise intensity during the intervention. Studies have shown that home-based walking has a positive effect on fatigue regardless of exercise frequency, duration, and intensity (17-20). Therefore, there is a need for further, well-methodized, home-based walking intervention studies in different patient groups to examine demographic, disease/medical and/or social determinants for a successful exercise program among these patients.

There are several approaches regarding how to develop or adapt clinical-based programs during the COVID-19 pandemic, including unsupervised, self-directed and supervised exercises (43). Similar to those in the literature, all four articles included in our systematic review provided training to participants through walking prescriptions, face-to-face training/counseling, telephone counseling and printed learning materials (17-20). The superior effect of supervised exercise programs over unsupervised ones may be that they provide participants with real-time, face-to-face environment. Supervised exercise programs ensure to select, prescribe and follow appropriate exercises, technique and intensities in order to achieve the desired physiological adaptations in patients (44). These programs are particularly important for cancer patients during and after treatment, as their physical and mental health status may change daily due to acute side effects of the disease (45,46). Because, understanding how to prescribe exercises, adapting their prescription according to daily changes or injuries, and interpreting the data obtained from relevant technologies are important in achieving the targeted outcomes (43). From this point of view, it can be said that patients may not get all the potential benefits of exercises in an unsupervised exercise program because they are not supervised by an exercise specialist (47). In the context, it is not surprising that studies have used counseling and additional materials in home-based walking programs to regulate exercise prescriptions and ensure participant safety (10, 17-20).

In our systematic review, no serious adverse effects of the home-based walking program were reported by participants in the intervention group in two studies (19, 20), while there was no report on the subject in one study (18). In contrast, another study reported a small to moderate adverse effect on arm use for those in the intervention group. However, since no measurement of arm strength or function was taken during the study, the underlying cause could not be determined in the study (17).

Adherence to intervention is important in the feasibility of physical activity programs. Despite the positive effects of home-based walking on fatigue, low compliance rates may have alleviated its effectiveness. Studies have found a wide range of variety in the adherence to intervention (18-20). Low rates of adherence to intervention indicate the need for strategies to promote compliance with home-based walking programs in the absence of an instructor. While a successful increase in walking volume is generally not achieved, it is encouraging that participants mostly adapt to moderate-intensity walking (19). These data are especially important in showing that they were able to reach the desired intensity levels without the need for any instructor or supervisor.

Recently, there is an increased interest in the self-directed, home-based exercise interventions in exercise oncology paradigm. In addition, the ever-evolving technology industry, exercise equipment, smartphones and watches, tablets and websites allow instant access to workouts, tracking and analysis of personal fitness and activity levels (43). At this point, the number of studies with high evidence value for home-based walking programs in breast cancer patients will increase in the forthcoming days.

4.1. Limitations

Our systematic review provides preliminary evidence for the benefits of home-based walking program among breast cancer patients. However, there are a few other limitations besides the inclusion of only English-language articles in the study. First, the sample consisted predominantly of young individuals, limiting its applicability to other age groups. Second, most of the included studies had low sample sizes, with one study even having less than 10 participants in the experimental group (19). In this context, a limited sample size may reduce the likelihood of reflecting a real effect. The third limitation is the small number of relevant randomised controlled trials and their variability in reporting outcome measures (fatigue, depression data, etc.), intervention procedures and measurement tools. This variability highlights the need to conduct more clinical studies in a more uniform manner to reach a stronger consensus on the effects of a home-based walking program on fatigue in women with breast cancer. However, depending on the treatment received, the effectiveness of the intervention may vary in women with breast cancer. In this context, it is recommended to examine the effectiveness of the intervention in women in different treatment processes. Additionally, further research with larger sample sizes and other cancer types is needed to confirm the preliminary findings from this study.

5. CONCLUSION

Outcomes of the home-based walking program are promising for women with breast cancer as it is a low-cost, unsupervised and simple intervention. Therefore, promoting walking can be a cost-effective and safe approach with meaningful benefits for women with breast cancer. As studies have shown, a home-based walking program combined with short face-to-face or telephone counseling or printed learning materials can result in clinically significant improvements in cancer patients. In particular, unlike previous home-based interventions, interventions that use printed learning materials, do not require additional phone calls or counseling with healthcare professionals, and do not impose an additional burden on healthcare staff can contribute to the current literature.

Physical exercise has numerous known benefits for breast cancer patients. As infection rates continue to increase due to COVID-19 variants, it is extremely important to encourage breast cancer patients to do physical exercises such as walking. Certain risks associated with inactivity, especially when combined with the risk of COVID-19 infection, can have unintended consequences in breast cancer patients. It is therefore important to support breast cancer patients/survivors to start and continue exercising even after the pandemic. There is a need to maintain and develop high-quality research in exercise oncology in this regard. These results have important implications: the main goal in public health interventions is to reach large numbers of people and implement effective programs that benefit them (breast cancer patients/survivors). In this context, our study is considered to increase the awareness of home-based walking program in breast cancer patients and be a reference for clinical practices.

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Clinical and Experimental Health Sciences

Amcasertib Increases Apoptosis While Decreasing Invasive and Migrating Abilities in Breast Cancer Stem Cells

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ABSTRACT

Objective: A relationship exists between breast cancer stem cells (BCSCs) and the chemo-resistance and recurrence of aggressive breast tumors. Amcasertib is a small chemical compound and multiple kinase inhibitor that inhibits downstream Nanog and other cancer stem signaling pathways in cancer stem cells by targeting several serine-threonine kinases. In this study, we aimed to investigate the cytotoxicity and anticancer effects of Amcasertib on BCSCs, gaining insight into the targetability of BCSCs.

Method: We used the combined xCELLigence-Real-Time Cell Analyzer (RTCA) equipment to analyze cytotoxicity and cell proliferation. We detected the IC_{50} dosages of Amcasertib at 24, 48, and 72 hours and examined its effects on apoptosis, cell cycle, invasion, and migration over 48 hours. We used flow cytometry for assays of apoptosis and cell cycle, and the CytoSelect 96-well Cell Migration and Invasion Assay Kit for evaluating invasion and migration.

Results: Our results showed that Amcasertib has cytotoxic properties, with an IC_{50} dosage of 1.9 µM at the 48th hour. In addition, Amcasertib significantly induced apoptosis in BCSCs, despite not affecting the cell cycle. Moreover, Amcasertib decreased BCSCs' invasion and migratory properties, part of epithelial-mesenchymal transition (EMT).

Conclusion: In conclusion, our findings provide crucial information for understanding the potential of Amcasertib in targeting BCSCs. In addition, we suggest that Amcasertib could be a beneficial drug for breast cancer treatment by targeting BCSCs.
Keywords: Amcasertib, small molecule inhibitor, stemness kinases, breast cancer stem cell

1. INTRODUCTION

Breast cancer is a significant global health issue because of its high rates of illness and death among women. In 2020, there were approximately 2.3 million new cases and 684,996 reported deaths, highlighting the high prevalence of the disease among women (1,2). The spread of cancer to other organs, known as metastasis, is responsible for more than 90% of deaths from breast cancer (3,4). Breast cancer, like other malignancies in humans, is both a hereditary and a sporadic disease (5). The most common causes of hereditary breast cancer are mutations in the BRCA1 and BRCA2 genes (6). High-risk genes for breast cancer include TP53, PTEN, STK11/LKB1, and CDH1, although these genes are associated with an increased rather than decreased chance of developing cancer in carriers (7). Histology and molecular features are used to classify the many different types of tumors that comprise breast cancer (8). Genetic profiling and immunohistochemical research have led to the classification of breast cancer into five subtypes: "luminal A," "luminal B," "HER2 overexpression," "basal-like," and "normal breast-like"(9). The gene expression patterns, clinical features, therapeutic response, and prognosis of these breast cancer molecular subgroups are distinct (10).

Cancer stem cells, which are also called tumor-initiating cells, can be found in a small number of malignancies. These cells can proliferate and differentiate (11,12). Biological activities of cancer stem cells, such as recurrence, metastasis, heterogeneity, multidrug resistance, and radiation resistance, are regulated by pluripotent transcription factors such as OCT4, Sox2, Nanog, KLF4, and c-Myc, which lead to treatment failure (12).

"Targeted therapies" are cancer treatments that are meant to kill only cancer cells without hurting any healthy tissue. Yet, conventional chemotherapy has the potential to kill healthy cells along with cancer cells (13). During the last 30 years, the FDA has approved 18 small-molecule drugs for various types of breast cancer, including those that are ER-positive, HR-positive, HER2-positive, BRCA-mutated, and PIK3CA-altered. These drugs; function as a microtubule-stabilizing agent, a prodrug of 5-FU, a DNA topoisomerase II inhibitor, an aromatase inhibitor, an estrogen receptor (ER) inhibitor,

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an estrogen receptor (ER) antagonist, a cyclin D-cyclin-dependent kinase 4/6 inhibitor, a HER2 and EGFR inhibitor, a HER2 inhibitor, a PARP inhibitor (14); on the other hand, there is no evidence to suggest that they are deliberately aiming for BCSCs. However, several small-molecule inhibitors and immunotherapeutic agents targeting BCSC have been reported in preclinical and clinical trials (15). These molecules target stemness markers (CD44, ALDH1, CD133, etc.), HIF family members inhibitors, EGFR/HER2 signaling axis, and several signaling pathways contributing stemness such as Notch, PI3K/AKT/mTOR, Wnt/β-Catenin, and Hedgehog (16).

Nanog is a stemness-related transcription factor that helps to maintain embryonic stem cell pluripotency and self-renewal. It is used as a marker to detect cancer stem cells and may also be used to identify these cells (17). Nanog is overexpressed in a variety of cancers, including those of the brain, breast, colon, ovary, and prostate (18). Nanog overexpression is associated with a more advanced stage of cancer, a lack of differentiation, a shorter overall survival, and the emergence of resistance to treatment. As a result, Nanog has been identified as a potential therapeutic target for treating cancer (19). In 2017, research into amcasertib, a first-in-class CSC-specific small-molecule multi-kinase inhibitor created by Boston Biomedical Inc., began in advanced adenoid cystic carcinoma (ACC) and advanced head and neck tumors. The orally active agent, well tolerated, works by inhibiting downstream cancer stem signaling pathways, including Nanog, by targeting several serine-threonine kinases (20,21). Amcasertib (Figure 1) is a potential anticancer drug investigated with just clinical trials, except for two preclinical studies (22,23).

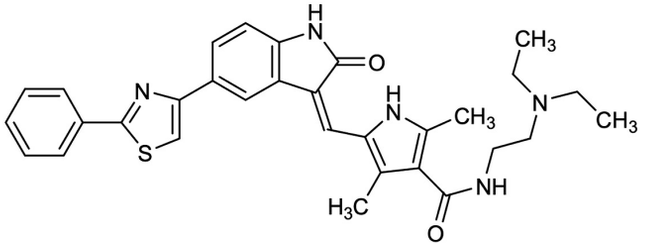

Figure 1. *The structure of amcasertib. Molecular formula is (C31H33N5O2S). Chemical name is N-[2-(diethylamino)ethyl]-2,4-dimethyl-5 {[2-oxo – 5-(2-phenyl-1,3-thiazol-4-yl)-1,2-dihydro-3H-indol – 3-ylidene]methyl}-1H-pyrrole-3 carboxamide (49).*

In this study, we aimed to investigate the cytotoxic, apoptotic, anti-invasion, and anti-migration effect of the multi – stemness kinase inhibitor Amcasertib directly on breast cancer stem cells (BCSC) in vitro.

2. METHODS

2.1. Cell Line and Chemicals

The breast cancer stem cell line (BCSC, #36102-29) was purchased from Celprogen (Torrance, United States). As stated in the datasheet, clonal selected stem cell line was derived from source of human breast cancer tissue (Triple negative; ER – negative, PR – negative, and HER2 – negative) and its positive markers are CD133, CD44, SSEA3/4, Oct4, Tumorigenicity (<1000 cells), Alkaline Phosphatase (ALP), Aldehyde Dehydrogenase (ALDH), Telomerase, Sox2 (24). BCSC cells were cultured in Human Breast Cancer Cell Line Complete Media with Serum (#M77002-07S) under a humidified atmosphere of 5% CO_2 in air at 37°C. Amcasertib (Cat no: S8572) purchased from Selleckchem (Planegg, Germany) was dissolved in DMSO and stored at – 80°C based on the manufacturer's recommendation.

2.2. Proliferation and Cytotoxicity Tests

Analyses of cytotoxicity and cell proliferation were carried out using the combined xCELLigence-Real-Time Cell Analyzer (RTCA) equipment, which allows for the continuous monitoring of cellular activities. To examine cell growth, a 96-well e-plate was seeded with densities ranging from 1×10^6 to 2×10^3 cells/ml. The e-plate was mounted on the RTCA system, and the culture of cells was allowed to continue for a full 96 hours. After incubation, the optimal cell proliferation numbers were found by choosing the required periods based on the cell index graphs found in the system. This was done after the cells had been allowed to proliferate. During the cytotoxicity test, the necessary cell number was seeded into 96-well plates after being determined by the cell proliferation test performed on the BCSC model. The microplates were then incubated in the RTCA system for 24 hours. After incubation, the cells were subjected to a treatment consisting of three repeats of amcasertib at a dosage ranging from 10 – 0.5 µM over 72 hours. The control group consisted of cells that had not been treated in any manner. After that, the cell index graphs that were produced in the system were used to estimate the IC_{50} dosages of amcasertib for the 24th, 48th, and 72nd hour time periods (25).

2.3. Apoptosis Test by Flow Cytometry

The Annexin V-FITC Apoptosis Detection Kit was used to analyze the apoptotic cell death of BCSC cells to amcasertib (BD Pharmigen, USA). BCSC cells in the number of cells identified as a result of the proliferation test were seeded in 6-well plates, and the microplates were then incubated for 24 hours. After incubation, the cells were subjected to a treatment with amcasertib at the IC_{50} dose and for the time specified. Untreated cells were used as control. Trypsin was used to detach the BCSCs, and then they were washed twice with PBS. After the stage of washing, one hundred microliters of binding buffer diluted one hundred times was applied to the cells. After the addition of 5 µl of FITC-Annexin V and 5 µl of propidium iodide (PI) antibody to the cells, while they were in the binding solution, the cells were left to incubate at room temperature and in the dark for 15 minutes. Following incubation, 400 µl of binding buffer was added to the cells, and then the cells were examined using CFlow Plus software on a flow cytometer (DB Accuri C6, Becton Dickinson, USA).

2.4. Cell Cycle Test by Flow Cytometry

Amcasertib's influence on the cell cycle was studied using a model of breast cancer stem cells and the BD Cycletest Plus DNA Kit, which was used following the protocol provided

for the kit. After seeding BCSC cells into 6-well plates and determining the cell number based on the results of a proliferation study, the plates were left to incubate for 24 hours. After incubation, BCSC cells were exposed to treatment with amcasertib at the dosage and period determined by the IC_{50}. Untreated cells were used as control. Trypsin was used to detach the BCSCs, and then they were washed twice with 1× PBS. The approach consisted of suspending the cells in 1 ml of buffer solution, centrifuging them at 300×g for 5 minutes, and then repeating this process twice. After adding 250 µl of Solution A to the cells and incubating them for 10 minutes at room temperature, the cells were taken out of the dish. After the incubation, 200 µl of Solution B was added to the cells. Subsequently, the cells were incubated again for 10 minutes at room temperature. After that, 200 µl of cold Solution C was added to the cells, and the cells were left to incubate on ice in the dark for ten minutes. Reading cells in flow cytometry allowed for the calculation of the percentages of cells in each stage of the cell cycle (BD Accuri C6, Becton Dickinson, USA).

2.5. Invasion and Migration Analysis

The CytoSelect 96-well Cell Migration and Invasion Assay Kit (Cell Biolabs, USA) was used to evaluate the effect of amcasertib on invasion and migration in a breast cancer stem cell model. The evaluation was performed according to the protocol provided with the kit. The IC_{50} dosage of amcasertib was administered to BCSC cells for 24 hours. We used the cells in the sample that had not been treated with amcasertib as a control group. After the treatment of the kit methodology to the control cells as well as the cells that had been treated with amcasertib, the relative fluorescence units (RFU) were measured using a fluorescent plate reader with a 485/538 nm filter.

2.6. Statistical Analysis

The results were analyzed with GraphPad Prism v.10.1.0 software using a two-tailed unpaired Student's t-test for calculating the *p*-value as the level of significance of comparison of treated and untreated cells. The p value < .05 was considered statistically significant. Data were presented as mean ± SD of three biological replicates.

3. RESULTS

3.1. Amcasertib Suppresses Breast Cancer Stem Cell Growth

As a result of the experiment involving cell proliferation, the optimal cell concentration in the logarithmic phase for BCSC cells was found to be 2×10^4 cells/ml. Amcasertib was shown to have a cytotoxic effect on BCSC cells, and the extent of that effect was found to be dependent both on the dosage administered and the amount of time that the treatment was allowed to take effect. In the BCSC cell line, the IC_{50} value of amcasertib was found to be 2.9 µM at 24 hours, 1.9 µM at 48 hours, and 1.8 µM at 72 hours. These values were established after the drug was tested for the appropriate period (Figure 2).

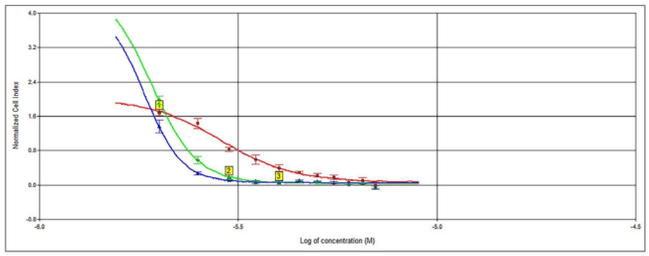

Figure 2. *The cytotoxic effects of Amcasertib in the BCSC cells. It shows dose-response curves for the 24th hour with "1" (red line), for the 48th with "2" (green line), and for the 72nd hour with "3" (blue line).*

3.2. Apoptosis is Induced in Breast Cancer Stem Cells by Amcasertib

After 48 hours of treatment with amcasertib at a concentration of 1.9 µM, a significant increase in apoptotic effect was observed in breast cancer stem cells (BCSCs). The percentage of total apoptotic cells in BCSCs rose from 18.6% in the control group to 97.2% in the alisertib-treated group, indicating a 5.24-fold increase compared to the control condition (Figure 3).

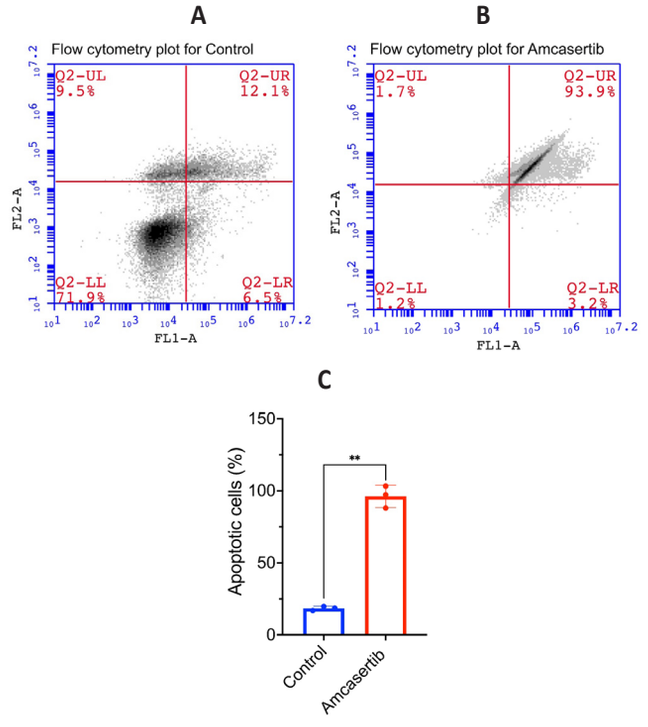

Figure 3. *Apoptotic effects of Amcasertib for 48 h in BCSC cells. Flow cytometry plots show apoptosis in cells without amcasertib treatment (A) and cells with amcasertib treatment (B) (FL2-A, PI fluorescence channel; FL1-A, FITC fluorescence channel; LL, live cells; LR, early apoptotoic cells; UR, late apoptotoic cells; UL, necrotic cells). C)The plot of the distribution percentage of apoptotic cells shows that apoptosis significantly increases in cells with amcasertib treatment compared to the cells without amcasertib treatment according to the two-tailed unpaired Student's t-test. Data shows as mean ± SD of n:3 replicates (**, p < .01).*

3.3. Cell Cycle Progression is Unaffected by Amcasertib in Breast Cancer Stem Cells

After 48 hours of treatment with amcasertib at the IC_{50} concentration of 1.9 µM, the proportion of breast cancer stem cells (BCSCs) in the G1 phase experienced a slight increase from 67.4% in the control group to 69.9% in the amcasertib-treated group. However, this increase was not statistically significant. (Figure 4).

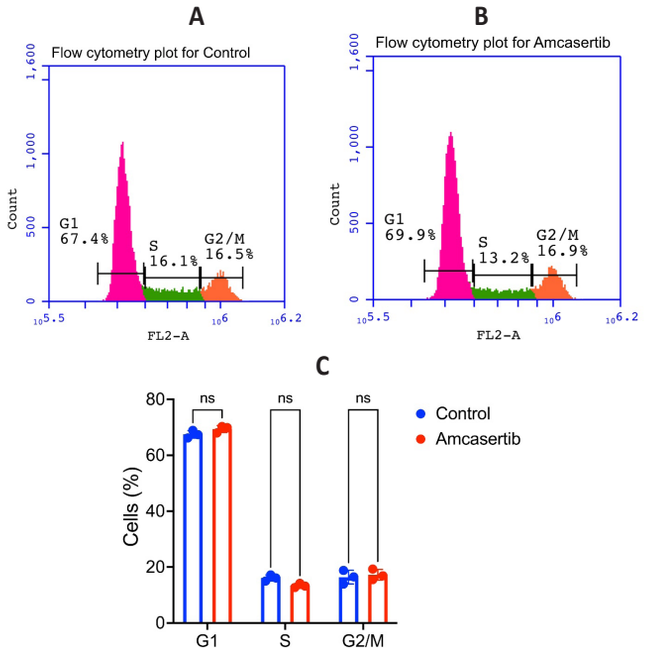

Figure 4. *Effects of Amcasertib on the cell cycle phases of BCSC cells for 48 hours. Flow cytometry plots show the distribution of cells without amcasertib treatment (A) and cells with amcasertib treatment (B) in cell cycle phases (FL2-A, PI fluorescence channel). C) The plot of the distribution percentages of cells in cell cycle phases shows no significance between cells with and without amcasertib treatment according to the two-tailed unpaired Student's t-test. Data shows as mean ± SD of n:3 replicates (ns, no significance).*

3.4. Amcasertib Reduces Invasion and Migration in Breast Cancer Stem Cells

Our investigation revealed a significant reduction in the cell invasion and migration activities of BCSCs subjected to a 48-hour treatment with 1.9 µM amcasertib (Figure 5).

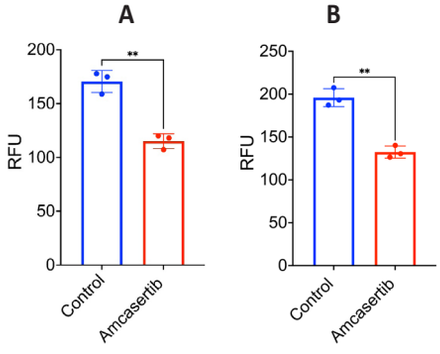

Figure 5. *Effects of Amcasertib on migration and invasion of BCSC cells for 48 hours. The plots of relative fluorescence units (RFU) for invasion (A) and migration (B) show that both invasion and migration significantly decrease in cells with amcasertib treatment compared to the cells without amcasertib treatment according to the two-tailed unpaired Student's t-test. Data shows as mean ± SD of n:3 replicates (**, p < .01).*

4. DISCUSSION

Since their identification in 2003, breast cancer stem cells (BCSCs) have been an important study issue for elucidating the malignancy of breast cancer, a complex disease with many etiology (26,27). Twenty percent of individuals with BCSCs show signs of chemoresistance and recurrence (28). One of the biggest challenges in treating breast cancer is the development of resistance to the drugs now in use; hence, efforts to develop a BCSC-targeted approach to combat this problem have been highlighted (29).

The anti-cancer effects of small molecules on breast cancer have been studied in vivo and in vitro, with many studies reporting that the small compounds specifically targeted BCSCs. Rather than using a dedicated BCSC cell line, researchers in these studies demonstrated the drugs' impact on BCSC by assessing alterations in the CD44+CD24- and/or $CD44^{+}CD24^{-}ALDH^{+}$ cell populations within murine or human breast cancer cell lines (triple negative or HR-positive), xenograft models, or patient tumor tissues (30–37). Nevertheless, in our research, we utilized directly a BCSC cell line, isolated from triple-negative breast cancer tissue, that has been verified by the manufacturer and was made available commercially. We explored the anticancer impact of amcasertib on BCSCs because, unlike other small medicines that target a particular cellular molecule, amcasertib targets selectively multiple cancer stemness kinases (20). Amcasertib has been studied extensively in individuals with advanced cancers other than breast cancer (phase I, phase Ib, phase Ib/II, and phase II trials all completed, NCT01781455, NCT02232633, NCT02279719, NCT02354898, NCT02483247, NCT02432326) (38–43).

Two distinct preclinical investigations have been conducted to assess the pharmacological impact of amcasertib. In the first study, focused on investigating the correlation between Iroquois-class homeodomain protein 4 (IRX4) and EGFR-tyrosine kinase inhibitor resistance, the inhibitory effects of amcasertib (10-0.3125 M, 1:2 serial dilution) for 48 hours on cell growth in gefitinib-resistant non-small cell lung cancer PC-9 cells (PC-9/GR) were observed in a dose-dependent manner (22). The second study, undertaken by our research team, aimed to explore the anticancer properties of amcasertib in ovarian cancer (23). Amcasertib's IC_{50} was found to be 2.9 µM after 24 hours, 1.9 µM after 48 hours, and 1.8 µM after 72 hours of treatment of BCSCs. Considering that other studies with data about triple negative BCSC population and using small molecule inhibitors may be seen much lower IC_{50} doses for relevant small molecule inhibitors. However, it may be based on being used triple-negative cell lines (TNBC) with a heterogeny population rather than a just BCSC niche. For example, exportin-1 or CRM1 inhibitor LFS-1107 was reported to exhibit an IC_{50} value of

40.80 nM in the MDA-MB-231 TNBC cell line for 72 hours (34). In a study investigating anticancer effects of Fluorine-Incorporated Gold I Compound (3F1) on human breast cancer cells by using commercial cell lines MDA-MB-231, MCF-7 (ER^+PR^+) and BCSC cell line (#36102-29, Celprogen), IC_{50} dose of 3F1 for 24 hours was reported 10.17 µM in BCSC, whereas 8.44 µM and 6.17 µM in MDA-MB-231 and MCF-7, respectively (44). Therefore, we'd like to point out that amcasertib exhibited an effective cytotoxicity on BCSCs. In addition, we found that amcasertib promoted apoptotic cell death with a significant increase over the control group. Thus, our current study exhibits in parallel our findings in the recent study demonstrating significant antiproliferative effects and apoptosis induction by amcasertib in ovarian cancer cells (MDAH-2774 and OVCAR-3) and ovarian cancer stem cells (OCSC). Nevertheless, we found that it did not lead to cell cycle arrest in BCSCs, like in OCSCs (23). Some studies emphasizing that different small molecule inhibitors targeted BCSC population in TNBC cells were reported to increase the sub-G1 cell population as depend on increasing apoptotic effect and induce G2/M phase arrest in MDA-MB-231 cells in a dose-dependent manner (35,36). We didn't evaluate sub-G1 cell population. However, abcasertib may have caused increasing in the sub-G1 cell population as depended on the strong apoptotic effect detected, although it exhibited an insignificant effect on the cell cycle phase. Notably, like amcasertib exhibited the ability to inhibit invasion and migration in MDAH-2774 and OCSC cells, when we compared the effects of amcasertib to the control group, we found that it greatly reduced invasion and migration in BCSCs (23). CSCs exhibit an EMT profile, involving enhanced invasive properties and migratory capacity, and resistance to apoptosis (45,46). Reportedly, Oct-4 and Nanog increased the EMT of BCSCs, which was linked to a poor prognosis in breast cancer patients (47). Studies report that in drug-resistant MDA-MB-231 cells constructed, the EMT profile is reduced, and drug resistance is also overcome, in correlation with inhibition of stemness markers (Nanog, OCT4, SOX2, and Kif4) (37,48). Also, by validating with experiments of silencing of Nanog, amcasertib was indicated to inhibit Nanog and CD133 expression, and so to increase the growth-inhibitor effect of gefitinib in PC-9/GR cells (22). Thus, we conclude that amcasertib decreased the EMT profile of BCSCs via inhibiting CD133, Nanog, and stemness-kinases, we would like to emphasize its potential ability for drug resistance to overcome.

