Clinical and Experimental Health Sciences

Contents

RESEARCH ARTICLES

Contents

Clinical and Experimental Health Sciences

Pain and Malnutrition in Elderly Cancer Patients: Examples of Southern Turkey

Sema Aytaç[1], Özlem Ovayolu[1], Sibel Serçe[1], Nimet Ovayolu[2]

[1] Gaziantep University, Faculty of Health Science, Department of Nursing, Gaziantep, Türkiye.
[2] Sanko University, Faculty of Health Science, Department of Nursing, Gaziantep, Türkiye.

Correspondence Author: Sema Aytaç
E-mail: aytac.sema27@gmail.com
Received: 24.04.2021 **Accepted:** 01.02.2023

ABSTRACT

Objective: The prevalence of geriatric cancer disease is gradually increasing. Both the cancer diagnosis and many accompanying symptoms affect individuals bio-psycho-socially and impair the quality of life. This study was conducted to evaluate pain and nutritional status in geriatric cancer patients.

Methods: This descriptive study was conducted with geriatric cancer patients receiving treatment in the ambulatory chemotherapy outpatient clinic of a university and oncology hospital. The population of the study consisted of cancer patients receiving treatment in the hospital and the sample consisted of 215 geriatric cancer patients who were voluntary to participate in the study. The data of the study were collected using a questionnaire, the Visual Analog Scale (VAS), and the Mini Nutritional Assessment.

Results: The mean age of the patients was 69.3±4.7 years. Respiratory tract cancers ranked first (28.8%). 42.8% of the patients were at stage four and they frequently experienced symptoms such as fatigue, loss of appetite and poor nutrition. VAS mean score was 4.8±2.8, Mini nutritional assessment mean score was 9.2±3.0, and there was a negative significant correlation between the VAS and Mini nutritional assessment mean scores ($p<0.001$).

Conclusion: It was determined that geriatric cancer patients experienced “moderate” pain, had a risk for malnutrition and as their pain levels increased, the risk for malnutrition increased.

Keywords: Geriatric Nursing, Cancer Pain, Nutrition Assesment

1. INTRODUCTION

Today the downward trend in the population growth rate and the increase of average life expectancy, have led to an increase rate in the elderly population within the general population and have caused our world to gradually enter a demographic ageing process. Thus, old age has remained on the agenda in both developed and developing countries and is becoming more and more important every passing day. As is known, chronic illnesses increase along with the increase of elderly population and average life expectancy in the world and in Turkey (1,2). Among the chronic diseases, 50% of cancer cases are encountered in people over 65 years of age, and cancers are in the second place among the causes of death in people over 65 years of age, after heart diseases. (3). Both the cancer diagnosis and many accompanying symptoms affect individuals bio-psycho-socially and impair the quality of life. Among the symptoms affecting patients negatively, “pain” comes first. Pain in cancer patients may impair their quality of life, lead to despair and prevent them from coping with the disease (2,4). Therefore, the treatment and management of symptoms related to cancer become more important (5). Pain in geriatric cancer patients also appears as an important problem. The pain prevalence has been reported to be 28% in patients who have recently been diagnosed with cancer, 50-70% in patients receiving cancer treatment actively, and 64-80% in patients with advanced cancer (5). Pain in cancer is encountered at the rate of 50% in the early period and diagnosis of disease and at the rate of 75-80% in the advancing periods of disease. In the literature it is indicated that cancer pain significantly affects patients’ quality of life and becomes a greater source of fear than the death itself for patients and relatives in more than 70% of advanced cancer cases (6). However, the first step of pain control is pain assessment. This assessment should be made by a multidisciplinary health care team. Playing a key role in this team, nurses are an important and supplementary element of cancer care.

What makes nurses more important and distinctive in pain control than other team members is that they spend more time with the patient than other team members (7).

One of the factors affecting cancer patients negatively in many aspects is their nutritional status. Weight loss is the first

DOI: 10.33808/clinexphealthsci.927643

sign of an impaired nutrition and is frequently observed in geriatric patients (8). It has been reported that malnutrition is encountered in 40-80% of these patients during diagnosis (9), the malnutrition prevalence ranges from 25% to 70% (10-13) and this rate rises up to 83% in geriatric cancer patients (14).

Thus, early evaluation and rapid intervention of nutritional status are of prime importance to prevent morbidity and mortality in this patient group (9). As is known, cancer patients intensely experience symptoms related to treatments such as anorexia, cachexia, taste changes, pain and malnutrition, besides symptoms caused by the disease process (15-17). Especially pain may prevent nutrition and lead to poor nutrition and malnutrition (18). Pain and malnutrition in elderly cancer patients should be routinely evaluated by nurses with appropriate assessment tools specific to the elderly individual, and pharmacological and non-pharmacological methods should be used in treatment (3). Therefore, this study was conducted to evaluate pain and nutritional status in geriatric cancer patients and contribute to the management of pain and nutritional problems.

2. METHODS

2.1. Design and Sample

This descriptive study was conducted with geriatric cancer patients receiving treatment in the ambulatory chemotherapy outpatient clinic of a university hospital and in an oncology hospital. The results of a previous study were used to calculate the sample size of the study (5,18). Necessary minimum sample size was calculated to be 215 with α=0.05 and the test power of (1-β) 0.80.

Prior to starting the study, a mini mental test was applied to the individuals and the patients who obtained 24 points and above, were over the age of 65 years, were diagnosed with cancer, could communicate, and were voluntary to participate in the study, were included in the study. However, the patients who got less than 24 points from the mini mental test, were under the age of 65 years, and refused to participate in the study, were not included in the study.

2.2. Data Collection Process

The data of the study were collected using a questionnaire, the Visual Analog Scale (VAS) and Mini Nutritional Assessment Questionnaire-Short Form (MNA).

Visual Analog Scale: The scale is applied by marking on a straight line with a pen. On this line, the point of 0 cm indicates no pain and the point of 10 cm indicates worst pain. In the literature, it is stated that VAS is a reliable tool to be used in evaluating the pain level (19).

Mini Nutritional Assessment Questionnaire-Short Form (MNA): This form is used in identifying malnutrition in both clinic and outpatient clinic patients and evaluating the outcomes of nutritional support treatments. It is accepted to be a valid measurement tool not only for revealing malnutrition in geriatric patients, but also for predetermining the risk for malnutrition. Also the European Society for Clinical Nutrition and Metabolism recommends this measurement tool particularly for the elderly (20,21). Turkish validity study of this form was conducted by Sarıkaya (2013). In the form, 0-7 points indicate “malnutrition”, 8-11 points indicate “risk for malnutrition”, and 12-14 points indicate “normal nutritional status” (22).

2.3. Procedure

The researchers applied the questionnaires to the patients who agreed to participate in the study via the face-to-face interview method in the clinic setting.

It took approximately ten minutes to apply the questionnaires. None of the patients wanted to leave the study or refused to answer the questions in the questionnaire.

2.4. Data Assessment

Statistical analyzes were reported using the SPSS 22.0 statistical software. The descriptive statistics were indicated via median and standard deviation values. In addition, the Student’s t-Test, Kruskal-Wallis, Mann-Whitney U Test, and Spearman’s correlation analysis were used. The value of $p<0.05$ was accepted to be statistically significant.

2.5. Ethical Considerations

Before starting the study, informed consent form and necessary permissions from the ethics committee and the institution were obtained from the patients.The study approval was obtained from Gaziantep University Clinical Trials Ethics Committee (approval number: 2017/163)

3. RESULTS

Two hundred fifteen patients completed the study. It was determined that the mean age of the geriatric cancer patients was 69.35±4.74 years. Of the patients, 43.3% were female, 44.2% were primary school graduate, 90.2% were married, 40.5% were unemployed, and 74.9% had a middle economic situation. In addition, When gender was evaluated with VAS mean scores; the women felt more pain than men and the difference between them was statistically significant ($p<0.05$). The mean VAS score of geriatric cancer patients was 4.8±2.8, and the mean score of mini nutritional assessment was 9.2±3.0. However, the patients, who were university graduate, married, freelancer and had a good, had higher MNA mean scores ($p>0.05$) (Table 1).

Table 1. *Comparison of Socio-demographic characteristics and pain and mini nutritional assessment mean scores of the patients*

Characteristics	n (%)	VAS Mean±SD	MNA Mean±SD
Gender			
Female	93 (43.3)	5.51±2.7	9.40±3.04
Male	122 (56.7)	4.33±2.80	9.21±3.14
p		**0.002**[a]	0.673[a]
Educational Background			
Illiterate	62 (28.8)	5.12±2.76	9.22±3.17
Literate	26 (12.1)	5.11±2.83	8.30±2.51
Primary Education	95 (44.2)	4.87±3.05	9.26±3.27
High School	27 (12.6)	3.96±2.2	10.33±2.80
University	5 (2.3)	4.20±0.83	10.40±1.34
p		0.373[b]	0.129[b]
Marital Status			
Married	194 (90.2)	4.73±2.80	9.41±3.14
Single	21 (9.8)	5.90±2.89	8.23±2.50
p		0.072[c]	0.054[c]
Occupation			
Worker	12 (5.6)	4.16±2.94	10.16±3.71
Civil Servant	7 (3.3)	3.57±1.61	10.28±1.25
Freelancer	30 (14.0)	4.30±3.16	10.33±3.00
Unemployed	87 (40.5)	5.54±2.88	8.91±3.18
Other	79 (36.7)	4.50±2.56	9.10±2.98
p		**0.036**[b]	0.105[b]
Economic Situation			
High	9 (4.2)	3.33±1.73	10.77±2.2
Middle	161 (74.9)	4.78±2.80	9.59±3.02
Low	45 (20.9)	5.37±2.97	7.95±3.16
p		0.111[b]	0.005[b]
Residence Place			
District	90 (41.9)	4.62±2.55	9.01±2.85
Province	125 (58.1)	5.00±3.00	9.50±3.25
p		0.254[a]	0.138[a]
Total	**215 (100.0)**	**4.8±2.8**	**9.2±3.0**

[a]: : Independent sample t test [b]:Kruskal-Wallis test c: Mann Whitney U

Of the patients who participated in the study, 28.8% suffered from respiratory tract cancer, 42.8% were at stage four, 60.9% underwent chemotherapy, 61.9% had metastasis, 18.6% had another cancer patient in family, and 34.4% had comorbidities. When comparing some characteristics of the patients and VAS mean scores, it was determined that there was a significant difference between the disease stage, presence of metastasis and VAS mean scores ($p<0.05$). There was no statistically significant difference between MNA mean scores and disease stage. However, as the disease stage advanced, the MNA mean score decreased ($p>0.05$) (Table 2).

Table 2. *Comparison of some characteristics and pain and mini nutritional assessment mean scores of the patients*

Characteristics	n(%)	VAS Mean±SD	MNA Mean±SD
Cancer Type			
Respiratory Tract	62 (28.8)	4.4±3.07	9.58±3.26
Digestive System	61 (28.4)	4.72±2.75	8.60±3.25
Reproductive System	35 (16.3)	5.71±2.35	9.31±2.68
Urinary System	9 (4.2)	5.00±2.00	8.11±3.33
Lymphoma	23 (10.7)	4.60±3.08	10.04±3.22
Breast	25 (11.6)	5.12±2.90	10.00±2.38
p		0.372[a]	0.170[a]
Duration of Disease (Month)			
1-12	104 (48.4)	4.57±2.94	9.06±3.13
13-24	39 (18.1)	5.48±2.55	9.30±3.37
25-36	37 (17.2)	5.21±2.86	9.18±2.90
37 months and above	35 (16.3)	4.54±2.66	10.08±2.85
p		0.226[a]	0.504[a]
Stage			
1	27 (12.6)	3.33±2.63	10.07±2.38
2	56 (26.0)	4.41±2.77	9.67±3.31
3	40 (18.6)	5.25±2.62	9.60±3.16
4	92 (42.8)	5.38±2.83	8.70±3.05
p		**0.002**[a]	0.099[a]
Treatment Type			
Chemotherapy (CT)	131 (60.9)	5.06±2.87	9.58±3.15
Radiotherapy (RT)	5 (2.3)	4.60±2.70	9.20±1.64
CT+RT	26 (12.1)	4.92±2.62	8.19±3.57
RT+Surgery	3 (1.4)	7.33±2.88	8.33±4.04
CT+Surgery	29 (13.5)	3.82±2.66	9.00±2.97
CT+RT+Surgery	21 (9.8)	4.47±2.80	9.42±2.27
p		0.224[a]	0.494[a]
Metastasis			
Available	133 (61.9)	5.33±2.75	8.94±3.10
N/A	82 (38.1)	4.06±2.77	9.86±3.01
p		**0.001**[b]	0.034[b]
Cancer Patient in Family			
Available	40 (18.6)	4.90±2.98	9.45±3.70
N/A	175 (81.4)	4.83±2.79	9.26±2.95
p		0.895[b]	0.767[b]
Comorbidities			
Available	73 (34.0)	5.06±2.83	9.78±2.97
N/A	142 (66.0)	4.73±2.82	8.96±3.14
p		0.410[b]	0.122[b]
Total	**215 (100.0)**		

[a]: Kruskal-Wallis test [b]: Independent sample t test

In this study, 33.9% had pain in the abdominal area, 47.4% experienced pain for 6-11 months, 25.6% had a condition triggering pain, 77.2% had pain at intervals, 36.7% had tingling pain, 45.1% took medications to decrease the pain, and 36.7% took nonopioid analgesics. When comparing the pain-related characteristics and VAS mean scores of the patients, it was found that the patients, who had pain in the waist-back area, described 'constant' and 'stabbing' pain

as pain frequency and took strong opioids, had higher VAS mean scores (p<0.05). It was determined that the patients, who had pain in the extremity and abdominal area, had experienced pain for a year or more and took strong opioids, had lower MNA mean scores (p<0.05) (Table 3).

Table 3. *Comparison of pain-related characteristics and pain and mini nutritional assessment mean scores of the patients*

Characteristics	n(%)	VAS Mean±SD	MNA Mean±SD
Area of Pain			
Chest	25 (11.6)	5.48±2.46	9.40±3.29
Waist-Back	49 (22.8)	5.87±2.54	9.22±2.60
Extremities	28 (13.0)	5.14±2.67	8.64±3.49
Abdomen	74 (33.9)	5.67±2.19	8.78±3.05
No Specific Area	39 (18.1)	1.35±1.73	10.7±2.99
p		**0.000**[a]	**0.011**[a]
Duration of Pain (Month)			
0-5	102 (20.0)	5.36±2.51	9.27±3.11
6-11	31 (47.4)	5.61±2.40	8.77±2.88
12 months and above	39 (14.4)	6.43±2.03	8.38±3.04
Constant	43 (18.1)	1.62±1.87	10.55±2.93
p		**0.000** [a]	**0.008** [a]
Frequency of Pain			
Constant	49 (22.8)	7.63±2.11	7.67±2.80
Intermittent	166 (77.2)	4.02±2.46	9.77±3.02
p		**0.000**[b]	**0.000**[b]
Type of Pain			
Throbbing	20 (9.3)	4.70±2.02	9.10±3.43
Tingling	79 (36.7)	5.67±2.60	9.46±2.64
Stabbing	55 (25.6)	5.98±229	8.25±3.43
Burning	22 (10.2)	5.31±2.14	8.90±2.75
Undescribable	39 (18.1)	1.38±1.78	10.74±2.94
p		**0.000**[a]	**0.005**[a]
Situations Decreasing Pain			
Taking Medications	97 (45.1)	6.29±2.29	9.02±3.04
Changing Position	4 (1.9)	6.50±2.51	9.00±2.70
Resting	48 (22.3)	5.29±2.14	7.95±2.91
N/A	66 (30.7)	2.28±2.18	10.69±2.83
p		**0.000** [a]	**0.000** [a]
Situations Increasing Pain			
Cold Weather	5 (42.8)	7.20±2.38	9.40±2.88
Moving	63 (2.3)	6.46±2.22	8.39±2.91
Stress	28 (13.0)	5.92±2.03	8.89±3.28
Going to the Toilet	9 (4.2)	4.66±1.50	10.00±2.69
Eating	18 (8.4)	5.77±2.53	8.22±3.07
N/A	92 (42.8)	3.11±2.61	10.17±3.02
p		**0.000** [a]	**0.004** [a]
Medications Taken for Pain			
Nonopioids	79 (36.7)	4.03±1.78	9.89±2.99
Weak Opioids	25 (11.6)	6.00±1.84	8.60±2.84
Strong Opioids	66 (30.7)	7.60±1.75	7.89±3.07
N/A	45 (20.9)	1.57±1.57	10.68±2.56
p		**0.000** [a]	**0.000** [a]

[a]: *Kruskal-Wallis test* , [b]: *Independent sample t test*

Of the patients, 90.7% had fatigue, 71.6% had loss of appetite, 60.5% were sufferring from poor nutrition, 47.4% had nausea-vomiting, and 58.6% had sleeplessness. It was determined that the patients who had loss of appetite, nausea-vomiting, malnutrition, poor personal care and sleeplessness, had higher VAS mean scores and lower MNA mean scores. This difference was statistically significant in all situations except for fatigue (p<0.05). The geriatric cancer patients describing pain also experienced many other symptoms (Table 4).