5. CONCLUSION

We determined that amcasertib suppressed the EMT of BCSCs acting on breast cancer recurrence and drug resistance. We suggest that amcasertib is a potential agent in the therapy of patients with breast cancer, particularly in overcoming drug resistance and recurrence.

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Ethics Committee Approval: *This study doesn't require ethical approval because of the use of a commercial cell line. We submitted the Declaration Form That The Article Does Not Require Ethics Committee Permission.*

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Author Contributions:

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

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Clinical and Experimental Health Sciences

The Effect of Parents' Fear of COVID-19 and Some Variables on Their Childhood Vaccination Attitudes According to the Health Belief Model: A Cross-Sectional Study

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ABSTRACT

Objective: The study sought to explore the relationship of parents' fear of COVID-19 and some variables with childhood vaccination attitudes according to the Health Belief Model during the COVID-19 pandemic.

Methods: This research employed a descriptive – correlation design, with 263 participants consisting of parents with children aged 0-6 included in the study. An online survey was used to collect data.

Results: The mean COVID-19 fear score of the participants was 19.71±6.46. As the COVID-19 fear score of the participants increased, their perceived benefit (β=.12; p=.03) and health motivation (β=.14; p=.02) scores also increased.

Conclusion: Parents' COVID-19 fear level increased, their perceived benefit and health motivation attitudes improved. Parents should be provided with education and counselling by health professionals to develop a positive attitude towards childhood vaccinations.

Keywords: Childhood, fear of COVID-19, health belief model, parents, vaccination.

1. INTRODUCTION

Childhood vaccines are important in terms of significantly reducing morbidity and mortality caused by many infectious diseases and protecting public health (1). Nevertheless, the COVID-19 pandemic that emerged in late 2019 has disrupted routine immunization programs (2, 3). The World Health Organization (WHO) and the UNICEF have documented significant disruptions in routine immunization programs, affecting nearly 80 million children in at least 68 countries (2). Interruption in childhood vaccinations could result in epidemics of preventable infectious diseases (3, 4).

It is stated that the reasons for the interruption in immunization programs include factors such as fears of parents, lockdown measures, a shift in healthcare personnel's priorities towards COVID-19, and logistical challenges in vaccine distribution. Furthermore, parents' concerns about potentially exposing their children to COVID-19 during routine check-ups and the subsequent decline in vaccination rates are also cited as contributing factors (5). Providing and maintaining routine childhood vaccinations play an important role in preventing outbreaks of vaccine-preventable diseases amid the pandemic (6).

Pandemic negatively affects the lifestyle, social life, close relationships and income levels of individuals, and the risk of a pandemic can cause fear in people (7). Fear of COVID-19 emerges with anxiety as the pandemic causes many uncertainties in the lives of individuals (8). The COVID-19 infection was encountered for the first time and the disease caused extraordinary situations, which are the reasons for fear. The whole world has been affected psychologically due to the fact that the disease has threatened human life and there have been unknown aspects of the disease (9). It is stated that the fear arising as a result of the pandemic has caused a change in the negative perspective towards vaccines (9).

One study revealed that parents' social security status, education level and professional characteristics are effective

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in their attitudes towards vaccination, and individuals with high education level and those who are knowledgeable about vaccinations are more sensitive about mandatory vaccinations (10). Another study reports that the mother's educational attainment is positively correlated with her vaccine-related knowledge level (11). It is stated that parents make the vaccination decision for their children according to the protection, effectiveness and reliability of the vaccine (12).

Existing literature suggests that parents have placed greater emphasis on childhood vaccination amid the COVID-19 pandemic, with the majority expressing a desire for their children to receive vaccines (6) 84.1% of parents stated that they find vaccination necessary, and 95% had their children vaccinated regularly (13, 14). Aygün and Tortop (15) reported that parents with children under the age of five have a high level of hesitation towards vaccines and their negative attitudes are due to their religious beliefs and lack of knowledge. The prevalence of uncertainty about routine childhood vaccines was reported as 6.1% in a study (16). Parents' attitudes and behaviors towards vaccination have a direct and significant impact on the decision to vaccinate their children (17).

No studies were found in the literature that evaluated parents' approaches to vaccination o based on the COVID-19 fear and Health Belief Model during the pandemic. Thus, this research was conducted with the objective of identifying the relationship of parents' fear of COVID-19 and some variables with their childhood vaccination attitudes during the pandemic according to the Health Belief Model.

Research Questions

1. What are the childhood vaccination attitudes of parents based on COVID-19 and the Health Belief Model?

2. Do childhood vaccination attitudes of parents differ according to the fear of COVID-19 and the Health Belief Model?

3. What is the relationship of parents' fear of COVID-19 and some variables with their childhood vaccination attitudes according to the Health Belief Model?

2. METHODS

2.1. Study Design

The research was structured as a descriptive – correlational study and followed the guidelines outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist (18).

2.2. Study Setting and Participants

The research involved parents with children aged 0-6 years in Turkey. The inclusion criteria encompassed the following conditions: (a) having children between the ages of 0-6, (b) expressing willingness to participate in the study, and (c) possessing proficiency in speaking and understanding Turkish. Exclusion criteria were defined as follows: (a) having a physical or mental disability that would prevent the use of mobile phones and computers, and (b) withdrawing from the study at any stage.

2.3. Study Population and Sample Size

The participants were parents with children aged 0-6 living in different cities of Turkey. To establish the study's sample size, a power analysis was performed employing the G-power 3.1.9.7 software. Utilizing the mean scores and standard deviation (16.05±2.95) of the Public Attitude Toward Vaccination Scale-Health Belief Model sub-dimension, as reported in Kaydirak et al. (19), a minimum sample size of 263 was computed with a power of 95%, a significance level of 0.05, and an effect size of 0.2. The sample was reached using snowball sampling, which is one of the nonprobability sampling selection methods (20).

2.4. Measures

The online survey comprised the Parent Information Form, the COVID-19 Fear Scale, and the Public Attitude Toward Vaccination Scale-Health Belief Model.

2.4.1. Parent Information Form

The form was prepared by the researchers in line with the literature (19, 21) and encompasses 15 questions to explore the parental and child-related characteristics (age, educational background, employment status, economic status, place of residence, the number of children in the family, and age, gender, and chronic disease status of the child) and childhood vaccination characteristics (having the child vaccinated before the pandemic, having sufficient knowledge about vaccines, believing that vaccines are useful and beneficial, changing the decision to have vaccines during the pandemic, having the child vaccinated during the pandemic).

2.4.2. The COVID-19 Fear Scale

The scale was developed by Ahorsu et al. (22), underwent Turkish validation and reliability assessment made by Satici et al. (23). This scale comprises 7 items, each rated on a five-point Likert scale (1 = strongly disagree, 5 = strongly agree) and single factor. The scores on the scale can vary between 7 and 35, where a higher score indicates a higher level of COVID-19 fear. In the Turkish validation and reliability study, the scale demonstrated a Cronbach's alpha coefficient of .85 (23). In the present study, the Cronbach's alpha was determined to be .88.

2.4.3. The Public Attitude Toward Vaccination Scale-Health Belief Model (PAVS-HBM)

The scale was formulated by Kocoglu-Tanyer et al. (21) with the objective of gauging society's stance towards vaccination through the lens of the Health Belief Model. The scale comprises 26 items, each rated on a five-point Likert scale, from 1= strongly disagree to 5= strongly agree, and encompasses five sub-dimensions: perceived susceptibility, perceived severity, perceived benefit, perceived barriers, and health motivation. The scale does not yield a total score; the perceived susceptibility and perceived severity sub-dimensions encompass four factors each (scoring between a minimum of 4 and a maximum of 20 points), while the perceived benefit and health motivation sub-dimensions comprise five factors each (ranging from a minimum of 5 to a maximum of 25 points). The perceived barriers sub-dimension involves eight factors, with scores ranging from a minimum of 8 to a maximum of 40 points. In this context, a lower score in the perceived barriers sub-dimension reflects a positive attitude, whereas higher scores in the other sub-dimensions indicate a positive attitude as well. The Cronbach's alpha coefficient for the scale was reported as .89 (21). In the present study, the Cronbach's alpha was determined to be .77.

2.5. Data Collection

Data were gathered between April and August 2021 using the online survey link generated by the researchers via a web-based platform (http://www.surveey.com/survey/). The online survey was sent to either the mother or father via an e-mail, or a message was sent through social media. Following their consent to partake in the study, participants were requested to finalize the survey. Additionally, they were encouraged to share the online survey link with other parents within their acquaintance.

2.6. Statistical Analysis

The data were analyzed using IBM SPSS Statistics Version 22. To assess the data's adherence to a normal distribution, the Kolmogorov-Smirnov test, skewness, and kurtosis values were examined. Descriptive statistics were employed to summarize variables related to sociodemographic details and vaccination characteristics. For evaluating the internal consistency of the scales, Cronbach's alpha analysis was applied. Descriptive statistics included the use of numbers, percentages, means, standard deviations, and minimum-maximum values. To compare variables associated with parental childhood vaccination attributes and the sub-dimension mean scores of the Fear of COVID-19 Scale and the Public Attitude Toward Vaccination Scale-Health Belief Model, an independent sample t-test was conducted.

A multiple linear regression analysis was performed using the stepwise method to assess the impact of variables (comprising parents' childhood vaccination characteristics and fear of COVID-19) on parents' childhood vaccination attitudes in accordance with the Health Belief Model. For all dichotomous variables, a dummy variable recoding was applied (e.g., 0 or 1). The outcomes of the analyzed model were reported as B (95%CI), standard error (SE), estimated β, the adjusted R2, F-test, and p-value for each variable. Statistical significance was considered at the $p<.05$ level.

2.7. Ethical Consideration

This research followed the ethical guidelines in the Declaration of Helsinki. Ethical approval (IRB: 2021/34) was obtained from the Selçuk University where the researchers are affiliated. Additionally, prior to commencing the study, approval was granted by the Ministry of Health Services General Directorate Scientific Research Platform (MoH: 2021-03-11T17_16_52). An informed consent document, which elucidated the study's objectives, was attached to the initial page of the online survey. Participants were granted permission to complete the questionnaire once their consent had been provided.

3. RESULTS

3.1. Sample Characteristics

The study sample consisted of 263 parents and the characteristics of the participants are given in Table 1. The mean age of the parents is 32.44±5.94 years, and the mean age of the children is 35.40±22.53 months. 93.2% of the participants were mothers, 61.6% were university graduates, 57.0% were unemployed, 60.1% had income equal to their expenses, 76.8% were living in the city, and 51.0% had one child (Table 1).

The mean COVID-19 fear score of the parents was found to be 19.71±6.46. The PAVS-HBM scale sub-dimension mean scores of the parents were as follows: perceived susceptibility 17.16±2.69, perceived severity 16.43±3.04, perceived benefit 20.78±3.45, perceived barriers 16.33±6.35, and health motivation 21.54±2.98 (Table 2).

The distribution of the mean score on the COVID-19 Fear Scale and the mean scores for the sub-dimensions of the PAVS-HBM Scale, based on parents' childhood vaccination characteristics, is presented in Table 3. As indicated in the table, 95.1% of the participants believe in the benefit of childhood vaccines, 74.9% have sufficient knowledge about childhood vaccines, 98.1% had their child vaccinated before COVID-19, 97.3% did not change their decision to have childhood vaccinations during the pandemic, and 82.9% reported that they wanted to have childhood vaccinations in the pandemic (Table 3).

The analysis revealed a statistically significant difference between the COVID-19 Fear Scale mean scores of parents who believe that childhood vaccines are beneficial compared to those who do not (p=.012). However, no statistically significant distinction was discerned in relation to other variables associated with childhood vaccination (p=.492,

p=.653, p=.904, p=.217, respectively). A statistically significant difference was observed among parents who believe in the benefits of childhood vaccines, possess adequate knowledge about vaccines, and desire childhood vaccination during the COVID-19 pandemic in relation to their PAVS-HBM Scale sub-dimension mean scores (p<.001). Significant differences were noted in the mean scores of the parents who had childhood vaccinations before COVID-19 compared to those who did not, specifically in the perceived severity and health motivation sub-dimensions (p=.035, p<.001, respectively). However, the differences between the mean scores for perceived susceptibility, perceived benefit, and perceived barriers sub-dimensions were not statistically significant (p=.257, p=.311, p=.084, respectively) (Table 3).

Table 1. *Characteristics of the sample (N= 263).*

Characteristics	n	%	Mean	SD
Age of parents, years			32.44	5.94
Parents				
Mother	245	93.2		
Father	18	6.8		
Education level				
Elementary-secondary school	42	16.0		
High school	59	22.4		
Bachelor's degree	162	61.6		
Employment status				
Yes	113	43.0		
No	150	57.0		
Perceived income status				
Income<expenses	48	18.3		
Income = expenses	158	60.1		
Income>high	57	21.7		
Place of residence				
City	202	76.8		
County	50	19.0		
Village	11	4.2		
Number of children				
One	134	51.0		
Two and more	129	49.0		
Age of children, months			35.4	22.53
Sex of children				
Female	130	49.4		
Male	133	50.6		
Chronic diseases in the children				
Yes	21	8.0		
No	242	92.0		

SD, Standard deviation

Moreover, while statistically significant differences were observed in the mean scores of perceived susceptibility, perceived benefit, perceived barriers, and health motivation for parents who maintained their decision to have childhood vaccinations during the COVID-19 pandemic versus those who altered their decision (p=.010, p=.009, p=.004, p=.016), no statistically significant difference was found in the mean perceived severity score (p=.164) (Table 3).

3.2. Determinants Affecting Parents' Childhood Vaccination Attitudes according to The Health Belief Model

The results of the multiple regression analysis are presented in Table 4. In the examined model, the statistical values for the variance inflation factors (VIF) were within an acceptable range (VIF 1-1.08), indicating the absence of multicollinearity issues in the dataset.

The results indicated that the regression models developed had a significant impact on the mean scores of the sub-dimensions (F(3, 262)=28.30, p<.001; F(3, 262)=18.97, p<.001; F(4, 262)=19.17, p<.001; F(4, 262)= 21.02, p<.001; F(3, 262)= 32.28, p<.001). The variables integrated into the model accounted for 24% of the variance in the perceived susceptibility, 17% in the perceived severity, 22% in the perceived benefit, 23% in the health motivation, and 26% in the perceived barriers sub-dimension.

According to the model, believing that childhood vaccines are beneficial, having sufficient knowledge about vaccines, and wanting to get routine childhood vaccination during the pandemic are the significant predictors variables. A one-point increase in these predictors variables increases the perceived susceptibility, severity, benefit and health motivation scores, while decreasing the perceived barriers score. Additionally, the increase in the COVID-19 fear score stands out as a significant determinant, contributing to the enhancement of perceived benefit (β=.12; p=.03) and health motivation (β=.14; p=.02) (Table 4).

Table 2. *The mean COVID-19 fear score and the mean PAVS-HBM subscale scores of the parents.*

	n (%)	Mean (SD)	Min-Max
Fear of COVID-19	263 (100)	19.71 (6.46)	7.0-35
PAVS-HBM	263 (100)		
Perceived Susceptibility		17.16 (2.69)	9.0-20
Perceived Severity		16.43 (3.04)	8.0-20
Perceived Benefit		20.78 (3.45)	5.0-25
Perceived Barriers		16.33 (6.35)	8.0-40
Health Motivation		21.54 (2.98)	11.0-25

PAVS-HBM, Public Attitude Toward Vaccination Scale – Health Belief Model; COVID-19, coronavirus disease 2019; SD, standard deviation

Table 3. *COVID-19 fear and PAVS-HBM mean scores according to parents' childhood vaccine characteristics.*

		Fear of COVID-19	Perceived Susceptibility	Perceived Severity	Perceived Benefit	Perceived Barriers	Health Motivation
	n (%)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Believing that childhood vaccinations are useful							
Yes	250 (95.1)	19.94 (6.46)	17.37 (2.54)	16.62 (2.98)	21.03 (3.33)	15.86 (6.1)	21.72 (2.93)
No	13 (4.9)	15.31 (4.85)	13 (2.27)	12.85 (1.86)	16 (1.91)	25.38 (4.05)	18.08 (1.85)
t		2.543	6.082	4.523	5.401	-5.561	4.443
p		**.012**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
Having sufficient knowledge about childhood vaccines							
Yes	197 (74.9)	19.55 (6.24)	17.62 (2.55)	16.86 (2.98)	21.37 (3.29)	15.12 (6)	22.05 (2.85)
No	66 (25.1)	20.18 (7.14)	15.77 (2.67)	15.18 (2.91)	19.05 (3.33)	19.95 (6.02)	20.03 (2.9)
t		-0.688	5.037	3.980	4.939	-5.657	4.967
p		.492	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
Having childhood vaccinations before the COVID-19 pandemic							
Yes	258 (98.1)	19.68 (6.44)	17.18 (2.71)	16.49 (3.02)	20.79 (3.48)	16.24 (6.32)	21.57 (3.01)
No	5 (1.9)	21 (8.46)	15.8 (1.48)	13.6 (3.05)	20.2 (1.1)	21.2 (6.83)	20.2 (0.45)
t		-0.451	1.137	2.119	1.110	-1.736	4.998
p		.653	.257	**.035**	.311	.084	**<.001**
Change in decision to have childhood vaccinations in the COVID-19 pandemic							
Yes	7 (2.7)	20 (8.02)	14.57 (3.15)	14.86 (2.97)	17.43 (3.87)	23.14 (6.49)	18.86 (3.08)
No	256 (97.3)	19.7 (6.44)	17.23 (2.65)	16.48 (3.04)	20.88 (3.4)	16.15 (6.26)	21.62 (2.96)
t		0.121	-2.600	-1.395	-2.640	2.915	-2.435
p		.904	**.010**	.164	**.009**	**.004**	**.016**
Wanting to have childhood vaccinations in the COVID-19 pandemic							
I will	218 (82.9)	19.93 (6.49)	17.58 (2.55)	16.89 (2.87)	21.28 (3.37)	15.22 (5.83)	22.06 (2.83)
I will not/ I am undecided	45 (17.1)	18.62 (6.31)	15.11 (2.47)	14.22 (2.9)	18.36 (2.75)	21.73 (6.06)	19.04 (2.45)
t		1.238	5.946	5.673	5.468	-6.778	6.655
p		.217	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**

n= 263; SD, Standard deviation; t, Independent sample t test

**p<.05*

Table 4. *Determinants of parents' childhood vaccination attitudes.*

	Susceptibility				Severity				Benefit				Health Motivation				Barriers			
Variables	**B**	**SE**	**β**	**p**	**B**	**SE**	**β**	**p**	**B**	**SE**	**β**	**p**	**B**	**SE**	**β**	**p**	**B**	**SE**	**β**	**p**
Believing that childhood vaccinations are useful (Yes)	3.30	0.69	0.27	**<.001**	2.68	0.81	0.19	**.01**	3.39	0.91	0.21	**<.001**	2.02	0.78	0.15	**.01**	-6.59	1.60	-0.23	**<.001**
Having sufficient knowledge about childhood vaccines (Yes)	1.27	0.34	0.21	**<.001**	1.12	0.41	0.16	**.01**	1.74	0.45	0.22	**<.001**	1.53	0.38	0.22	**<.001**	-3.49	0.80	-0.24	**<.001**
Wanting to have childhood vaccinations in the COVID-19 pandemic (Yes)	1.90	0.39	0.27	**<.001**	2.20	0.47	0.27	**<.001**	2.19	0.51	0.24	**<.001**	2.45	0.44	0.31	**<.001**	-5.22	0.91	-0.31	**<.001**
Fear of COVID-19									0.07	0.03	0.12	**.03**	0.06	0.03	0.14	**.02**				
R	0.50				0.42				0.48				0.50				0.52			
Adj. R2	0.24				0.17				0.22				0.23				0.26			
F and p value	28.301		**<.001**		18.966		**<.001**		19.171		**<.001**		21.016		**<.001**		32.281		**<.001**	

*n= 263; ***p<.001, **p<.01, *p<.05. Abbreviations: B, Regression coefficient; SE, Standard error of regression coefficient; β, Standard regression coefficient; CI, Confidence interval; Adj. R^2, Adjusted predicted value. Note. Multiple linear regression analysis performed with the Stepwise Method*

4. DISCUSSION

The primary aim of this study was to offer insights into parents' perspectives regarding their concerns about COVID-19 and their attitudes towards childhood vaccination during the pandemic in Turkey when the COVID-19 cases reached their peak. The study was undertaken to assess the impact of parents' COVID-19 fears and various factors on their attitudes toward childhood vaccination, utilizing the Health Belief Model, during the COVID-19 pandemic. The findings showed that when parents' COVID-19 fear level increased, their perceived benefit and health motivation attitudes improved. The results also indicated that parents' enhanced belief in the advantages of childhood vaccines, their adequate knowledge about vaccines, and their willingness to have their children vaccinated during the COVID-19 pandemic are significant predictors associated with increased perceived susceptibility, perceived severity, perceived benefit, and health motivation attitudes. These factors are also linked to a decrease in the perceived barriers attitude.

The majority of the participants in the study participants belonged to the medium socioeconomic status category and had a high level of education. It was observed that their fear levels regarding COVID-19 were notably increased. This observation aligns with the results reported by Bakioğlu et al. (24). Moreover, Baghdadi et al. (4) reported that parents commonly harbor concerns about the risk of exposing themselves or their children to COVID-19 during routine check-ups for healthy children. In addition, it is thought that the increase in parents' fear levels may be attributable to the high mortality rates due to COVID-19 infection and the uncertainty about the long-term effects of COVID-19 on social life and health.

The vaccination attitudes subscale mean scores of the parents showed similarity with the study of Kaydirak et al. (19). In the study, the increase in the scores of parents who believe that childhood vaccines are beneficial, who have sufficient knowledge about vaccines and who want to have childhood vaccines during the COVID-19 pandemic increased the perceived susceptibility, perceived severity, perceived benefit and health motivation attitude scores of the parents. Concurrently, it led to a reduction in the perceived barriers attitude score. The study found that 17.1% of the parents did not consider or were undecided about having childhood vaccinations. Recent findings suggest that the global execution of vaccination programs has been hindered by the COVID-19 pandemic (2, 3, 25). Bell et al. (6) reported that parents are afraid of contracting COVID-19 or they do

not want their children to contract COVID-19 due to the risk of encountering people while going to the health center for vaccination. It can be thought that this situation has affected childhood vaccination practices.

This study showed that the increase in parents' COVID-19 fear levels is a significant predictor that increases the perceived benefit and health motivation attitude. Increasing COVID-19 fear level led to an increase in the perceived benefit score. This indicated an enhancement in parents' awareness regarding the advantages of the recommended health protective measures for themselves and their children, resulting in a favorable attitude (21). As the level of COVID-19 fear increases, parents attach more importance to their own and their child's health, and as a result, they can develop a more attentive and positive attitude towards taking the necessary precautions. From this perspective, the heightened level of COVID-19 fear can be regarded as a protective factor and may be considered an anticipated response.

5. CONCLUSION

The study indicates that parents have high COVID-19 fear levels and increased fear levels improve perceived benefit and health motivation attitudes based on the Health Belief Model. Furthermore, an increase in the scores of parents who hold the belief in the benefits of childhood vaccines, possess adequate knowledge about vaccines, and express the willingness to have their children vaccinated during the COVID-19 pandemic leads to an enhancement in their attitudes based on the Health Belief Model. This includes an increase in perceived susceptibility, perceived severity, perceived benefit, and health motivation attitudes, while decreasing the perceived barriers attitude. This shows the positive attitude of the parents towards childhood vaccines. Parents should be provided with education and counselling by health professionals to develop a positive attitude towards childhood vaccinations. Other factors (concerns about vaccination safety) that may cause hesitancy towards childhood vaccines should be investigated.

The study presents certain limitations. To begin with, the web-based nature of the research may potentially hinder the generalizability of the results to the entire population. Moreover, a limitation is that the sample is primarily comprised of mothers and individuals with university degrees. Additionally, the data collection tools rely on self-report, which might introduce social desirability bias. At the same time, the generalization of all childhood vaccines in the data collection tool is another limitation of the study. Finally, this study with a cross-sectional design may prevent establishing a cause-effect relationship. It may be recommended to conduct a prospective longitudinal study.

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Clinical and Experimental Health Sciences

Examination of Health Data of Elderly Individuals Registered with Family Medicine: A Retrospective Study

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ABSTRACT

Objective: The research aims to determine the utilization of health services by elderly individuals registered in family medicine according to their sociodemographic characteristics and mobile service usage.

Methods: The population of the research consists of 417 individuals aged 65 and over who are registered with a family health center in the west of Türkiye. The retrospective research method was used in this research. In the study, the information of elderly people aged 65 and over registered with family medicine was used.

Results: In the study, it was determined that there was a statistically significant relationship between old age and gender, educational status, place of residence, and mobile service availability. It has been determined that mobile services are the most used in middle age (55.2%). Additionally, it was determined that 97.8% of mobile service users had not been vaccinated (Influenza Vaccine, Hepatitis B Vaccine, Tetanus-Diphtheria Vaccine).

Conclusion: The results revealed that mobile health services are inadequate for elderly individuals. As a solution, it is recommended to increase the number of days of mobile health service for rural areas or to establish a family medicine unit in these villages.

Keyword: Elderly, family medicine, health services, mobile health services.

1. INTRODUCTION

As life expectancy at birth increases in society, related diseases are increasing, which causes many health problems in old age (1,2). While the number of elderly people aged 60 and over worldwide was 600 million in 2000, it is expected to double in 2025 and triple in 2050 (17%, meaning an average of 1.6 billion) (3,4). Likewise, it is seen that the elderly population has increased in Türkiye, especially after the 1960s (5–7). According to the report of the Turkish Health Institutes Presidency (TÜSEB), in Türkiye, the elderly population aged 65 and over is close to 8 million in 2020, while the proportion of the population in the total population is 9.5% (1,8). Moreover, according to studies, the elderly population in Türkiye is expected to be 10.2% in 2023, 16% in 2040, 20.8% in 2050, over 25% in 2060 (at least 24 million), and 27.7% in 2075. If this happens, Türkiye will be in the group of countries with very elderly populations in 2075 (1,4,8).

In the study conducted by the Ministry of Health of the Republic of Türkiye in 2021 on non-communicable diseases and risk factors; It has been reported that 46% of elderly individuals aged 65 and over have more than two chronic diseases (1,9). Chronic diseases are among the diseases that most commonly cause disability or death in the world and account for 70% of deaths (9,10). In Türkiye, the mortality rate of chronic diseases is 89%, while the premature death rate (under 70 years of age) is 16% (10). For these reasons, it is thought that it is important to examine the health status of elderly individuals living in Türkiye.

The workload of chronic diseases is quite high at all levels of healthcare, especially primary healthcare (11,12). Since the follow-up and treatment of these diseases are long-term, monitoring them in primary health care is very important in terms of process management. It aims to monitor chronic diseases by providing health services for individuals and society with the “Chronic Diseases Monitoring in Primary Health Care Services” and “Health Transformation Program” carried out by family physicians in Türkiye (1,13).

Preventive health services: ensure that the individual, family, and society are more beneficial physically, psychologically,

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economically, and socially. As a result, while individuals are encouraged to have a healthy lifestyle, health services improve, and by providing a more effective and efficient service, both the health status of society increases, and the welfare level of the society increase (1,14,15). Protective and preventive health services play a very important role in eliminating all kinds of problems that pose a risk to protect and improve public health at the highest level. Preventive health services for individuals and society in Türkiye; It is provided in Family Health Centers (FHC) and Community Health Centers (CHM) (14,15). With immunization programs, which are among the preventive health services, to elderly individuals; Individuals are protected from diseases by administering vaccines such as tetanus-diphtheria, pneumococcal, influenza, and COVID-19 vaccines (16,17). Considering the COVID-19 epidemic, which has caused the death of millions of people around the world, especially people aged 65 and over and those with chronic diseases are listed as risk groups (18). Research results show that the case fatality rate from COVID-19 is higher in patients aged 65 and over (19–21).

In addition, mobile health services are provided within the scope of family medicine. Mobile health service refers to the health service that the family physician and family health personnel will provide on-site by going to settlements such as remote neighborhoods, towns, villages and hamlets determined by the directorate, in accordance with the procedures and principles determined by the Public Health Institution of Türkiye (22).

The research aims to determine the utilization of health services by elderly individuals registered with family medicine according to their sociodemographic characteristics and mobile service usage and to contribute to health policymakers and the literature.

2. METHODS

2.1. Design and Sample

In the study, health and sociodemographic data of elderly individuals aged 65 and over who were registered to the Family Medicine Unit at a Family Health Center in the west of Türkiye were examined. Sample selection was made using convenience sampling. A family medicine clinic in the region where the researchers lived was selected. This research is a descriptive retrospective type of research. The population of the research consists of 417 individuals aged 65 and over, recorded in the registration system of this family practice. The entire population was included in the research without choosing a sample. In the family health unit where the research was conducted; A family doctor and a midwife were on duty, serving a population of 3450, serving 5 remote villages and those in the city. In the family health unit where the research was conducted; immunization, diagnosis, treatment, monitoring of chronic diseases, pregnant monitoring, maternity monitoring, baby monitoring, reproductive health, monitoring of women aged 15-49, elderly health monitoring, and mobile health services are provided.

2.2. Data Collection Tools

Research data was collected between March 1 and May 31, 2023. Research data was collected with a data collection form prepared by the researcher. The research form consists of two parts: sociodemographic characteristics and health status characteristics. In the surname demographic characteristics section, there are 5 statements including the individual's age, gender, education level, place of residence, and mobile service availability. In the health status characteristics section, the individual's acute and chronic diseases, vaccination status, mobile service availability, etc. It consists of 18 statements. Family Medicine Information Management System was used to collect data.

2.3. Statistical Analysis

The data analysis of the research was evaluated using the SPSS (IBM SPSS Statistics 27) package program. Frequency tables and descriptive statistics were used to interpret the findings. "Pearson-$\chi 2$" cross-tabulations were used to examine the relationships between two qualitative variables. In the study, $p< .05$ value was considered statistically significant.

2.4. Ethical Considerations

Ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Kütahya Health Sciences University (dated 11.01.2023 and numbered 2023/01-25) and institutional permission numbered 2021/14 was obtained from the Kütahya Governorship Provincial Health Directorate. While conducting the research, the Declaration of Helsinki was followed, and scientific and universal ethical principles were followed.

3. RESULTS

It was determined that 50.7% of the youngest-old age group was male, 59.0% of the middle-old group, 73.3% of the elderly group were female, and 53.5% of the total elderly individuals were female. It was determined that 59.1% of the young-old age group were primary school graduates, 73.3% of the middle-aged group and 100.0% of the elderly group did not attend any school. It was determined that there were no elderly individuals whose education level was secondary school or high school, and 49.6% of the elderly individuals in total did not attend any school. While the rate of people living in the district in youngest-old age was 53.9%, it was determined that this rate increased to 73.3% during the old age period. Additionally, it was determined that 54.2% of the elderly lived in the village. Considering the status of receiving mobile service; It has been determined that mobile services are mostly used in middle age (55.2%), while the rate of mobile service usage in youngest-old age is 39.0%, this rate is 46.7% in old age. Additionally, it was determined that 43.6% of elderly individuals use mobile services (Table 1).

Table 1. *The relationship between sociodemographic characteristics and old age periods*

Sociodemographic Characteristics	Old Age Period								Statistical analysis* Possibility
	Youngest-old (65-74)		Middle-old (75-84)		Oldest-old (85 and older)		Total		
	n	%	n	%	n	%	n	%	
Gender									
Female	139	49.3	62	59.0	22	73.3	223	53.5	χ^2=8.050 **p= .018**
Male	143	50.7	43	41.0	8	26.7	194	46.5	
Educational status									
Without any graduation	100	35.5	77	73.3	30	100.0	207	49.6	χ^2=77.245 **p< .001**
Elementary school	167	59.1	27	25.7	-	-	194	46.6	
Middle school	12	4.3	1	1.0	-	-	13	3.1	
High school	3	1.1	-	0.0	-	-	3	0.7	
Living place									
County	152	53.9	31	29.5	8	26.7	191	45.8	χ^2=23.084 **p< .001**
Village	130	46.1	74	70.5	22	73.3	226	54.2	
Mobile service usage status									
Yes	110	39.0	58	55.2	14	46.7	182	43.6	χ^2=8.315 **p= .016**
No	172	61.0	47	44.8	16	53.3	235	56.4	

** In examining the relationships of two qualitative variables "Pearson-χ2" cross table.*

Table 2. *Relationship between mobile service usage and vaccination*

Variables	Mobile service usage situation						Statistical analysis* Possibility
	Yes		No		Total		
	n	%	n	%	n	%	
Vaccination Status							
Yes	4	2.2	46	19.6	50	12.0	χ^2=29.348 **p< .001**
No	178	97.8	189	80.4	367	88.0	
Vaccination Administered							
Influenza Vaccine	3	100.0	44	93.6	47	94.0	χ^2=0.204 p= .903
Hepatitis B Vaccine	-	-	1	2.1	1	2.0	
Tetanus-Diphtheria Vaccine	-	-	2	4.3	2	4.0	
Status of getting COVID-19 vaccine							
Yes	140	76.9	184	78.3	324	77.7	χ^2=0.112 p= .738
No	42	23.1	51	21.7	93	22.3	
COVID-19 Vaccine Dosage							
1 or 2 doses	15	10.7	26	14.1	41	12.6	χ^2=12.829 **p= .002**
3 doses	124	87.9	139	75.1	263	80.7	
4 doses	2	1.4	20	10.8	22	6.7	

** In examining the relationships of two qualitative variables "Pearson-χ2" cross table.*

In this research, it was determined that the distribution of old age periods differs according to gender. According to these findings, the rate of men in youngest-old age is 50.7%, the rate of men in middle-old is 41%, and the rate of men in oldest-old is 26.7% (p<.05).

In this research, it was determined that the educational status of individuals varies according to their old age. According to these findings, the rate of those who did not graduate from any school in their youngest-old age is 35.5%, the rate of those who did not graduate from any school in their middle-old is 73.3%, and the rate of those who did not graduate from any school in their oldest-old age is 100% (p< .001).

In this research, it was determined that the residential situation of individuals varies according to their old age. According to these findings, the rate of people living in the village in their youngest-old age is 46.1%, the rate of people living in the village in their middle-old is 70.5%, and the rate of people living in the village in their oldest-old age is 73.3% (p< .001).

In this research, it was determined that individuals' use of mobile services varies depending on their age. Additionally, it was determined that 97.8% of mobile service users had not been vaccinated (Influenza Vaccine, Hepatitis B Vaccine, Tetanus-Diphtheria Vaccine) (p< .05). According to these findings, the rate of using mobile services in the youngest-old age is 39%, the rate of using mobile services in the middle-old age is 55.2%, and the rate of using mobile services in the oldest-old age is 46.7% (p<.05).

A statistically significant relationship was determined between the use of mobile health services by elderly individuals and

Table 3. *Disease and drug use status according to old age*

Disease and drug use	Old Age Period								Statistical analysis* Possibility
	Youngest-old (65-74)		Middle-old (75-84)		Oldest-old (85 andolder)		Total		
	n	%	n	%	n	%	n	%	
Chronic disease									
Yes	218	77.3	90	85.7	27	90.0	335	80.3	χ^2=5.336 p= .069
No	64	22.7	15	14.3	3	10.0	82	19.7	
Number of chronic diseases									
One disease	65	29.8	21	23.3	6	22.2	92	27.5	χ^2=1.789 p= .774
Two diseases	46	21.1	20	22.3	6	22.2	72	21.5	
Three or more diseases	107	49.1	49	54.4	15	55.6	171	51.0	
Regular medication use									
Yes	217	77.0	89	84.8	27	90.0	333	79.9	χ^2=4.970 p= .083
No	65	23.0	16	15.2	3	10.0	84	20.1	
Multiple drug use									
Yes	154	54.6	69	65.7	21	70.0	244	58.5	χ^2=5.643 p= .060
No	128	45.4	36	34.3	9	30.0	173	41.5	

** In examining the relationships of two qualitative variables "Pearson-χ2" cross table.*

their vaccination status and COVID-19 vaccine doses. It has been determined that 97.8% of individuals using mobile health services have not been vaccinated. It was determined that 87.9 percent of those using mobile health services had received 3 doses of the COVID-19 vaccine (p< .001) (Table 2). It was determined that 75.1% of those who did not receive mobile health services had 3 doses of COVID-19 vaccine (p<.05) (Table 2).

Chronic disease and medication use status by age groups are shown in Table 3. There is no statistically significant relationship between chronic disease status according to old age groups (p> .05). 83.7% of the youngest-old, 87.6% of the middle-old, and 83.3% of the oldest-old had at least one chronic disease. When the number of chronic diseases in elderly individuals was examined, it was determined that 51% had three or more chronic diseases, 25.5% had a single chronic disease, and 21.5% had two chronic diseases.

There is no statistically significant relationship between regular drug use according to age groups (p> .05). It was determined that 77% of the youngest-old, 84.8% of the middle-old, and 90% of the oldest-old used their medications regularly. There is no statistically significant relationship between polypharmacy status according to age groups (p> .05). It was determined that 54.6% of the youngest-old, 65.7% of the middle-old, and 70% of the oldest-old used multiple medications.

4. DISCUSSION

The research aims to determine the utilization of health services by elderly individuals registered in family medicine according to their sociodemographic characteristics and mobile service usage.

In this study, it was determined that the rate of people using mobile health services to receive 3 doses of COVID-19 vaccine was higher than those who did not use mobile health services (p< .05). The reason for this situation may be that the vaccination service was brought to the region where people live in a planned manner during the COVID-10 pandemic period.

In this research, it was determined that mobile service usage was highest in middle age. It has also been determined that most elderly individuals use mobile services (p< .05). Additionally, it was determined that 97.8% of mobile service users had not been vaccinated (p< .05). The Ministry of Health has planned to provide mobile health services once or twice a week, depending on the population, for towns, villages, hamlets, remote neighborhoods, and similar settlements where access to health services is difficult (22). It is advantageous for individuals living in those settlements to go to settlements far from the center to provide mobile service outside the FHC. However, the results of this research show that the vaccination rate of mobile service recipients is very low. The reason for this situation suggests that the mobile health service provided once or twice a week is insufficient.

This study determined that the status of having a chronic disease did not change according to older age groups. Additionally, most elderly individuals had at least one chronic disease, and more than half had three or more chronic diseases. In their study on elderly individuals living in nursing homes, Özcan and Alpaslan determined that 75% of elderly individuals had at least one chronic disease (23). Şahan et al. it was determined that 72% of elderly individuals had chronic diseases (24). Kaçan and Değer found that 78.3% of elderly individuals had hypertension (25). It seems that our results are compatible with the literature results.

In this study, it was determined that the majority of elderly individuals used their drugs regularly. Özcan and Alparslan found that 78.3% of elderly individuals used medication regularly (23). Ünlü and Olgun determined that 60% of elderly

individuals use medication regularly. The results of our research are like the results of research in the literature (23-25).

In this study, it was determined that most elderly individuals used multiple medications. Hsu et al. determined that the prevalence of polypharmacy in older adults ranged from 7-45% (26). Wastesson et al. determined that 45% of elderly individuals use multiple medications (27). In our study, the rate of polypharmacy appears to be higher than in other studies.

The study shows that there is a statistically significant relationship between old age and gender. It has been determined that the elderly and total elderly individuals in advanced old age are mostly women, and the proportion of men gradually decreases with age ($p<0.05$). In different studies conducted in Türkiye, it has been determined that women are the majority in the gender distribution of elderly individuals (18,28). According to the 2022 data from the Turkish Statistical Institute, the rate of the female population in Türkiye is 52.2% in the 60-74 age group and 72.4% in the 90 and over age group (29). It appears that the results of our research are like other results in the literature.

In this research, it was determined that the rate of not going to any school increases with age, there are no elderly people who are secondary school or high school graduates, and most elderly individuals do not go to any school ($p< .05$). In a study conducted in Türkiye, it was determined that the education level of most elderly individuals was primary school (30). Another study reported that the proportion of illiterate elderly was higher (31). Considering the elderly individuals over the age of 65 in our research, considering the conditions of the region and individuals in the past, the inadequacies in educational institutions, and the society's perspective on reading, it is a predictable situation that the rate of illiterate elderly is high.

The research shows that there is a statistically significant relationship between old age and place of residence and that the proportion of elderly people living in villages increases as they get older ($p< .05$). Turkish society is getting older, so chronic diseases are becoming an important problem related to aging (32). Elderly individuals' need for healthcare is a public health priority (33). Therefore, we recommend increasing public health practices in rural areas. It can be thought that it will be difficult for elderly individuals living in rural areas to access health services due to distance, transportation, low-income levels, and lack of health service providers, especially in areas requiring expertise.

5. CONCLUSION

In this study, it was determined that among individuals over the age of 65, the proportion of men decreased with age. Future studies may examine why men live shorter lives than women. In this research, it was determined that most elderly people live in villages. In addition, it has been determined that mobile health services are provided in villages with difficult access and that the vaccination rate of elderly individuals receiving mobile services is low. As a solution, the number of days of mobile health service can be increased in these regions or a family medicine unit can be established in these villages. The results of this study and studies conducted in other countries differ in terms of polypharmacy rates. In future studies, cross-country comparisons can be made regarding polypharmacy. Since our research was conducted in a family medicine unit in the west of T**ürkiye**, it cannot be generalized about the utilization of primary health care services by individuals aged 65 and over throughout the country.

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Clinical and Experimental Health Sciences

The Effect of Using Virtual Reality Glasses on Pain During Fistula Cannulation in Hemodialysis Patients: A Randomized Controlled Trial

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ABSTRACT

Objective: Fistula cannulation is performed very frequently for hemodialysis patients. Cannulation carried out repeatedly causes significant pain. In this study, it was aimed to determine the effect of watching videos with virtual reality glasses on reducing the pain during fistula cannulation in hemodialysis patients.

Methods: The study was carried out with a total of 47 patients. The patients in the intervention group were shown a video for about five minutes during the procedure using virtual reality glasses as the intervention. The control group patients went through only the standard cannulation procedure.

Results: There was no statistically significant difference between the first pain measurement scores of the intervention and control groups. There was a statistically significant difference in the mean pain score of the patients in the intervention group. Also, when the first and second pain measurements were compared within the groups, the second measurement scores were statistically significantly lower than the first in the intervention group.

Conclusion: The study results revealed that virtual reality can reduce the pain experienced during fistula cannulation in hemodialysis patients.

Keywords: Arteriovenous fistula, hemodialysis, pain, virtual reality

1. INTRODUCTION

Chronic kidney disease (CKD) is a common disease that affects all age groups, has high morbidity and mortality rates, reduces the quality of life, and can be prevented and/or its advanced stages can be slowed down with early diagnosis (1). CKD is defined as the chronic, progressive and irreversible deterioration of the kidneys due to the balance in the body's fluid-electrolyte and metabolic endocrine functions not being able to be adjusted with a decrease in glomerular filtration rate (GFR) (2).

Some symptoms become obvious in the body due to a decrease happening in kidney functions over time in patients who develop kidney disease. Renal Replacement Therapy (RRT) methods should be applied to the patient when conservative treatments are not sufficient, and the picture of uremia cannot be corrected. Hemodialysis (HD), peritoneal dialysis (PD) or kidney transplantation, among the RRT methods, is started according to the patient's condition (2,3).

Permanent vascular access that can be used for months or years is required for HD treatment to be sufficient for patients with End-Stage Renal Disease (ESRD) to survive and improve their quality of life. Therefore, arteriovenous fistula (AVF), graft or catheter should be always available to provide adequate blood flow in patients who would have the HD treatment (4,5). CKD patients with HD treatment go through 300-320 AVF cannulations per year on average. The diameter size of the fistula needles and their length, the puncturing process in the skin, the progress of the needles into the tissue during the procedure, the angle of insertion of the

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needles and the insertion techniques cause the patients to experience pain during the procedure (3,4,6-8).

Nurses are considered the primary healthcare providers for patients having hemodialysis treatment. Predicting the pain experienced during cannulation and planning appropriate interventions to reduce this pain are among the roles that nurses should fulfill (9). In patients receiving HD treatment, many non-pharmacological interventions are used by nurses to reduce the symptoms caused by the disease process and hemodialysis treatment. These non-pharmacological methods, included in independent nursing practices, have been preferred in nursing practices in recent years due to their safe and easy application and minimal side effects. Nurses use many non-pharmacological methods such as distraction, massage, hot and cold application, and aromatherapy to reduce the pain experienced during hemodialysis AVF cannulation (10).

Distraction, which is one of the non-pharmacological methods, is a method that enables patients to control and reduce the symptoms they experience by focusing their attention on a different point (11). Methods such as listening to music, drawing pictures, watching TV, solving puzzles, daydreaming, deep breathing and coughing exercises, sphygmomanometer blowing, active listening, touching, blowing up a balloon, distraction cards, and use of virtual reality glasses are used for this purpose. The use of virtual reality (VR) has been determined to be effective in reducing pain and anxiety (12-16).

The individuals concentrate their attention on the image they watch and feel like they are in another world thanks to these five-dimensional glasses by getting away from the environment they are in with the glasses connected to the device worn on their heads and the sounds coming from the headsets. The most basic feature distinguishing virtual reality glasses from similar applications is that it gives people the feeling of being real. The use of virtual reality glasses, which are easy to apply and use, have no side effects and can be effective in physical, psychological and social recovery, is an intervention that can be preferred in nursing practices (15). Karaman and Taşdemir (2021) reported, in their study with patients who underwent breast biopsy, that virtual reality intervention reduced the pain and anxiety of the participants (16). In the study conducted by Şen (2020) with hemodialysis patients, the use of virtual reality glasses during the cannulation procedure was noted to reduce the pain of the patients and increased patient satisfaction (15).

It was aimed in this study to determine the effect of using virtual reality glasses during fistula cannulation applied to hemodialysis patients on their pain experienced.

2. METHODS

2.1. Design

The study was carried out with patients in the hemodialysis unit of a hospital in Istanbul between September and November 2022. This was a single-centred parallel-design randomized controlled intervention trial with a pre-test and post-test. CONSORT were used in reporting the study. The study was registered as a Clinical Trial on January 23, 2023, with ClinicalTrials.gov ID: NCT05693584

2.2. Setting and Participants

The study population consisted of patients (n=82) in the hemodialysis unit of a private hospital and the sample included patients who met the inclusion criteria and agreed to participate in the study (n=47).

2.2.1. Inclusion Criteria

Patients who agreed to participate in the study, who were 18 years or older, had hemodialysis treatment administered via AVF, without vision, hearing and perception problems, were open to communication and could speak Turkish, without any signs of infection such as redness, swelling or open wound at the site of the intervention, had cannulation procedure performed with a 16 G AVF (Proximal) needle and a single needle insertion attempt was made, and those who had not taken any analgesics in the last three hours were included in the study.

2.2.2. Sample Size, Randomization and Blinding

The sample size of the study was calculated using the G*Power 3.1.9.7 program. The sample size calculation was performed for the t-test in independent groups, taking the study design with two groups (Intervention Group, Control Group) and two measurements (Pretest, Posttest) into account. The sample size was calculated as seven for each group by taking the with a 5% margin of error ($\alpha = 0.05$) and 95% power ($1-\beta = 0.95$), according to reference study (14,17).

Patients who met the inclusion criteria were randomized into two groups, the intervention group (n= 28) and the control group (n=28). After nine patients were excluded from the study since seven of them did not continue the study and two of them were not feeling well during the study, it was completed with a total of 47 patients, 23 in the intervention group and 24 in the control group. In the post hoc analysis, the power of the study was determined as 99% (17).

Computer-assisted simple randomization (https://www.randomizer.org/) was used to determine the groups. The data were collected by one of the researchers and the analysis and evaluations were performed by the other researcher. Thus, blinding was used during data collection and analysis.

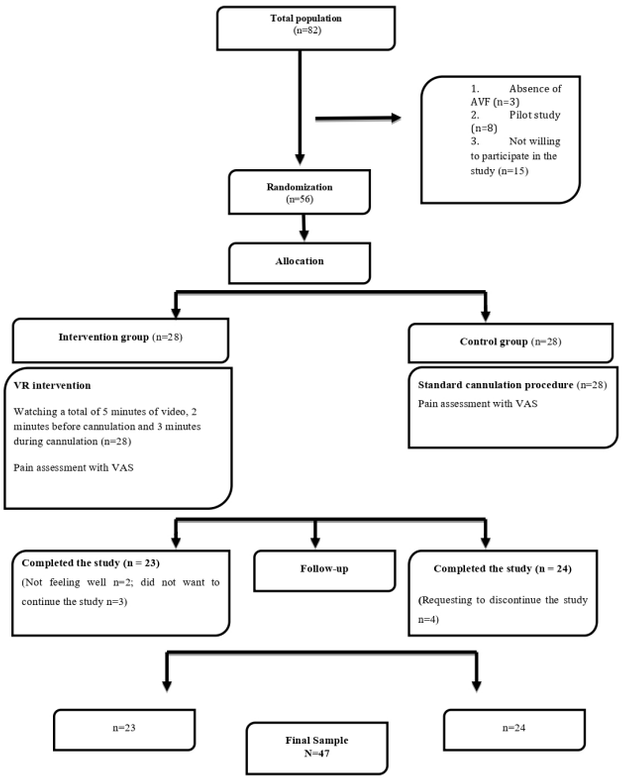

Figure 1. *Flow diagram of the study*

2.3. Instruments

The data collection was carried out with the Personal Information Form and Visual Analogue Scale (VAS).

• *Personal Information Form:* The form prepared by the researchers includes questions about participants' age, gender, educational status, employment status, disease, and hemodialysis.

• *Visual Analogue Scale (VAS):* One of the scales used for the assessment of pain is the VAS. This scale, developed by Price et al. (1983), is used for the measurement of subjective experiences (18). VAS is a scale evaluated by individuals marking their levels of pain on a horizontal or vertical line of 10 cm or 100 mm, with one end of the scale indicating that the patient is very good, meaning no pain (0 points), and the other end indicating that the patient is feeling very bad, meaning unbearable pain (10/100 points). It is used in various patient populations to assess the severity of acute pain, especially the efficacy of treatment/intervention. Its sensitivity has been reported to be higher than other methods in the assessment of pain severity (19).

Virtual reality glasses: Bobo VR Z4 brand virtual reality glasses with 5.7 inches with 1440x2560 pixel screen resolution and James Bullough Lansing (JBL) brand wireless headsets were used.

2.4. Interventions

*Standard Care:*There were a total of 40 hemodialysis machines and beds in the hemodialysis unit where the study was conducted, and a total of nine nurses and three doctors were working. Patients are admitted to hemodialysis sessions in four groups: morning and afternoon groups visiting on Monday-Wednesday-Friday, and morning and afternoon groups visiting on Tuesday-Thursday-Saturday. During the study, there were 82 patients in total, three of whom did not have AVF.

The patients were welcomed by the unit staff on the day of the session, then they were taken to their beds following their weight measurements, and cannulation was performed by the inpatient nurses after the hemodialysis machine and materials were prepared. A cannula size 16 was used for the AVF cannulation. Rope ladder technique is used for cannulation and all nurses use the same technique. The nurses in the dialysis unit had at least two years of experience in this field.

*Pilot study:*To test the understandability of the Personal Information Form and VAS and the applicability of the intervention, a pilot study was carried out with a total of eight patients, representing 10% of the individuals included in the sample, and these eight patients included in the pilot study were excluded from the study. As a result of the pilot study, the intervention and the materials used were determined to be suitable.

Intervention: One week before the intervention, the pain experienced by the patients during AVF cannulation was assessed with VAS (First measurement).

- *Intervention group:* First, the Personal Information Form was filled. Then, the patients were shown a video of nature, forest, and seaside images with relaxing background music, an average of five minutes, two minutes before the AVF cannulation procedure and three minutes during the procedure, using virtual reality glasses(https://www.youtube.com/watch?v=pXfUhkK_QRQ&t=8s). The cannulation procedure was performed by the inpatient nurse. Pain assessment was performed by researcher after intervention immediately **(**Second measurement). The researcher did not actively participate in the cannulation process. To prevent interaction among the participants during the virtual reality intervention, a curtain was placed between the patients in the intervention and control groups. The VR glasses and headsets used were cleaned with alcohol-based disinfectant wipes before use for each patient.
- *Control group:* First, the Personal Information Form was filled. No intervention was performed on the patients other than the routine practices of the unit. As in the intervention group, needle insertion into the fistula was performed by clinical nurse. Pain assessment was performed by the researcher after intervention immediately **(**Second measurement). The researcher did not actively participate in the cannulation process.

2.5. Data Analysis

In this study, the data were analyzed in the SPSS program. A normality test was done with skewness and kurtosis. While mean,

standard deviation and number-percentage distributions were used in the data analysis, the Independet Sample T-test, Mann-Whitney U test, and Chi-square test were used for repeated measurements. Cohen-d was used to determine the effect size.

2.6. Ethical Consent

The ethical approval from the Fenerbahçe University Ethics Committee (29.2022fbu,14.09.2022) and institutional permission were obtained from the relevant hospital management to be able to conduct the study. As per the Declaration of Helsinki's Privacy and Confidentiality Principle, all precautions were taken to manage the confidentiality of the personal information of the volunteers participating in the study. Informed consent was obtained from all participants.

3. RESULTS

When the distribution of the personal information of the patients in both groups was compared, the difference between the groups was determined not to be statistically significant and both groups were similar to each other in terms of variables (p>.05) (Table 1).

Table 1. *Distribution of the personal information of patients in the intervention and control groups*

Characteristics	Intervention		Control		Test Statistic	P Value
	X±SD		X±SD		t	P
Age	59.73 ± 11.48		60.54 ± 11.40		-0.240	.811
Duration of dialysis(year)	10.30±9.20		8.12±8.45		0.846	.402
	N	**%**	**N**	**%**	**X^2**	**P**
Gender						
Female	8	34.8	7	29.2	0.170	.680
Male	15	65.2	17	70.8		
Marital Status						
Unmarried	4	17.4	2	8.3	0.865	.416
Married	19	82.6	22	91.7		
Education						
Primary school	14	60.9	10	41.7	7.314	.120
Secondary school	3	13	1	4.2		
High school	2	8.7	10	41.7		
Associate degree	2	8.7	1	4.2		
Undergraduate	2	8.7	2	8.3		
Working status						
Yes	4	17.4	2	8.3	0.865	.416
No	19	82.6	22	91.7		
Status of having a hemodialysis patient in their family						
Yes	4	17.4	9	37.5	1.475	.225
No	19	82.6	15	62.5		
Vascular access site						
Right brachial	4	17.4	2	8.3	0.865	.416
Left brachial	19	82.6	22	91.7		

t: Independet Sample T – test
X^2: Fisher's Chi-Square

The pain experienced during the cannulation procedure by the patients included in the study was assessed one week before the intervention, and the mean pain score of the intervention group patients was 3.69±2.63 and it was 3.37±1.05 for the patients in the control group. There was no statistically significant difference between the groups (p>.05).