Table 4. *Comparison of some symptoms and pain and mini nutritional assessment mean scores of the patients*

Problems	n(%)	VAS Mean±SD	MNA Mean±SD
Fatigue			
Available	195(90.7)	5.01±2.79	9.21±3.14
N/A	20(9.3)	3.20±2.60	10.10±2.46
p		0.008[a]	0.216[a]
Loss of Appetite			
Available	154(71.6)	5.46±2.54	8.63±2.95
N/A	61 (28.4)	3.29±2.90	10.96±2.82
p		**0.000**[b]	**0.000**[b]
Nausea-Vomiting			
Available	102(47.4)	5.75±2.83	8.26±3.05
N/A	113(52.6)	4.02±2.56	10.23±2.84
p		**0.000**[b]	**0.000**[b]
Poor Nutrition			
Available	130(60.5)	5.46±2.69	8.66±2.90
N/A	85 (39.5)	3.90±2.78	10.27±3.15
p		0.000[b]	0.000[b]
Poor Personal Care			
Available	65(30.2)	6.32±2.64	8.06±3.14
N/A	150(69.8)	4.20±2.66	9.83±2.92
p		**0.000**[b]	**0.000**[b]
Negative Effect on Quality of Life			
Yes	125(58.1)	5.43±2.63	8.69±3.08
No	90(41.9)	4.03±2.89	10.13±2.93
p		**0.000**[b]	**0.000**[b]
Sleeplessness			
Available	126(58.6)	5.98±2.48	8.76±3.23
N/A	89(41.4)	3.23±2.48	10.05±2.73
p		0.000[b]	0.002[b]
Desire to Cry			
Available	69(32.1)	6.23±2.49	8.18±3.16
N/A	146(67.9)	4.19±2.74	9.82±2.93
p		**0.000**[b]	**0.000**[b]
Unwillingness to Talk			
Available	69(32.1)	6.78±2.02	8.27±2.94
N/A	146 (67.9)	3.93±2.69	9.78±3.06
p		**0.000**[b]	**0.001**[b]
Sense of Burnout			
Available	73(34)	6.21±2.42	8.17±3.08
N/A	142(66)	4.14±2.76	9.87±2.95
p		**0.000**[b]	**0.000**[b]
Decrease in Relations with Other People Around (Such as Coworkers. Social Friends)			
Available	56(26)	6.50±2.13	8.03±3.34
N/A	159(74)	4.26±2.81	9.74±2.88
p		**0.000**[b]	**0.001**[b]

[a]: *Mann Whitney U,* [b]: *Independent sample t test*

The patients' VAS mean score was 4.84±2.82, which was stated as "moderate" and MNA mean score was 9.29±3.09, which was stated as "risk for malnutrition". It was determined that there was a negative correlation between VAS mean score and MNA mean score (p<0.01) (Table 5).

Table 5. *Correlation between the pain and mini nutritional assessment mean scores of the patients*

	r	MNA p
VAS	-.327	0.000[a]

a: Spearman correlatıon Coefficient

4.DISCUSSION

The population of elderly patients is growing with increasing prevalence of cancer diagnoses and cancerrelated pain syndromes. Cancer pain occur at any time in the disease's progression. It is a multidimensional and complex phenomenon that need proper assessment, management and evaluation based on current nursing knowledge and practices (24). Playing a key role in this team, nurses are an important and supplementary element of cancer care (5).

Also, they have important duties and responsibilities in determining the risk for malnutrition and improving the nutritional status (25). Accordingly, this study aimed to assesment the pain and nutritional status of geriatric cancer patients.

As geriatric patients had more complex health issues than young patients, serious difficulties are faced in evaluating and managing pain in geriatric cancer patients. Despite present treatments, geriatric patients are unable to receive adequate treatment for cancer pain (26) and at least 42% of patients complain about pain that cannot be treated as required (27). In a study comprising a nursing home for people over the age of 65 years, it was stated that as age advanced, the opportunity for patients not to receive adequate treatment increased and more than one quarter of these patients took no analgesic agents especially over the age of 85 years or in case of decreased cognitive functions (28). In the study conducted by Kutluturkan et al., with 106 geriatric cancer patients, the most frequent symptoms experienced by the patients were reported to be weakness (83%), dryness of the mouth (71.7%) and pain (62.3%) (29).

In a cohort study with 292 patients, the prevalence of pain in geriatric cancer patients was found to be 65% (30). In this study, it was determined that the patients' VAS mean score was 4.84±2.82 and the most frequent symptoms they experienced were fatigue (90.7%), loss of appetite (71.6%), and malnutrition (60.5%), respectively.

Malnutrition is a clinical condition that is not regarded much by most clinicians and does not receive attention for treatment when identified. However, it is common especially among the geriatric population and has a proven effect on the morbidity and mortality of patients (31). Uncontrollable malnutrition may worsen the tolerance of treatment, including a greater possibility for relapse or death during or after the treatment and prevent the completion of treatment (32). Its prevalence might be 23-62% for the elderly in the hospital environment and rise up to 85% for the patients in nursing homes (31).

In a study conducted in Turkey it was determined that 28% of the patients applying to geriatric outpatient clinic had a poor nutritional status, 69% of hospitalized patients had a risk for malnutrition, and 12% had a malnutrition rate (33,34).

In a study conducted in a nursing home, it was found that according to the MNA, 63% of the elderly had a risk for malnutrition and 9.6% had malnutrition (35). In another study, it was determined that the risk for malnutrition was 31% and rate of malnutrition was 13% in patients applying to outpatient clinic; whereas, the risk for malnutrition was 39% and rate of malnutrition was 25% among hospitalized patients (36). In the Turkish Nursing Homes Nutritional Status Evaluation Project conducted by the Academic Geriatrics Society, it was found that the risk for malnutrition was 38.3% and the malnutrition rate was 11.9% (37). These results indicated that malnutrition was frequently encountered in cancer patients. The severity of malnutrition varies according to the type, area and stage of cancer (17,38).

In their study, Hamaker et al., found that the malnutrition prevalence in geriatric cancer patients was 46% (30). In a review examining multiple studies, it was reported that malnutrition or risk for malnutrition in geriatric cancer patients ranged from 27% to 83% (39). In this study, it was determined that the patients' MNA mean score was 9.29±3.09 and 46.9% had a risk for malnutrition and 28.1% had malnutrition. In addition, the patients had problems such as loss of appetite (71.6%), malnutrition (60.5%) and nausea-vomiting (47.4%). As is known, loss of appetite may lead to weight loss, malnutrition, morbidity and mortality in geriatric patients. In their study, Kutluturkan et al., determined that the severest symptom experienced by geriatric cancer patients was loss of appetite (29).

Pain frequency varies according to the stage of disease, might be around 25-50% in early-stage patients and patients receiving active cancer treatment and rises up to 70-80% in metastatic patients (40).

In this study, it was determined that advanced staged cancer patients, patients feeling constantly pain and metastatic patients had higher VAS mean scores and lower MNA mean scores.

Thus, it is thought that nurses giving care to advanced stage cancer patients should begin to evaluate patients' pain as from the early period, follow their nutrition and weight with a multidisciplinary team approach and support them before malnutrition develops. In the studies it has been reported that geriatric patients experience fatigue more often due to cancer and reasons not related to cancer (41,42). Especially untreated cancer may cause fatigue and reduce or cease physical, social, interpersonal and recreational activities, prevent household, family, work and educational

performance and affect all living spaces such as psychosocial and spiritual well-being.

It may cause significant declines in productivity, self – esteem, physical functionality and quality of life and also pose a distress in sticking to treatment regimes. In addition, it may delay the treatment and cause a dose limitation or cessation of the treatment (3,42,43). Thus, it is of prime importance to define fatigue in the geriatric patient group very well and apply necessary nursing interventions. Also in this study, it was determined that the most frequent symptom experienced by geriatric cancer patients was fatigue and this symptom was accompanied by many other problems. Accordingly, it is of particular importance to evaluate fatigue and other related problems in geriatric patients regularly. As is known, cancer patients, no matter how old they are, typically experience multiple symptoms at the same time. Cancer itself, direct or indirect outcomes of cancer, early or late side effects of the treatment or comorbidities may cause these symptoms (44). In a study, it was reported that nearly one third of the elderly (31.2%) had pain, fatigue, sleeplessness and mood disorders at the same time (45).

In the cancer report published by the World Health Organization in 2020, it was reported that 20-50% of patients could show symptoms such as pain, fatigue and nutritional problems, have a difficulty in expressing their pain depending on fatigue, and geriatric patients could face a risk for malnutrition under the effect of symptoms such as nutritional difficulty and loss of appetite (32). In this study, it was determined that the patients most frequently experienced symptoms such as fatigue, loss of appetite, malnutrition and sleeplessness in addition to pain.

In the study, it was found that the patients taking strong opioids for analgesics, had the highest levels of pain and the lowest malnutrition mean scores. This showed that the patients still had pain and their nutritional problems continued despite taking strong opioids. In cancer patients malnutrition is a frequently encountered situation due to nausea-vomiting and loss of appetite, depending on the burdens caused by the disease and treatment.

In case of loss of appetite, symptoms such as changes in sense of taste, presence of nausea-vomiting, pain and depression should be questioned.

It is recommended that changes related to sense of taste can be controlled by adding a little salt and spice to the food. Removing any odor or view, increasing nausea-vomiting from the environment, before nutrition in order for nausea and vomiting not to affect nutrition is among possible interventions (46).

5. CONCLUSION

It was determined that geriatric cancer patients experienced “moderate” pain, had a risk for malnutrition and as their pain levels increased, the risk for malnutrition increased. The patients who had fatigue, loss of appetite, nausea-vomiting, malnutrition, poor personal care and sleeplessness, had higher VAS mean scores and lower MNA mean scores.

In addition, the pain experienced by the patients was accompanied by fatigue, loss of appetite, nausea-vomiting, malnutrition, poor personal care, sleeplessness, desire of crying and sense of burnout. In accordance with these results, it is recommended to evaluate geriatric cancer patients in terms of pain and malnutrition in the treatment process, take necessary precautions before their symptoms advance, follow other problems that may accompany pain and support patients.

The most important limitation of the study was that pain and nutritional status were evaluated only via a questionnaire and results are limited to the research group only.

Procurement of pain management and nutritional support is crucial for intended clinical outcomes in geriatric cancer patients. It is suggested to follow up pain and malnutrition of patients using appropriate assessment tools. Nurses at this point play a key role.

Thus pain, malnutrition and accompanying problems of patients should be managed with a multidisciplinary team approach.

Acknowledgments: *The researchers would like to thank the patients who participated in the study.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Clinical Trials Ethics Committee of Gaziantep University (2017/163)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: SA, ÖO, SS, NO
Design of the study: SA, ÖO, SS, NO
Acquisition of data for the study: SA, SS
Analysis of data for the study: SA, ÖO, SS, NO
Interpretation of data for the study: SA, ÖO, SS, NO
Drafting the manuscript: SA, ÖO, NO
Revising it critically for important intellectual content: SA, ÖO, SS, NO
Final approval of the version to be published: SA, ÖO, SS, NO

REFERENCES

[1] Tekin ÇS, Kara F. Dünyada ve Türkiye’de yaşlılık. Uluslararası Bilimsel Araştırmalar Dergisi 2018;3(1):219-229 (Turkish)
[2] Çetin A, Arslan İ, Tekin O, Sarı O. Yaşlıların yaşadığı yerin ve sosyodemografik özelliklerinin yaşam kalitesi üzerine etkisi. Journal of Medicine and Palliative Care. 2020;1(2):34-40 (Turkish)
[3] Karadağ E. Yaşlı kanser hastalarında yorgunluk. Dokuz Eylül Üniversitesi Hemşirelik Fakültesi Elektronik Dergisi 2018;11(4): 328 – 333 (Turkish)
[4] Barutcu CD. Kronik hastalığı olan yaşlı bireylerin ağrı kesici tercihleri. Geleneksel ve Tamamlayıcı Tıp Dergisi 2020;3(1):69-76 (Turkish)
[5] Mutluay E. Yaşlı kanser hastalarında ağrı. Türk Onkoloji Dergisi 2015;30(2):90-95 (Turkish)

[6] Durmuş E, Uğur Ö. Kanser ağrısının yönetiminde bakım verenlerin ağrı kesicileri kullanım engelleri ve etkileyen faktörlerin incelenmesi. Hemşirelikte Araştırma Geliştirme Dergisi 2018;20(2/3):22-33 (Turkish)

[7] Eti Aslan F. Ağrı doğası ve kontrolü. 1. baskı, İstanbul: Avrupa Tıp Kitapevi; 2006. (Turkish).

[8] Alan Ö, Gürsel Ö, Ünsal M, Altın S, Kılçıksız S. Geriatrik hastalarda onkolojik yaklaşım. Okmeydanı Tıp Dergisi 2013;29(Ek sayı 2):94-98 (Turkish)

[9] Özel V, Alphan E. Yetişkin hematolojik kanserli hastaların malnütrisyon düzeylerinin farklı tarama araçları ile değerlendirilmesi. Sağlık ve Yaşam Bilimleri Dergisi 2019;1(1):21-29 (Turkish)

[10] Hébuterne X, Lemarié E, Michallet M, de Montreuil CB, Schneider SM, Goldwasser F. Prevalence of malnutrition and current use of nutrition support in patients with cancer. J Parenter Enteral Nutr. 2014;38(2):196-204.

[11] Planas M, Álvarez-Hernández J, León-Sanz M, Celaya-Pérez S, Araujo K, De Lorenzo A.G. Prevalence of hospital malnutrition in cancer patients: a sub-analysis of the PREDyCES® study. Support Care Cancer. 2016;24(1):429-435.

[12] Pressoir M, Desné S, Berchery D, Rossignol G, Poiree B, Meslier M, Meuric J. Prevalence, risk factors and clinical implications of malnutrition in French Comprehensive Cancer Centres. Br J Cancer. 2010;102(6):966-971.

[13] De Melo Silva FR, de Oliveira MGOA, Souza ASR, Figueroa JN, Santos CS. Factors associated with malnutrition in hospitalized cancer patients: a croos-sectional study. Nutrition Journal 2015;14(1):123.

[14] Caillet P, Liuu E, Simon AR, Bonnefoy M, Guerin O, Berrut G, Paillaud E. Association between cachexia, chemotherapy and outcomes in older cancer patients: a systematic review. Clin Nutr. 2017;36(6):1473-1482.

[15] Ovayolu Ö, Ovayolu N. Onkolojide semptom yönetiminde kullanılan kanıt temelli tamamlayıcı yöntemler ve etkileri. ERÜ Sağlık Bilimleri Fakültesi Dergisi. 2013;1(1):83-98 (Turkish)

[16] Kutlu R, Çivi S, Börüban M, Demir A. Kanserli hastalarda depresyon ve yaşam kalitesini etkileyen faktörler. Selçuk Üniv Tıp Derg. 2011;27(3):149-153 (Turkish).

[17] Kocatürk C, Kalafat, S. Akciğer kanserli hastalarda malnütrisyon ve perioperatif nütrisyon desteği. Toraks Cerrahisi Bülteni 2015;9(4):297-302 (Turkish)

[18] Silva PB, Trindade LC, Gallucci MC, Schirr RA. Prevalence of malnutrition and pain in patients admitted by the screening service of an oncologic hospital. Rev Dor. 2013;14(4):263-266

[19] Güzeldemir ME. Ağrı değerlendirme yöntemleri. Sendrom. 1995;6(7):11-21 (Turkish)

[20] Guigoz Y. The mini nutritional assessment (mna) review of the literature—what does it tell us? J Nutr Health Aging. 2006;10(6):466-485.

[21] Van Nes MC, Herrmann FR, Gold G, Michel JP, Rizzoli R. Does the mini nutritional assessment predict hospitalization outcomes in older people? Age Ageing. 2001;30(3):221-226.