While the mean pain score assessed while watching a video with virtual reality glasses during the cannulation of the patients of the intervention group was 2.47±1.95, it was 3.58±1.14 for the patients in the control group who only had standard cannulation. The difference between the groups was determined to be statistically significant (p< .05) (Table 2).

Table 2. *Intragroup and intergroup comparisons of VAS mean scores*

	Intervention (n=23)	Control (n=24)	Test and Significance	
	X±SD	X±SD	t/Z	p
First measurement	3.69±2.63	3,37±1.05	– 0.489	.625
Second measurement	2.47±1.95	3.58±1.14	– 2.021	**.043***
	Z= – 2.416	Z= – 1.414		
	p=0.016*	p=0.157		

t: Independet Sample T-test
Z:Mann Withney U Test

Table 3. *Effect of virtual reality glasses on fistula cannulation pain intensity*

	Intervention d (n=23)	Control d (n=24)	First measurement	Second measurement
First measurement & Second measurement	0.53	-	-	-
Intervention & Control	-	-	-	0.69

d= Cohen's d effect size (Cohen's d effect size: Small 0.2; Medium 0.5; Large 0.8; Very large 1.3)

When the intra-group pre-test and post-test measurements were compared, the first measurement, which was the pre-test of the intervention group, was 3.69±2.63 and the second measurement, which was the measurement during the intervention, was 2.47±1.95, and there was a significant difference between the measurements. The second pain score measurement of the intervention group was significantly lower than the first one (p<.05). In the measurements of the control group, the mean pain score at the first measurement was 3.37±1.05 and it was 3.58±1.14 at the second one, and there was no statistically significant difference between the measurements (p>.05).

When the effect size was calculated according to the measurements with a significant change, it was d=0.53 according to the difference between the first and second measurements of the intervention group and it was d=0.69

according to the difference between the intervention and control groups in the second measurements. Considering Cohen's d effect size, watching videos with virtual reality was determined to have a medium effect on reducing pain (Cohen's d effect size: Small 0.2; Medium 0.5; Large 0.8; Very large 1.3) (18).

4. DISCUSSION

It was aimed in this study to determine the effect of using virtual reality glasses during fistula cannulation applied to hemodialysis patients on their pain experienced. The mean pain scores of the intervention and control groups during fistula cannulation before the intervention were 3.69±2.63 and 3.25±1.15, respectively in our study and these scores were similar to the pain scores found in the studies of Bagheri et al. (2014) and Sabitha et al.(2008) (8,20).

The main factors that virtual reality is assumed to contribute to reducing pain are related to the degree of virtual reality and the level of interaction with the virtual environment. A high level of interaction with the virtual world has been suggested to reduce pain by leading to a block in visual and auditory pain stimuli present in a clinical setting (21,22). Patients report less pain because their attention is temporarily distracted from the painful stimulus through virtual reality glasses (23). In our study, there was a significant decrease in the level of pain experienced by the intervention group patients, who watched a video with virtual reality glasses during fistula cannulation, compared to the control group. In their study in which they evaluated the effectiveness of using virtual reality glasses in reducing pain during fistula cannulation in hemodialysis patients, Nasirzadeh et al. (2019) concluded that virtual reality glasses had a positive effect on pain and reduced the felt pain (24). In their study conducted in 2017 on the effect of relaxing music chosen by the patient as a distraction method on pain experienced due to fistula cannulation in hemodialysis patients, Shabandokht-Zarmi et al. reported that the pain felt during the procedure was less in the intervention group patients (25). In a meta-analysis study examining the effectiveness of virtual reality goggles in relieving pain and anxiety in pediatric patients, it was concluded that virtual reality goggles are effective in managing pain by drawing pediatric patients into a virtual world (26). A recent systematic review and meta-analysis of randomized controlled trials using virtual reality to reduce the sensation of pain noted that virtual reality effectively reduces pain during medical procedures in both children and adults (26). The difference of this study from other studies is that it was conducted with adult hemodialysis patients.

Many studies conducted in different sample groups support that distraction with virtual reality glasses significantly reduces pain compared to standard care (21,27-29). However, some studies did not find a statistically significant difference between virtual reality distraction and standard care procedures (30-32). In our study, it was found that the use of VR glasses significantly reduced pain during fistula cannulation in hemodialysis patients pain compared to standard care.

4.1. Limitations

There are some limitations of our study. First, the study was conducted only in one hemodialysis unit, which may affect the generalizability of its results to other settings. Secondly, it was not possible to control the effect of participants' anxiety and stress levels on their pain intensity. Third, the fact that it was not possible that the fistula cannulation to be performed by the same nurse for all participants might have negatively affected the study results. Finally, during intervention, interaction may occur even if there are curtains between patient beds.

5. CONCLUSION

According to the results of this study, the intervention group patients, who watched a video with virtual reality glasses, were determined to feel less pain during fistula cannulation than the patients in the control group. Therefore, virtual reality may be an effective therapeutic option that can be used to reduce pain experienced due to fistula cannulation in hemodialysis patients. Since hemodialysis patients are regularly cannulated, they are constantly exposed to pain and different methods should be used to reduce pain. With this study, the use of virtual reality glasses can be used as a different method to reduce pain.

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Clinical and Experimental Health Sciences

The Validity-Reliability Study of Turkish Version of Electronical Symptom Screening Tool (8-18) in SSPedi-Pediatric Patients with Cancer

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ABSTRACT

Objective: The first step in symptom management is symptom screening which is necessary to keep the symptom under control. This study aimed to determine the validity and reliability of the Turkish version of the Electronic Symptom Screening Tool in Pediatrics for children aged 8-18 (SSPedi 8-18) with cancer.

Methods: The research was conducted between September 2020 and June 2021. The sample in our study consists of 80 children (8-18 years) with cancer. For content validity, the scale was first translated into Turkish and then adapted using Davis' correspondence analysis technique. The construct validity of the scale was assessed using the Barlett test and Kaiser-Mayer Olkin. For the scale's reliability, Cronbach Alpha Reliability Coefficient, Equivalent Forms Method, bisection method and Item Total Score Correlation Coefficient correlation tests were analyzed. Before starting the study, ethics committee approval and written permits from institutions and individuals were obtained.

Results: The content validity index was found between 0.93 and 1. The reliability of the scale of the equivalent form method was r:0.57, and bisection method was r:0.85 and the Cronbach internal consistency coefficient of scale=0.86. The correlation coefficient between the items and the total score was greater than r=0.20. The Kaiser-Meyer-Olkin coefficient was 0.82. The $\chi 2$ value (Bartlett test) was statistically significant (p= .001).

Conclusion: The SSPedi 8-18 is a reliable and valid tool that can be used to assess the symptoms of Turkish children with cancer. Nurses' use of the scale while providing nursing care to children diagnosed with cancer and their families will guide them in objectively determining symptoms, understanding the relationship between symptoms, and managing symptoms.

Keywords: Cancer, children, nurse, symptom, scale, validity

1. INTRODUCTION

Childhood cancer is a growing chronic health problem worldwide. Childhood cancers refer to cancers seen in children aged 0-19 years (1). Childhood cancers consist of 0.5%-4.6% of all cancers. Depending on the developments in cancer treatment in recent years, early diagnosis and appropriate treatment have improved the prognosis of childhood cancers (2,3). While 5-year survival rates were 58% in children diagnosed with cancer in the 1970s, this rate has increased to 80% today (4). However, cancer treatment is a long-term treatment that includes various methods (chemotherapy, radiotherapy, transplants, surgical procedures, targets in cancer therapy, etc.) (5-7). Children could show many physical and psychosocial symptoms related to the diagnosis and treatment of cancer (8,9). The most general physical symptoms in children can be listed as fatigue, loss of comfort, weakness, nausea, pain, constipation, diarrhea, mouth sores, lack of appetite, taste changes, numbness in the hands and feet, and hair loss (7,10,11). Psychosocial symptoms, on the other hand, are feeling sad, anxious, or angry, stress, fear, sleep difficulties, and anxiety (9,12,13).

Symptoms can inhibit the child's physical, emotional development, and psychosocial. They may negatively affect their ability to participate in activities and the quality of life of their families and themselves. Studies show that only one-dimensional study of cancer-related symptoms is evaluated, and they are tried to be managed accordingly (14,15). Initial studies assessing symptoms related to cancer or its treatment usually focus on a single symptom. This has provided a detailed understanding and management of specific symptoms (nausea, vomiting, pain, fatigue, anxiety, difficulty sleeping, etc.) (16).

On the other hand, treatment and care focused on a single symptom could not provide symptom control at the desired level (9). Symptoms that cannot be evaluated and managed effectively may lead to a prolonged hospital stay, interruption

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of treatment, changes in drug doses, protocols, or treatment (17,18). Other symptoms often accompany symptoms and have a synergistic effect (17,19-21). Therefore, identifying symptoms that affect and are affected by each other may lead to a better understanding of the complexity of symptoms and the development of interventions for their management (22).

Symptom management is essential for healthcare outcomes of the children and their families since the continuity of treatment, the life span and quality of the child, morbidity, and mortality are affected by symptoms in children with cancer (23,24). Assessing individual symptoms and their relationship to each other is the first step in symptom management (16). The symptoms experienced by the child should be evaluated objectively and from a holistic perspective (25). The number of adequate measurement tools is limited in the world, which collectively evaluates the symptoms in children followed up with the diagnosis of cancer. There are scales generally used to assess symptoms around the world, such as Advanced Symptom Management System, Memorial Symptom Assessment Scale (MSAS) 7–12, MSAS 10–18, Symptom Distress Scale, Sitaresmi, Therapy-Related Symptom Checklist for Children, Rotterdam Symptom Checklist, SSPedi (8-18), and SSPedi (4-7) (13,26). The Memorial Symptom Assessment Scale (MSAS) 10-18 is a multi-symptom assessment tool with validity and reliability in Turkey. MSAS is a scale that can be used for 10 to 18-year-olds and filled in on paper (27). The Symptom Screening Tool in Pediatric Patients (SSPedi), one of these measurement tools, has been developed by Tomlinson et al. to evaluate the symptoms seen in children aged 8 to 18 years with oncological problems. The tool evaluates 15 symptoms in terms of frequency and severity (11). SSPedi (8-18) can be filled on paper or electronically (13). It is thought that the broad age range and electronic use of SSPedi 8-18 will provide the opportunity to evaluate the child's symptoms 24/7 and in any environment. Considering the place of technology in our lives and the passion of children and young people for technology, it is clear that there is a need for multiple symptom assessment tools that can be used electronically in the assessment of symptoms in children with cancer (28).

2. METHODS

2.1. Aim

This study aimed to conduct the reliability and validity of the Turkish version of the Electronic Symptom Screening Tool in Pediatrics for children aged 8-18 with cancer.

2.2. Design

Linguistic and content validity were tested in this methodologic study.

Linguistic validity was tested using the translation-back translation method for the SSPedi 8-18. First, a native Turkish translator with good English skills translated the tool. Two English-speaking academics independently checked the translated texts of SSPedi. Then, the translator translated the tool into English again.

Content validity was determined by getting feedback from eight experts about the content of the tool. Experts were from the fields of pediatric nursing as the professor (2), assistant professor (4), research assistant in Ph.D. level (1), and pediatric nurse (1). They were asked to evaluate the intelligibility and relevance of each item for measurement using a 4-point rating scale (4=very relevant, 3=quite relevant, 2=somewhat relevant and 1=not relevant) (29). The original version of the tool was compared with its English version by the researchers. After experts' feedback, a pilot study was conducted with ten children aged between 8 and 18.

2.3. Sample

Eighty-three children and their families who met the research criteria were interviewed between September 2020 and June 2021. Three families declined to participate in the study. The sample in our study consists of 80 children (8-18 years) with cancer. The sample of the study consisted of 80 children diagnosed with cancer. For reliability and validity studies, it is recommended that the sample size should be 5-10 times the number of items (30,31). Therefore, in our study, the sample size for the 15-item tool was determined as 80. Inclusion criteria were: a) Cancer diagnosis and follow-up, b) 8-18 years of age, c) Turkish speaking, (d) chemotherapy treatment started (at least five days ago), (e) voluntary participation (children and their parents), and (f) children with an android phone, tablet or computer. Exclusion criteria were: (a) organic brain syndrome, etc., cognitively or visually impaired, (b) diagnosed medically with psychological problems, and (c) end-of-life in children.

2.4. Data Collection

The researcher filled out the descriptive data form with the data obtained from the child, family, and child's hospital records. The children were asked to fill out the MSAS (10-18) scale and an electronic version of the SSPedi 8-18 tool by themselves in their hospital room. The equivalent forms method was used instead of re-test because the symptoms can change instantaneously. MSAS (10-18) is the only valid and reliable multi-symptom assessment tool in Turkey, so this scale was used as an equivalent form. MSAS (10-18) and SSPedi (8-18) were requested to be filled out consecutively in the same time period by the child because symptoms can change quickly. After the child filled out the MSAS written form, the researchers collected a scale from the child. Since the number of items of the scales differed from each other and in order to perform correlation analysis between the scales, the two scales were sent to 7 different expert opinions in order to ensure item compatibility. In this second expert opinion, which was different from the content validity, the compatible items and the compatibility scores for the items were determined. On the other hand, after SSPedi was filled in by the children on their phones or tablets, the data was immediately seen on the results screen of the researchers. Children were informed that if they needed help while filling the scales, they would be helped. During the scales filling process, no request for assistance was required from the children. Each participant completed the scales in approximately 15 minutes.

2.5. Data Instrument

The Descriptive Data Form, MSAS (10-18), and SSPedi (8-18) were used as data instruments.

The Descriptive Data Form consisted of 14 items related to gender, age, child and parent education, child illness and treatment, and was developed by the researchers in accordance with the literature. (diagnosis of the child, age at diagnosis, duration of treatment, etc.) (11,13,32,33). Data about the illness and treatment of the child was checked from the hospital records.

The Memorial Symptom Assessment Scale (MSAS 10-18) was developed by Collins et al. (2000) to assess the frequency, severity, and bother of the symptoms experienced in children aged 8-18 with cancer. There are 30 items on the scale that children are asked to evaluate according to their experiences in the last week. Each item is of 4 or 5 Likert types to measure a symptom's frequency, severity, and discomfort. However, eight symptoms (weight loss, etc.) that are not suitable for frequency questioning were evaluated only in terms of severity and discomfort. In the study of Collins et al., Cronbach's alpha coefficients of the Physiological, Psychological, and General Condition Index sub-dimensions were determined as 0.87, 0.83, and 0.85, respectively (12). The Cronbach's alpha coefficients of the Physiological, Psychological, and General Condition Index sub-dimensions of the Turkish reliability and validity study of the scale by Atay et al. were found to be 0.92, 0.93, and 0.93, respectively (27). The total MSAS score is obtained by averaging the symptom scores of thirty symptoms. The total MSDS score and its sub-dimensions can be a minimum of 0 and a maximum of 4. As the score increases, the symptoms' frequency, severity, and discomfort increase.

Electronic Symptom Screening Tool in Pediatrics (SSPedi 8-18) validity study was made by Dupuis et al. (2018) to evaluate the symptoms experienced in children aged 8-18 years receiving cancer treatment. The tool consists of 15 symptoms that assess the symptoms that the children have experienced today and yesterday. A 5-point Likert scale is used to rate each symptom. The tool has a form that can be filled both on paper and electronically (11,13). In 2018, Dupuis et al. did a validity and reliability study of the electronic form of SSPedi in children aged 8-18 years who had cancer diagnosis follow-up. The validity and reliability of Cronbach's alpha coefficient for the electronic form of the screening tool were found to be 0.88. The tool score range varies between 0 (none) and 60 (worst possible) (13).

2.6. Ethics Consideration

Permission to conduct a reliability and validity study of the electronic SSPedi 8-18 was obtained from Dr. Lillian Sung via email. The study was approved by the Gazi University Ethics Committee (No: E-77082166-604.01.02-251580) of the university and the hospital. Both the children and their parents provided written informed consent.

2.7. Data Analysis

Data were analyzed using SPSS for Windows version 21.0 (SPSS, Chicago, IL, USA) at a significance level of .05. Validity analyses were confidently conducted using content and construct validity. The Davis technique was confidently employed to ensure content validity. The minimum value in the content validity index is taken as 0.70 (34). While calculating the content validity index, the total score of each item was divided by the total number of experts. Kaiser-Meyer-Olkin (KMO) and Bartlett's sphericity tests were used to determine power and sample size, respectively.

Equivalent forms method, bisection method and internal consistency were used in reliability analyses. For the reliability of the tool, Cronbach's alpha reliability coefficient and item-total score correlation coefficient correlation tests were calculated. To determine the consistency of the tool over time, we also tested the equivalent forms method (MSAS 10-18 and SSPedi 8-18) using Spearman's correlation coefficient (total instrument score p< .001). In addition, bisection method used to calculate the reliability coefficient of the whole test.

3. RESULTS

3.1. Descriptive Variables

The descriptive characteristics of children are given in Table 1. The mean ages of children who participated in the study were 12.65 ± 2.90, and the mean treatment duration (month) of children was 15.34±11.96. and the age of diagnosis of children was 11.56±3.90. In the study, 46 (57.5%) were male and 32 (39.9%) were diagnosed with lymphoma. The majority of the children had no relapse (77.5%). Previous treatments of the children participating in the study included chemotherapy, radiotherapy, and surgery (41.25%).

Table 1. *Descriptive characteristics of children (n=80)*

Descriptive properties	Mean ± SD	Min-Max
Age (years)	12.65±2.90	8-18
Treatment duration (month)	15.34±11.96	2-60
Age of diagnosis (month)	11.56±3.90	5-25
	n	**%**
Gender		
Female	34	42.5
Male	46	57.5
Income status*		
Income lower than expenses	47	58.8
Income equal to expenses	29	36.2
Income higher than expenses	4	5.0
Diagnosis		
Leukemia (ALL, AML)	18	22.5
Lymphoma	32	40
Solid tumors	30	37.5
Relapse status		
Yes	18	22.5
No	62	77.5
Previous treatment		
Chemotherapy+radiotherapy+surgery	33	41.25
Chemotherapy+radiotherapy	21	26.25
Chemotherapy+surgery	7	8.75
Chemotherapy	19	23.75

**The income status is as declared by the children.*

3.2. Content Validity

Item content validity index ranged from 0.93 to 1.00, while total scale content validity index was 0.96. Word and verb conjugation changes were made after expert opinions such as "nervous instead of angry." No item was omitted during the content validity process.

3.3. Construct Validity

The Kaiser-Meyer-Olkin coefficient demonstrated a high level of sampling adequacy (KMO=0.825). Additionally, Bartlett's test indicated a statistically significant χ2 value, supporting the suitability of the data for analysis (p< .001). In this study, the eigenvalues between the first and second factors were 5.67 (33.81%) to 1.75 (11.64%) in the exploratory factor analysis.

3.4. Reliability

The Cronbach's alpha value and the correlations between the items and the total score of the SSPedi (8-18) are shown in Table 2. The total item correlations of SSPedi is ranged between 0.15 and 0.72. The Cronbach's alpha coefficient is 0.86 (p< .01). Item-total score matching of the SSPedi (8-18) and MSAS (10-18) scales, which are used as equivalent scales, is given in Table 3. According to correlation tests of SSPedi and MSAS, correlations ranged from 0.17 to 0.67 (Table 4). Other items have a statistically significant correlation, except for items 11 and 12. The item-total score correlation coefficient is above r= 0.20 (p< .01). The correlation (SSPedi and MSAS) results of the relationship between the total scale score and its items are shown in Table 4.

Table 2. *Item Cronbach values and analysis of SSPedi (n = 80)*

		M	SD	Total item correlation (p <.001)	Scale coefficient of reliability if item deleted (p <.001)
Item 1	Feeling disappointed or sad	1.20	0.84	0.55	0.85
Item 2	Feeling scared or worried	1.15	0.88	0.56	0.85
Item 3	Feeling cranky or angry	1.11	0.82	0.43	0.86
Item 4	Problems with thinking or remembering things	0.62	0.80	0.45	0.86
Item 5	Changes in how your body or face look	2.50	0.72	0.67	0.85
Item 6	Feeling tired	2.56	0.82	0.72	0.84
Item 7	Mouth sores	2.22	0.72	0.54	0.85
Item 8	Headache	2.10	1.26	0.67	0.84
Item 9	Hurt or pain (other than headache)	2.20	1.12	0.64	0.85
Item 10	Tingly or numb hands or feet	0.99	0.72	0.44	0.86
Item 11	Throwing up or feeling like you may throw up	2.60	0.66	0.51	0.85
Item 12	Feeling more or less hungry than you usually do	1.89	0.82	0.51	0.85
Item 13	Changes in taste	1.70	0.96	0.63	0.85
Item 14	Constipation (hard to poop)	2.29	1.04	0.15	0.87
Item 15	Diarrhea (watery, runny poop)	1.04	1.16	0.34	0.86
	Total Items	**26,17**	**8.06**	**Total α = 0.86**	

Abbreviations: M = Mean, SD = Standart Deviation

Table 3. *Item matching of the SSPedi and MSAS scales*

Item number	Item text	Item number	Item text	Expert opinion suitability score Total score: 0.95
SSPedi 1	Feeling disappointed or sad	**MSAS 15**	Feeling sadness?	0.93
SSPedi 2	Feeling scared or worried	**MSAS 17**	Worrying?	1
SSPedi 3	Feeling cranky or angry	**MSAS 5**	The feeling of being nervous?	0.93
SSPedi 4	Problems with thinking or remembering things	**MSAS 1**	Difficulty concentrating or paying attention?	0.89
SSPedi 5	Changes in how your body or face look	**MSAS 29**	"I don't look like myself."	0.93
SSPedi 6	Feeling tired	**MSAS 3**	Lack of energy?	1
SSPedi 7	Mouth sores	**MSAS 23**	Mouth sores?	1
SSPedi 8	Headache	**MSAS 2**	Pain	1
SSPedi 9	Hurt or pain (other than headache)	**MSAS 2**	Pain	0.93
SSPedi 10	Tingly or numb hands or feet	**MSAS 9**	Numbness/tingling or pins and needles feeling in hands or feet?	0.96
SSPedi 11	Throwing up or feeling like you may throw up	**MSAS 7** **MSAS 12**	Nausea or feeling like you could vomit? Vomiting or throwing up?	0.96
SSPedi 12	Feeling more or less hungry than you usually do	**MSAS 19**	Lack of appetite or not wanting to eat?	0.93
SSPedi 13	Changes in taste	**MSAS 24**	Change in the way food tastes?	0.96
SSPedi 14	Constipation (hard to poop)	**MSAS 27**	Constipation or uncomportability because bowel movements are less frequent?	0.93
SSPedi 15	Diarrhea (watery, runny poop)	**MSAS 14**	Diarrhea or loose bowel movement?	0.96

Table 4. *Correlation tests (SSPedi and MSAS) results of the relationship between total scale score and its items*

		SSPedi 1	SSPedi 2	SSPedi 3	SSPedi 4	SSPedi 5	SSPedi 6	SSPedi 7	SSPedi 8	SSPedi 9	SSPedi 10	SSPedi 11	SSPedi 12	SSPedi 13	SSPedi 14	SSPedi 15	**TOTAL SSPedi**
MSAS 15	r(p)	.321(,004)															
MSAS 17	r(p)		.257(.021)														
MSAS 5	r(p)			.274(.014)													
MSAS 1	r(p)				.437(.001)												
MSAS 29	r(p)					.243(.030)											
MSAS 3	r(p)						.394(.001)										
MSAS 23	r(p)							.247(.027)									
MSAS 2	r(p)								.409(.001)								
MSAS 2	r(p)									.517(.001)							
MSAS 9	r(p)										.334(.002)						
MSAS 7	r(p)											.017(.879)					
MSAS 12	r(p)											.061(.588)					
MSAS 19	r(p)												.059(.603)				
MSAS 24	r(p)													.343(.002)			
MSAS 27	r(p)														.649(.001)		
MSAS 14	r(p)															.674(.001)	
TOTAL MSAS	r(p)																**.57(.001)**

4. DISCUSSION

Identifying and measuring symptoms in children with cancer can positively improve the prognosis of treatment and care and the child's health (17,22). Evaluating the symptoms individually and using the scales in paper form may cause them not to be assessed in every setting and all the time. Therefore, using multiple assessment tools in the electronic environment is essential in evaluating and managing symptoms (9,13). The SSPedi can be filled in by the child herself/himself electronically; previous data can be easily accessed and evaluated, which can contribute positively to the child's self-care and self-evaluation.

This study determined the reliability and validity of the Turkish version of the Electronic Symptom Screening Tool in Pediatrics for children aged 8-18, which was applied in electronic form by Dupuis et al. to 502 participants (13). The sample in our study consists of 80 children who have been diagnosed with cancer. In the original study by Dupuis et al. the exploratory factor analysis suggested that one factor was appropriate, as the eigenvalues between the first and second factors decreased from 5.23 to 1.34 in the scree plot. Therefore, factor analyses were not performed (13). The eigenvalues between the first and second factors were 5.67 to 1.75 in the exploratory factor analysis in this study. Adequate sampling is indicated if the KMO value is greater than 0.70 (34). The KMO value was 0.825 in this study. For this reason, this study continued as a single factor without performing factor analysis.

If the alpha coefficient, which measures the internal consistency of scale items, is in the range of $0.79 > \alpha > 0.60$, the scale is considered highly reliable, and if $\alpha > 0.80$, the scale is considered very reliable (35). The validity and reliability of the Cronbach alpha coefficient for the electronic form of the screening tool of the original one was found to be 0.88 (13). In our study, Cronbach alpha of the SSPedi 8-18 was 0.86. This value shows that it is quite reliable. SSPedi 8-18 is validated in multiple languages, including Spanish, French, Brazilian, and Australian. Invalidity and reliability studies show that the scale is valid and reliable in different cultures and religions (26,36). The use of valid and reliable tools in symptom assessment can contribute to obtaining objective and evidence-based data. Thus, effectively managed symptoms will have a positive impact on the child's health care outcomes.

Item-total correlations were examined for each item to identify the item distinctiveness of the scale. In this study, the item-total correlation of more than 0.30 was regarded as the criterion for item retention (37,38). In our study, the correlations between the items and the total score ranged from 0.15 to 0.72. In this study, the item-total score correlation value is above 0.30, except in item 14. However, since all items showed a statistically significant correlation ($p< .01$), item 14 was not removed.

"Equivalent Forms Method" was used to determine the reliability coefficient between MSAS 10-18 and SSPedi 8-18. Expert opinions were obtained about the paired items of the scales used as the equivalent form. The consistency of the items of the paired scale sent to 7 experts was between 0.89 and 1, and the total consistency score was determined to be 0.95. Dupuis et al. applied the original scale to 502 participants 1. day and 282 participants 4 days after the first measurement and reported a test-retest correlation coefficient of 0.88 (13). In our study, the correlation coefficient of equivalent forms was 0.57. The correlation coefficient (*r*) shows the relationship between two variables, and values are interpreted as follows: $1.00 > r > 0.90$= very high correlation, $0.89 > r > 0.70$ = strong correlation, and $0.69 > r > 0.50$ = moderate correlation (39). Correlation coefficient effect size (r) ranges from – 1 to +1 (40). There is a moderate and positive linear correlation between SSPedi (8-18) and MSAS (10-18) ($r= 0.57$) ($p< .01$). Therefore, bisection method used to calculate the reliability coefficient of the whole test, this coefficient is modified based on the split form by Spearman-Brown formula. After analysis with this method, the Spearman-Brown coefficient value was found to be 0.85. These results showed that SSPedi 8-18 could be used in cancer-diagnosed children for clinical and research purposes. The SSPedi 8-18 can positively contribute to the field due to children's self-reporting, ease of understanding, wide age range, and electronic use.

4.1. Strengths and Limitations

In Turkey, MSAS (10-18) was only used to assess multiple symptoms. No scale can be used both on paper and electronically in Turkey. All children could easily complete the electronic version of SSPedi 8-18 in this study. The SSPedi 8-18 is the first symptom assessment tool that can be used electronically in Turkey. Tomlinson et al. managed the original scale for children receiving cancer treatment and undergoing hematopoietic stem cell transplant, but we applied it only to children receiving cancer treatment (11). The results of this study cannot be generalized due to its sample size of one hospital setting. Since the retest of the SSPedi could not be performed due to the rapid change of symptoms, the equivalent forms method was used and expert opinion was obtained for the compatibility of similar items.

5. CONCLUSION

The results show that the SSPedi 8-18 is culturally and linguistically adaptable. It is considered to be a valid and reliable tool. The tool could be used to assess the symptoms of children with cancer aged 8-18 in Turkish. The new translation and adaptation version has been named the Electronic SSPedi 8-18-TR. The scale is thought to contribute to the determination and control of symptoms due to its easy use and multiple symptom content.

Electronical SSPedi 8-18-TR can be used everywhere and every time. Nurses' use of the scale while providing nursing care to children diagnosed with cancer and their families will guide them in objectively determining symptoms,

understanding the relationship between symptoms, and managing symptoms. Early diagnosis of symptoms could guide pediatric oncology nurses to plan, practice, and evaluate nursing care. This would enable effective screening and management of symptoms. It would also improve the quality of life of children and their families.

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Clinical and Experimental Health Sciences

Anatomical Variations of the Canalis Sinuosus: A CBCT Study

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ABSTRACT

Objective: Canalis sinuosus (CS) is a bony canal separated from the infraorbital nerve containing the anterior superior alveolar vessel-nerve bundle. This study aimed to assess the anatomical variations of the canalis sinuosus from cone-beam computed tomography (CBCT) images.

Methods: CBCT images of 568 patients (328 females and 240 males; aged between 18 and 81 years old) were evaluated retrospectively. Axial, sagittal, coronal, and cross-sectional images with 0.5 mm slice thicknesses were used to evaluate the presence of CS and associated accessory canal (AC).

Results: Bilateral CS was detected in the entire sample (n=568, 100%). A total of 340 ACs were detected, including at least one AC in 41.9% of the patients. The median value of AC diameter was calculated as 0.89 mm both for females and males. ACs were found in 135 females and in 103 males. One up to five ACs were found per patient. However, the majority of the patients had one AC. ACs were mostly located at tooth region 11 (17.9%) and tooth region 12 (16.4%). Only 59.71% of ACs had a radiographically observed foramen.

Conclusion: In conclusion, all patients had CS and ACs were in nearly half of the patients. Knowledge about these structures aid to correct radiographic diagnosis of these canals and minimize the risk of complications during surgical procedures.

Keywords: Canalis sinuosus, cone-beam computed tomography, maxilla, maxillary nerve, radiology

1. INTRODUCTION

Two-dimensional conventional imaging methods, such as periapical and panoramic radiography, may be insufficient in the diagnosis for because of superposition, distortion, and magnification. Cone-beam computed tomography (CBCT) is one of the important advances in imaging the maxillofacial region (1). CBCT is one of the preferred imaging methods in dentistry because it shows hard tissues well, allows one-to-one measurements, and achieves images with a lower radiation dose compared to medical computed tomography (2).

Surgical operations are commonly performed in the maxillary anterior region (3). Vascular damage causes a risk of bleeding and nerve damage can significantly affect the patient's quality of life due to hyperesthesia, paraesthesia, or pain (4,5). Therefore, the major neurovascular structures and anatomical variations in this region should be well known. One of the anatomical structures that have not been sufficiently investigated in this region is canalis sinuosus (CS).

CS was first described by Frederic Wood Jones in 1939 as a bony canal of approximately 2 mm in diameter, separating from the infraorbital nerve (ION) and running beside the nasal cavity, containing nerve and blood vessels (6). It was named CS because of its double-curved course. CS runs forward and downward in the inferior wall of the orbita, lateral to the infraorbital canal. Afterwards, it passes under the infraorbital foramen and curves medially toward the anterior wall of the maxillary sinus. It then follows the inferior edge of the pyriform aperture and opens lateral to the nasal septum in front of the incisive canal (6). This canal, with a course of approximately 55 mm in the maxilla, contains the anterior superior alveolar nerve (ASAN) and vessels of the same name (7). Although CS is a normal anatomical structure, the accessory canal (AC) continuing in the anterior maxilla are not well known.

CS and AC may not be adequately visualized on conventional radiographs. The superposition of AC on tooth roots may

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mimic periapical lesion (8) and root resorption (9-11), resulting in misdiagnosis. Implants may cause pain due to their association with CS (12-14) and traumatic neuromas may originate from ASAN (15,16). Also, the maxillary bone anterior to the CS is thin, therefore in midface fractures, the integrity of the CS may be compromised, and the ASAN may become more susceptible to injury (17). Therefore, being aware of the course and anatomical variations of CS is particularly important in terms of diagnosis and treatment planning. Thus, we aimed to evaluate the anatomical variations of CS from CBCT images.

2. METHODS

The study was conducted by the principles defined in the Declaration of Helsinki, including all regulations and revisions. Access to the data used was restricted to the principal researcher only. The Ethical Committee of the University approved this work (date: 07.01.2020 number: 91610558-604.01.02-). The CBCT images of patients who applied the Department of Oral and Maxillofacial Radiology between 2015 and 2018 for various dental reasons were evaluated retrospectively.

CBCT scans of the maxilla, including the bilateral maxillary sinuses and the orbital floor up to the lower border of the maxillary alveolar process, with erupted incisors, canines, and premolars were selected. The total sample size was found to be at least 294 according to the power analysis (Critical x^2 = 50.9985, total sample size = 294, actual power = 0.8011).

The exclusion criteria were as follows: underage patients, artifacts preventing the evaluation of anatomical structures, missing tooth, implant and graft, supernumerary/impacted tooth, the presence of lesion in the anterior maxilla, maxillary surgery, syndrome or malformation, and poor quality CBCT scans. A total of 2327 CBCT images, including the maxilla, were examined. 568 CBCT images meeting the inclusion criteria were assessed.

The CBCT images were obtained using a Planmeca Promax 3D Mid (Planmeca, Helsinki, Finland) device with 16.0 × 9.2 cm FOV, 90 kVp, 12 mA, 13.5 sec, 0.4 mm voxel, or 16.0 × 16.3 cm FOV, 90 kVp, 12 mA, 13.5 sec, 0.4 mm voxel. The images were displayed with the original software Romexis 4.6.2.R (Planmeca, Helsinki, Finland) of the CBCT device. All images were analyzed on the same 24-inch medical monitor (Philips, Luchu Hsiang, Taiwan) with an ideal screen display (resolution: 1920 × 1080 pixels) provided with an NVIDIA QUADRO FX 380 graphics card. All examinations in the study were carried out by a researcher in the Department of Oral and Maxillofacial Radiology, in a quiet environment with reduced light, and approximately 50 cm away from the monitor. The brightness and contrast of the images were adjusted with the image manipulation tool in the computer software for optimum visualization. Axial, coronal, and sagittal planes were aligned to ensure standardization before the images were evaluated.

CBCT scanning to identify the CS and associated AC was performed according to the anatomical descriptions stated in the previous literature (3,18,19). Studies on the visibility of canals in the anterior maxilla at different slice thicknesses have shown that optimal visualization is achieved at 0.5 mm and 1 mm slice thickness (20). In this study, axial, sagittal, coronal, and cross-sectional images with 0.5 mm slice thicknesses were used to evaluate the presence of CS and associated ACs. All images were scanned in the inferior direction from the border of the orbital floor to the lower border of the alveolar process, and in the horizontal direction from the midline to the distal of the premolar teeth.

First, the presence of CS was evaluated. The largest diameter measured in the axial sections formed from the origin of the CS to the termination of its course was accepted as the diameter of the CS (19,21). Second, the presence of an AC in the anterior maxilla was examined in patients with CS. Structures suspected to be ACs but smaller than 0.5 mm in diameter were excluded from the evaluation. The AC location was increased by modifying the regions given as reference in De Oliveira-Santos et al. (18). ACs were localized according to adjacent teeth (according to the FDI tooth numbering system) and incisive foramen.

It has been reported that the ASAN shows different variations in its course and number (22-24) thus, the trunk number of CS containing ASAN was evaluated as a single trunk or multiple trunks in coronal, sagittal, and axial sections in CBCT images with a slice thickness of 0.5 mm.

Chi-square analysis was used to examine the differentiation of the two categorical variables. In examining the differentiation of a continuous variable at the level of the categorical variable, the assumption of normal distribution was first evaluated. Unrelated sample t-test was used when the normal distribution was achieved, and the categorical variable level was two. In cases where the normal distribution was not achieved, the Mann-Whitney U test was used when the categorical variable level was two, and the Kruskal–Wallis H test was used when the categorical variable level was more than two. P value was taken as .05.

3. RESULTS

CBCT images belonging to 568 patients (240 males, and 328 females) were assessed. The ages of the participants ranged from 18 to 81 years (mean:42.51, standard deviation±14.43). Ages were grouped as 18-29, 30-39, 40-49, 50-59 and ≥ 60. The number of patients in the age groups was similar in terms of gender ($p > .05$). CS diameter according to age groups is shown in Table 1.

Table 1. *Canalis sinuosus diameter (mm) in age groups*

Age	18-29 Median (Min.-Max.)	30-39 Median (Min.-Max.)	40-49 Median (Min.-Max.)	50-59 Median (Min.-Max.)	≥60 Median (Min.-Max.)
Right	2.00 (1.13-4.33)	2.00 (1.13-4.33)	2.26 (0.89-4.00)	2.15 (1.13-3.96)	2.00 (1.13-4.56)
Left	2.00 (1.13-4.33)	2.15 (1.13-3.42)	2.15 (1.20-4.00)	2.26 (1.13-3.77)	2.02 (0.89-3.69)

**Kruskal Wallis H-Test*
Min: Minimum Max: Maximum

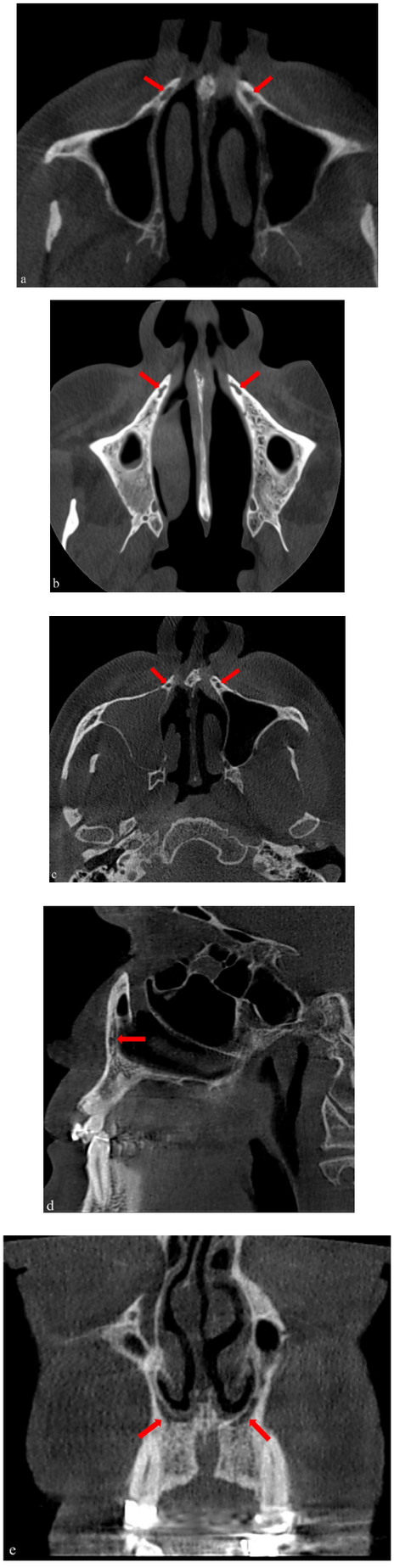

Figure 1. *CBCT (cone-beam computed tomography) images of the CS (Canalis sinuosus) course; a-c) The axial plane, d) The sagittal plane, and e, f) The coronal plane. c, d, and f) CBCT images showing double trunk CS*

Bilateral CS was detected in all patients (Figure 1). 91% of the CS on the right side had a single trunk, while 9% had a double trunk. Similarly, on the left side, 92% of the CS had a single trunk, while 8% had a double trunk. There was no significant difference between the right and left CS diameters of the patients ($p > .05$). When CS diameters were examined in terms of gender, both right and left CS diameters were larger in males than in females ($p < .001$, Table 2).

Table 2. *Canalis sinuosus diameter (mm) and gender*

Gender	Female Median (Min.-Max.)	Male Median (Min.-Max.)	Z*	*p*
Right	2.00 (1.13-3.96)	2.26 (0.89-4.56)	6.199	.000
	Female $\bar{X}$ ±ss	Male $\bar{X}$ ±ss	t**	*p*
Left	2.06±0.49	2.34±0.59	6.033	.000

**Mann Whitney U-Test*
***Independent Sample T-Test*
Min: Minimum Max: Maximum

In total, ACs were found in 238 (41.9%) patients (135 females, 103 males). 182 ACs were present in 135 females and 158 ACs were present in 103 males. A total of 340 ACs were found in the entire sample. One up to five ACs were seen in the patients. The majority of the females and males had one AC. Details are shown in Table 3.

Table 3. *Presence of accessory canals according to gender*

Total accessory canal number	Right (n %)		Left (n %)		Total (n %)	
	Female	Male	Female	Male	Female	Male
1	87 (%26.7)	67 (%27.9)	61 (%18.8)	47 (%19.6)	90 (%27.3)	63 (%26.3)
2	10 (%3)	10 (%4.2)	7 (%2.1)	9 (%3.8)	44 (%13.6)	29 (%12.1)
3	0 (%0)	2 (%0.8)			0 (%0)	8 (%3.3)
4					1 (%0.3)	2 (%0.8)
5					0 (%0)	1 (%0.4)

N: Number

A total of 340 ACs were detected, including at least one AC in 41.9% of the patients (Figure 2). AC diameter was between 0.5-1 mm in 70.9% of the patients and bigger than 1 mm in 29.1% of the patients. The median values of AC diameter in females were calculated as 0.89 mm (range=0.57–1.70 mm) and 0.89 mm (range=0.57–2.00 mm) on the right and left sides, respectively. In males, median values of the AC diameter were calculated as 0.89 mm (range=0.57–2.00 mm) and 0.89 mm (range=0.57–1.27 mm) on the right and left sides, respectively. Both the right and left AC diameters did not show a statistically significant difference according to the gender and age of the patients (p > .05, Table 4).

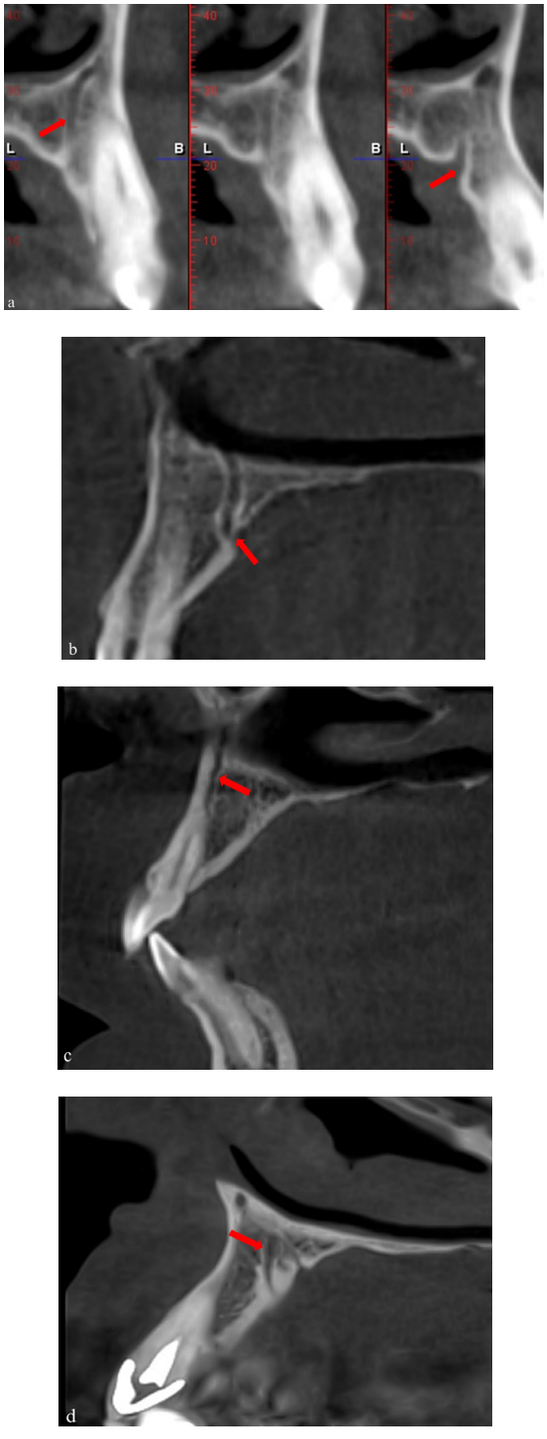

Figure 2. *Cropped CBCT (cone-beam computed tomography) images of different patients showing the course of AC (accessory canal)*

Table 4. *Accessory canal diameter (mm) according to gender and age*

Age/Gender		18-29 Median (Min.-Max.)	30-39 Median (Min.-Max.)	40-49 Median (Min.-Max.)	50-59 Median (Min.-Max.)	≥60 Median (Min.-Max.)	x^2 *(sd = 4)	*p*
Female	Right	0.89 (0.57-1.65)	0.89 (0.69-1.70)	0.80 (0.57-1.60)	0.87 (0.57-1.70)	0.89 (0.80-1.20)	4.332	.363
	Left	0.89 (0.57-1.65)	1.06 (0.57-1.65)	0.89 (0.57-1.26)	0.89 (0.57-2.00)	0.85 (0.57-1.26)	2.004	.735
Male	Right	0.89 (0.57-1.60)	0.89 (0.57-2.00)	0.89 (0.57-1.27)	0.89 (0.57-1.44)	0.89 (0.57-1.34)	.442	.979
	Left	0.80 (0.73-1.26)	0.89 (0.57-1.20)	0.80 (0.80-1.26)	0.89 (0.57-1.27)	0.80 (0.57-1.26)	3.282	.512

**Kruskal Wallis H test*
Min: Minimum Max: Maximum

ACs were mostly located at tooth region 11 (17.9%), tooth region 12 (16.4%), tooth region 21 (13.8%), tooth region 22 (10.9%), and the anterior of the incisive foramen (10.6%). Details are shown in Table 5. All ACs did not have a radiographically observed foramen. 137 AC ended in the alveolar process or near the tooth roots and 203 AC ended with a foramen in the palatal, buccal or connected to the nasopalatine canal (NPC). A foramen was detected only in 59.71% of the ACs. 49.12% of the ACs had a palatal foramen, 1.47% had a buccal foramen, and 9.12% were associated with NPC (Table 6).

Table 5. *Number of the accessory canals according to location in maxilla*

Location*	Number of accessory canals
11	61 (%17.9)
Between 11-12	27 (%7.9)
12	56 (%16.4)
Between 12-13	6 (%1.8)
13	18 (%5.3)
Between 13-14	2 (%0.6)
14	0 (%0.0)
21	47 (%13.8)
Between 21-22	19 (%5.6)
22	37 (%10.9)
Between 22-23	6 (%1.8)
23	14 (%4.1)
Between 23-24	3 (%0.9)
24	1 (%0.3)
Anterior of the incisive foramen	36 (%10.6)
Posterior of the incisive foramen	0 (%0.0)
Lateral of the incisive foramen	7 (%2.1)
Total	340 (%100)

**Teeth are numbered according to the FDI system*

Table 6. *Accessory canals foramen and its location*

Location of accessory canals foramen*	Location of foramen			
	Presence of foramen	Palatal	Buccal	Nasopalatine canal
11	38 (%11.18)	29 (%8.53)	1 (%0.29)	8 (%2.35)
Between 11-12	15 (%4.41)	15 (%4.41)	0 (%0.0)	0 (%0.0)
12	28 (%8.24)	28 (%8.24)	0 (%0.0)	0 (%0.0)
Between 12-13	4 (%1.18)	4 (%1.18)	0 (%0.0)	0 (%0.0)
13	16 (%4.71)	15 (%4.41)	1 (%0.29)	0 (%0.0)
Between 13-14	2 (%0.59)	2 (%0.59)	0 (%0.0)	0 (%0.0)
14	0 (%0.0)	0 (%0.0)	0 (%0.0)	0 (%0.0)
21	22 (%6.47)	17 (%5.00)	0 (%0.0)	5 (%1.47)
Between 21-22	12 (%3.53)	12 (%3.53)	0 (%0.0)	0 (%0.0)
22	24 (%7.06)	24 (%7.06)	0 (%0.0)	0 (%0.0)
Between 22-23	4 (%1.18)	4 (%1.18)	0 (%0.0)	0 (%0.0)
23	13 (%3.82)	12 (%3.53)	1 (%0.29)	0 (%0.0)
Between 23-24	3 (%0.88)	3 (%0.88)	0 (%0.0)	0 (%0.0)
24	1 (%0.29)	1 (%0.29)	0 (%0.0)	0 (%0.0)
Anterior of the incisive foramen	16 (%4.71)	1 (%0.29)	2 (%0.59)	13 (%3.82)
Posterior of the incisive foramen	0 (%0.0)	0 (%0.0)	0 (%0.0)	0 (%0.0)
Lateral of the incisive foramen	5 (%1.47)	0 (%00)	0 (%0.0)	5 (%1.47)
Total	203 (%59.71)	167 (%49.12)	5 (%1.47)	31 (%9.12)

**Teeth are numbered according to the FDI system*

4. DISCUSSION

Periapical and panoramic radiographs, which are frequently used in dentistry, often can't describe, and show structures such as CS in detail (8,25). In particular, with the increase in the use of CBCT, better visualization of bony canals has caused CS to attract attention. There is great variation in the prevalence of CS due to the use of different methodologies in studies. (17,19,23,25-29).

Manhães júnior et al. (25) detected CS in 36.20% of the patients, Aoki et al. (19) detected CS in 66.5% of the patients and Wanzeler et al. (27) detected this anatomic structure in 88% of the patients in CBCT scans. In our study, the entire course of CS after separation from the ION was comprehensively evaluated and the presence of bilateral CS was detected in the entire sample regardless of age and gender. Ghandourah et al. (29), Baena-Caldas et al. (28) and Gurler et al. (26) reported similar findings. In contrast, Aoki et al. (19) and Manhães junior et al. (25) reported a lower prevalence because they evaluated the prevalence of continuing CS only in the maxillary anterior region. In previous cadaver studies, researchers stated that ASAN could be of dual origin (22,23). In a study conducted with panoramic radiographs by Scarfe et al. (24) it was mentioned that the anterior superior dental plexus can be dual and triple. Some studies mention the presence of bifurcation and trifurcation during the course of CS (28). Radiological studies investigating the CS trunks are limited and usually mention the presence of furcation (28). In cadaver studies, the presence of ASAN with multiple trunks has been shown (22,23).

In our study, CS was evaluated in terms of the presence of single and multiple trunks in CBCT images. According to our results, 91% of the right side CS had a single trunk, while 9% had a double trunk. Similarly, on the left side, 92% of the CS showed a single trunk, while 8% showed a double trunk. The fact that the studies by Heasman (22) and Robinson and Wormald (23) were cadaver studies may explain the difference in the CS trunk pattern compared with our study.

As far as we know, there is no clear data describing the CS diameter in the literature. Jones described the CS as a bony canal approximately 2 mm in diameter and mentioned that the ASAN is at least one-third the size of the main trunk (6). Heasman stated that the diameter of the ASAN is between one-half, and one-third of the ION and that the ASAN is a larger structure than both the posterior superior alveolar nerve and the medial superior alveolar nerve (22). Gurler et al. (26) found no statistically significant difference in canal diameter according to age but, significantly higher mean canal diameters in males compared to females. In our study, both right and left CS diameters showed a statistically significant difference according to gender. A larger CS diameter in males may be related to wider anatomical structures in males than in females (26).

Radiographically visible ACs can contain neurovascular structures (3,18,30,31). Various designations have been used in the literature for these canals, including the lateral

incisive canal (32), neurovascular anatomical variations in the anterior palate (18), AC (3), and terminal extension of the CS.

In studies conducted with CBCT, the prevalence of AC in the anterior maxilla was found to be 27.8% by Von Arx et al. (3), 15.7% by De Oliveira-Santos et al. (18), 51.7% by Machado et al. (33), and 32.9% by Temmerman et al. (34). In studies conducted in the Turkish population, the prevalence of AC was found to be 22.3% by Sekerci et al. (35), 70.8% by Orhan et al. (36), 34.66% by Tomrukçu et al. (31), 8.17% by Şalli and Öztürkmen (37), and 35.5% by Alkis et al. (38). In our study AC were observed in 41.9% of the sample. Von Arx et al. (3) used limited CBCT images and evaluated canals with a minimum diameter of 1 mm. Temmerman et al. (34) examined only the canine region in their study. The fact that the area examined in these two studies is more limited than in our study may explain the higher prevalence of ACs in our study. De Oliveira-Santos et al. (18) and Sekerci et al. (35) evaluated patients with an additional foramen of the palate at least 1 mm in diameter. In our study, ACs associated with CS with diameter greater than 0.5 mm were evaluated. This may explain the higher prevalence of AC in our study compared with studies evaluating canals larger than 1 mm in diameter. In addition, the different prevalence seen in the studies may be due to the different methods used by the researchers, different device and imaging features, the variability of voxel sizes and examination areas, and the examination of patient populations with different ethnic origins.

Von Arx et al. (3) found the mean diameter of the AC as 1.31 mm (median=1.23 mm, range=1.01–2.13±0.26 mm) and that gender and age did not significantly affect the diameter. De Oliveira-Santos et al. (18) measured the palatal foramen opening of the canal in the anterior palate and found the mean diameter to be 1.4 mm (range=1–1.9 mm). Machado et al. (33) found that the mean diameter of the AC was 1.19 mm (median=1.15 mm, range=1.00–2.58±0.22 mm). In this study, the relationship between age and the number of ACs, the relationship between age and the diameter of the ACs, and the relationship between the number of ACs and gender were found to be feeble (33). Sekerci et al. (35) found that the mean diameter was 1.12 mm (range=1–1.7±0.26 mm). In these studies, only the diameter of canals with a diameter greater than 1 mm was measured. Tomrukçu et al. (31) found a median diameter of 1.07 mm (range=0.53–2.72±0.35 mm) for AC with a diameter greater than 0.5 mm. In Machado et al. (33), 20% of the ACs was at least 1 mm in diameter. In our study, 70.9% of the ACs had a diameter between 0.5 and 1 mm, while 29.1% had a diameter of 1 mm or more. In our study, there was no statistically significant difference in the diameter of both the right and left AC according to gender and age.