[22] Sarıkaya D. Geriatrik Hastalarda Mini Nütrisyonel Değerlendirme (MNA) Testinin uzun ve kısa (MNA-sf) Formunun Geçerlilik çalışması, Tıpta Uzmanlık Tezi 2013, Hacettepe Üniversitesi, Ankara (Turkish)

[23] T.C Sağlık Bakanlığı. Türkiye Halk Sağlığı Kurumu Kronik Hastalıklar, Yaşlı Sağlığı ve Engelliler Daire Başkanlığı, Türkiye Bulaşıcı olmayan hastalıklar çok Paydaşlı eylem planı, 2017-2025. Accessed [01September 2020] https://www.euro.who.int/__data/assets/pdf_file/0006/346695/BOH_TR.pdf

[24] Khalil AZ, Meawad EB, Abd Elhameed SH. Nurses' attitude, practices and barriers towards pain management of elderly patients with cancer. Mansoura Nursing Journal 2022;9(1):23-30.

[25] Baz S, Ardahan M. Yaşlılarda malnütrisyon ve hemşirelik yaklaşımları. Balıkesir Sağlık Bilimleri Dergisi 2016;(5):147-153 (Turkish)

[26] Caltagirone C, Spoletini I, Gianni W, Spalletta G. Inadequate pain relief and consequences in oncological elderly patients. Surg Oncol. 2010;19(3):178-83.

[27] Uyar M. Eyıgör C. Geriatride kanser ağrısına yaklaşım. Ege Tıp Dergisi 2014;53(Ek sayı):38-45 (Turkish)

[28] Barford KL, D'Olimpio JT. Symptom management in geriatric oncology: Practical treatment considerations and current challenges. Curr Treat Options Oncol. 2008;9(2-3):204-214.

[29] Kutlutürkan S, Sözeri Öztürk E, Böke Erdoğan S, İyimaya Ö, Bay F, Gül F. Yaşlı kanser hastalarında yaşam kalitesinin ve semptomların değerlendirilmesi. Van Tıp Dergisi 2018;26(4):418-426 (Turkish)

[30] Hamaker ME, Buurman BM, van Munster BC, Kuper IM, Smorenburg CH, de Rooij SE. The value of a comprehensive geriatric assessment for patient care in acutely hospitalized older patients with cancer. The oncologist. 2011;16(10):1403

[31] Akademik Geriatri Derneği, Yaşlılarda Malnütrisyon Kılavuzu, Grup 1 Malnütrisyonun önemi tarama ve tanı. Published [January 2013]. Accessed [01January 2020] https://www.akademikgeriatri.org/files/thn-kitap.pdf (Turkish)

[32] World Health Organization. WHO report on cancer: setting priorities, investing wisely and providing care for all. Accessed [01 January 2020]. https://repository.gheli.harvard.edu/repository/13173/

[33] Ülger Z, Halil M, Kalan I, Yavuz BB, Cankurtaran M, Güngör E, Arıoğul S. Comprehensive assessment of malnütrition risk and related factors in a large group of community-dwelling older adults. Clin Nutr. 2010;29(4):507-511.

[34] Kuyumcu ME, Yeşil Y, Oztürk ZA, Halil M, Ulger Z, Yavuz BB, Arıoğul S. Challenges in nutritional evaluation of hospitalized elderly; always with mini-nutritional assessment? Eur Geriatr Med. 2013;4(4):231-236

[35] Ekici E, Yetim Çolak M, Kozan EH. Huzurevinde yaşayan yaşlıların beslenme durumları ve günlük yaşam aktivitelerinin belirlenmesi. Online Türk Sağlık Bilimleri Dergisi. 2019;4(4):506-518 (Turkish)

[36] Saka B, Kaya O, Ozturk GB, Erten N, Karan MA. Malnütrition in the elderly and its relationship with other geriatric syndromes. Clin Nutr. 2010;29(6):745-748.

[37] Cankurtaran M, Saka B, Sevnaz S, Varlı M, Döventaş A, Yavuz BB, Halil M, Çurgunlu A, Ülger Z, Öztürk GB, Tufan F, Tekin N, Akçiçek F, Karan MA, Atlı T, Beğer T, Erdinçler DS, Arıoğul S. 2012. Türkiye huzurevleri ve bakımevleri nutrisyonel değerlendirme projesi. Accessed [01 January 2020]. https://www.akademikgeriatri.org/files/thn-kitap.pdf (Turkish).

[38] Thomas PA, Berbis J, Falcoz PE, Le Pimpec-Barthes F, Bernard A, Jougon J, Alauzen M. National perio – perative outcomes of pulmonary lobectomy for cancer: the influence of nutritional status. Eur J Cardiothorac Surg. 2014;45(4):652-659

[39] Caillet P, Laurent M, Bastuji-Garin S, Liuu E, Culine S, Lagrange JL, Paillaud E. Optimal management of elderly cancer patients:

usefulness of the comprehensive geriatric assessment. Clin Interv Aging. 2014;9:1645

[40] Yekedüz E, Utkan G, Ürün Y. Ağrili kemik metastazlarinda onkolojik yaklasimlar. Nükleer Tıp Seminerleri 2020;6:11-16 (Turkish)

[41] Berger AM, Gerber LH, Mayer DK. Cancer-related fatigue: implications for breast cancer survivors. Cancer 2012;118(S8):2261-2269

[42] Eyigor S, Eyigor C, Uslu R. Assessment of pain, fatigue, sleep and quality of life (QoL) in elderly hospitalized cancer patients. Archives of Gerontology and Geriatrics 2010;51(3):57-61

[43] Luctkar-Flude MF, Groll DL, Tranmer JE, Woodend K. Fatigue and physical activity in older adults with cancer: a systematic review of the literature. Cancer Nurs. 2007;30(5):35-45.

[44] Henson LA, Maddocks M, Evans C, Davidson M, Hicks S, Higginson IJ. Palliative care and the management of common distressing symptoms in advanced cancer: Pain, breathlessness, nausea and vomiting, and fatigue. Journal of Clinical Oncology 2020;38(9):905-914

[45] Cheng KK, Lee DT. Effects of pain, fatigue, insomnia, and mood disturbance on unctional status and quality of life of elderly patients with cancer. Crit Rev Oncol Hematol. 2011;78(2):127-137.

[46] Yurtsever S, Çavuşoğlu E, Onkoloji hastalarının palyatif bakımında semptom yönetimi, 2. Uluslararası Multidisipliner Çalışmalar Kongre Kitabı; Adana,Türkiye; 2018. Pp.1-12 (Turkish)

How to cite this article: Aytaç S, Ovayolu O, Serçe S, Ovayolu N. Pain and Malnutrition in Elderly Cancer Patients: Examples of Southern Turkey. Clin Exp Health Sci 2023; 13: 1-8. DOI: 10.33808/clinexphealthsci.927643

Clinical and Experimental Health Sciences

Effect of Online Case-Based Teaching Method on Professional Development of Nursing Students

Derya Evgin[1], Adem Sümen[2]

[1] Kayseri University, Faculty of Health Sciences, Department of Child Development, Kayseri, Türkiye.
[2] Akdeniz University, Kumluca Faculty of Health Sciences, Department of Public Health Nursing, Antalya, Türkiye.

Correspondence Author: Derya Evgin
E-mail: deryaevgin@kayseri.edu.tr / evginderya@gmail.com
Received: 24.05.2021 **Accepted:** 19.10.2022

ABSTRACT

Objective: This study was conducted to determine the effect of online case-based education, as part of distance education, on nursing students' professional values, professional behavior, problem-solving, critical-thinking skills, and self-directed learning skills.

Methods: The study was conducted using a single-group pretest-posttest quasi-experimental design. A total of 58 3rd grade nursing students participated in this study The data before the study were collected electronically with the Google forms application using the personal information forms and scales (Self-Directed Learning Skills Scale, Problem Solving Inventory, California Critical Thinking Disposition Scale, Nurses Professional Values Scale, Nursing Students Professional Behaviors Scale).

Results: After online case-based nursing process teaching, the difference in the total mean score of the students in all scales was found to be statistically significant ($p< 0.01$). It was found that, after the training had been provided through case-based education, the score average of students' professional values, professional behaviors, problem-solving, critical thinking skills and self-directed learning increased.

Conclusion: Use of online case-based teaching is useful for increasing nursing students' nursing skills in distance education.

Keywords: Care, distance education, nursing student, online case-based teaching, professional development

1. INTRODUCTION

The coronavirus disease (COVID-19), which has spread across the world in a short period since its emergence in Wuhan, China, and which has caused the mobilization of international health authorities due to its effects, is now accepted as a pandemic by the World Health Organization (WHO) (1,2). For months, COVID-19 has caused death, disease, and socioeconomic problems (2,3) and has also caused serious disruptions in educational processes, both in Turkey, and globally (3). In this context, universities in Turkey suspended their provision of education from 12th March 2020, based on the decision of the Higher Education Council in Turkey. As the coronavirus pandemic continues to affect the world unimpeded, the Higher Education Council has stated that it has decided to continue the 2019–2020 spring term education via distance education only, open education, and digital education opportunities, and that face-to-face education will not be provided in Turkey (4). The distance-education process, which has become mandatory across all higher education in Turkey due to the coronavirus epidemic, is also affecting nursing education. In order to continue learning, information technologies will be particularly beneficial when face-to-face training is impossible. Nurses use technology as a tool of change by guiding this process and policies for the provision of good-quality, qualified, and low-cost care to individuals and communities (5). It is noted that distance education, which contributes significantly to the widespread use of lifelong education and which has the capability of reaching a large student population, independently from time and place (6), and which focus on individual learning (7), can be performed by web support using different teaching techniques (8). Within this process, with the exception of existing methods that teaching methods that would keep the student active, reinforce learning, and increase individual responsibility-undertaking in learning have been researched and used in line with possibilities available to students within nursing programs.

The aim of nursing education is to teach cognitive, psychomotor and attitudinal behaviors to the student (9,10). Especially, clinical practice training aims to improve students' critical thinking, psychomotor, communication and management skills and to strengthen their sense of self-confidence (11). Team studies are recommended for increasing the learning effectiveness among students, and

DOI: 10.33808/clinexphealthsci.942370

case-based teaching is an example of one of these methods (12). Furthermore, it is noted that self-efficacy perception can also be improved with case-based teaching (13). Self-efficacy is shown to be one of those individual characteristics that forms the professional identity of nurses (14). Training nurses with self-efficacy is an important issue, one that is also included in nursing education programs. The aim of nursing undergraduate education is to develop individuals who follow developments and updates within their field; who use lifelong learning, problem solving, and critical thinking skills; and who participate in those activities that will contribute to their professional development (15). Self-efficacy is an important factor for nurses to successfully perform the practices required by their profession (16,17). Furthermore, it is noted that nurses' self-efficacy is directly related to professional autonomy (18), and that nurses with high self-efficacy more often perceive threats as opportunities (19). It is essential for nurses to meet current needs and cope with contemporary problems (20). Thanks to a qualified nursing education provided with innovative and active learning methods, the quality of healthcare services will also increase (21).

This study was conducted to determine the effect of case-based education provided within the scope of a pediatrics course within the process of distance education, on the nursing students' professional values, professional behavior, problem solving, critical thinking, and self-directed learning skills.

2.METHODS

Hypotheses of Study

H_1: After the trainings provided with case-based education in the distance education process, the score average of students' professional values increases.

H_2: After the trainings provided with case-based education in the distance education process, the score average of students' professional behaviors increases.

H_3: After the trainings provided with case-based education in the distance education process, the score average of students' problem-solving increases.

H_4: After the trainings provided with case-based education in the distance education process, the score average of students' critical thinking increases.

H_5: After the trainings provided with case-based education in the distance education process, the score average of students' self-directed learning increases.

2.1. Study design

This research was conducted using a single group pretest–posttest quasi-experimental design.

2.2. Setting and sample

This study was performed in the Nursing Department of a Faculty of Health Sciences during the spring semester of the 2019–2020 academic year. Originally, the study was to be carried out with 61 students who were studying in the third year of nursing and who participated in the practice of Pediatric Nursing course. The study sample comprised 58 students who agreed to take part in the study, who participated in training full-time, and who answered the questionnaires completely. The participation rate in this research study was 95%.

2.3. Measures

Personal Information Form: This comprises nine questions on the students demographic characteristics, feelings toward the nursing profession, studying system, and academic achievements.

***Self-Directed Learning Skills Scale (SDLSS)*:** This scale was developed by Koçdar et al. (2018), comprises 30 items, and has a five-point Likert-type structure. Responses to the Scale items answered by the participants are rated between "I strongly disagree" and "I strongly agree". The scale measures the participants' skills in regard to setting goals, seeking help, developing self-study strategies, managing their physical environment, and managing their efforts (22). The Cronbach's alpha reliability coefficient of the scale was found to be 0.93. In this study, the Cronbach's alpha reliability coefficient was found to be 0.93 in the pretest and 0.96 in the posttest.

Problem Solving Inventory (PSI): Developed by Heppner and Peterson (1982), this inventory is a six-point Likert-type scale comprising 35 items that measure the self-perception of adolescents and adults toward problem-solving skills. Each scale item asks the responder how often they undertake a certain behavior, with responses being rated between "I always behave like this" and "I never behave like this". The lowest score obtainable from the scale is 32, and the highest obtainable score is 192. Higher total scores obtained from the scale indicate that the individual perceives themselves to be insufficient in regard to their own problem-solving skills. In other words, as the score obtained from the scale increases, problem-solving skill decreases. The total scale comprises six subscales, including four effective problem solving methods, i.e., "self-confident", "thinking", "evaluating" and "planned" approaches, and two ineffective problem-solving methods, i.e., "hasty" and "avoidant" approaches. In the study conducted by Şahin et al. (1993) on 244 university students, the Cronbach's Alpha reliability coefficient of the PSI was found to be 0.88 (23). The current study, the Cronbach's Alpha reliability coefficient of the PSI was found to be 0.83 in the pretest and 0.87 in the posttest.

California Critical Thinking Disposition Scale (CCTDS): This scale, developed by Facione et al. (1990), was used to evaluate the critical thinking level of the individual responder (24). The scale comprises a total of 51 items and six subscales: truth seeking, open-mindedness, analyticity, systematicity,

self-confidence, and inquisitiveness. California Critical Thinking Disposition Scale (CCDTS) is a six-point Likert-type scale. Items are rated on a 6-point rating scale ranging from 1–6 (ranging from "strongly disagree": 1 point, to "strongly agree": 6 points). To evaluate the subscales, the total score obtained from each question within the subscale is divided by the number of in that subscale before being multiplied by 10. The minimum score possible from each of the subscales is 10 and the maximum score possible is 60. The sum of the subscales scores then gives the critical-thinking disposition score. A subscale score below the value of 40 indicates low critical thinking disposition, while a score above 50 points indicates high critical thinking disposition. In this context, a total CCDTS score below 240 points indicates a low critical thinking disposition, and a total Scale score of more than 300 points indicates a high critical thinking disposition. The Turkish validity and reliability study of the scale was conducted by Kökdemir (2003) and the Cronbach's alpha coefficient of the Scale was found to be 0.88 (25); in the current study, the Cronbach's alpha reliability coefficient of the Scale was found to be 0.82 in the pretest and 0.84 in the posttest.

Nurses Professional Values Scale-Revised (NPVS-R): The NPVS-R was developed by Weis and Schank (2009) (26), and a validity and reliability study of the Scale in Turkish as conducted by Acaroğlu (2014) (27). The scale, which is used to evaluate nurses and nursing students' professional values, is a five-point Likert-type scale (whereby responses are rated between 1= "not important" to 5= "very important") comprising 26 items and three sub-dimensions: care, professionalism, and confidence. The total score obtained from the Scale is determined by adding up those numerical values corresponding to the answers given. The lowest score obtainable from the Scale is 26, and the highest score obtainable is 130. High scores indicate a strong compliance with professional values (27). The total Cronbach's alpha value of the Scale for this study was found to be 0.92; the Cronbach's alpha value of the Scale was found to be 0.94 in the pretest and 0.91 in the posttest in the current study.

Nursing Students Professional Behaviors Scale (NSPBS): This scale, used to determine the professional behaviors of nursing students, was developed by Göz and Geçkil (2010) (28). The scale is a five-point Likert-type measurement tool that comprises 27 items and includes three sub-dimensions, i.e., healthcare practices, activity practices, and reporting. Items in the scale are rated between "very inadequate" (1 point) and: "satisfactory" (5 points). The total score that can be obtained from the scale is 27–135. The high scores of the scale indicate that the students' level of professional behavior is high. The total Cronbach's alpha value of the scale for this study was found to be 0.95 and was calculated as being 0.93 for both the pretest and the posttest.