In the study by De Oliveira-Santos et al. (18), most of the ACs were observed in the alveolar process near to the incisor or canine teeth. Sekerci et al. (35) found that ACs were most commonly located on the palatal of the left and right lateral incisors. Tomrukçu et al. (31) found ACs most frequently in the right lateral incisor region. The researchers found that the regions with the lowest number of ACs were the region between teeth 14 and 15 and between teeth 24 and 25 (31). Machado et al. (33) found that the ends of the AC trajectories were most frequently located in the palatal region of the anterior maxillary teeth and less frequently in the buccal and transversal positions. Of the ACs in our study, 49.12% had palatal foramen and 1.47% had buccal foramen. Previous studies have reported that the presence of ACs was associated with NPC (18,35). In our study, 9.12% of the ACs were associated with the NPC.

59.71% of the ACs had a foramen, whereas 40.29% of the ACs terminated in the apical region or around the tooth roots in the alveolar process. In our study, not only canals with palatal openings but also all ACs were associated with CS in the anterior maxilla. The voxel size in this study was 0.4 mm. This voxel size is larger than many studies, which may have limited the visualization of the terminal portions of the ACs. Additionally, most studies have evaluated canals of only 1 mm or larger (3,18,35). ACs with a diameter of less than 1 mm were also evaluated in our study. This may also have allowed the evaluation of ACs smaller than 1 mm in diameter terminating around tooth roots.

Within the CS is the anterior superior alveolar vascular nerve bundle. While cadaver studies focused more on ASAN, radiological studies focused on the prevalence, course, and variations of CS. The limitation of this study was the examination of only CBCT images of the patients.

5. CONCLUSION

In conclusion, all patients had CS on both the right and left side of the cranium. ACs were in nearly half of the patients. Insufficient knowledge about these structures may lead to misdiagnosis and complications during surgical procedures. For this reason, physicians should know the major anatomical structures and the variations.

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Clinical and Experimental Health Sciences

Evaluation of Nutrition, Pressure Ulcer and Fall Risk Status and Related Factors in Individuals Receiving Home Health Care Services

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ABSTRACT

Objective: In this study, the aim is to evaluate the factors associated with nutrition, pressure ulcers, and fall risks in individuals receiving home health care.

Methods: A retrospective study was designed by examining the files of the patients registered in the Home Health Care. Mini Nutritional Assessment Short Form, Modified Norton Scale and Itaki Fall Risk Scale scores were recorded.

Results: The study included 334 patients. 197 (59%) of them were female and the mean age was 74.15±16.12 (min: 18, max: 101). The most common chronic diseases was hypertension (HT) (n=167, 50%), whereas the two most common comorbidities were DM and HT (n=77, 23.1%). Patients receiving home health care were found to be at risk for malnutrition, pressure ulcers and falls. Being semi-bedridden and wheelchair bound were found to be predictors for the risk of developing pressure ulcers according to regression analysis results. Also it was identified that the age and dependency levels of the patients receiving home health care explained 12% of the falling behaviours of the older people.

Conclusion: Necessary warnings should be given at every visit, so that receiving home health care should not be neglected in terms of risks and preventive measures should be taken for this patient group.

Keywords: Home care services, falls, malnutrition, pressure ulcer.

1. INTRODUCTION

Home health care (HHC) services are defined as a service model that ensures examination, treatment, follow-up, and rehabilitation services provided by a professional team in the homes of individuals who cannot maintain their daily care independently. It also provides the necessary emotional and physical assistance to these individuals and their families (1). HHC services support individuals with chronic, malignant, congenital diseases, or those in recovery, relieving the burden on family members and serving as a bridge between the patient and hospital services. It plays a critical role in the management and treatment of common chronic diseases such as hypertension (HT), and diabetes mellitus (DM). Regular monitoring, correct medication use, lifestyle changes, and patient education help health professionals control disease progression and improve patients' quality of life (2).

Home health care services can serve patients of all ages, and the patient population generally consists of adults of advanced age (1). Today, developing technology and increasing treatment options have led to a prolongation of human life and an increase in the proportion of the older adults in the total population. According to the Turkish Statistical Institute, the older people population constituted 9.1% of the total population in 2019 and is predicted to reach 12.9% by 2030 and 22.6% by 2060 (3). This demographic shift is associated with a higher incidence of chronic diseases, disability, and dependency, thereby increasing the demand for health services, including HHC.

Malnutrition is a crucial health problem that causes gradual weight loss, especially with a decrease in lean body mass, as a result of inadequate food intake, may impair physical and/or cognitive functions, and may occur as a result of aging or a disease (4). Since early diagnosis and treatment of malnutrition improves the patients' quality of life, nutritional screening is a critical step in patients at risk (5). Studies on malnutrition in the literature have generally focused on older adults, with prevalence rates varying widely (5,6).

Pressure ulcers are localized tissue damage on the skin and subcutaneous tissues caused by pressure on bony prominence regions or friction. Risk factors include advanced age, being bedridden, malnutrition, and chronic diseases (7). Falls, defined as an unintentional descent to a lower level than the person, can occur at any age and are independent

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of acute events like presyncope, syncope, and seizures (8). Factors increasing fall risk include age, medication, chronic diseases, immobility, and unconsciousness (9). Patients receiving HHC services often have multiple factors increasing their fall risk.

Malnutrition, pressure ulcers and falls may affect mortality and morbidity in patients receiving HHC, but complications can be prevented with early diagnosis or preventive measures. Thus, regular screenings are performed for malnutrition, pressure ulcers and risk of falling in our HHC unit.

To the best of the authors' knowledge, most studies determining the risks associated with malnutrition, pressure ulcers, and falls have focused on healthy older individuals living in the community (10), those attending outpatient clinics (11), those hospitalized in nursing homes (6). Despite the small number of research on nutrition, pressure sores, and the risk of falls in HHC patients, these studies highlighted the high incidence of the concerns described and the importance of comprehensive and protective interventions to reduce these risks (2).

The objective of this research is to look into the prevalence of malnutrition, pressure ulcers, and fall risk in people receiving home health care services, as well as the relationship between these risks. Furthermore, it seeks to assess how chronic diseases and older age influence these health issues. The study's goal is to highlight the need of early detection of these hazards and preventive measures in those receiving home health care services. Therefore, it is planned to test the following hypotheses;

1. Malnutrition percentage, pressure ulcer prevalence and fall risk are high in individuals receiving home health care services.
2. Malnutrition, pressure ulcers and fall risk are interrelated.
3. Chronic diseases and advanced age are the main factors that increase the prevalence of these three health problems.

2. METHODS

2.1. Study Design, Population and Sample

A single-centered, retrospective and descriptive study was designed by examining the files of the patients registered in the HHC unit of the Hassa State Hospital. The files of 556 patients who were assessed in HHC services of Hassa State Hospital between 01/02/2020 and 31/01/2021 were examined.

The files of patients aged 18 years and over, who were followed up by HHC for at least 6 months and whose data that planned to be analysed in the study were complete were included in the study. Due to the different clinical characteristics and care needs of the paediatric patient group, files of patients under 18 years of age were excluded. Furthermore, the files of patients whose need for home health care services lasted less than 6 months were excluded from the study since it was believed that sufficient data could not be obtained during this time and long-term results could not be assessed. Patients with missing data in their patient files were also excluded from the study because the necessary information for analysis could not be obtained. The files of 222 patients who did not match the inclusion criteria were excluded from the study.

Data of 334 patients who met the inclusion criteria were included in the study. Ethics committee approval was obtained from Hatay Mustafa Kemal University with the decision no. 15 on 24/09/2020.

2.2. Data Collection Tools

Data on socio-demographic characteristics, chronic diseases, medical history, state of consciousness, nutritional information, mobility and use of assistive devices (walker, cane, air mattress, etc.) were recorded by retrospective examination of the diagnosis-treatment and follow-up forms prepared by the Ministry of Health for the evaluation of patients receiving HHC.

Mini Nutritional Assessment Short Form (MNA-SF), Modified Norton Scale (MNS) and Itaki Fall Risk Scale (IFRS) scores were recorded.

Although the air mattress is a protective method that prevents the development of pressure ulcers, it has been accepted as an assistive device in the study because it was included in the assistive device class, not as a protective method in the file data.

2.2.1. Mini Nutritional Assessment Short Form (MNA-SF): Mini Nutritional Assessment (MNA**)** was developed by Guigoz in 1994 (12). It is a valid nutritional screening tool for older populations which can be completed in 10-15 minutes. As MNA is used infrequently in some care settings due in part to the time needed to complete it to reduce this short time burden further, Rubenstein and colleagues developed a six question MNA short-form (MNA-SF) by identifying a subset of questions from the full MNA that had high sensitivity, specificity and correlation to the full MNA (13). MNA-SF consists of six questions including anthropometric measurement (body mass index), appetite, weight loss during the last 3 months, mobility, whether the patients are suffering from psychological stress of acute disease in the past 3 months and neuropsychological problems. Each question gets a certain point based on the answer. The points from 12 to 14 are considered as normal nutritional status, from 8 to 11 points as risk of malnutrition and from 0 to 7 as malnourished. The Turkish validity and reliability study of the MNA short form was performed (14).

2.2.2. Modified Norton Scale (MNS): The patients' risk for developing pressure ulcers assessed by modified Norton scale (15) comprising 7 subscales with four items each (mental condition, physical activity, mobility, food intake, fluid intake, incontinence, and general physical condition).

Each component receives a score ranging from 1 to 4, with 1 indicating no function and 4 indicating typical function.

2.2.3. Itaki Fall Risk Scale (IFRS): In 2011, a fall risk scale specific to our country, understandable and easy to apply and to be used in adult patients was developed by Department of Health Productivity, Quality and Accreditation under the Ministry of Health of the Republic of Türkiye (16). There are 11 minor and 8 major risk factors in the scale consisting of a total of 19 risk factors. As minor factors age, state of consciousness (unconsciousness) , history of falling, chronic diseases (hypertension, diabetes mellitus, neurological diseases etc.), urinary-fecal incontinence, visual status, need for physical support while walking, number of medications, use of less than 3 patient care equipment (drain, pacemaker, intravenous infusion, etc.) and physical obstacles in the walking area are evaluated. As major factors state of consciousness (conscious but uncooperative), balance problems while standing/walking, dizziness, orthostatic hypotension, visual impairment, physical disability, use of 3 or more patient care equipment and risky drug use in the last 1 week (antidiabetic, benzodiazepine, anticoagulant, etc.) are questioned. Each minor factor is scored as 1 point and each major factor is scored as 5 points. If the total score is below five, the risk of falling is considered low, and if it is five or more, the risk of falling is considered high (16).

2.3. Statistical Analysis

All statistical analyses were carried out using SPSS statistical software version 25.0. Descriptive statistics of the evaluation results were given as numbers and percentages for categorical variables, and mean, standard deviation, minimum and maximum values for numerical data. Pearson Chi-Square test was used to compare independent groups and to investigate differences between participants; in cases where Pearson Chi-Square test could not be applied, Fisher's Exact Chi-Square test was applied. Statistical relationship between continuous and discrete numerical variables was investigated using Spearman's correlation test. Multivariate logistic regression analysis was performed. The statistical significance of the alpha level was accepted as $p < .05$.

3. RESULTS

3.1. Sociodemographic Data

The study included 334 patients. 197 (59%) of them were female and 137 (41%) of them were male. The mean age was 74.15±16.12 (min: 18, max: 101). Figure 1 shows the age distribution histogram of the patients. The age characteristics of the patients did not follow a normal distribution. Because health and care demands vary with age, the patients were separated into three age groups and examined. According to the results in Table 1 there were 63 patients (18.9%) between the ages of 18-64, 114 people (34.1%) between the ages of 65-79, and 157 people (47%) aged 80 and over. 120 (35.9%) of the patients had previously worked as blue-collar workers. 199 (59.6%) were primary school graduates.

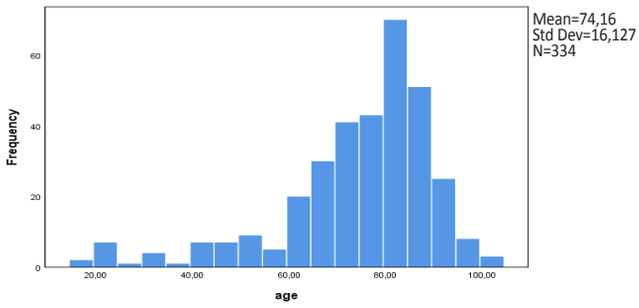

Figure 1. *Histogram graph of age distribution of patients*

Table 1. *Descriptive characteristics of patients in the study*

		n (%)
Gender	Woman	197(59)
	Man	137(41)
Age	18-64	63(18.9)
	65-79	114(34.1)
	80 and above	157(47)
Occupation	Blue Collar	120(35.9)
	None	212(64.1)
Educational Status	Illiterate	199(59.6)
	Below primary school	75(22.5)
	Primary school and above	60(18)
State of consciousness	Conscious	313(93.7)
	Confused /lethargic	14(4.2)
	Mental Retardation	7(2.1)
Bed Dependecy	Bedridden	125(37.4)
	Semi-bedridden	195(58.3)
	Independent	14(4.2)
Assistive device use	None	144(43.1)
	Wheelchair	18(5.4)
	Walker	37(11.1)
	Air mattress	26(7.8)
	Walking stick	81(24.3)
	Other	28(8.3)

3.2. Characteristics of Patients and Their Diagnoses

In this study, 'bedridden' refers to patients confined to bed and unable to perform daily activities independently. 'Semi-bedridden' patients are those with significant mobility limitations who are not entirely confined to bed but require assistance for some activities. These patients can move their upper limbs and support their weight with help from another person or by using walls or furniture.

Independent patients typically do not require any assistive devices for daily activities. Semi bedridden patients require assistive devices such as a walker, walking stick, and wheelchair, whilst bedridden patients require assistive devices such as an air mattress. The goal is to improve the patient's mobility, make it easier to meet everyday demands, and avoid consequences like falls and pressure ulcers (17,18). In the study, patients who did not use assistive devices were categorised as "patients who did not use the device because

they were independent" and "bedridden/semibedridden patients who needed the device but did not use it".

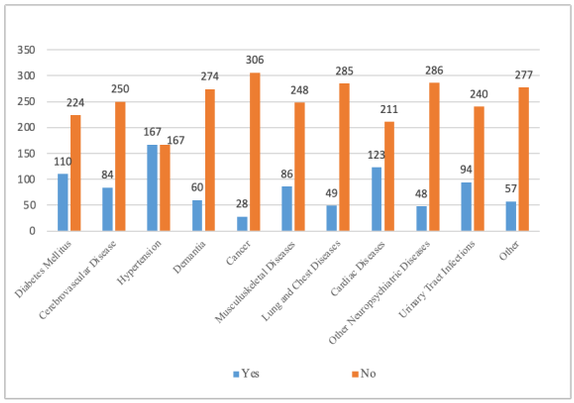

Figure 2. *Chronic disease distribution of the participants*

As shown in Figure 2; the five most common chronic diseases were hypertension (HT) (n=167, 50%), diabetes mellitus (DM) (n=110, 32.9%), cerebrovascular disease (CVD) (n=84, 25%), dementia (n=60, 17.9%), and cancer (n=28, 8.3%). All other diseases are classified according to systems. The most common system disease was cardiovascular system diseases (n=123, 36.8%). The two most common comorbidities were DM and HT (n=77, 23.1%)

3.3. Evaluation of MNA-SF, MNS, IFRS and Related Factors

According to MNA-SF the nutritional status of 112 people (33.6%) was normal, 137 people (41.0%) were at risk of malnutrition, and 85 (25.4%) people were found to be malnourished. 175 people (52.4%) were at risk of developing pressure ulcers with respect to MNS, while 319 people (95.5%) were in the high-risk group for falls according to IFRS.

No statistically significant relationship was found between gender, educational status and occupation and MNA-SF, MNS and IFRS (p< .05).

3.3.1. Assessment of Factors Associated with MNA-SF

A statistically significant relationship was found between MNA-SF and age, DM, HT, dementia, cancer, musculoskeletal diseases (rheumatoid arthritis, hip fractures, muscular dystrophy, etc.), state of consciousness, bedridden status and using assistive devices for mobility (p< .05). Therefore, the risk of malnutrition was found to be higher in those aged between 65 and 79 years, with DM, HT, musculoskeletal diseases and those who were semi-bedridden. Patients diagnosed with dementia, cancer and patients with impaired consciousness (lethargy, confusion, mental retardation) were found to be significantly more malnourished than conscious patients and those without dementia and cancer (Table 2).

Table 2. *Factors associated with MNA-SF, MNS and IFRS*

		MNA-sf n N (%)				**Modified Norton Scale N (%)**			**Itaki Fall Risk Scale N (%)**		
		Normal	Risk of Malnutrition	Malnourished	**p**	At risk	No Risk	p	Low Risk	High Risk	**p**
Age	18-64	24 (38.1)	20 (31.7)	19 (30.2)		30 (47.6)	33 (52.4)		5 (7.9)	58 (92.1)	
	65-79	43 (37.7)	53 (46.5)	18 (15.8)	**.031**	63 (55.3)	51 (44.7)	.621	1 (0.9)	113 (99.1)	.056
	80 and over	45 (28.7)	64 (40.8)	48 (30.6)		82 (52.2)	75 (47.8)		9 (5.7)	148 (94.3)	
Diabetes Mellitus	Yes	40 (36.4)	56 (50.9)	14 (12.7)	**.001**	50 (45.5)	60 (54.5)	.075	2 (1.8)	108 (98.2)	.157[&]
	No	72 (32.1)	81 (36.2)	71 (31.7)		125 (55.8)	99 (44.2)		13 (5.8)	211 (94.2)	
Hypertension	Yes	69 (41.3)	74 (44.3)	24 (14.4)	**.000**	77 (46.1)	90 (53.9)	**.021**	5 (3.0)	162 (97.0)	.187
	No	43 (25.7)	63 (37.7)	61 (36.5)		98 (58.7)	69 (41.3)		10 (6.0)	157 (94.0)	
Cerebrovascular Disease	Yes	21 (25.0)	41 (48.8)	22 (26.2)	.127	58 (69.0)	26 (31.0)	**.000**	3 (3.6)	81 (96.4)	.769[&]
	No	91 (36.4)	96 (38.4)	63 (25.2)		117 (46.8)	133 (53.2)		12 (4.8)	238 (95.2)	
Demantia	Yes	12 (20.0)	23 (38.3)	25 (41.7)	**.003**	33 (55.0)	27 (45.0)	.656	12 (4.4)	262 (95.6)	.738[&]
	No	100 (36.5)	114 (41.6)	60 (21.9)		142 (51.8)	132 (48.2)		3 (5.0)	57 (95.0)	
Cancer	Yes	7 (25.0)	8 (28.6)	13 (46.4)	**.029**	20 (71.4)	8 (28.6)	**.035**	1 (3.6)	27 (96.4)	1.000[&]
	No	105 (34.3)	129 (42.2)	72 (23.5)		155(50.7)	151 (49.3)		14 (4.6)	292 (95.4)	
Musculoskeletal Diseases	Yes	38 (44.2)	36 (41.9)	12 (14.0)	**.007**	37 (43.0)	49 (57.0)	**.043**	4 (4.7)	82 (95.3)	1.000[&]
	No	74 (29.8)	101 (40.7)	73 (29.4)		138 (55.6)	110 (44.4)		11 (4.4)	237 (95.6)	
Disordered Consciousness	Yes	110 (35.1)	131 (41.9)	72 (23.0)	**.000**	159 (50.8)	154 (49.2)	**.024**	15 (4.8)	298 (95.2)	.611[&]
	No	2 (9.5)	6 (28.6)	13 (61.9)		16 (76.2)	5 (23.8)		0 (0.0)	21 (100)	
Bedridden Status	Bedridden	31 (24.8)	48 (38.4)	46 (36.8)		91 (72.8)	34 (27.2)		4 (3.2)	121 (96.8)	
	Semi-bedridden	74 (38.3)	84 (43.5)	35 (18.1)	**.003**	78 (40.4)	115 (59.6)	**,000**	8 (4.1)	185 (95.9)	**.017**
	Independent	7 (43.8)	5 (31.3)	4 (25.0)		6 (37.5)	10 (62.5)		3 (18.8)	13 (81.3)	
Assistive Device Use*	No	37 (27.8)	51 (38.3)	45 (33.8)		90 (67.7)	43 (32.3)		3 (2.3)	130 (97.7)	
	Wheelchair	9 (52.9)	6 (35.3)	2 (11.8)		5 (29.4)	12 (70.6)		1 (5.9)	16 (94.1)	
	Walker	18 (48.6)	16 (43.2)	3 (8.1)		14 (37.8)	23 (62.2)		1 (2.7)	36 (97.3)	
	Air Mattress	4 (15.4)	11(42.3)	11 (42.3)	**,000**	22 (84.6)	4 (15.4)	**,000**	2 (7.7)	24 (92.3)	.763
	Cane/Crutch	34 (42.0)	35 (43.2)	12 (14.8)		33 (40.7)	48 (59.3)		4 (4.9)	77 (95.1)	
	Other	3 (11.5)	14 (53.8)	9 (34.6)		7 (26.9)	19 (73.1)		1 (3.8)	25 (96.2)	

Pearson Chi-Square,&Fisher's Exact Test, Independent patients who did not need assistive devices were excluded during the statistical analysis. (n=320).*

When independent patients who do not need to use any assistive devices are excluded, the risk of malnutrition was found to be significantly higher in patients who did not use any assistive device although they needed it and in patients who used a cane/crutch (Table 2).

As seen in Table 3, a statistically significant positive correlation was found between nutritional condition and risk for pressure ulcers of patients (r=0.437, p< .001). However, no statistically significant correlation was found between nutritional condition and fall risk of patients (r=-0.055, p= .313).

Table 3. *Correlation results for the relationships of MNA-SF, MNS and IFRS*

		MNA-sf	IFRS	MNS
MNA-sf	r	1	-	-
IFRS	r	-0.055	1	-
	p	.313		-
MNS	r	.437*	-.164*	1
	p	< .001	.003	

** Correlation is significant at the level (Spearman correlation test),*
*** Correlation is significant at the level (Spearman correlation test)*

In Table 4, the results of multiple logistic regression analysis between various variables, including being malnourished, high-risk to develop pressure ulcers and high-risk to fall. A significant correlation was found between being malnourished and the variables used in the model, HT (OR=0.42, p= .007), dementia (OR=2.39, p= .016) and cancer (OR=3.47, p= .015). Malnutrition rates were 0.42 times higher in patients with HT than in those without HT, 2.39 times higher in patients with dementia than in those without dementia, and 3.47 times higher in patients with cancer than in those without cancer. It was discovered that the variables in the model described 29.5% of the factors influencing the existence of malnutrition (R^2=0.295). This suggests that the variables in the model play a major role in determining malnutrition, although other factors influencing malnutrition may also exist.

3.3.2. Assessment of Factors Associated with MNS

A statistically significant correlation was found between the risk of developing pressure ulcers and HT, CVD, cancer, musculoskeletal diseases, state of consciousness, bedridden status and using assistive devices for mobility (p< .05). Accordingly, a statistically significant negative correlation was found between HT and musculoskeletal diseases and the risk of developing pressure ulcers.

Risk of developing pressure ulcers was found to be significantly higher in patients with CVD, cancer, disordered consciousness and those who were bedridden and not using any assistive device for mobility (Table 2).

As seen in Table 2, a statistically negative and significant correlation was found between MNS and IFRS (r=-0.164 p= .003).

Table 4. *Multivariate logistic regression analysis results between various variables and malnutrition, pressure ulcer risk and high fall risk*

	MNA-sf (Malnourished)		Pressure Ulcer (At Risk)		Fall Risk (High)	
Variables	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
Diabetes Mellitus	0.51(0.25-1.06)	.073	-	-	-	-
Hypertension	0.42 (0.22-0.79)	.007	0.67 (0.41-1.10)	.120	-	-
Demantia	2.39 (1.18-4.86)	.016	-	-	-	-
Cerebrovascular Disease	-	-	2.10 (1.14-3.87)	.017	-	-
Cancer	3.47 (1.27-9.50)	.015	2.87 (1.09-7.55)	.033	-	-
Musculoskeletal Diseases	0.54 (0.25-1.13)	.104	0.85 (0.49-1.49)	.586	-	-
Consciousness	0.44 (0.15-1.26)	.126	0.59 (0.17-1.98)	.394	-	-
Bedridden Status		.572		.009		.033
Independent	1.00		1.00		1.00	
Semi-Bedridden	2.25 (0.48-10.45)	.298	5.74 (1.51-21.77)	.010	8.68 (1.64-45.93)	.011
Bedridden	1.88 (0.40-8.86)	.422	2.48 (0.65-9.40)	.179	5.56 (1.20-25.79)	.028
Assistive Device		.102		.014		-
None	1.00		1.00		-	
Wheelchair	0.30 (0.06-1.49)	.141	0.18 (0.05-0.56)	.003	-	-
Walker	0.29 (0.07-1.22)	.093	0.61 (0.24-1.50)	.285	-	-
Air Mattress	1.83 (0.65-5.14)	.247	1.48 (0.44-4.98)	.518		
Cane/crutch	0.61 (0.23-1.60)	.324	0.66 (0.32-1.38)	.275	-	-
Other	1.55 (0.54-4.44)	.407	0.293 (0.10-0.80)	.017	-	-
Age		.101		-		.129
18-64	1.00		-	-	1.00	-
65-79	0.54 (0.21-1.34)	.188	-	-	9.40 (1.03-85.08)	.046
80 and over	1.18 (0.52-2.67)	.678	–	-	1.27 (0.38-4.19)	.687
	R^2=0,295 – 2 Log likelihood=304,448		R2=0,248 – 2 Log likelihood=393,547		R^2=0,121 – 2 Log likelihood=109,765	

**p<0.05 is statistically significant*

In Table 3, a significant correlation was found between the risk of developing pressure ulcers according to the MNS score, and the variables used in the model, such as CVD (OR=2.10, p= .017), cancer (OR=2.87, p= .033), bedridden status (OR=5.74, p= .010), and wheelchair bound status (OR=0.18, p= .003). This indicates that the rate of pressure sore development is 2.1 times higher in patients with CVD, 2.87 times higher in cancer patients, 5.74 times higher in semibedridden patients and 0.18 times higher in wheelchair bounds. It was seen that 24.8% of the factors that determine the risk of developing pressure ulcers were explained by the variables in the model (R^2=0.248).

3.3.3. Evaluation of Factors Associated with IFRS

A statistically significant relationship was found between the risk of falling and bedridden status and it was stated that bedridden patients had the highest risk (p< .05) (Table 2).

There was no statistically significant relationship between age classification and fall risk of patients (p = .056). As age is clinically associated with falling (19) and the p value is < .200 – .250 the relationship between age and falling risk was analyzed in logistic regression analysis. In Table 4, being semi-bedridden (OR = 8.68, p = .011), bedridden (OR = 5.56, p = .028), and aged between 65–79 years (OR = 9.40, p = .046) were found to be substantially linked with the variables employed in the model. Being semi-bedridden increases the risk of falls 8.68 times and being bedridden increases the risk of falls 5.56 times compared to independent patients, and being 65-79 years of age increases the risk of falling 9.4 times more than being 18-64 years old. The variables in the model were found to explain 12.1% of the factors indicating high fall risk (R^2=0.121).

4. DISCUSSION

Our study revealed that 41.0% of the patients receiving home health care services were at risk of malnutrition, 52.4% were at risk of developing pressure ulcers, and 95.5% had a high risk for falling. It has been demonstrated that malnutrition, pressure ulcers and risk of falling are common in patients receiving HHC. HT, dementia and cancer increase the risk of being malnourished, cancer and SVO increase the risk of developing pressure ulcers and being bedridden increases the risk of developing pressure ulcers and the risk of falling.