2.4. Practice

In the current study, the effectiveness of case-based teaching of nursing process – which was conducted by distance education method – was evaluated based on 10 cases prepared in the pediatric nursing field. After the courses in the faculty have been theoretically taught in classrooms, they are applied in basic skills laboratories and clinical/field areas. Educational activities within the faculty are carried out according to the classical method of education. Innovative teaching methods, such as simulation, mobile learning, and online learning, are also used to teach courses. Teaching on the subject of the "nursing process" in the faculty is performed theoretically and practically within the scope of the vocational courses. The form, developed on the basis of Gordon's Functional Health Patterns, is used as part of the nursing education process taught within each department. Information on face-to-face teaching concerning the nursing process as part of the Pediatric Nursing course is provided in the table below (Table 1).

Table 1. *Information on teaching of the nursing process in face-to-face education in the Pediatric Nursing course*

Course content information	Time	Explanation
Nursing process theoretical and classroom hours	4 hours	The course is conducted as 4 hours theoretical, 2 hours laboratory and 10 hours clinical practice per week. Clinical practice continues along with theoretical course topics. A half-day case discussion is held every week throughout the practice. The received care plan is reviewed by the instructor and delivered to the student with written feedback. The form developed based on Gordon's Functional Health Patterns is used to collect data in the clinic.
Weekly laboratory practice hours	2 hours	
Weekly clinical practice time	10 hours	
Total clinical practice time	14 weeks	
Case discussion/ rotation	Half-day case discussion every week throughout the practice	
Number of care plans that a student is expected to deliver	14 pcs	
The way how the instructor evaluates the care plan	A note is taken on the delivered care plan and the student is given feedback.	

Within the scope of the Pediatric Nursing course, the subject of the nursing process was taught to those students in the classroom environment in the form of face-to-face education; this comprised 4 hours of theoretical lessons and 4 hours of a sample case discussion. After the face-to-face education was suspended during the epidemic process, it was decided to continue the practice in the form of distance education (course notes, homework, case analysis). In this context, relevant course notes, case presentations, and sample articles that supported the learning of the course subject were uploaded to the online course system. Before the commencement of the online course, all students were prepared for the relevant case according to the practice

calendar. For case-based nursing process teaching the practice, an online case discussion was conducted over the program Zoom under the moderation of the instructor of the course for at least 4 hours per week over a 10-week period. Additionally, the WhatsApp social media platform was used to create a group including all students and instructors, which was used by the instructor to answer students; questions throughout the preparation process. After the case discussions had been held online on those dates and times specified in the practice calendar, students were asked to write down the nursing care details they learned in practice (Table 2). Students were then expected to answer the following five questions in the care plan.

- Which nursing diagnoses did you identify?
- What data did you use to identify this issue? How did you get this data?
- What nursing initiatives did you apply for this diagnosis?
- What change did you expect in the patient as a result of the nursing intervention or interventions you applied?
- How did you assess the effectiveness of the nursing intervention you used?

Table 2. *Online teaching of nursing process and practice calendar in the distance education process*

Case Topics	*Practice Date and Time*	*Method*	*Instruction*
NEWBORN RDS CARE	23.03.2020 18:30 – 22:30	Question-Answer Discussion	• Distance education practices are made between the dates and hours announced by the instructor. • Preparation for the relevant topic is made before the practice day. • The practice subject is discussed on the date specified in the program. • Students record their notes on care in their notebooks after the course. • At the end of the practice, students send their care plans to the instructor.
ASTHMA CARE	30.03.2020 18:30 – 22:30	Question-Answer Discussion	
CONGESTIVE HEART FAILURE CARE	06.04.2020 18:30 – 22:30	Question-Answer Discussion	
DIABETIC KETOACIDOSIS CARE	13.04.2020 18:30 – 22:30	Question-Answer Discussion	
NECROTIZING ENTEROCOLITIS (NEC) CARE	20.04.2020 18:30 – 22:30	Question-Answer Discussion	
NEPHROTIC SYNDROME CARE	27.04.2020 18:30 – 22:30	Question-Answer Discussion	
EPILEPSY CARE	04.05.2020 18:30 – 22:30	Question-Answer Discussion	
SICKLE CELL ANEMIA CARE	11.05.2020 18:30 – 22:30	Question-Answer Discussion	
ACUTE LYMPHOBLASTIC LEUKEMIA CARE	18.05.2020 18:30 – 22:30	Question-Answer Discussion	
OBESITY CARE	25.05.2020 18:30 – 22:30	Question-Answer Discussion	

2.5. Data Collection

Pretest data were collected electronically through the Google forms application by using the personal information forms and many scales (SDLSS, PSI, CCTDS, NPVS-R, NSPBS) created by researchers as a result of a literature review.

Students were informed that that their participation in this research was entirely voluntary. The voluntary consent requirement was specified at the beginning of the questionnaire, and those students who agreed to participate in this research confirmed this by electronically responding to the questionnaire questions. Thus, students were enabled to fill out the data collection forms without interacting with one another. All participants were asked to provide a specific nickname in the form. The questionnaire took approximately 30–35 minutes to complete. Online practices and initiatives were given over a period of 4 hours a week and continued for a total of 10 weeks. At the end of the practice, students were then asked to send their care plans to the instructor. One week after the end of the practice, the forms were submitted again and filled out by the student participants. Students were asked to write the nickname they had used in previous questionnaires on this questionnaire.

2.6. Data Analysis

The research data was analyzed using the IBM SPSS 23.0 (IBM Statistical Package for the Social Sciences Corp.; Armonk, NY, USA) software program. In the data analysis, descriptive statistics (number, percentage, average, standard deviation) were used in the characteristics of variables, while the dependent t-test (paired t-test) was used to compare the pretest and posttest values of the scales. The normality of the data for numeric variables was analyzed using the Shapiro–Wilk test, histogram, and *Q-Q* graphics. Results were then evaluated at 95% confidence interval according to a significance level of $p<0.05$. In addition, Cohen's d (effect size) was used to determine the effect size of the difference, which was significant in paired comparisons (d= 0.2 small, d= 0.5 medium, d= 0.8 high, d≥ 1 very high).

2.7. Ethical Considerations

For this research, permission to use the study scales was obtained from the scale creators, institutional permission was granted by the faculty in which the research was conducted, ethical approval was granted by the Clinical Research Ethics Committee affiliated to the state university in the region (Decision number: 70904504/287 dated 29/04/2020), and informed consent was obtained from all the study participants.

3. RESULTS

Sociodemographic characteristics

Of those students included in the study 63.8% were female; the average age of all participating students was 21.06±0.83

years. It also was determined that 62.1% of the students chose the nursing department willingly, 65.5% liked their profession, and 43.1% wanted to change their profession because they don't like the nursing department. Most students stated that they wanted to become academic nurses (37.9%) or clinical nurses (36.2%) after graduation. Students reported that they studied more during the examination time, that they worked an average of 3.79±2.46 hours per day, and reported their academic success as being 43.1% good and 39.7% moderate (Table 3).

Table 3. *Descriptive characteristics of the students*

Characteristics	n	%
Age	21.06±0.83	
Gender		
Female	37	63.8
Male	21	36.2
Willing choice of nursing department		
Yes	36	62.1
No	22	37.9
Fondness of nursing profession		
Yes	38	65.5
No	20	34.5
Desire to change nursing profession		
Yes	25	43.1
No	33	56.9
Studying system		
During examination time	52	89.7
Regularly	6	10.3
Study time per day	3.79±2.46	
Academic achievement		
Very good	2	3.4
Good	25	43.1
Moderate	23	39.7
Poor	8	13.8
Post-graduation plan		
Become an executive nurse	7	12.1
Become a clinical nurse	21	36.2
Become an instructor nurse	8	13.8
Become an academic member	22	37.9

Scales-level differences

Students' total mean SDLSS scores were determined as 97.68±19.05 in the pretest and 103.98±22.64 in the posttest; the difference between these two scores was found to be statistically significant ($p < 0.01$). The score increases in the seeking help ($p < 0.01$), self-study strategies ($p<0.05$), and managing the physical environment ($p < 0.01$) sub-dimensions of the scale were also found to be statistically significant.

Students' total mean PSI score was determined as 121.29±10.33 in the pretest and 102.22±15.79 in the posttest; this difference was found to be statistically significant ($p<0.001$). The score decreases in the self-confident approach ($p<0.001$), evaluating approach ($p<0.01$), planned approach ($p<0.01$), hasty approach ($p<0.001$), and avoidant approach ($p<0.001$) sub-dimensions of the scale were also found to be statistically significant (Table 4).

The total mean CCTDS score was determined as 222.52±24.82 in the pretest and 238.90±24.03 in the posttest; this difference was also found to be statistically significant ($p<0.001$). Furthermore, it was determined that the student's average scores increased in regard to the analyticity ($p<0.01$), self-confidence ($p <0.01$), inquisitiveness ($p<0.05$) sub-dimensions of the scale; this increase was also found to be statistically significant (Table 4).

Students' total NPVS-R mean score was found to be 104.67±16.09 in the pretest and 114.27±11.03 in the posttest; this difference was also found to be statistically significant ($p<0.01$). It was also found that the increases in the care ($p<0.01$), professionalism ($p<0.05$), and confidence ($p<0.01$) subscales of the NPVS-R Scale were statistically significant (Table 4).

Students' total mean NSPBS score was determined as 113.12±15.72 in the pretest and 121.58±12.56 in the posttest; this difference was also found to be statistically significant ($p< 0.01$). It was also determined that the score increases in the healthcare practices ($p< 0.05$), activity practices ($p< 0.001$), and reporting ($p< 0.001$) sub-dimensions of the NPVS-R Scale were statistically significant (Table 4).

Table 4. *Students' mean scale scores pre-test and post-test*

MEAS and its subscales	Pre-test		Post-test		*t*	*p*	*d*
	Mean	SD	Mean	SD			
Self-Directed Learning Skills Scale	**97.68**	**19.05**	**103.98**	**22.64**	**3.016**	**0.004****	**0.295**
Setting goal	17.32	2.95	17.03	3.85	0.586	0.560	0.084
Seeking help	27.91	6.39	30.24	7.49	3.189	0.002**	0.329
Self-study strategies	26.44	6.97	28.32	7.80	2.348	0.022*	0.252
Managing the physical environment	19.60	6.04	21.94	6.41	3.202	0.002**	0.376
Managing efforts	6.39	2.49	6.43	2.48	0.142	0.887	0.014
Problem Solving Inventory	121.29	10.33	102.22	15.79	7.050	0.000***	1.439
Self-confident approach	25.60	3.14	21.31	5.39	5.129	0.000***	0.974
Thinking approach	22.03	3.06	21.63	3.46	0.611	0.544	0.121
Evaluating approach	12.55	2.82	10.05	5.30	3.378	0.001**	0.580
Planned approach	16.70	2.70	14.48	3.79	3.541	0.001**	0.676
Hasty approach	34.44	5.13	27.37	7.67	5.943	0.000***	1.081
Avoidant approach	9.94	4.05	7.36	2.75	4.000	0.000***	0.746

MEAS and its subscales	Pre-test		Post-test		t	p	d
	Mean	SD	Mean	SD			
California Critical Thinking Disposition Scale	222.52	24.82	238,90	24.03	3.774	0.000***	0.670
Seeking truth	32.83	8.22	35.71	7.43	1.888	0.064	0.367
Open-mindness	28.69	6.62	30.63	10.05	1.247	0.218	0.228
Analyticity	44.37	6.78	47.89	5.46	2.950	0.005**	0.572
Systematicity	34.62	6.54	34.59	6.16	0.024	0.981	0.005
Self-confidence	40.24	7.71	44.33	6.03	2.908	0.005**	0.592
Inquisitiveness	41.74	9.35	45.72	7.00	2.568	0.013*	0.483
Nurses Professional Values Scale	104.67	16.09	114.27	11.03	3.433	0.001**	0.700
Care	60.10	9.68	66.05	7.81	3.405	0.001**	0.677
Professionalism	32.01	5.54	34.39	3.66	2.541	0.014*	0.509
Confidence	12.55	2.22	13.82	1.54	3.295	0.002**	0.669
Nursing Students Professional Behaviors Scale	113.12	15.72	121.58	12.56	3.321	0.002**	0.594
Health-care practices	77.03	10.36	81.00	9.76	2.245	0.029*	0.394
Activity practices	28.20	4.95	31.25	3.09	3.965	0.000***	0.739
Reporting	7.87	1.81	9.32	0.96	5.161	0.000***	1.004

Mean: Average, SD: Standard Deviation
*t: Paired t-test, *p<0.05, **p<0.01, ***p<0.001, d: effect size*

4. DISCUSSION

Considering the importance of nursing and its contribution to the health system – and in order to make those contributions visible and improve nursing-related reports – nurses need to be entrepreneurial, adopt technological advances, and follow the world agenda closely (5). When technology is used correctly within nursing education, it supports an effective classroom environment and clinical learning. Learning; the millennial generation is particularly open to new educational methods (8).

Considering that web-based teaching can be conducted regardless of temporal and spatial restrictions (4,29) and enables individual learning (29), it is thought that students will follow the course according to their own learning speed (29,30), and their participation within an environment in which they feel comfortable will facilitate their learning. It is one of the aims of those institutions that provide nursing undergraduate education in Turkey to determine the nursing care needs of healthy or sick individuals in every environment, as well as to educate professional nurses who can plan, implement, and evaluate the nursing care necessary for meeting these requirements to professional standards (15). In order to be able to use the nursing process effectively and correctly in their professional lives, nursing students must correctly and effectively use the nursing process in clinical practice, starting with their undergraduate education (31).

In the current study, the total mean score of students' professional values was determined as being 104.67±16.09 in the pretest and 114.27±11.03 in the posttest, and this difference was found to be statistically significant. Consequently, it can be suggested that case-based teaching has a positive effect on students' professional values. One of the education methods recommended for the improved education of nurses is the case-based teaching method (32). Case-based teaching is a method based on the realization of learning by analyzing a previously prepared situation, one that has already been experienced or that is likely to be experienced. This method is based on the theory of "constructivism" (33), and facilitates student-centered learning, strengthens individual decision-making, and it is supported by studies in the literature (34,35). It was also seen that the score increase in the NPVS-R scale sub-dimensions—those of care, professionalism, and confidence—was also statistically significant. The current study also showed that case-based teaching had a positive effect on these results, and that it is effective for helping nurses to reflect on the facts and act professionally. Furthermore, it is stated that the case-based teaching method is enjoyable and strengthening, that it improves skills and diagnostic ability, reduces stress levels, and supports the gaining of professional competence (36). Moyo et al. (2016) reviewed 50 studies on the personal and professional values of nurses, doctors and other healthcare professionals. They reported that the professional values of healthcare professionals were related to their critical thinking skills, problem-solving skills, and professionalism (37). Professional values also affect the problem-solving and critical-thinking skills of nurses, and therefore have a significant effect on the quality of care provided (38). In the current study, the total mean score of professional behaviors of the students was determined as 113.12±15.72 in the pretest and 121.58±12.56 in the posttest; the difference between these two values – and the score increase in the sub-dimensions of healthcare practices, activity practices, and reporting – were also found to be statistically significant. According to case-based teaching, nursing students can the technique being taught, look integrally, and conduct in-depth learning by discussing the technique with their instructor (39).

The effect of case-based teaching on the persistence of knowledge in nursing is also mentioned (40), and it is explained that critical-thinking skills and problem-solving skills develop faster in teamwork trainings (12). In the current study, it was discovered that the problem-solving skill levels of the students increased (p<0.01) after training had been given through case-based education as part of the distance-education process, and so it can be concluded that the method used was effective. Other studies note that the use of case-based teaching method is recommended

for the development of problem-solving skills (33). Case-based learning is involves team working (12) while also being a method based on problem-solving (33). A nurse must be able to cope with nursing problems and identify complex patient-care needs. They need to develop critical-thinking and problem-solving skills to identify patient care needs and provide systematic care (41). In order to achieve this, it is suggested that the application of case-based teaching—a method that develops critical thinking—instead of traditional teaching methods will prove more effective. The current study also determined that students' total mean critical-thinking skills score was 222.52±24.82 in the pretest and 238.90±24.03 in the posttest, and that this difference was statistically significant. Furthermore, it was discovered that the score increase in the analyticity, self-confidence, and inquisitiveness sub-dimensions of the CCTDS scale was also a statistically significant. It seems that the method used is effective in increasing of critical thinking skills. The level of critical thinking should reflect students' ability to question, research, analyze, and work out the clinical situation they face, or else their clinical decision-making that is, in practice, related to these skills in the nursing discipline. In nursing, it is important for students to gain the ability to think critically (34,35); this is because nurses should be able to solve patient problems, an ability that can be gained during student life (42). A nurse who can think critically will be able to solve problems more easily and produce faster and more accurate solutions to the problems of their patient. For this reason, students should learn to think critically throughout the education period, and should be able to solve problems using this ability (43). Furthermore, nurses should be able to think critically, seek information, question, find solutions to problems, and have social sensitivity in order to respond to the health needs of the society (44). It was seen that critical thinking significantly affected the perception of nursing care (45) and that it increased the quality of care (46). Studies in the literature suggests that case-based teaching increases critical thinking in nursing students (32,33,36,47).

Case-based teaching also involves teamwork as it is planned in groups. Therefore, it is reported that both critical thinking (47) and a sense of self-efficacy can be developed when using the case-based teaching method (13). It is also noted that the improvement in self-efficacy perception in nurses positively reflects on clinical practices and the development of management skills, and self-efficacy should be supported during student life in order to increase the student's self-efficacy (48). It is also emphasized that, as the experience in nursing increases, self-efficacy also increases and that the teaching method used for self-efficacy development is important (49).

The current study also determined that the total mean score of the students' self-directed learning skill (103.98±22.64) after the practice was higher than the pre-practice value (97.68±19.05), and that this difference was statistically significant ($p< 0.01$). Therefore, it can be argued that nursing process education supported by online case discussions is effective in increasing students' self-directed learning skills. Student readiness is also considered to be important for the successful realization of e-learning practices, which are rapidly becoming more pervasive in education and training applications. It is noted that student readiness is influences factors such as self-orientation and interaction with the learning environment (50). In the current study, the score increase in the seeking help ($p< 0.01$), self-study strategies ($p< 0.05$), and managing the physical environment ($p< 0.01$) sub-dimensions of the scale were also found to be statistically significant. In order for the individual to be ready for self-directed learning, and for them to be able to acquire self-learning skills, it is necessary to have an academic background in a certain area (51). It is also reported that knowledge has a positive effect on increasing self-efficacy (52). The high level of knowledge and skills of a nursing student supports their self-confidence and greater clinical compliance (32). In addition, it was reported that self-efficacy skills could be improved via e-learning methods (53). The communication skills, success, and motivation of nurses can also be improved by increasing nurses self-efficacy (54).

In an information society, there are certain characteristics that individuals should possess: problem solving, critical thinking, questioning, information literacy, effective communication and collaboration skills, entrepreneurship, etc. To ensure students gain these characteristics, individuals of the information society should be equipped with those skills they need to access information, instead of information being directly transferred to them. In this context, learners who can manage their own learning processes and meet their own learning needs are therefore required (55).

5. CONCLUSIONS

After online case-based teaching practice, the difference in the total mean scores of all students in all scales was found to be statistically significant ($p< 0.01$). After the training have been provided with case-based education via the distance education process, the scoring average of students' professional values, professional behaviors, problem-solving, critical-thinking, and self-directed learning increased. All study hypotheses (H_1, H_2, H_3, H_4, H_5) have therefore been accepted. Use of online case-based teaching is useful in increasing nursing students' nursing skills in distance education. It is therefore important to use new approaches that improve professional skills in nursing education.

One of the limitations of the research is that the data was collected through online forms instead of face-to-face interviews due to the social distance rule and curfews after the COVID-19 pandemic process during the research phase. Another limitation in terms of generalization of the research results is that the results of the research include 3rd grade students of the nursing department of a school selected for the sample group.

Acknowledgments: *The authors are grateful to all the nursing students for their cooperation in this study.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics committee of Akdeniz University Medical Faculty Clinical Research Ethics Committee (Document ID: 70904504/287 dated 29/04/2020)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: DE
Design of the study: DE
Acquisition of data for the study: DE, AS
Analysis of data for the study: AS
Interpretation of data for the study: DE, AS
Drafting the manuscript: DE, AS
Revising it critically for important intellectual content: DE
Final approval of the version to be published: DE, AS

REFERENCES

[1] Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, Lessler J. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Annals of internal medicine 2020.
[2] World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19. Published (11 March 2020). https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19
[3] Huang L, Xu F, Liu H. Emotional responses and coping strategies of nurses and nursing college students during COVID-19 outbreak. MedRxiv. 2020. DOI: 10.1101/2020.03.05.20031898
[4] Telli SG, Altun D. Coronavirüs ve çevrimiçi (online) eğitimin önlenemeyen yükselişi. Üniversite Araştırmaları Dergisi 2020;3(1):25-34.(Turkish) DOI: 10.32329/uad.711110
[5] Karaöz S, Üstün B, Eroğlu K, Doğan S. The situation of nursing education in Turkey during the coronavirus 19 pandemic. Journal of Education and Research in Nursing 2022;19(1):56-64. DOI: 10.5152/jern.2022.21181
[6] Gunawardena CN, McIsaac MS. Distance education. In Handbook of research on educational communications and technology. Routledge; 2013: 361-401.
[7] Clark, JT. Distance education. In: Clinical engineering handbook. Academic Press; 2020: 410-415.
[8] Culha I. Active learning methods used in nursing education. Journal of Pedagogical Research 2019;3(2):74-86. DOI: 10.33902/JPR.201.925.4174
[9] Lee BO, Liang HF, Chu TP, Hung CC. Effects of simulation-based learning on nursing student competences and clinical performance. Nurse Education in Practice 2019;41(2019):102646. DOI:10.1016/j.nepr.2019.102646
[10] Çayır A, Özkal F. The use of simulation in nursing education: The example of myocardial infarction. Research on Education and Psychology 2017;1(1):38-46.
[11] Özkan İ, Taylan S, İlaslan E. The experiences of nursing students towards distance education during the COVID-19 pandemic. International e-Journal of Educational Studies 2021;5(10):106-117. DOI: 10.31458/iejes.942443
[12] Thistlethwaite JE, Davies D, Ekeocha S, Kidd JM, MacDougall C, Matthews P, Purkiss J, Clay D. The effectiveness of case-based learning in health professional education. A BEME systematic review: BEME Guide No. 23. Medical teacher 2012;34(6):421-444. DOI:10.3109/0142159X.2012.680939
[13] George LE, Locasto LW, Pyo KA, Cline TW. Effect of the dedicated education unit on nursing student self-efficacy: A guasi-experimental research study. Nurse Education in Practice 2017; 23: 48-53. DOI: 10.1016/j.nepr.2017.02.007
[14] El Seesy NA, Sofar SM, Al-Battawi JAI. Self-directed learning readiness among nursing students at King Abdulaziz University, Saudi Arabia. IOSR Journal of Nursing and Health Science 2017; 6: 14–24.
[15] HUÇEP. Hemşirelik Ulusal Çekirdek Eğitim Programı, 2014. Retrieved:22.07.2020 (In Turkish).
[16] Lee TW, Ko YK. Effects of self-efficacy, affectivity and collective efficacy on nursing performance of hospital nurses. Journal of Advanced Nursing 2010; 66(4):839-848.
[17] Tyler S, Bourbon E, Cox S, Day N, Fineran C, Rexford D, Rinas J, Shutame K, Ward-Smith P. Clinical competency, self-efficacy, and job satisfaction: Perceptions of the staff nurse. Journal for Nurses in Professional Development 2012;28(1):32-35. DOI: 10.1097/NND.0b013e318240a703
[18] Smith JS, Kirksey KM, Becker H, Brown A. Autonomy and self-efficacy as influencing factors in nurses' behavioral intention to disinfect needleless intravenous systems. Journal of Infusion Nursing 2011; 34(3):193-200. DOI: 10.1097/NAN.0b013e31821478e7
[19] Ançel G. Problem-solving training: Effects on the problem-solving skills and self-efficacy of nursing students. Eurasian Journal of Educational Research 2016;64:231-246. DOI: 10.14689/ejer.2016.64.13
[20] Kaya H. Hemşirelik eğitimi programlarında kuramsal eğitim sorunsalı. Florence Nightingale Journal of Nursing 2017;24(3):175-180. DOI:10.17672/fnhd.88235 (Turkish)
[21] Bvumbwe T. Enhancing nursing education via academic–clinical partnership: An integrative review. International Journal of Nursing Sciences 2016;3(3):314-322. DOI: 10.1016/j.ijnss.2016.07.002
[22] Koçdar S, Karadeniz A, Bozkurt A, Büyük K. Measuring self-regulation in self-paced open and distance learning environments. International Review of Research in Open and Distributed Learning 2018;19(1):24-43. DOI: 10.19173/irrodl.v19i1.3255
[23] Şahin N, Şahin NH, Heppner PP. Psychometric proporties of the problem solving inventory in a group of Turkish university students. Cognitive Therapy and Research 1993;4(17):379-396.
[24] Facione P. California Critical Thinking Skills Test – College level. Technical Report 1. Experimental Validation and Content Validity. Millbrae: California Academic Press; 1990.
[25] Kökdemir D. Eleştirel düşünme ve bilim eğitimi. PiVOLKA. 2003;2(4):3-5. Erişim adresi: http://www. elyadal. org/pivolka/04/PiVOLKA_04_01. pdf. (Turkish)
[26] Weis D, Schank MJ. Development and psychometric evaluation of the nurses professional values scale-revised. Journal of Nursing Measurement 2009;17(3):221-231. DOI: 10.1891/1061-3749.17.3.221
[27] Acaroğlu R. Reliability and validity of Turkish version of the nurses professional values scale-revised. Florence Nightingale Journal of Nursing 2014;22(2):8-16. https://dergipark.org.tr/en/pub/fnjn/issue/30811/333281
[28] Göz F, Geçkil E. Nursing students professional behaviors scale (NSPBS) validity and reliability. Pakistan Journal of Medical Sciences 2010;26(4):938-941.

[29] Ng CF. The physical learning environment of online distance learners in higher education – A conceptual model. Frontiers in Psychology 2021;12:635117. DOI: 10.3389/fpsyg.2021.635117
[30] Oğuz I, Tengilimoğlu D, Tekin PŞ, Tosun N, Zekioğlu A. Evaluation of students' opinions regarding distance learning practices in Turkish universities during the Covid-19 pandemic. Turkish Academy of Sciences 2021;11(3): 607-616. DOI: 10.2399/yod.20.006000
[31] Özveren H, Özden D, Gülnar E. Determination of nursing students' perception states in nursing diagnosis. International Journal of Caring Sciences 2019;12(2):1049-1055.
[32] Chan AW, Chair SY, Sit JW, Wong EM, Lee DT, Fung OW. Case-based web learning versus face-to-face learning: A mixed-method study on university nursing students. The Journal of Nursing Research 2016;24(1):31-40. DOI: 10.1097/jnr.000.000.0000000104
[33] Yoo MS, Park JH. Effect of case-based learning on the development of graduate nurses' problem-solving ability. Nurse Education Today 2014;34(1):47-51. DOI: 10.1016/j.nedt.2013.02.014
[34] Kantar LD, Massouh A. Case-based learning: What traditional curricula fail to teach. Nurse Education Today 2015;35(8):e8-e14. DOI: 10.1016/j.nedt.2015.03.010
[35] Kanbay Y, Okanlı A. The effect of critical thinking education on nursing students' problem-solving skills. Contemporary Nurse 2017;53(3):313-321. DOI: 10.1080/10376.178.2017.1339567
[36] Gholami M, Moghadam PK, Mohammadipoor F, Tarahi MJ, Sak M, Toulabi T, Pour AH. Comparing the effects of problem-based learning and the traditional lecture method on critical thinking skills and metacognitive awareness in nursing students in a critical care nursing course. Nurse Education Today 2016;45(2016):16-21. DOI: 10.1016/j.nedt.2016.06.007
[37] Moyo M, Goodyear-Smith FA, Weller J, Robb G, Shulruf B. Healthcare practitioners' personal and professional values. Advances in Health Sciences Education, 2016;21(2):257-286. DOI 10.1007/s10459.015.9626-9
[38] Kim K, Han Y, Kim J. Korean nurses' ethical dilemmas, professional values and professional quality of life. Nursing Ethics 2015;22(4):467-478. DOI: 10.1177/096.973.3014538892
[39] Peixoto T, Peixoto N. Critical thinking of nursing students in clinical teaching: An integrative review. Revista de Enfermagem Referência 2017;13(2017):125-138. DOI:10.12707/RIV16029
[40] Aluisio AR, Daniel P, Grock A, Freedman J, Singh A, Papanagnou D, Arquilla B. Case-based learning outperformed simulation exercises in disaster preparedness education among nursing trainees in India: A Randomized Controlled Trial. Prehospital and Disaster Medicine 2016;31(5):516-523. DOI:10.1017/S1049023X16000789
[41] Ludin SM. Does good critical thinking equal effective decision-making among critical care nurses? A cross-sectional survey. Intensive and Critical Care Nursing 2018;44(2):1-10. DOI: 10.1016/j.iccn.2017.06.002
[42] Orgun F, Karaoz B. Epistemological beliefs and the self-efficacy scale in nursing students. Nurse Education Today 2014;34(6):e37-e40. DOI:10.1016/j.nedt.2013.11.007
[43] Choi E, Lindquist R, Song Y. Effects of problem-based learning vs. traditional lecture on Korean nursing students' critical thinking, problem-solving, and self-directed learning. Nurse Education Today 2014;34(1):52-56. DOI: 10.1016/j.nedt.2013.02.012
[44] Dirimeşe E, Dicle A. Hemşirelerin ve hemşirelik öğrencilerinin eleştirel düşünme eğilimlerinin değerlendirilmesi. Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi 2012;15(2):89-98. (Turkish)
[45] Siles-Gonzalez J, Solano-Ruiz C. Self-assessment, reflection on practice and critical thinking in nursing students. Nurse Education Today 2016;45(2016):132-137. DOI:10.1016/j.nedt.2016.07.005
[46] Ward M, Knowlton MC, Laney CW. The flip side of traditional nursing education: A literature review. Nurse Education Practice 2018;29(2018):163-171. DOI: 10.1016/j.nepr.2018.01.003
[47] Hong S, Yu P. Comparison of the effectiveness of two styles of case-based learning ımplemented in lectures for developing nursing students' critical thinking ability: A randomized controlled trial. International Journal of Nursing Studies 2017;68(2017):16-24. DOI: 10.1016/j.ijnurstu.2016.12.008
[48] Johnson CO, Petterson J, Rybing J, Nilsson H, Prytz E. Short simulation exercises to ımprove emergency department nurses' self-efficacy for ınitial disaster management: Controlled before and after study. Nurse Education Today 2017;55(2017):20-25. DOI: 10.1016/j.nedt.2017.04.020
[49] Park S. Effects of an intensive clinical skills course on senior nursing students' self-confidence and clinical competence: A quasi-experimental post-test study. Nurse Education Today 2018;61(2018):182-186. DOI:10.1016/j.nedt.2017.11.028
[50] Kaymak Z, Horzum MB. Relationship between online learning readiness and structure and interaction of online learning students. Educational Sciences: Theory and Practice 2013;13(3):1792-1797. DOI: 10.12738/estp.2013.3.1580
[51] Karataş K, Başbay M. Öz yönetimli öğrenmeye hazırbulunuşluk düzeyinin eleştirel düşünme eğilimi, genel öz yeterlik ve akademik başarı açısından yordanması. İlköğretim Online 2014;13(3):916-933. (Turkish).
[52] Van Hooft SM, Becque YN, Dwarswaard J, Van Staa A, Bal R. Teaching self-management support in Dutch bachelor of nursing education: A mixed methods study of the curriculum. Nurse Education Today 2018;68(2018):146-152. DOI:10.1016/j.nedt.2018.06.005
[53] Priesack A, Alcock J. Well-being and self-efficacy in a sample of undergraduate nurse students: A small survey study. Nurse Education Today 2015;35(5):e16-e20. DOI: 10.1016/j.nedt.2015.01.022
[54] Zhang ZJ, Zhang CL, Zhang XG, Liu XM, Zhang H, Wang J, Liu S. Relationship between self-efficacy beliefs and achievement motivation in student nurses. Chinese Nursing Research 2015;2(2-3):67-70. DOI: 10.1016/j.cnre.2015.06.001
[55] Tekkol İA, Demirel M. Öğretmen adaylarının öz-yönetimli öğrenme becerilerine ilişkin görüşlerinin incelenmesi. International Journal of Curriculum and Instructional Studies 2017;6(12):151-168. (Turkish)

How to cite this article: Evgin D, Sümen A. Effect of Online Case-Based Teaching Method on Professional Development of Nursing Students. Clin Exp Health Sci 2023; 13: 9-17. DOI: 10.33808/clinexphealthsci.942370

Clinical and Experimental Health Sciences

Evaluation of Eating Habits and Nutrient Intake in Adolescents with and without Suspected Eating Disorders in Iran

Mansooreh Sadat Mojani-Qomi[1,2], Monireh Hatami[2], Parisa Ziarati[1]

[1] İslamic Azad University, Tehran Medical Sciences, Nutrition and Food Sciences Research Center, Tehran, İran.
[2] İslamic Azad University, Tehran Medical Sciences, Faculty of Pharmacy, Department of Food Science and Technology, Tehran, İran.