Malnutrition rates in older patients in primary care were reported to be between 2.1% and 11%, with a risk of malnutrition ranging from 5-25% (11, 20). In studies conducted in patients receiving HHC, the malnutrition rate was found to be between 28.8%-52.6% whereas the risk of malnutrition was between 30.2% and 39.3% (21, 22). The prevalence of malnutrition in patients receiving HHC was higher than in the normal population. It can be said that receiving HHC is a risk factor for malnutrition (23). Among the reasons for this, the fact that patients receiving HHC are mostly dependent on daily living activities such as nourishment increases the risk of being malnourished in this patient group (24). In addition, as seen in our study, the fact that the majority of patients receiving HHC is older (mean age 74.15±16.12 years) and the negative effects of old age on nutrition (decreased sense of taste and smell, dental problems, chewing and swallowing difficulties, etc.) may be another reason (5).

The prevalence of malnutrition is increasing with the increase in chronic diseases. In one study, it was found that the most common chronic diseases in patients receiving home health care (HHC) were HT, cardiovascular diseases, DM, and dementia. In addition, the risk of malnutrition increases in the presence of dementia and fracture (22). Diseases such as DM, COPD, atherosclerosis, chronic renal failure, fracture and Alzheimer's disease adversely affect the metabolic and nutritional status and increase the risk of malnutrition by triggering inflammation processes in elderly individuals (25).

The study found that the risk of malnutrition was higher in DM, HT and musculoskeletal-joint diseases, while malnutrition was significantly more prevalent in patients with dementia and cancer. Regression analysis revealed that both HT and cancer are risk factors for malnutrition. Malnutrition is a prevalent complication among cancer patients, particularly those with gastrointestinal malignancies. Cancer cachexia can arise as a result of tumor-induced inflammation, reduced food intake, and metabolic abnormalities caused by cancer (26).

Hypertension can cause heart failure and renal failure, impairing appetite and food absorption. DM causes gastroparesis as well as inflammation, delaying gastric emptying and decreasing food intake. Drugs used in connection with these diseases and side effects related to drugs are other factors that increase the risk of malnutrition (23, 27). These findings show the importance of chronic disease follow-up and side effect management of the drugs used to reduce the risk of malnutrition for patients receiving HHC.

It is known that factors such as age, malnutrition, skin condition, perfusion-related diseases (DM, HT, vascular diseases, edema, etc.), hematological measures (low albumin, anemia, etc.) and skin moisture (urinary, fecal incontinence) predispose to pressure ulcer development (7). Although more than one factor plays a role in the development of pressure ulcers, poor perfusion and immobilization are direct risk factors. Prolonged periods of time lying or sitting, wheelchair use and being a sedentary elderly put the individuals at risk (28).

Multivariate logistic regression analysis in the study showed that the presence of CVD and cancer are predictors of pressure ulcer risk. In a systematic review lack of mobility/activity was found as an independent predictors of pressure ulcer development (29). Patients with a history of CVD constitute the group of immobile patients who tend to stay in the same position for a long periods due to neurological deficits, and therefore, pressure ulcers can be expected (30).

More than half of cancer patients develop pressure ulcers. Malnutrition, anorexia, cachexia, anaemia and decreased

mobility, which occur especially in advanced cancers, are common complications in cancer patients and these conditions increase the risk of pressure ulcers by causing skin disorders (31).

In the study, a relationship was found between musculoskeletal diseases and pressure sores. The risk of pressure ulcers may be increased due to the fact that diseases such as rheumatoid arthritis and hip fractures, which are included in this group, both affect mobility and the effects of drugs such as steroids used in the treatment on the skin (32, 33).

In the study, the risk of developing pressure ulcers increased with the level of bedridden status. Pressure ulcers, also referred to as bedsores in the literature, may have a higher risk of developing in bedridden patients due to the reasons listed above (34). Although the rates of pressure ulcers are higher in bedridden patients, it was not found to be a predictor for the risk of developing pressure ulcers in the study. Being semi-bedridden and wheelchair bound were found to be predictors for the risk of developing pressure ulcers according to regression analysis results.

Semi-bedridden patients are more inclined to sit in the same position due to the loss of pain sensation or the ability to feel pressure due to sitting for a long time. They use their support systems less because of their little mobility, or do not use any protective measures to prevent the risk of pressure in assistive devices such as wheelchairs. The risk of developing pressure ulcers may increase due to such reasons (34).

In the study, all air mattress users consisted of bedridden patients, and the rate of air mattress usage was found to be lower (7.8%) compared to the literature (35). Air mattresses are protective methods developed to reduce the risk of compression, and their use rate is much higher in bedridden patients (35). However, it has been shown in the study that semi-bedridden patients, who are less likely to develop pressure ulcers due to their limited mobility, are also at risk. For this reason, to reduce the risk of PU in semi-dependent patients receiving HHC, support systems should be used for pressure redistribution as they tend to sit in prolonged periods, and patients should be provided with a pressure reducing position at certain intervals and times (36). In recent years, pressure sensors have been developed to reduce pressure ulcers in wheelchair users. Thanks to these sensors, the pressure values in the chair are recorded in the application on the phone or computer so that the person can see it and also the sensors can give a warning when sitting in the same position for a long time is detected (34).

Malnutrition has strong effects on pressure ulcer development and delays wound healing (37). In a study conducted with 2.327 patients over 65 years of age, the relationship between the chronic diseases and malnutrition was evaluated and it was found that the frequency of malnutrition was highest in individuals with pressure ulcers (38). In a study, it was observed that malnourished patients who were treated in the hospital had approximately 2.5 times higher risk of developing pressure ulcers than those who did not (39).

In the study, a strong and positive correlation was found between MNA-SF and MNS scores, indicating that a high risk in one measure tends to coincide with a high risk in the other. However, it is important to emphasize that correlation does not imply causation. Another study found that malnutrition screening can also be used as a predictor for pressure ulcer risk in HHC patients. It was observed that a score below 8 on the MNA test predicted the development of pressure ulcers more strongly than the Braden scale (40). Nevertheless, these findings should be interpreted with caution, and additional studies should explore whether malnutrition directly causes an increased risk of pressure ulcers or if other confounding factors may be at play.

Falls are a significant public health issue because they are common in adults with advanced age, have high death and morbidity rates, impose an economic burden, decrease the older people's quality of life, and generate psychological impacts such as dread of falling in the future (41). Studies have found that factors such as ageing and decreased mobility with age, the presence of chronic diseases such as cancer or hypertension, the number of medications used and their side effects such as dizziness and impaired balance, impaired functionality or the need for home care are significantly associated with the risk of falls (42, 43).

Malnutrition may increase the risk of falls due to its negative effects on muscle strength and balance and through comorbidities such as sarcopenia and frailty (44, 45). Studies have shown that malnutrition increases the likelihood of a future fall (45).

In a study conducted by Adly et al. in hospitalised patients aged 60 years and older, the relationship between MNA-SF scores and various fall scales was evaluated. In the study, malnutrition was shown to be associated with a high risk of falls, but no relationship was found between nutritional status and some fall risk tools such as Schmid-FRAT. This was attributed to the low prevalence of the Schmid-FRAT and that it may not reflect the relationship between nutritional status and fall risk in certain populations (46). Similarly, the lack of a significant relationship between MNA-SF and IFRS may be due to the inadequacy of IFRS in establishing a relationship with nutritional status. This situation is important in terms of showing that different fall risk tools may differ in their relationship with nutritional status and that each tool may not be equally valid in every population.

In a retrospective study in which 1692 patients aged 65 years and older who received HHC were retrospectively analysed, the fall risk was found to be 63.7% and the fall rates were found to be 66.3% between the ages of 65-74 years, 73.6% between the ages of 75-84 years and 75% between the ages of 85-94 years (47). According to the regression analysis performed in the study, being between the ages of 65 and 79 was found to be a predictor of high fall risk. This finding shows the necessity of fall risk assessment and strategies to reduce the risk of falls especially in this age group.

Our study revealed that the risk of falling was found to be higher in bedridden patients than in semi-bedridden and independent patients. According to the regression analysis, both being bedridden and semi-bedridden were found to be predictors of falling and being semi-bedridden has a higher predictive value in determining the risk of falling. In Japan, it has been shown that the dependency status of patients determined by bedridden rank is very useful in predicting in-hospital fall risks. Accordingly, the highest predictive value for falls was found to be in chair-bound patients who were semi-bedridden, followed by housebound and finally bedridden patients who were fully dependent (48).

Another study discovered that older adults with gait problems and concomitant conditions had a higher risk of falling and a better sense and knowledge of the risk of falling than healthy senior patients. Being aware of their conditions and being aware that they are at risk of falling may lead to them adopting fall prevention behaviours and reducing the frequency of their falls (43). However, there are also studies in which a decrease in mobility increases the level of dependency and an increased level of dependency is associated with an increased risk of falling (49).

When the studies studying the factors related with the risk of falling in the literature are analyzed, it is found that, in contrast to our study, the investigations were usually conducted in healthy older persons (10, 11, 21). However our study identified that the age and dependency levels of the patients receiving HHC explained 12% of the falling behaviors of the older people. When the literature was searched, there were no studies examining the relationship between bedridden status and fall risks in home care patients. Comprehensive and predictive studies on this subject are needed because bed dependency and age have a 12% effect on the risk of falling in patients receiving HHC.

5. CONCLUSION

In our study patients receiving HHC were found to be at risk for malnutrition, pressure ulcers and falls. Although it is very difficult to explain the cause of the three complications with a single cause, it was found that the presence of chronic diseases such as HT, dementia and cancer was associated with the development of malnutrition in patients receiving HHC, and the risk of pressure ulcers was high in an individual with nutritional deficiency. The presence of chronic diseases such as CVD, cancer and using a wheelchair were found to be associated with the development of pressure sores.

Although a significant difference was observed between dependency level and the risk of pressure ulcers and falls, one of the most important results of our study was that being semi-bedridden was also shown to be a risk factor for developing pressure ulcers and falling. Necessary warnings should be given at every visit, so that semi-bedridden patients receiving HHC should not be neglected in terms of risks and preventive measures should be taken for this patient group.

The limitations of the study are that it was carried out retrospectively and included patients in a specific region. At the same time, no scale was used while evaluating the dependency levels of the patients and it was determined by the clinician's decision during the patient examination. In future studies, the use of scoring systems evaluating the addiction levels of patients may minimise the potential for clinician bias. In addition, conducting prospective studies with a larger patient population may facilitate the establishment of a causal relationship.

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Clinical and Experimental Health Sciences

The Mediating Role of Resilience in the Relationship Between Self-Efficacy and Demographic Variables in Parents of Children with Cystic Fibrosis

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ABSTRACT

Objective: In genetic diseases such as Cystic Fibrosis, parental self-efficacy is an important variable that increases the child's compliance with treatment. The aim of this study is to determine the mediating role of resilience in the relationship between self-efficacy and demographic variables in parents of children with Cystic Fibrosis.

Methods: The participants of the research were 269 children with Cystic Fibrosis and their parents living in Turkey. Parents were reached via WhatsApp communication tools of the Cystic Fibrosis Association (KIFDER). The data of the research were gathered online between September 1 and December 20, 2020. Child with Cystic Fibrosis and Parent Identification Form, General Self-Efficacy Scale and Resilience Scale for Adults were used as data collection tools. Descriptive statistics and linear regression were utilized to analyze the data, while Hayes' PROCESS was utilized to analyze the mediating role.

Results: Demographic variables that predict parents' self-efficacy; It was determined as the number of children, maternal income level, gender and the need for information about Cystic Fibrosis. Parents' mean self-efficacy score was 30.00±.62. Self-perception (β=.252,SE=.054,95%CI [0.144-0.360], p<.01) and future perception sub-scales (β=.147,SE=.043,95%CI) of the resilience scale [0.061-0.233, p<.01) had an indirect mediating effect on parental self-efficacy.

Conclusion: In parents of children with Cystic Fibrosis, parental self-efficacy is negatively affected by the increase in the number of children they have. Another variable in increasing parental self-efficacy is increasing parental psychological resilience.

Keyword: Self-Efficacy, Parents, Children, Cystic Fibrosis, Resilience

1. INTRODUCTION

Cystic fibrosis (CF) is a genetically inherited chronic disease with a high mortality rate that occurs because of mutation in the gene encoding the cystic fibrosis transmembrane regulator (CFTR) protein and affects multiple organs and glands (1). Before screening programs, cystic fibrosis was mostly diagnosed with recurrent lung infections that did not respond to treatment. In the last 10 years, the first diagnosis is made with heel prick blood in newborn screening programs as CF symptoms are observed (2). While the life expectancy of infants with Cystic Fibrosis (CF) has considerably improved thanks to recent advancements in therapy (3), daily routine treatments have become more complex, burdensome and time-consuming (4). In particular, the maintenance of the airway and drug management are stress sources for parents (5). When parents are not given assistance for stress management, the disease is harder to control, and psychosocial issues may develop (6). The quality of life for children with CF is adversely impacted by the psychosocial issues of their parents (7). Studies on the quality of life of children with persistent illnesses highlight the link between parental self-efficacy and the effectiveness of therapy (8-9). Parental self-efficacy plays an important role in childcare, health management and activities of daily living in children with chronic diseases such as cystic fibrosis (10-11).

Self-efficacy has positive effects on self-management of disease in parents who are primarily responsible for the care and treatment of the child in chronic diseases (10). It has been suggested that raising parents' self-efficacy

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is a successful strategy for lessening the intensity of the illness and enhancement the quality of life of children (12). Home attendance protocol in cystic fibrosis makes the child dependent on parents for treatment (13). Demographic variables affecting parental self-efficacy of children with chronic diseases are reported as age, education, economic status and number of children (14). In the literature, there are conflicting studies on the variables affecting parents' self-efficacy levels. (15-16). For this reason, there is a need to determine the relationship between parents' self-efficacy and the variables affecting it as it will guide the planning of nursing care.

Planning strategies to boost parents' self-efficacy in the delivery of care is advised for practical uses for pediatric nurses (17). Results of a systematic review dealing with parental self-efficacy report that parental self-efficacy is affected by modifiable factors such as the number of children, education, work situation and number of births. Although the importance of increasing self-efficacy in parents of children with CF was emphasized in the same study, it was noted that there was little evidence on how to increase it and what it is affected by (16).

The level of resilience is another important factor in coping with the difficulties faced by parents during chronic diseases such as CF that are life-threatening and involve numerous uncertainties (18-19). Resilience is defined as the capacity of individuals to cope with stressful and traumatic events and the ability to adapt positively to changing challenging factors (20). While resilience allows parents to effectively utilize their assets (21), Additionally, it lessens the caregiving burden on parents, helps children grow positive emotions, and improves their quality of life (22). By enhancing parents' coping mechanisms and problem-solving abilities, resilience also boosts the clinical decision-making and therapy in the context of chronic illnesses (23).

It is important to understand the mediating effect of resilience on demographic variables affecting self-efficacy in parents of children with Cystic Fibrosis. Because self-efficacy affects how these parents can provide better care for their children and how they can maintain their own quality of life. In the literature, it is reported that parents with high psychological resilience can cope with stress more effectively. It is reported that they develop multiple coping strategies and are associated with effective use of social support networks. The aim of this study is to determine the mediating role of resilience in the relationship between self-efficacy and demographic variables in parents of children with Cystic Fibrosis.

H1 Demographic variables influence parental self-efficacy.

H2 Psychological resilience variable significantly mediates the relationship between parental self-efficacy and demographic variables.

2. METHODS

The aim of this research was to create a model to dentification the mediating role of demographic parameters affecting parental self-efficacy and parental psychological resilience. It is reported that parental self-efficacy is affected by variables such as number of children, child age, child gender, parent education, age, gender, income level (16). From this perspective, all variables in this study's regression analysis will be included in the model, and the variables with a meaningful association will be assessed individually in the model. Secondary aim of the study can be expressed as is to verify the connection among the demographic characteristics, self-efficacy and psychological resilience of parental with a child with CF.

2.1. Sample

The data of the research were gathered through communication with parents between April 1st and July 31st, 2022, via WhatsApp accounts created online by KIFDER (Cystic Fibrosis Association). The population of the study consisted of 750 parents registered to KIFDER WhatsApp groups. The population of the study consisted of 750 parents registered to KIFDER WhatsApp groups. The sample calculation method for groups with known population was used to determine the sample size of the study. According to Yazıcıoğlu and Erdoğan (2004), with a sampling error of 0.05, probabilities of $p = 0.5$, $q = 0.5$ and confidence interval $\alpha = 0.05$, at least 254 parents should be sampled in a population of 750 parents (24). The study was completed with 269 parents who met the inclusion criteria and voluntarily agreed to participate in the study. The population of the study consisted of 750 parents registered to KIFDER WahtsApp groups.

2.2. Data Collection Tools

The data of the study were collected by using (1) the *Child with CF and Parent Identification Form*", (2) *"General Self-Efficacy Scale"* and (3) "*Resilience Scale for Adults.*

The Child with CF and Parent Identification Form

The Identification Form for Child with CF and Parent have collected data about the age, gender, education level, working status, income level of parents, place of residence, number of children, number of children with CF, educational needs related to the disease of child and parent (10-14).

General Self-Efficacy Scale

General Self-Efficacy Scale' developed by Schwarzer & Jerusalem (1995) determines the general self-efficacy of parents. The self-efficacy level of Parents was collected with the *General Self-Efficacy Scale* (25). The scale has 10 items and is scored on a 4-point Likert scale. The scale score was accepted as the total score of the scale. Scores between 10 and 40 are obtained from the scale and high scores are accepted as high self-efficacy perception. The Turkish validity and reliability study of the scale was carried out by Aypay

(2010) and alpha coefficient was determined as 0.83 (26). In this study, the Cronbach's alpha reliability coefficient of the *General Self-Efficacy Scale* was found as .90.

Resilience Scale for Adults

The level of resilience in parents was determined by using the *Resilience Scale for Adults* The Resilience Scale for Adults was developed by Friborg et al. (2009). (27). The scale consists of 6 sub-scales as follows: Structured Style, Planned Future, Family Cohesion, Perception of Self, Social Competence, and Social Resources. In the scale, structural style (3,9,15,21) and future perception (2,8,14,20) are measured with 4 items each; family cohesion (5,11,17,23,26,32), self-perception (1,7,13,19,28,31) and social competence (4,10,16,22,25,29) with 6 items each; and social resources (6,12,18,24,27,30,33) with 7 items. There are 33 questions in total. Questions 1-3-4-8-11-12-13-14-14-15-16-23-24-25-27-27-31-33 will be reverse questions. If it is desired that psychological resilience increases as the scores increase, the answer boxes should be evaluated as 12345 from left to right. In this research, it was scored as 12345 from left to right. The minimum score in the questionnaire is 33 and the maximum score is 165. The Turkish validity and reliability study of the scale was carried out by Basım & Çetin (2011), and the alpha coefficient was determined as 0.86 (28). In this study, the Cronbach's alpha reliability coefficient of the Resilience Scale for Adults was found as .89.

2.3. Data Collection

After the research ethics committee permission was obtained, the research procedure and purpose were explained to the parents in an online meeting organized through KIFDER. After the first information meeting, parents were sent a research invitation and information text via WhatsApp application. Parents who responded to the invitation were informed about the study by the researchers via WhatsApp application. It was made clear that participation in the study was completely voluntary and that participants could withdraw from the study at any time. Parents who met the inclusion criteria and voluntarily accepted to participate in the study were contacted. Pre-interviews were conducted with 5 parents to test the applicability of the data collection forms and these parents were not included in the sample of the study. Interview times were arranged according to the convenience of the parents. Each interview lasted on average 20-25 minutes. During the interview, the 'Child with CF and Parent Identification Form', 'General Self-Efficacy Scale' and 'Resilience Scale for Adults' prepared through the Google Form application were sent to the parents and they were asked to fill them in. The responses were recorded. Information messages continued to be sent until the sample size was reached.

2.4. Data Analysis

Number, percentage, mean and standard deviation are given for the descriptive statistics of the study data. The conformity to normal distribution of numerical data was evaluated by Skewness (between – .56 and – .25) and Kurtosis (between – .70 and .24) test and data was determined to have a normal distribution. Generalized linear models and backward multiple linear regression analysis were used to identify potential demographic variables that might influence scores on the General Self-Efficacy Scale. The significance level was accepted as $p<.05$. General Self-Efficacy Scale scores of parents in the primary analyzes were evaluated with PROCES method was used to determine the mediator effect of resilience on the relationship between parental self-efficacy and demographic variables. PROCESS is a macro extension of the SPSS program, which is used in moderation, mediation and conditional process analysis (29). The SPSS macro-PROCESS model 4 was used in this research. PROCESS uses the regression-based or ordinary least squares approach to evaluate moderation trends.

2.5. Ethical Aspect of the Study

Approval for the research was given by the Ethics Committee of Necmettin Erbakan University Health Sciences Scientific Research (meeting and Decision Number: 05 dated 05.07.2020). Before starting the data gathered process, the parents were informed about the aim and context of the investigation and their online consent was obtained, indicating that they agreed to participate in the research.

The limitation of the study is that the data are collected online. The fact that the parents of children with CF who are WhatsApp users participated in the research constitutes our first limitation in terms of generalization. Secondly, the mediator effect of resilience on self-efficacy was examined in this study. Further evidence is needed to explain the relationship between parental self-efficacy and resilience, considering all variables affecting parental self-efficacy.

3. RESULTS

3.1. Descriptive Characteristics of Participants

In the research, 50.9% of children with CF were girls, 31.6% were between the ages of 7 and 12, 40.5% had received preschool education, and 36.8% had a teacher coming to the home for education. 79.9% of the parents who answered the questionnaire were mothers. 53.9% of the mothers were between the ages of 31-40, 41.3% had primary education and 82.9% were not working. Demographic characteristics of the children with CF and their parents who participated in the study are given in Table 1.

When the General Self-Efficacy Scale (GSE) scores of the parents of the CF children were investigated, the mean general self-efficacy score of the parents was 30.00±62, and the mean adult resilience score was 37.33±.62 (Table 1).

Table 1. *Descriptive Characteristics of Children with Cystic Fibrosis and Their Parents (n= 269).*

Descriptive Characteristics	n	%
Gender of the child		
Female	137	50.9
Male	132	49.1
School Attended by Child		
Preschool / Kindergarten	109	40.5
Primary school	64	23.8
Middle school	50	18.6
High school	42	15.6
University	4	1.5
Type of education		
The teacher is coming home	99	36.8
Going to school	170	63.2
Age Group of Children		
1-3 age group	55	20.4
4-6 age group	65	24.2
7-12 age group	85	31.6
13-18 age group	64	23.8
Questionnaire Respondent		
Mother	215	79.9
Father	54	20.1
Mother Age Group		
21-30 age group	60	22.3
31-40 age group	145	53.9
41-50 age group	60	22.3
51-60 age group	4	1.5
Father Age Group		
21-30 age group	15	5.6
31-40 age group	148	55.0
41-50 age group	87	32.3
51-60 age group	19	7.1
Education Level of Mother		
Illiterate	5	1.9
Primary education	111	41.3
High school	87	32.3
University	66	24.5
Education Level of Father		
Primary education	95	35.3
High school	91	33.8
University	83	30.9
Working Status of Mother		
Not working	223	82.9
Working	46	17.1
Working Status of Father		
Not working	26	9.7
Working	243	90.3

Table 1. Continues: *Descriptive Characteristics of Children with Cystic Fibrosis and Their Parents (n= 269).*

Descriptive Characteristics	n	%
Number of Children		
1 child	50	18.6
2 children	118	43.9
3 children	77	28.6
4 children and more	24	8.9
Number of Children with Cystic Fibrosis		
1 child	240	89.2
2 children	25	9.3
3 children	4	1.5
Social Security of the Family		
Yes	236	87.7
No	33	12.3
Income-Expenditure Status of the Family		
My income is less than my expenses	110	40.9
My income is equal to my expenses	125	46.5
My income is more than my expenses	34	12.6
Place of Family Living		
Provincial center	180	66.9
District center	70	26.0
Town/village	19	7.1
Lack of Knowledge / Need for Information about Cystic Fibrosis		
Yes	226	84.0
No	43	16.0
Scales	**Min-Max**	**$\overline{X}$ ±SD**
Self-Efficacy Total	11.00-40.00	30.00±.62
Resilience Total	18.20-50.00	37.33±.62

3.2. Change in Parent Self-Efficacy Scale Scores According to Demographic Variables

Model 1 and Model 2 examined the relationship between demographic variables and self-efficacy of parents with children with CF in Table 2. The findings of Model 1 are presented below. Gender was determined as an effective variable on self-efficacy scores ($p<.05$). Parents of girls had 2.382 points higher self-efficacy scores than those of boys. The number of children affected self-efficacy scores ($p<.05$). The self-efficacy scores of parents with one or two children were 1.898 points higher than those of parents with three or more children. Income was determined as an effective variable on self-efficacy ($p<.05$). The self-efficacy scores of parents with negative income were – 2.072 points lower than those of parents with neutral or positive income. The need for information about CF was found to be an effective variable on parental efficacy ($p<.05$). Parents who did not need information about CF had 2.385 points higher self-efficacy scores than parents who needed information. The child's education level, age, type of parent, age, education level, employment status, number of children with CF, presence of social security, and place of residence did not affect self-efficacy scores ($p>.05$) (Table 2).

Model 2 was reanalyzed by removing statistically insignificant variables. Gender was determined as an effective variable on self-efficacy scores (p<.05). The self-efficacy score of the parents of girls was 2,640 points higher than that of the parents of boys. The number of children was found to be an effective variable on self-efficacy (p<.05). The self-efficacy scores of parents with one or two children were 1.640 points higher than those of parents with three or more children. Income affected self-efficacy (p<.05). The self-efficacy scores of parents with negative income were 1.920 points higher than those of parents with neutral or positive income. The need for information about CF was an influential variable on parental efficacy (p<.05) Parents who did not need information about CF had 2.480 points higher self-efficacy scores than parents who needed information (Table 2).

Table 2. *Comparison of Generalized Self-Efficacy Scale Mean Scores According to Sociodemographic Characteristics of Children with Cystic Fibrosis and Their Parents According to Generalized Linear Models (n= 269)*

Descriptive Characteristics	Model 1					Model 2				
			95% Wald Confidence Interval					95% Wald Confidence Interval		
	B	Std. Error	Lower	Upper	p	B	Std. Error	Lower	Upper	p
(Intercept)	27.872	2.4888	22.994	32.750	**.000**	26.060	0.790	24.510	27.620	**.000**
Gender of the child										
Female	2.382	.7363	.939	3.825	**.001**	2.64	0.720	1.230	4.060	**.000**
Male	ref	.	.	.	.	Ref	.	.	.	.
School Attended by Child										
≤ Primary school	-3.141	1.6502	-6.375	.094	.057					
≥ High School	ref	.	.	.	.					
Number of Children										
1-2 child	1.898	.8724	.188	3.608	**.030**	1.64	.750	.016	31.200	**.030**
≥ 3 children and more	Ref	.	.	.	.	Ref	.	.	.	.
Income-Expenditure Status of the Family										
My income is less than my expenses	-2.072	.7681	-3.577	-.566	**.007**	1.920	.740	0.046	.337	**.010**
My income is equal to/more than my expenses	Ref	.	.	.	.	Ref	.	.	.	.
Lack of Knowledge / Need for Information on Cystic Fibrosis										
I don't need information	2.385	.9879	.449	4.322	**.016**	2.480	.980	.055	4.420	**.012**
I need information	Ref	.	.	.	.	Ref				
Age Group of Children										
1-3 age group	-.774	1.6860	-4.079	2.530	.646					
4-6 age group	1.940	1.5886	-1.174	5.054	.222					
7-12 age group	.851	1.5116	-2.112	3.813	.574					
13-18 age group	ref	.	.	.	.					
Questionnaire Respondent										
Mother	1.364	.8992	-.398	3.127	.129					
Father	ref	.	.	.	.					
Mother Age Group										
21-30 age group	.811	1.4891	-2.108	3.729	.586					
31-40 age group	1.173	1.0341	-.853	3.200	.257					
≥ 41age	ref	.	.	.	.					
Father Age Group										
21-30 age group	1.104	2.0222	-2.859	5.068	.585					
31-40 age group	-.008	.9645	-1.898	1.882	.993					
≥ 41age	ref	.	.	.	.					
Education Level of Mother										
≤ Primary education	-.604	.9574	-2.480	1.272	.528					
≥ High school	ref	.	.	.	.					
Education Level of Father										
Primary education	-.026	.9597	-1.907	1.855	.978					
≥ High school	ref	.	.	.	.					