Correspondence Author: Mansooreh Sadat Mojani-Qomi
E-mail: mansooreh.moujani@gmail.com
Received: 30.07.2021 **Accepted:** 30.01.2023

ABSTRACT

Objective: Increasing the incidence of eating disorder (ED) attitude during adolescence may lead to inadequate nutrient intake and consequently to growth disorders. This study aimed to compare eating habits and nutrient intakes of adolescents with high risk and low risk of developing eating disorders in high schools in Tehran, Iran.

Methods: In the current cross-sectional study, the Eating Attitude Test (EAT-26) was applied to 299 students (185 females and 114 males) aged 15-18 years for the determination of ED attitudes. Food habits and nutrient intake were assessed by a self-administered questionnaire and a validated Food Frequency Questionnaire (FFQ), respectively. Nutrient adequacy ratio (NAR) and mean adequacy ratio (MAR) were calculated using nutrient's Dietary Reference Intake (DRI) for age and sex.

Results: Of all students, 17.7% were at risk of developing ED. The results showed that girls, unlike boys, consumed more energy than they needed, but this difference between the ED group in both sexes was not significant. The distribution of sweetened drinks, candy, chocolate, and fast food frequency was significantly different between the two groups of girls ($P<0.05$). In contrast, boys at risk of developing ED tended to eat healthier followed by higher vegetable intake and nutritional components. NAR scores were above 1 for all nutrients excluding potassium and vitamin D. The mean MAR in ED groups of boy and girl participants was 2.1% and 13.2% higher than non-ED subjects, respectively.

Conclusion: Eating habits and accordingly nutrient intake can be affected by eating disorder attitudes in girls and boys adolescents.

Keywords: Eating disorder (ED), Eating Habit, Dietary Intake, Adolescents

1. INTRODUCTION

Eating disorder is a public health problem that affects adolescents around the world. The new definition and criteria for diagnosing eating disorders (EDs) are based on the Fifth Diagnostic and Statistical Manual of Mental Disorders (DSM-5), which refers to different types of EDs including anorexia nervosa (AN), bulimia nervosa, binge-eating disorder (BED) and other specified feeding or eating disorders (OSFED) including atypical anorexia nervosa, purging disorder, night eating syndrome, and bulimia nervosa and binge-eating disorder of low frequency and/or limited duration. Moreover, unspecified feeding or eating disorder (UFED) is known as another type that does not meet the criteria for other eating disorders. Pica and rumination disorder, which is a preventive/restrictive eating disorder (ARFID), is a type of eating disorder related to infancy or early childhood (FDIEC) (1). Due to nutritional transition in the Iranian community, eating disorder attitudes became a significant concern in adolescents. In a recent study among adolescents in Iran, the weighted prevalence of all types of eating disorders was estimated at 0.89 (0.81–1.10) with more frequency among girl's participants in urban areas (2). One of the things that should be considered in patients with eating disorders, especially AN, is having different and obsessive eating habits, which can lead to excessive weight loss, and as a result, imbalances in the intake of macronutrients, lack of micronutrients, and specific medical consequences (3, 4). Similarly, a phenomenon related to obesity-eating disorders (binge eating) individuals is food impulsivity, a multidimensional personality trait, that cause uncontrolled and excessive food intake (5), which can cause nutritional ailments and would be alarming (6).

Few studies have demonstrated the eating habits and nutrient intake of Iranian adolescents; Azadbakht et al. (2014) evaluated the Healthy Eating Index (HEI) among 265 girls and found that it was 6.15±1.61 out of 10 points, and except for vitamin D, other nutrients were adequately consumed by the

DOI: 10.33808/clinexphealthsci.976389

adolescents (7). An assessment of eating habits in another study of 3207 teenage girls revealed that more than half of students have daily intake of fruits, while only around 46 % had a consumption of vegetables each day. Dairy products were used by one-third of adolescents daily, and the most skipped meal by individuals was breakfast (15.8%) (8).

Therefore, the unbalanced diet of Iranian teenagers on the one hand, and the increasing prevalence of eating disorders in developing countries on the other hand show the necessity of conducting more research to link these two phenomena to identify possible deficiencies in time. As far as we know, although such studies have been conducted in Iran for the general adolescent population, eating disorders have not been well considered. Thus, the present study was designed to evaluate eating habits and nutrient intake in adolescents with and without suspected eating disorders by gender in Tehran.

2. METHOD

2.1. Participants and Procedure

In the present cross-sectional study, both girl and boy high school students aged 15 to 18 years were selected by random multistage stratified sampling method in Tehran, the capital city of Iran, between October 2019 and September 2020. Upon completion of the written consent form, all participants were eligible to continue. The appropriate sample size was determined by the highest available prevalence of eating disorder attitude (18.9%) in a previous study conducted in Iran (9). The following formula was used to calculate the sample size: $n=(Z1\text{-}a/2)^2pq/d^2$ (n=sample size, $Z_{1-\alpha/2}$ =1.96 (the confidence interval constant at 95 percentile confidence interval), P=the estimate of disordered eating prevalence (9), d=0.05 (determining precision value). Therefore, a total of 236 cases were determined, while a larger sample size was used to control any sample loss. Finally, 299 students` data were eligible for analysis.

2.2. Measurements

Anthropometric parameters were evaluated according to the World Health Organization (WHO) manual for collecting physical measurements (10). Height and weight were measured twice by a trained researcher using pre-calibrated equipment; including a digital scale (Seca-813) to the nearest 0.1 kg for weight and a stadiometer (Seca Model 217) to the nearest 0.1cm for height. To calculate body mass index (BMI), weight (kg) divided by squared height (m^2) and its standard deviation scores (BMI z-scores) derived using the age and sex-specific 2007 WHO percentiles reference data (11).

2.3. Questionnaires

Eating disorder behavior among adolescents was measured by Eating Attitude Test (EAT-26); this self-administered questionnaire is generally used as a screening tool for primary diagnosis of eating disorder (12). EAT-26 was previously translated and validated among Iranian students, the reliability of this test (Cronbach's Alpha) was 0.86 (13), with a cut-off point of the total score of 20 or higher for diagnosis of having eating disorder attitudes (14).

Food habits were obtained from a self-administered questionnaire to determine the frequency of intake of fruits, vegetables, soft and sweetened drinks, chips or french fries, candy/chocolate, fast food and milk, and chips per week (15). A valid 168-item questionnaire (FFQ) (16) was selected to determine the usual diet in adolescents. Data were collected by trained nutritionists through face-to-face and group interviews, all students were trained on how to fill out the questionnaires before completing them. Therefore, the consumption data of each food item in the last year was obtained and the daily intake grams were calculated using the manual of household measures. The collected data were analyzed using Nutritionist IV software and the amount of calories and nutrient intake was evaluated. To assess nutrient adequacy, the average intake of each nutrient was compared with the DRI (17). Nutrient adequacy ratio (NAR) and mean adequacy ratio (MAR) were used as two indicators to evaluate nutrient adequacy compared to the reference value (7); The MAR is based on the calculated by averaging the NAR, a measure that expresses an individual's intake of a nutrient as a ratio or percentage of the corresponding recommended allowance for that nutrient (18). These ratios are specific to age and gender and reflect a reliable explanation of the quality of the diet (19). Meanwhile, this index shows the overall nutritional adequacy of a population based on an individual's diet using a specific nutrient. One strength of MAR is focusing on a population's overall nutritional adequacy, rather than one specific nutrient alone (18).

In this work, NAR values were determined for protein, K, Ca, Mg, Zn, Fe, vitamins A, C, and D, thiamine, riboflavin, niacin, vitamin B6, folate, and vitamin B12. Then, the sum of the NAR of nutrients was divided by the number of nutrients (n=15) to calculate the mean adequacy ratio (MAR). For both NAR and MAR, a value of 1 is ideal, meaning that the intake is equal to the DRI; therefore, a value greater or less than 1 indicates less or more intake than the requirement. The total energy requirements for individuals were calculated by the following formulas (17):

Boys: 88.5 – (61.9 3 Age [yr]) + PA × (26.7 × Weight [kg] + 903 × Height [m]) + 25 kcal

Girls: 135.3 – (30.8 × Age [yr]) + PA × (10.0 × Weight [kg] + 934 × Height [m]) + 25 kcal

2.4. Data Analysis

All data were analyzed by SPSS version 25 (SPSS, Inc, Chicago, Illinois, USA). The data were presented as mean±standard deviation (SD) for continuous variables and numbers and percentages for categorical variables. The normality distribution was tested by the Kolmogorov-Smirnov test. Paired sample t-test was performed to evaluate energy consumption with their energy requirements. One sample t-test was recruited to check the adequacy of nutrient intake with their reference values. To compare the mean of nutrient intakes, NAR, and MAR values between eating disorder groups, independent samples t-test were used, and a Chi-squared test was applied for analysis of the categorical data. Statistical tests were analyzed at the significant level of $P<0.05$.

2.5. Ethical Approval

All participants filled out a written consent form, and they ensured that their information was confidential. The study protocol was approved by the Research Ethical Committee of Tehran Medical Sciences of Islamic Azad University (IR.QUMS.REC.1399.246/15.09.2020).

3. RESULTS

The mean age of students was 15.67 years (SD 0.84) with a mean BMI of 23.78 kg/m^2 (SD 5.19). Fifty-two students (17.7%) were suspected to have EDs, and the prevalence of eating disorder was not statistically significant between boys and girls (*P*=0.81) (Table 1).

Table 1. *Prevalence of disordered eating, weight status, and mean total Eating Attitude Test score of adolescents*

	Total (n=299) Mean ± SD	**Girls (n=185)** Mean ± SD	**Boys (n=114)** Mean ± SD
Age (years)	15.67±0.84	15.40±0.46	16.13±5.19
Body Mass Index (kg/m2)	23.78±5.19	23.02±4.56	24.98±5.88
BMI z-score	0.66±1.28	0.46±1.16	0.92±1.37
	n(%)	n(%)	n(%)
Eating disorder attitude Yes No	 53 (17.7) 246 (82.3)	 33 (17.8) 152 (82.2)	 20 (17.5) 94 (82.5)
Total Eat Score	12.53±9.42	12.43±8.91	12.70±10.21

Table 2. *Eating habits of two categories of adolescents with and without eating disorder by sex in the past week*

	Girls			**Boys**		
Frequency of intake	**EAT-26<20**	**EAT-26≥20**	**Test Value/p**	**EAT-26<20**	**EAT-26≥20**	**Test Value/p**
Fruit						
≤ 2 times/week	13 (8.5)	7 (21.2)	5.42/0.06	4 (4.2)	1 (5.0)	0.47/0.65
3–4 times/week	21 (13.8)	2 (6.1)		15 (16)	2 (10.0)	
≥5 times/week	118 (77.7)	24 (72.7)		75 (79.8)	17 (85.0)	
Vegetable						
≤ 2 times/week	31 (20.4)	7 (21.2)	0.87/0.66	30 (31.9)	1 (5.0)	8.24/**0.03**
3–4 times/week	34 (22.4)	5 (15.2)		16 (17.02)	2 (10.0)	
≥5 times/week	87 (57.2)	21 (63.6)		48 (51.1)	17 (85.0)	
Soft drink						
≤ 2 times/week	132 (86.9)	25 (75.8)	3.02/0.22	57 (60.6)	13 (65.0)	8.56/**0.03**
3–4 times/week	13 (8.5)	6 (18.2)		23 (24.5)	0 (0.0)	
≥5 times/week	7 (4.6)	2 (6.1)		14 (14.9)	7 (35.0)	
Sweetened drink						
≤ 2 times/week	124 (81.6)	28 (84.8)	6.27/**0.04**	70 (74.5)	13 (65.0)	3.07/0.81
3–4 times/week	25 (16.5)	2 (6.1)		14 (14.9)	2 (10.0)	
≥5 times/week	3 (1.9)	3 (9.1)		10 (10.6)	5 (25.0)	
Chips or French Fries						
≤ 2 times/week	104 (68.6)	20 (60.6)	1.45/0.47	67 (71.3)	12 (60.0)	1.60/0.62
3–4 times/week	28 (18.3)	6 (18.2)		13 (14.8)	5 (25.0)	
≥5 times/week	20 (13.1)	7 (21.2)		14 (14.9)	3 (15.0)	
Candy/Chocolate						
≤ 2 times/week	69 (45.4)	17 (51.5)	7.70/**0.02**	58 (61.7)	16 (80.0)	2.42/0.11
3–4 times/week	42 (27.6)	2 (6.1)		14 (13.8)	2 (10.0)	
≥5 times/week	41 (27)	14 (42.4)		22 (23.4)	2 (10.0)	
Fast food						
≤ 2 times/week	101 (66.5)	14 (42.5)	7.61/**0.02**	66 (70.2)	10 (50.0)	3.20/0.24
3–4 times/week	35 (23)	11 (33.3)		13 (13.8)	4 (20.0)	
≥5 times/week	16 (10.5)	8 (24.2)		15 (16)	6 (30.0)	
Milk						
≤ 2 times/week	82 (54)	8 (24.2)	11.80/**0.003**	37 (39.4)	5 (25.0)	1.89/0.49
3–4 times/week	26 (17.1)	13 (39.4)		24 (25.5)	5 (25.0)	
≥5 times/week	44 (28.9)	12 (36.4)		33 (35.1)	10 (50.0)	
Breakfast frequency						
Skippers	73 (48.1)	16 (48.5)	1.03/0.60	36 (38.3)	6 (30.0)	1.72/0.42
(0-2 days/week)	18 (11.8)	2 (6.1)		11 (11.7)	1(5.0)	
Semi-skippers	61 (40.1)	15 (45.4)		47 (50.0)	13 (65.0)	
(3-4 days/week)						
Non-skippers						
(5-7 days/week)						

Data are presented as numbers (%). Significant P values of the chi-squared test were bolded in the table, the df was 2 in all tests. EAT: Eating Attitude Test

Table 3. *Energy consumption and nutrient Intake of two categories of adolescents with and without eating disorder by sex*

	Girls			Boys		
	EAT-26<20	EAT-26≥20	Test Value/p	EAT-26<20	EAT-26≥20	Test Value/p
Total Energy Intake (kcal)	2560.85±600.76	2595.81±812.36	-0.23/0.80	2313.50±626.95[c]	2413.35±602.82[b]	-0.55/0.55
Carbohydrate (g)	334.91±78.21	328.78±92.56	0.35/0.71	300.76±77.95	325.17±85.28	-1.07/0.29
%Calorie	52.68±4.92	51.56±6.20	-	52.61±6.54	54.11±6.64	
Total Fiber	41.93±17.46[c]	37.69±12.86[c]	1.58/0.13	38.97±15.62	46.44±1685	-1.64/0.10
Total Fat (g)	104.15±32.44	110.56±48.37	-0.97/ 0.39	93.73±38.78	92.08±34.46	0.15/0.85
%Calorie	36.17±5.43	37.24±6.89	-	35.68±7.17	34.05±6.92	
Saturated Fat (g)	34.65±13.60	37.08±16.37	-0.89/0.41	30.52±15.54	26.85±13.40	0.83/0.40
%Calorie	12.04±3.53	12.54±3.52	-	11.36±3.42	9.73±3.17	-
Protein (g)	85.12±19.18[c]	87.15±21.29[c]	-0.53/0.60	82.78±20.87[c]	90.49±23.18[c]	-1.26/0.21
%Calorie	13.54±2.31	13.84±2.02	-	14.60±2.48	15.08±1.97	
NAR	1.85±0.42	1.89±0.46		1.59±0.40	1.74±0.45	
Sodium (g)	3.90±0.30[c]	3.920±1.07[c]	-0.11/ 0.91	3.87±0.82[c]	4..08±0.64[c]	-0.92/0.38
Potassium (g)	3.83±1.00[c]	4.03±1.07[c]	-1.01/0.32	4.11±1.50[b]	4.82±1.81	-1.60/0.11
NAR	0.81±0.21	0.86±0.23		0.87±0.32	1.02±0.39	
Calcium (mg)	1219.43±402.86[a]	1226.86±444.77	-0.09/0.89	1238.11±488.10	1432.17±484.48	-1.38/0.16
NAR	0.94±0.31	0.94±0.34		0.95±0.37	1.10±0.37	
Magnesium (mg)	412.29±115.00[c]	412.59±114.68[a]	-0.01/ 0.99	413.59±134.56	473.23±172.82	-1.47/0.12
NAR	1.14±0.32	1.15±0.30		1.01±0.33	1.15±0.42	
Zinc (mg)	11.95±2.83[c]	12.17±3.33[c]	-0.38/0.72	11.65±3.24	12.89±4.14	-1.27/0.16
NAR	1.33±0.31	1.35±0.37		1.06±0.29	1.17±0.38	
Iron (mg)	35.25±23.56[c]	35.93±25.02[c]	-0.15/ 0.88	37.74±28.01[c]	45.67±28.65[b]	-0.98/0.32
NAR	2.35±1.57	2.39±0.1.67		3.34±2.55	4.15±2.60	
Vitamin A (mcg)	643.38±222.99	706.54±184.40	-1.51/ 0.13	753.11±304.64	903.34±382.92	-1.64/0.07
NAR	0.92±0.32	1.01±0.26		0.83±0.34	1.00±0.42	
Vitamin D (IU)	83.87±47.82	93.82±53.16	-1.02/ 0.32	89.28±47.45	88.22±54.39	0.07/0.94
NAR	0.14±0.07	0.16±0.10		0.15±0.07	0.15±0.09	
Vitamin C (mg)	115.86±52.05[c]	127.17±48.66[c]	-1.13/ 0.26	146.02±88.22[c]	200.78±116.37[c]	-2.05/**0.048**
NAR	1.78±0.80	1.96±0.75		1.95±1.18	2.68±1.55	
Thiamin (mg)	1.98±0.67[c]	1.83±0.62[c]	-1.18/ 0.24	1.71±0.44[c]	1.92±0.61[b]	-1.20/0.18
NAR	1.98±0.67	1.83±0.62		1.43±0.37	1.60±0.51	
Riboflavin (mg)	1.96±0.45[c]	1.95±0.49[c]	0.17/0.85	1.96±0.57[c]	2.19±0.65[c]	-1.42/0.17
NAR	1.96±0.45	1.95±0.49		1.50±0.44	1.69±0.50	
Niacin (mg)	25.70±5.98[c]	25.28±5.82[c]	0.36/0.72	24.04±6.59[c]	25.03±6.61[c]	-0.52/0.62
NAR	1.83±0.43	1.81.041		1.50±0.41	1.56±0.41	
Vitamin B6 (mg)	1.95±0.36[c]	1.98±0.33[c]	-0.41/ 0.68	1.97±0.52[c]	2.23±0.58[c]	-1.68/0.11
NAR	1.63±0.30	1.65±0.28		1.51±0.40	1.71±0.44	
Folate (mcg)	562.54±145.29[c]	533.65±135.62[c]	1.04/0.29	519.19±121.63[c]	623.96±159.19[c]	-2.85/**0.005**
NAR	1.65±0.28	1.41±0.34		1.30±0.30	1.56±0.40	
Vitamin B12 (mcg)	3.89±1.62[c]	4.37±2.00[c]	-1.47/ 0.15	3.90±1.63[c]	3.87±1.91[a]	0.05/0.09
NAR	1.62±0.68	1.82±0.83		1.62±0.68	1.61±0.79	
MAR	1.44±0.28	1.47±0.32	-0.46	1.38±0.44	1.59±0.49	-1.64

An Independent sample t-test was used to compare between groups; significant p values were bolded in the table.
a P<0.05, b P<0.01, c P<0.001: Mean values were significantly different with DRI values by one sample t-test. NAR: Data are presented as mean±SD. Nutrient adequacy ratio, MAR: Mean adequacy ratio. EAT: Eating Attitude Test.

Table 2 shows adolescents' eating habits through frequencies of food or meal consumption in the past week. In girl adolescents, the differences in eating habits between the two groups of developing EDs and non-developing EDs were significant for sweetened drinks, candy/chocolate, fast foods, and milk between the two groups (*P*<0.05). Referring to the boys in the EDs group, 85% of students have eaten ≥5 times/week of vegetables, and 65% have consumed less than 2 times/week of soft drinks. There was no significant difference in other eating habits between the two groups of boys (*P>0.05*). The higher percentage of male students complying in the EDs group or non-EDs were breakfast non-skippers (50% and 65%, respectively).

The energy and nutrient intake of adolescents is demonstrated in Table 3. In terms of energy intake, both groups of girls consumed more calories than their needs, but this difference was not significant either for their needs or between the groups (*P>0.05*). Except for potassium and vitamin D, the NAR value for other nutrients was more than 1 in both girl groups (*P<0.05*). However, NAR for calcium was

less than 1 for girls with EAT<20. Regarding boy participants, no significant differences were observed in caloric and macronutrient intake between the high-risk group of EDs and low-risk participants (*P<0.05*), but boys in the two groups consumed significantly less energy than their needs (*P>0.05*). High-risk boys with disorders of eating had a higher intake of vitamin C compared to boys with EAT-26<20 (*P<0.05*). About NAR, similar to girls, all scores were above than 1 excluding potassium intake in non-disordered boys. Dietary fiber was consumed up to 16% higher in eating disordered boys than in their counterparts, but this difference was not statistically significant (*P=0.11*). Sodium intake was above the DRI values in all students (*P<0.05*). The mean MAR for students at risk of ED in both sexes was higher than the non-ED group, while the difference was not statistically significant (*P>0.05*).

4. DISCUSSION

Eating disorders recently became a health issue among adolescents in Iran (20) that can cause many changes in food consumption, eating habits, and nutrient intake of individuals. These changes may lead to a lack of nutrient storage and the appearance of chronic diet-related diseases (21). There seems to be a gap in the literature in this country, so we highlighted the eating habits and nutrient intake of Iranian adolescents with and without suspected eating disorders by gender in Tehran.

Regarding the prevalence of ED, we found a relatively equal prevalence in girls and boys (approximately 17%), whereas a previous study showed that 24.2% of their participants were at risk of ED with a higher prevalence in adolescent girls than boys. Also, the overall prevalence of eating disorders in Iranian children and adolescents was estimated to be 0.89 (0.81-1.10) by Mohammadi et al. (2020) (2). According to the different findings in the prevalence rate in Iran, this difference seems to be related to the region, ethnicity, age, and sample size.

Our results showed that in a population of Iranian adolescents, most students consumed more than five servings of vegetables and fruits per week, separately. The distribution of vegetable intake was significantly different among the two groups of boys; 85% of eating disordered boys were taken more than 5 times vegetables per week. About 76% of girls with high EDs consume milk more than 3 times a week. Similar to our results, Marashi et al. (2019) revealed that from 245 cases, 56.7% and 60.4% consumed more than 6 times per week from fresh fruit juice and vegetables, respectively (22). The frequencies of daily intake of fruits and vegetables were 60.9% and 33.5% in Iranian adolescents of the CASPIAN-V study, too (8).

Skipping breakfast was higher among girl students (about 48% in both EDs groups) than the boy participants; 65% of male participants with ED were non-skippers. Similarly, a meta-analysis including 24 studies among Iranian students (23) indicated that girls skipped breakfast more than boys (26% vs. 18%). Studies among US adolescents also specified that the decrease in consumption of breakfast usually happens in mid-adolescence, especially in girls (24). It was known that irregular and infrequent breakfast intake might be associated with poor metabolic control and increased ED pathology (25). Our results emphasize that boys with ED were less affected by breakfast skipping, and the prevalence of breakfast skipping was not significantly different among EDs and non-EDs participants. Another study has also highlighted that the omission of daily meals including breakfast considered unimportant among eating-disordered subjects (26).

According to our calculations, girls consumed more energy than they needed, while all boys received significantly less energy than their requirements. The interesting thing about our study is that, contrary to our expectations, there is no significant difference in energy consumption between the two ED groups of girls and boys, which was contrary to other studies (26, 27). Given macronutrients, boys consumed less amount of fat than girls; boys in the ED group consumed the least percentage of saturated fat (9.73±3.17) which is the nearest amount to the recommended intake for adolescents (28). All students, even those with EDs, consumed more protein than their recommended value (*P<0.001*). In contrast to our findings, Quiles-Marcos et al. (2011) showed that adolescents with EDs ate fewer meals and had more diets (26). The proportions of macronutrient intake in girl students with ED were similar to the study by Chang et al. (2011) (29).

As regards nutrient intake, it is believed that subjects with any kind of eating disorder behavior may develop nutritional deficiency (1, 30). For instance, a survey on the nutritional intake of Taiwanese adolescents reported that participants with irregular ED scores had a significantly lower intake of energy, protein, carbohydrate, zinc, and vitamins B6 and B12 compared to students with a normal score of Eat-26 (*P<0.05*); conversely, EDs participants consumed more dietary and crude fiber than their counterpart (29). Similarly, results from another study among female athlete adolescents showed that mean carbohydrate and protein intakes as well as some micronutrient intakes were below recommended levels of RDAs/DRIs (31). But, the mean intake of most micronutrients among our students, regardless of gender, was higher than the DRIs. Only potassium and vitamin D intake were not sufficiently used by the adolescents.

5. CONCLUSION

In brief, we conclude that eating habits may differ following eating disorder attitudes in girls and boys adolescents. In a representative sample of students in an urban city in Iran, there were no differences between the two EDs groups in energy intake in both genders. Suspected boys to EDs preferred healthier and nutrient-dense foods. Breakfast-skipping did not frequently take place in students with a risk of EDs in both sexes. Except for vitamin D and potassium, all other nutrients were adequately consumed by all adolescents aged 15-18 years.

We need to mention that there are some limitations in this work; first, because of the cross-sectional nature of this study, we could not express any causal inferences about the associations. Second, the use of additional questionnaires to determine the type of eating disorder in suspected teenagers could be more effective in our justification, which could not be evaluated due to the coronavirus pandemic. So, we recommend performing future longitudinal studies in different regions among girl and boy adolescents to determine the real approach to eating disorders in Iran. In addition, the potential risks and psychological aspects associated with food and dietary changes should be considered in the development of eating disorders. Last but not least, since healthy eating habits and adequate intake of nutrients are very important during adolescence, we emphasize regular dietary assessment and appropriate strategies to solve the nutritional problems of this age group.

Acknowledgments: *The authors would like to thank all study participants. Also, we appreciated the authorities of the Ministry of Education, Iran, and all principals of the selected school for their cooperation to do current work.*

Funding: *The article is taken from a research project approved by the Tehran Medical Sciences from Islamic Azad University on 16th June 2019, and its financial resources have been provided by the Islamic Azad University of Medical Sciences of Tehran.*

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

Ethics Committee Approval: *The study protocol was approved by the Research Ethical Committee of Tehran Medical Sciences of Islamic Azad University (IR.QUMS.REC.1399.246/15.09.2020).*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

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

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author upon request.

REFERENCES

[1] American Psychological Association. Diagnostic and statistical manual of mental disorders: DSM-5, 5th ed.; American Psychiatric Publishing: Arlington, VA, USA, 2013.

[2] Mohammadi MR, Mostafavi SA, Hooshyari Z, Khaleghi A, Ahmadi N, Molavi P, Armani Kian A, Safavi P, Delpisheh A, Talepasand S, Hojjat SK, Pourdehghan P, Ostovar R, Hosseini SH, Mohammadzadeh S, Salmanian M, Alavi SS, Ahmadi A, Zarafshan H. Prevalence, correlates and comorbidities of feeding and eating disorders in a nationally representative sample of Iranian children and adolescents. Int J Eat Disord. 2020;53(3):349-361. DOI: 10.1002/eat.23197

[3] Brambilla F, Monteleone P. Physical complications and physiological aberrations in eating disorders. Eating Disorders. Maj M, Halmi K, López-Ibor JJ, Sartorius N, editors. Wiley Online Library; 2003.6.p.139-222. DOI: 10.1002/047.086.7183.ch3

[4] Tremblay L, Lariviere M. The influence of puberty onset, body mass index, and pressure to be thin on disordered eating behaviors in children and adolescents. Eat Behav. 2009;10(2):75-83. DOI: 10.1016/j.eatbeh.2008.12.001

[5] Davis C. Psychobiological traits in the risk profile for overeating and weight gain. Int J Obes. 2009;33(2): S49-S53. DOI: 10.1038/ijo.2009.72

[6] Da Luz FQ, Hay P, Touyz S, Sainsbury A. Obesity with comorbid eating disorders: associated health risks and treatment approaches. Nutrients. 2018;10(7):829-837. DOI: 10.3390/nu10070829

[7] Azadbakht L, Akbari F, Esmaillzadeh A. Diet quality among Iranian adolescents needs improvement. Public Health Nutr. 2014;18(4):615-621. DOI: 10.1017/S136.898.0014000767

[8] Shayan-Moghadam R, Heidari-Beni M, Riahi R, Motlagh ME, Fesharaki S, Heshmat R, Daniali SSH, Kelishad R. Assessment of lifestyle and eating habits among a nationally representative sample of Iranian adolescent girls: The CASPIAN-V study. Arch Iran Med. 2020;23(8):522-529. DOI: 10.34172/aim.2020.54

[9] Jalali-Farahani S, Chin YS, Mohd Nasir MT, Amiri P. Disordered eating and its association with overweight and health-related quality of life among adolescents in selected high schools of Tehran. Child Psychiatry Hum Dev. 2015;46(3):485-492. DOI: 10.1007/s10578.014.0489-8.

[10] World Health Organization. WHO STEPS Surveillance Manual, Part 3: Data Collection, Section 5: Collecting Step 2 Data: Physical Measurements. Updated [26 January 2017]. Accessed [03 March 2019]. https://www.who.int/docs/default-source/ncds/ncd-surveillance/steps/steps-manual.pdf?sfvrsn=c281673d_5

[11] WHO (2007). The WHO Child Growth Standards. Accessed [12 April 2019]. https://www.who.int/tools/child-growth-standards.

[12] Mintz LB, O'Halloran MS. The Eating Attitudes Test: Validation with DSM-IV eating disorder criteria. J Pers Assess. 2000; 74(3):489-503. DOI: 10.1207/S15327752JPA7403_11

[13] Babaee S, Khodapanah MK, Sedghpour BS. Validity and reliability of Eating Attitude Test. JBS. 2008;1(1):61-68.

[14] Garner DM, Garfinkel PE. The Eating Attitudes Test: an index of the symptoms of anorexia nervosa. Psychol Med. 1979;9(2):273-279. DOI: 10.1017/S003.329.1700030762

[15] Hatami M, Taib MNM, Jamaluddin R, Saad HA, Djazayery A, Chamari M, Nazari M. Dietary factors as the major determinants of overweight and obesity among Iranian adolescents. A cross-sectional study. Appetite. 2014; 82:194-201. DOI: 10.1016/j.appet.2014.07.026

[16] Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Dietary patterns and markers of systemic inflammation among Iranian women. J. Nutrit. 2007;137(4):992-998. DOI: 10.1093/jn/137.4.992

[17] Trumbo P, Schlicker S, Yates AA, Poos M; Food and Nutrition Board of the Institute of Medicine, The National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc. 2002; 102(11):1621-1630. DOI: 10.1016/s0002-8223(02)90346-9.