Table 2. Continues: *Comparison of Generalized Self-Efficacy Scale Mean Scores According to Sociodemographic Characteristics of Children with Cystic Fibrosis and Their Parents According to Generalized Linear Models (n= 269)*

Descriptive Characteristics	Model 1					Model 2				
			95% Wald Confidence Interval					95% Wald Confidence Interval		p
	B	Std. Error	Lower	Upper	p	B	Std. Error	Lower	Upper	
Working Status of Mother										
Not working	1.241	1.0036	-.726	3.208	.216					
working	ref	.	.	.	.					
Working Status of Father										
Not working	-.551	1.3495	-3.196	2.094	.683					
working	ref	.	.	.	.					
Type of education										
The teacher is coming home	.492	.7824	-1.041	2.026	.529					
Going to school	ref	.	.	.	.					
Number of Children with Cystic Fibrosis										
1 child	.280	1.2340	-2.138	2.699	.820					
2-3 children	Ref	.	.	.	.					
Social Security of the Family										
Yes	-1.511	1.2224	-3.907	.885	.216					
No	Ref	.	.	.	.					
Family Living Place										
Provincial center	.283	1.4497	-2.558	3.124	.845					
District center	.904	1.5776	-2.188	3.996	.567					
Town/village	Ref	.	.	.	.					

Multiple regression analysis (backward method) was performed to evaluate the effect of the independent variables on the GSE scores of the parents. There is a strong correlation among the total points of the Adult Resilience Scale and the subscales of the scale (r: .68 to .84, p<.001) Tolerance for total score: .000, VIF: – could not be calculated). Therefore, in the regression analyses, the total resilience score was not included in the model due to autocorrelation. The subscale scores were included in the model. According to the correlation analysis and Collinearity statistics, no high level of autocorrelation was found among the remaining independent variables included in the regression model.

Of the independent variables included in the regression model, six independent variables, namely income-expenditure status, the structural style, social resources, family cohesion, social competence sub-scale scores of the Adult Psychological Resilience Scale, and the need to be informed about CF and its score, were excluded from the regression model respectively because they did not have sufficient effect on the total score of general self-efficacy (p>.05).

The order of importance (from the most important to the least important) of the five variables that had a significant effect on the General Self-Efficacy Scale score of the parents who remained in the model and had a child with CF, is as follows: total points for self-perception sub-scale of the Adult Resilience Scale (p<.001) and planned future sub-scale, gender of the child with cystic fibrosis (p<.01), educational status of the child and number of children in the family (p<.05) (Table 3). A 1-point increase in The Resilience Scale for Adults self-perception sub-scale increases parents' general self-efficacy scores by .24 points and a 1-point increase in the planned future sub-scale score by .15 points. The general self-efficacy score of parents whose children with cystic fibrosis are girls is .19 points higher than those whose children with cystic fibrosis are boys. The general self-efficacy score of parents whose children with CF were educated at high school and university level was .19 points higher than those whose children were educated at primary school or less. The general self-efficacy score of parents with one or two children is .14 points higher than those with three or more children.

It was found in the mediator effect analysis that the child's gender and the way of education did not have a mediator effect on self-efficacy. The mediating effect of perception of self and planned future sub-scales, and the number of children on self-efficacy are explained in Figure 2. When the mediating role of resilience between parental self-efficacy and the number of children among demographic variables was examined; it was determined that the model created was statistically significant (p<.05) and the R^2 value is 27.0%. The explanatory rate of the model is 27.0%.

It was observed in the model that the demographic variable of the number of children had a significant and direct effect on parental self-efficacy (β=.135, SE=.067, 95%CI [0.003-0.267], p=.043).

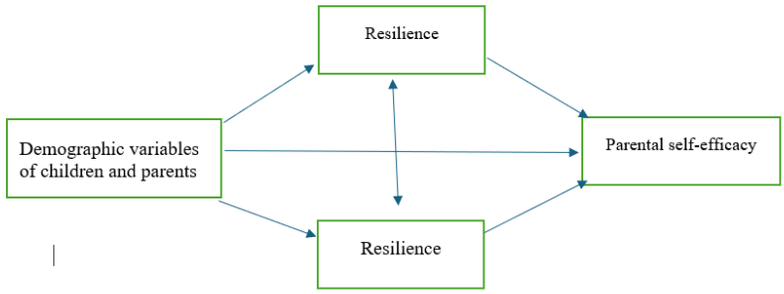

Figure 1. *Variable relationship to be examined in line with the hypotheses based on the Process model*

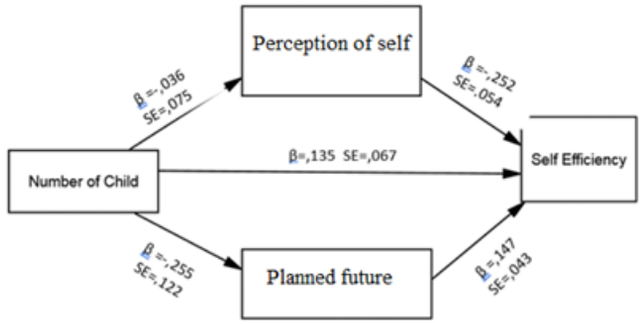

Figure 2. *Model 4 (Parallel mediation model) from Hayes 2013*

There was no correlation between the self-perception sub-scale of resilience and the number of children (β=-.036, SE=.075, 95%CI [-0.096-0.286], p=.635), but there was an indirect mediator effect on self-efficacy (β= .252, SE=.054, 95%CI [0.144-0.360], p=.001.

There was a significant and positive correlation between the planned future sub-scale scores of resilience and the number of children (β=.255, SE=.122, 95%CI [0.015-0.494], p=.036) and there was an indirect mediator effect on self-efficacy (β=.147, SE=.043, 95%CI [0.061-0.233], p=.001) (Table 4).

Table 3. *The Effect of Independent Variables on General Self-Efficacy Scale Scores of Parents: Results of Multiple Linear Regression Analysis (n= 269)*

Independent variables	B	S. Error	b	t	p	For B 95% Confidence Interval	
(Constant)	1.39	.16		8.690	.000	1.07	1.70
Resilience Perception of self	.24	.05	.30	4.528	.000	.14	.35
Resilience Planned Future	.15	.04	.23	3.406	.001	.06	.23
Gender of the child	.19	.06	.15	2.943	.004	.06	.31
Education of child	.19	.08	.12	2.296	.022	.03	.36
Number of children	.14	.07	.11	2.137	.034	.01	.27
R: .56 Adjusted R^2: .30 F: 23.51 p: .000 Durbin Watson: 1.38							

Table 4. *The mediating role of perception of self and planned future sub-scales between the number of children and self-efficacy of parents*

Outcome Variables	Predictor Variables	β	S.E.	t	p	%95 CI
Resilience Planned future	Number of children	.255	.122	2.094	**0.036**	0.015-0.494
Resilience Perception of self	Number of children	-.036	.075	-.474	0.635	-0.096-0.286
Self-Efficacy	Resilience Perception of self	.252	.054	4.638	**0.001**	0.144-0.360
Self-Efficacy	Number of children	.135	.067	2.021	**0.043**	0.003-0.267
Self-Efficacy	Resilience Planned future	.147	.043	3.390	**0.001**	0.061-0.233

4. DISCUSSION

In this study, it was found that parents' self-efficacy levels were affected by psychological resilience scale self-perception and planned future sub-scale scores, gender of the child with CF, school attendance status, and number of children. The results show that an increase in the psychological resilience scale perception of self and planned future sub-scale scores positively affects parents' self-efficacy. This result is similar to the studies in the literature showing that the increase in the future perception and planned future scores of the psychological resilience scale increases the self-efficacy of parents (30-31). Nurses caring for children with CF should evaluate and support parents' self-efficacy and factors affecting it within the scope of family-centred care.

Another result of this study is that the general self-efficacy levels of parents of children with CF are affected by some demographic characteristics. The general self-efficacy scores of parents who have a male child, who have 3 or more children and whose child with CF has an education level of primary school or below are lower. The result that parental self-efficacy levels decreased as the number of children increased in this study is similar to some studies in the literature showing that parental self-efficacy decreased as the number of children increased (32-33-34).

However, one study showed that parental self-efficacy increased as the number of children increased (35). In a systematic review in which the variables affecting parental self-efficacy were analysed, no definite conclusion was reached that there was a relationship between general self-efficacy of parents and demographic characteristics (16). These different results indicate that more studies are needed on the general self-efficacy of parents of children with CF and the factors affecting it. In this study, the result that the lower education level of the child and the higher number of children decreased the general self-efficacy of the parents may be associated with the fact that these children have more care needs.

In this study, it was found that psychological resilience scale planned future sub-scale scores played a mediating role in increasing the self-efficacy of parents with more children. Nurses can support the self-efficacy level of parents with more children by increasing the planned future sub-scale levels of the psychological resilience scale of parents of children with CF.. In order to increase the level of planned future resilience of parents, it may be recommended that nurses discuss their goals, plans and objectives related to the future and support their hopes for the future (28).

In the literature, it is reported that the perception of self sub-scale of psychological resilience is structurally associated with self-efficacy (36) and contributes positively to the development of self-efficacy (37)). Nurses should plan interventions for the development of psychological resilience perception of self-sub-scale to increase self-efficacy in parents of children with CF. From this point of view, it can be said that there is a need for interventions to improve parents' problem-solving and stress coping skills (28).

Another result of this study is that psychological health plays a mediating role in the relationship between self-efficacy and number of children. Self-perception and future perception, sub-scales of the psychological resilience scale, were found to play a mediating role in this relationship. Psychological resilience is the ability of an individual to resist the problems encountered (38). It is reported that psychological resilience leads to the development of protective factors such as self-efficacy in the process of coping with problems (39). In the literature, it is reported that the mediating role reduces the effect of the independent variable on the dependent variable and at the same time continues to maintain the emerging significant relationship. (40). In this study, it is seen that increased psychological resilience in parents reduces the negative effect of the number of children the parent has on parental self-efficacy. The number of children of parents is an uncontrollable variable, but the psychological resilience levels of parents can be improved. Thus, it may be possible to increase the general self-efficacy of parents who have many children by utilising the mediating role of increased psychological resilience.

Parental self-efficacy directly affects the management of the disease and health outcomes (41-42-43). İt is thought that increasing the self-efficacy of parents whose responsibilities are increasing in the home care process of children with CF will be effective in overcoming the disease. Nurses caring for children with CF will improve the quality of care by assessing parents' self-efficacy and factors affecting it within the scope of family-centred care and planning interventions to

improve it. The findings of this study will shed light on the development of guidance and counselling services offered by nurses to parents.

5. CONCLUSION

In chronic diseases such as CF, the treatment burden increases with age. It has been determined that this situation affects parental psychological resilience and indirectly parental self-efficacy. In addition, the number of children, which is one of the demographic variables of the parents, directly affects the parental self-efficacy. Nurses have responsibilities to evaluate parents, who ignore their own mental health and development during the intensive treatment process, in order to recognize their problems and to implement interventions that improve them. Interventional plans that develop effective coping and problem solving skills in parents can be planned to increase psychological resilience. Increasing psychological resilience will indirectly lead to an increase in parental self-efficacy. We believe that these improvements will be effective in achieving good clinical results and increasing the quality of life of children with CF. In this context nurses should routinely evaluate parents of children with CF and provide guidance and counseling.

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Clinical and Experimental Health Sciences

Prevalence of Norovirus Coinfection in *Clostridioides difficile* Toxin Positive Patients

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ABSTRACT

Objective: In this study we aimed to evaluate the prevalence of norovirus genogroups I and II and *C.difficile* coinfection among patients with gastroenteritis symptoms.

Method: A total of 76 patients with diarrhea were included in the study. Of these, 40 are children (<18y), 23 are adults between the ages of 18-65y, and 13 are patients older than 65 years. All these were *C.difficile* toxin GDH/Toxin A+B (BIOTEC, Spain) positive. In these toxin positive stool samples, Norovirus GI and GII antigen was studied by 2 methods; i.ELISA (R Biopharm, Darmstadt, Germany) and ii.polymerase chain reaction (RT – PCR). We compared the results of the antigen test (ELISA) with those of PCR for the detection of norovirus in stool specimens. SPSS 19.0 statistical program was used to evaluate the data of the research.

Results: Out of 76 stool samples tested, 3 (3.9%) were positive for norovirus by ELISA. Subsequent RT-PCR identified norovirus GI and GII in 7 samples (9.2%). Concerning RT-PCR, the sensitivity of the ELISA test was 42.8%, and the specificity was found as 96%.

Conclusion: The study identified a 9.2% rate of co-infection with *C. difficile* and norovirus, with this co-infection being particularly prevalent in children. This finding emphasizes the critical need to consider both co-infection and *C. difficile* infection as potential causes of diarrhea in hospitalized patients, especially those under 18 or over 65 years old.
Keywords: *C. difficile*, Coinfection, ELISA, Norovirus, PCR

1. INTRODUCTION

C. *difficile* is a Gram-positive, spore forming and toxin producing anaerobic bacteria. It is the most common cause of health care-associated infectious gastroenteritis and can cause a wide range of infections, from asymptomatic intestinal colonization to severe diarrhea, pseudomembranous colitis, and toxic megacolon. In recent years, a significant increase in morbidity and mortality due to *C. difficile* infections has been observed. CDC has placed this microorganism in a priority group for the prevention of health-related infections (1). The most important risk factors for the development of community-acquired *C. difficile* infection include being 65 years of age or older, while healthcare-associated *C. difficile* infection is also associated with hospitalization or nursing home residence. Immunosuppressive conditions such as hematologic malignancy. Although *C. difficile* infections are mostly healthcare-associated infections, they are increasingly occurring in the community among people without classic risk factors. Recently, some studies have shown that more than 35% of patients with community-acquired (CA) *C. difficile* infection (CDI) do not use antibiotics, and more than 50% of these patients report symptoms such as nausea or vomiting that are not present in classic symptoms of CDI (2).

This suggests that some symptoms may occur due to co-infections with other pathogens, especially viruses. Noroviruses are viruses that are common causes of acute gastroenteritis globally (3). Noroviruses are nonenveloped, single-stranded, positive – sense ribonucleic acid (RNA) viruses. The genogroups that infect humans are GI (8 genotypes), and GII are the most common cause of NoV outbreaks worldwide (4,5,6).

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Despite both *C. difficile* and NoVs posing major health threats, their potential interaction within the host is largely unexplored. By disrupting the intestinal microbiota or natural host defenses, viruses can create favorable environmental conditions for the colonization of *C. difficile* and thus cause co-infections (7,12). Although coinfections with both pathogens have been reported, the number of studies are limited in number.

Therefore, in this study, we aimed to detect the prevalence of norovirus co-infection in *C. difficile* toxin-positive patients with gastroenteritis using PCR and ELISA methods.

2.METHODS

2.1. Patients and Specimen Collection

Stool samples sent to Clinical Microbiology Laboratory between January 2019 and March 2020 from patients with gastroenteritis symptoms and were positive for *C. difficile* toxin were included in the study. Samples were examined macroscopically and microscopically. Direct examination results were recorded (13).

2.2. Detection of C.difficile Toxin

The stool samples were tested for the presence of *C. difficile* glutamate dehydrogenase antigen and toxins A and B using chromatographic immunoassay (GDH/Toxin A+B combo card test, Certest, Spain). Amorphous stool samples, which were negative with this method but inflammatory cells were present in direct microscopy, were directly inoculated in cycloserine, cefoxitin agar (CLO agar; BioMerieux, France) for *C. difficile.* Based on the colony's appearance, size, color, and other characteristics, the bacteria were identified by MALDI-TOF MS (VITEK MS®, bioMerieux, France). Colonies were inoculated into brain-heart infusion broth (BHIB; Becton Dickinson, Germany) and incubated in an anaerobic environment. After five days of incubation, the liquid medium was centrifuged at 3000 rpm for 20 minutes and the supernatant was removed and the presence of toxin was investigated by chromatographic immunoassay (GDH/Toxin A+B combo card test, Certest, Spain). Remaining stool samples were aliquoted and stored at −80°C.

2.3. Detection of Norovirus Antigen by ELISA Method

To detect norovirus antigen, an ELISA kit (RIDASCREEN, third generation, R-Biopharm, Darmstadt, Germany), which can determine GI and GII genotypes, was used. Stool samples stored at – 80°C were thawed at room temperature for 30 minutes, diluted 10% with the ELISA kit buffer, and centrifuged before following the manufacturer's protocol. The assay validity was confirmed by negative control OD < 0.2 and positive control OD > 0.5. Samples were considered positive if their OD values exceeded the calculated cut-off (negative control OD + 0.15) by 10% or more, otherwise negative. Kit documentation claims no cross-reactivity, 80% sensitivity, and 100% specificity (13).

2.4. Detection of Norovirus in Stool with RT-PCR Method

RIDA®GENE Norovirus I & II real-time PCR (R-Biopharm, Darmstadt, Germany), a real-time in vitro diagnostic kit, was used for the detection of norovirus by PCR in stool. For RNA extraction from feces, the HigherPurity™ Viral RNA Extraction Kit (Canvax, Mexico) was used according to the manufacturer's instructions. PCR was performed with Rotor-Gene 6000 Real-Time PCR (Corbett Research, Qiagen GmbH).

PCR procedures were carried out as follows: reverse transcription for 10 minutes at 58°C, initial denaturation for 1 minute at 95°C, denaturation for 15 seconds at 95°C and primer binding/extension for 30 seconds at 55°C, for a total of 45 cycles.

2.5. Clinical Epidemiology

In this study, we analyzed the epidemiologic factors to determine whether certain parameters were predictive of coinfection. Epidemiologic factors included age, sex, clinics (inpatient,outpatient), symptoms (diarrhea, nausea, vomiting, fever) history of the patient, antibiotic usage, history of chronic conditions (inflammatory bowel disease, malignancy, diabetes, autoimmune disease), antibiotic usage, laboratory findings (stool culture for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. , Rotavirus Ag, Adenovirus Ag positive with Rota-Adeno Card Test (CerTest, Biotec, Spain))

2.6. Data Analysis

SPSS 19.0 statistical program was used to evaluate the data of the research. Data were analyzed using frequency and percentage, descriptive statistical analysis. When comparing variables of categorical data such as clinical and demographic, the chi-square test was used. Statistical significance level $p \leq 0.05$ was determined.

Ethical Approval

The study was approved by Marmara University Ethics Committee, Noninvasive Clinic Ethics Committee (Approval date-no. 14.01.2019-13).

3. RESULTS

The study included a total of 76 patients 39 (51.3%) were males and 37 (48.6%) were females. Of these, 40 are children (<18), 23 are adults between the ages of 18-65, and 13 are patients older than 65. Twenty-one of the children are between the ages of 2-3 In microscopic examination, leukocytes were seen in 20 (26.3%) of the samples, while leukocytes and erythrocytes were seen in 13 (17.1%). Of the 76 *C. difficile*-positive samples, 70 were toxin-positive directly from the sample, while 6 were positive from toxigenic culture. No association was found between the detection of toxin positivity, either directly from the sample or through toxigenic culture, and norovirus positivity. Norovirus antigen was detected in 9.2% (n:7) of the samples by PCR, whereas the ratio was 3.9% (n:3) by ELISA. As a result, 3 samples were detected positive by both RT-PCR and ELISA, while 4 samples were only positive by RT-PCR. Using RT-PCR as a reference, the sensitivity of the ELISA test was found to be 42.8% and the specificity was 94.7% (Table 1). In our study, when adenovirus and rotavirus positivity was included, the viral co-infection rate was found to be 19.7% (n:15). Except for 7 patients with norovirus coinfection, rotavirus antigen was detected in 5 patients (6.5%) and adenovirus antigen in 3 patients (3.9%).

For bacterial coinfection, *Salmonella enterica* was the only isolated bacterial pathogen among the samples**.** Norovirus coinfection increased the likelihood of experiencing symptoms like nausea, vomiting, and fever, with a prevalence of 42.7% in coinfected patients compared to just 27.8% in non-coinfected patients. In the clinical history of the patients 18 patients were diagnosed with Inflammatory Bowel Disease, 17 patients were diagnosed with malignancy and 4 patients were followed up due to Cystic Fibrosis. 50% of the patients had an underlying condition (Table 2). Among prior medication exposures, antibiotherapy rate was very similar in each group. No statistically significant difference was observed in demographic characteristics such as age groups, gender and clinical findings of co-infected patients (p>0.05 for all groups).

Table 1. *Results of Norovirus antigen detected by RT-PCR and ELISA according to age groups*

Age	METHODS			
	RT PCR		ELISA	
	positive	negative	positive	negative
2-18	5	35	2	38
18-65	1	22	0	23
>65	1	12	1	12
TOTAL	7	69	3	73

Table 2. *Comparison of demographics, prior healthcare and medication exposures, and clinical characteristics between co-infected and non-co-infected patients*

Characteristics	Norovirus Co-infected cases (n/%) (7/9.2)	Norovirus Non-co-infected cases (n/%) (69/90.7)
Age group		
2-3	**0(0)**	**21(30.4)**
3-18	**5(71.4)**	**14((20.2)**
18-65	**1(14.2)**	**22(31.8)**
65 and over	**1(14.2)**	**12(17.3)**
Male/ Female	**3(42.8)/ 4(57.1)**	**41(59.4)/ 30(43.4)**
Inpatient/ Outpatient	**4(57.1)/ 3(42.8)**	**35(50.7)/ 36(52.1)**
Diarrhea	**7(100)**	**69(100)**
Nausea or vomiting	**1(14.2)**	**9(13.04)**
Fever	**2(28.5)**	**10(%14.4)**
Patient history		
Inflammatory bowel disease	**1(14.2)**	**17(24.6)**
Malignancy	**2(28.5)**	**15(21.7)**
Cystic fibrosis	**0(0)**	**4(5.7)**
Diabetes mellitus	**0(0)**	**4(5.7)**
Surgical procedure	**1(14.2)**	**5(7.2)**
Prior medication exposures		
Any antibiotics	**4(57.1)**	**40(57.9)**
Charlson comorbidity index ≥1	**6(85.7)**	**53(76.8)**
Laboratory findings		
Stool culture positive**	**1**	
Rotavirus Ag positive	**0(0)**	**5(7.2)**
Adenovirus Ag positive	**0(0)**	**3(4.3)**
Immunosuppressants*	**3(42.8)**	**29(42)**

**: Patients with a history of immunosuppressive medication or receiving chemotherapy. ** Salmonella enterica was reported for the stool culture*

4. DISCUSSION

In this study we aimed to determine the prevalence of *C. difficile*-norovirus coinfection in order to aid the clinical diagnosis and treatment for patient management. The pathogenesis of *C. difficile*-norovirus coinfection is not fully understood, but it is suggested that C*. difficile* toxins may alter intestinal homeostasis, predisposing to viral coinfections. Norovirus is one of the most leading causes of gastroenteritis in sporadic cases or epidemics and frequently studied virus in *C. difficile* coinfections (12,14,15). The prevalence of norovirus coinfection was detected as 9.2% in our study. Previous studies in a pediatric population reported coinfection rate as 12% and in adults this ratio ranged from 8.9% to 10% (16,17,18).

In a meta-analysis, 31 different studies conducted on children under the age of 18 suffering from diarrhea were examined. Of the total 10,201 patients, 16.1% were *C. difficile* positive and 10% of them were reported as norovirus co-infected (19). The literature shows that norovirus co-infection rates can reach higher levels during outbreak situations. In a study examining the Norovirus outbreak in Germany between 2002 and 2012, the results of 44 outbreaks in 5 different German hospitals were reported, and at least one of 9 outbreaks (20%) was reported to have *C. difficile* and norovirus co-infection (20). During our study period there was no evidence of norovirus outbreak. When we evaluated the laboratory epidemiological data, we found out that rotavirus antigen was also detected in 5 patients (6.5%) and adenovirus antigen in 3 patients (3.9%), in addition to the 7 patients with norovirus co-infection. Overall viral co-infection rate was found to be 19.7%. When frequently detected viral co-infection agents are evaluated, it is seen that norovirus incidence is followed by adenovirus and rotavirus, in line with world data (17). Since the detection of *C. difficile* under the age of two is generally considered colonization, this age group was not included in our study (21). Five of the patients with norovirus co-infection are in the 3-18 age group and 1 is over 65 years old. In our study, *C. difficile*-norovirus co-infection was detected in 12.5% (n:5) of those under 18 years of age. The rate we found appears to be above the literature values for this age group. In our study, the norovirus co-infection rate was found to be 7.6% (n:1) in adult patients over 65 years of age. The patient in this group had a history of multiple myeloma, and a sample was taken due to diarrhea that developed after clarithromycin and piperacillin/tazobactam treatment for pneumonia. Upon detection of coinfection, the patient was started on metronidazole and vancomycin treatment. It is known that the only patient between the ages of 18-65 had a history of meropenem treatment due to ventriculitis and was given metronidazole for the treatment of diarrhea.

C. difficile norovirus coinfection is more common in immunosuppressed patient groups. In a study conducted in China, *C. difficile* was reported in 68% (n:55) of 81 individuals living with HIV who complained of diarrhea, while *C. difficile*-norovirus co-infection was reported in 16.3% (9/55) of them (22). No HIV positive patients were included in our

study. However, 38.1% (n:29) of the patients were patients receiving immunosuppressive therapy. It is noteworthy that more coinfections with both norovirus and other viral agents (rotavirus, adenovirus) were detected in these patient groups.

When all patients included in the study were evaluated, it was observed that 65% (n:26) of the patients under the age of 18 had a history of previous antibiotic use and 42.5% were treated with metronidazole. It was observed that 47.2% of adult patients (n:17) had a history of antibiotic use, and 45.8% of them used metronidazole for the treatment of *C. difficile*. Four of the patients with norovirus co-infection had a history of previous antibiotic use (vancomycin, meropenem, clarithromycin and piperacillin/tazobactam), and all of these patients were treated by metronidazole.

As a result, when investigating the etiology of diarrhea in hospitalized patients, especially in patients under the age of 18 and over the age of 65, the presence of co-infection, as well as *C. difficile* infection, should be taken into consideration.

Another important outcome of our study; is the evaluation of the performance of ELISA and PCR, for the detection of Norovirus. In our study; the sensitivity of the ELISA test was 42.8% and the specificity was 96%, when RT-PCR was taken as the gold standard test. In a study, comparing the performance of ELISA and RT-PCR, the sensitivity of the ELISA test was reported as 66% and 86%, and the specificity was reported as 92.5% and 100%, respectively. Our results are compatible with the literature and suggest that PCR is more suitable for Norovirus.

In conclusion, in addition to *C. difficile* infection, the presence of co-infection should be considered when investigating the etiology of diarrhea in hospitalized patients, especially those under 18 and over 65 years of age. In recent years, panel tests have become increasingly important in the diagnostic approach to gastroenteritis. However, these tests are expensive and not cost-effective for every patient. Although the ELISA tests we used in our study are relatively cheaper tests, there were differences in sensitivity and specificity between them and molecular tests. This suggests that molecular methods, which are the gold standard for detecting norovirus, should also be used routinely outside of outbreak situations. There is still a need for cheaper, faster, and more accurate tests for the detection of co-infections.

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Author Contributions:

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Design of the study: HŞŞ, Aİ
Acquisition of data for the study: HŞŞ, Aİ
Analysis of data for the study: HŞŞ, FMA, ES, Aİ
Interpretation of data for the study: HŞŞ, FMA, ES, Aİ
Drafting the manuscript: FMA, ES, Aİ
Revising it critically for important intellectual content: FMA, ES, Aİ
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