[18] Steyn NP, Nel J, Labadarios D, Maunder EM, Kruger HS. Which dietary diversity indicator is best to assess micronutrient

adequacy in children 1 to 9 y? Nutrition. 2014;30(1):55-60. DOI: 10.1016/j.nut.2013.06.002

[19] Rouhani MH, Mirseifinezhad M, Omrani N, Esmaillzadeh A, Azadbakht L. Fast food consumption, quality of diet, and obesity among Isfahanian adolescent girls. Journal of Obesity. 2012; 597924. DOI; 10.1155/2012/597924

[20] Garrusi B and Baneshi M R. Body dissatisfaction among Iranian youth and adults. Cadernos de Saude Publica. 2017;33(9):e00024516. DOI: 10.1590/0102-311X00024516

[21] Simons M, Taft T H, Doerfler B, Ruddy J S, Bollipo S, Nightingale S, Siau K, van Tilburg M A. Narrative review: Risk of eating disorders and nutritional deficiencies with dietary therapies for irritable bowel syndrome. Neurogastroenterology & Motility. 2021;34:e14188. DOI:10.1111/nmo.14188

[22] Marashi T, Ramezankhani A, Dadipoor S, Safari-Moradabadi A. Health problem multiple behaviors in Iranian adolescents: A cross-sectional study. International Journal of Adolescence and Youth. 2019;24(2):224-233. DOI: 10.1080/02673.843.2018.1513851

[23] Ghafari M, Doosti-Irani A, Amiri M, Cheraghi Z. Prevalence of the skipping breakfast among the Iranian students: A review article. Iran J Public Health. 2017;46(7):882-889. PMID: 28845398; PMCID: PMC5563869.

[24] Mullan BA, Singh MA. Systematic review of the quality, content, and context of breakfast consumption. Nutrition & Food Science. 2010; 40(1): 81-114. DOI: 10.1108/003.466.51011015953

[25] Wisting L, Reas DL, Bang L, Skrivarhaug T, Dahl-Jørgensen K, Rø Ø. Eating patterns in adolescents with type 1 diabetes: Associations with metabolic control, insulin omission, and eating disorder pathology. Appetite. 2017; 114:226-231. DOI:10.1016/j.appet.2017.03.035

[26] Quiles-Marcos Y, Balaguer-Solá I, Pamies-Aubalat L, Quiles-Sebastián MJ, Marzo-Campos JC, Rodríguez-Marín J. Eating habits, physical activity, consumption of substances and eating disorders in adolescents. Span J Psychol. 2011;14(2):712-723. DOI: 10.5209/rev_sjop.2011.v14.n2.19

[27] Elran-Barak R, Sztainer M, Goldschmidt AB, Crow SJ, Peterson CB, Hill LL, Crosby RD, Powers P, Mitchell JE, Grange DL. Dietary restriction behaviors and binge eating in anorexia nervosa, bulimia nervosa and binge eating disorder: Trans-diagnostic examination of the restraint model. Eat Behav. 2015; 18:192-196. DOI: 10.1016/j.eatbeh.2015.05.012

[28] Mahan LK, Raymond JL. Nutrition in Adolescence. Food & The Nutrition Care Process. 14th ed: Elsevier; 2017. p. 331-351. DOI: 10.1111/pme 12679

[29] Chang Y-J, Lin W, Wong Y. Survey on eating disorder–related thoughts, behaviors, and their relationship with food intake and nutritional status in female high school students in Taiwan. J Am Coll Nutr. 2011;30(1):39-48. DOI: 10.1080/07315.724.2011.10719942

[30] Schmidt R, Hiemisch A, Kiess W, von Klitzing K, Schlensog-Schuster F, Hilbert A. Macro – and micronutrient intake in children with avoidant/restrictive food intake disorder. Nutrients. 2021;13(2). DOI: 10.3390/nu13020400

[31] 31. Beals KA. Eating behaviors, nutritional status, and menstrual function in elite female adolescent volleyball players. J Am Diet Assoc. 2002;102(9):1293-1296. DOI: 10.1016/S0002-8223(02)90285-3

How to cite this article: Mojani-Qomi MS, Hatami M, Ziarati P. Evaluation of Eating Habits and Nutrient Intake in Adolescents with and without Suspected Eating Disorders in Iran. Clin Exp Health Sci 2023; 13: 18-24. DOI: 10.33808/clinexphealthsci.976389

Clinical and Experimental Health Sciences

The Relationship between Pregnancy-Related Low Back Pain, Kinesiophobia, and Physical Activity in the Third Trimester

Büşra Kepenek Varol[1], Ayşen Aydoğdu[1], Esra Nur Temur[1],Göksu Fırat[2], Merve Selvi[1], Meltem Yazıcı Gülay[2], Hüseyin Aksoy[2]

[1] Nuh Naci Yazgan University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Kayseri, Türkiye.
[2] Çankırı Karatekin University, Faculty of Health Sciences, Department of Occupational Therapy, Çankırı, Türkiye.
[3] University of Health Sciences, Kayseri Health Practice and Research Center, Department of Obstetrics and Gynecology, Kayseri, Türkiye.

Correspondence Author: Büşra Kepenek Varol
E-mail: busrakepenek@gmail.com

Received: 19.08.2021 **Accepted:** 26.10.2022

ABSTRACT

Objective: The aim of this study is to examine the relationship between pregnancy-related low back pain, kinesiophobia, and physical activity during the third trimester of pregnancy.

Methods: Fifty-one pregnant women between the ages of 18-40, at the gestational age of 27 weeks and above were included in the study. The intensity of pregnancy-related low back pain was assessed with the Numerical Pain Scale, kinesiophobia with the Tampa Kinesiophobia Scale, and physical activity levels with the short form of the International Physical Activity Questionnaire.

Results: Only 4 (7.8%) subjects had high levels of physical activity, 12 (23.5%) had moderate, and 35 (68.6%) had low levels of physical activity. A strong positive correlation was found between low back pain and kinesiophobia in the third trimester of pregnancy ($r = 0.796$; $p < 0.001$). There was no statistically significant relationship between low back pain and physical activity or between kinesiophobia and physical activity ($r = -0.097$; $p = 0.498$ and $r = -0.212$; $p = 0.135$ respectively).

Conclusion: Pregnancy-related low back pain can cause kinesiophobia during pregnancy, and kinesiophobia may cause limitations of movements. Considering the negative effects of pregnancy-related low back pain and kinesiophobia during pregnancy, the evaluation of pain, and kinesiophobia by health professionals is important during this period.

Keywords: low back pain, kinesiophobia, physical activity, pregnancy

1. INTRODUCTION

Pregnancy is a natural phenomenon, during which women experience physical, physiological, biomechanical, and psychological changes (1-3). Physical problems experienced during pregnancy differ according to the characteristics of the mother, the pregnancy process, and the trimesters. While nausea-vomiting, nasal congestion, fatigue, and breast tenderness are observed in the first trimester, these problems stop in the second trimester but conditions such as constipation, headache, and hypotension can be observed. In the third trimester; fatigue, insomnia, edema in the lower extremities, varicose veins, shortness of breath, and joint pain are experienced due to the developing and growing fetus and the preparation of the mother's body for birth (1).

Low back pain is one of the most common musculoskeletal complaints during pregnancy, and pregnancy-related low back pain can have adverse effects on the pregnant woman's life (4). However, pregnancy-related low back pain increases in the later stages of pregnancy due to reasons such as weight gain, the growing fetus, and the displacement of the center of gravity (4-7). Kinesiophobia, which is also defined as the avoidance of movement, anxiety about movement, or fear of pain due to movement, may develop in patients with low back pain (8,9). Some activities during pregnancy can increase low back pain, and increased musculoskeletal pain brings on pain-related fear that leads to avoidance of activities (2,10,11). Considering that pregnancy-related low back pain in pregnant women will negatively affect daily life activities, the fear of movement may increase as pregnancy progresses (2). There is limited literature investigating kinesiophobia in pregnancy and almost all of the studies investigating the relationship between pregnancy-related low back pain and kinesiophobia focus on the postpartum period (2,10,11). In a study investigating pregnant women in the postpartum period, higher levels of kinesiophobia were reported in moderately disabled cases due to pregnancy-related low back pain (10). In another study by Gutke et al. (11), it was shown that disability due to postpartum lumbopelvic pain was associated with kinesiophobia. To the best of our knowledge, only one study questioned kinesiophobia related

DOI: 10.33808/clinexphealthsci.984617

to pregnancy-related low back pain in late pregnancy and reported that kinesiophobia negatively affected depressive symptoms 1 month after delivery (2).

Physical activity decreases for many different reasons during pregnancy and this risk increases in the later stages of pregnancy (12). De Sousa et al. (13) reported that sedentary pregnant women experienced more severe pain than active women. On the other hand, it is possible that the risk of low back pain increases in cases where physical activity decreases irrespective of the pregnancy (14), and that kinesiophobia is observed in patients with low back pain (15,16). Based on this information, we hypothesized that increasing pregnancy-related low back pain increases kinesiophobia and decreases physical activity among pregnant women in their third trimester. This study aims to examine the relationship between pregnancy-related low back pain, kinesiophobia, and physical activity in the third trimester of pregnancy.

2. METHODS

This cross-sectional study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Erciyes University under protocol number 96681.246.2019/727. Written informed consent was obtained from all subjects included in the study.

2.1. Participants

The pregnant women followed up for antenatal care in the Kayseri City Hospital, Gynecology and Obstetrics Clinic formed the study sample. Inclusion criteria were being aged between 18-40 years, having a spontaneous pregnancy, having low back pain due to pregnancy, and having a gestational age of 28 weeks and above. The exclusion criteria were high-risk pregnancy detected by the physician, pregnancy with assisted reproductive techniques, having a different type of pain other than pregnancy-related low back pain, having gestational diabetes, having chronic neurological, endocrine, orthopedic, and rheumatological diseases before pregnancy, and low back pain longer than 3 months due to any reason before pregnancy. The low back pain was diagnosed by the obstetrician according to the International Classification of Functioning (ICF) criteria (17).

Assessments

2.2.1. Demographic Variables

Socio-demographic characteristics such as age, education level, occupation, smoking, alcohol use, being on medication, previous pregnancies and the number of children, the gestational week, a multiple pregnancy, and weight gain during pregnancy were recorded.

2.2.2. Numerical Rating Scale

The Numerical Rating Scale (NRS) is a scale containing 11, 21, or 101 points, and one end of the scale reflects “no pain” while the other end represents “the worst pain possible”. It is one of the most frequently clinically used scales due to its ease of administration in the assessment of pain intensity. In practice, the patient is asked to choose the number that best reflects the severity of their pain on a scale with numerical values (18). In our study, an 11-point NRS (0= no pain, 10= unbearable pain) was used to evaluate the pregnancy-related low back pain levels of the women. The subjects were informed about the use of the scale before evaluation and were asked to rate and circle the number that reflected their pain on the scale from 0 to 10.

2.2.3. Tampa Kinesiophobia Scale

The Tampa Kinesiophobia Scale (TKS) is a questionnaire used for rating the fear of movement. A 4-point Likert scoring system (1= strongly disagree, 4= strongly agree) is used for each of the 17 items on the scale. The total score ranges from 17 to 68 where higher scores indicate increased severity of kinesiophobia. A total score is calculated after the inversion of the scores of items 4, 8, 12, and 16 (9,19). The fear of movement of the pregnant women included in the study was evaluated using the Turkish version of the TKS (9). The subjects were asked to mark the option that suited them best for each item, and the total score was calculated.

2.2.4. International Physical Activity Questionnaire – Short Form

The International Physical Activity Questionnaire (IPAQ) is a tool developed by researchers from various countries with the support of the World Health Organization to measure physical activity. The Turkish validity and reliability of the questionnaire, which has a long form consisting of 27 items and a short form of 7 items, was conducted by Sağlam et al. (20). The short form of IPAQ was used in this study, which assesses physical activity across a variety of domains including walking, moderate and vigorous activity in the past week (or last 7 days), and estimated time spent sitting per week. The vigorous, moderate, and walking scores were calculated by reported minutes within each category by an average metabolic equivalent (MET) score (3.3 MET for walking, 4 MET for moderate activity, 8 MET for vigorous activity), and the total physical activity score was calculated by summing the results of all categories. The question about sitting time (sedentary behavior), which is not a part of the summed physical activity score, is not included in the physical activity total score (20,21) in our study either. Based on IPAQ total score, physical activity levels were classified as high (>3000 MET-min/week), moderate (600-3000 MET-min/week), and low level (<600 MET-min/week).

2.3. Statistical Analysis

Correlation coefficients ranging from 0.293 and 0.604 were reported for the relationship between kinesiophobia (evaluated with the TKS) and low back pain (15,22). In this study, we hypothesized to detect a significant relationship

between kinesiophobia and low back pain with a correlation coefficient of 0.400 in pregnant women. The sample size was determined as 46 subjects but was increased by 10% in case of possible missing values to a total of 51 subjects to detect this relationship with a 95% confidence level and 80% power (23). Qualitative variables are presented as percentages and quantitative variables are shown as means with standard deviations. Data analyses were performed using the SPSS V.20 (SPSS Inc., USA) program. The Kolmogorov–Smirnov and Shapiro-Wilk tests were used to evaluate whether the data had a normal distribution. Spearman correlation coefficient and statistical significance were calculated to detect the relationships between variables. The significance level was accepted as $p< 0.05$.

3. RESULTS

None of the pregnant women included in the study were on medication other than supplementary vitamin/mineral pills used under physician supervision during pregnancy. Although none of the pregnant women used alcohol, 5 of them (10%) were smokers. Only 1 case had multiple (twin) pregnancy. Other demographic and clinical characteristics of the women included in the study are shown in Table 1. The intensity of low back pain, kinesiophobia scores, physical activity scores and levels are shown in Table 2.

Table 1. *Demographic and clinical characteristics*

	X ± SD	MIN – MAX
Age (year)	28 ± 5.19	19 – 38
Gestational age (week)	36.3 ± 2.8	28 – 40
	n	**%**
Educational level		
Primary school	13	25.5
Secondary school	17	33.3
High school	14	27.5
University medium degree	5	9.8
University higher degree	2	3.9
Occupation		
Housewife	43	84.3
Official	3	5.9
Self-employed	3	5.9
Other	2	3.9
Weight gain (kg)		
5 – 10	3	5.9
11-15	15	29.4
16-20	25	49
≥ 21	8	15.7
Number of previous pregnancies		
0	14	27.5
1	17	33.3
2	15	29.4
≥ 3	5	9.8
Number of miscarriages		
0	47	92.1
1	3	5.9
2	1	2

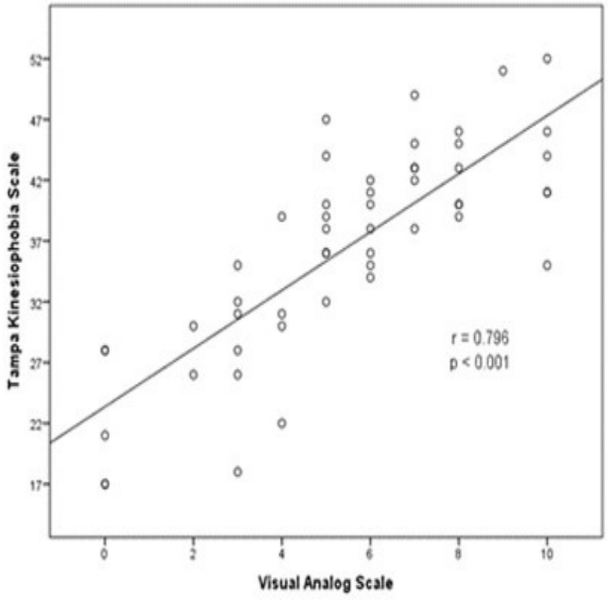
X ± SD = mean ± standard deviation; MIN – MAX = minimum – maximum

As a result of the study, a strong positive correlation ($r= 0.796$; $p< 0.001$) was found between low back pain and kinesiophobia in pregnant women. There was no statistically significant relationship between low back pain and physical activity ($r= – 0.097$; $p= 0.498$) and between kinesiophobia and physical activity ($r= – 0.212$; $p= 0.135$) (Figure 1).

Table 2. *Pregnancy-related low back pain, kinesiophobia and physical activity scores and physical activity levels of subjects*

	X ± SD	MIN – MAX
VAS	5.47 ± 2.87	1 – 10
TKS	36.5 ± 8.56	17 – 52
IPAQ (MET-min/week)		
Vigorous	23.5 ± 168	0 – 1200
Moderate	206.7 ± 734.2	0 – 5040
Walking	586.9 ± 943.3	0 – 2772
IPAQ Total	807.3 ± 1216	0 – 5544
Sitting	365.3 ± 226.7	45 – 900
	n	**%**
The physical activity level according to IPAQ		
Low	35	68.6
Moderate	12	23.5
High	4	7.8

X ± SD = mean ± standard deviation; MIN – MAX = minimum – maximum; VAS = Visual Analog Scale; TKS = Tampa Kinesiophobia Scale; IPAQ = International Physical Activity Questionnaire; MET = metabolic equivalent task



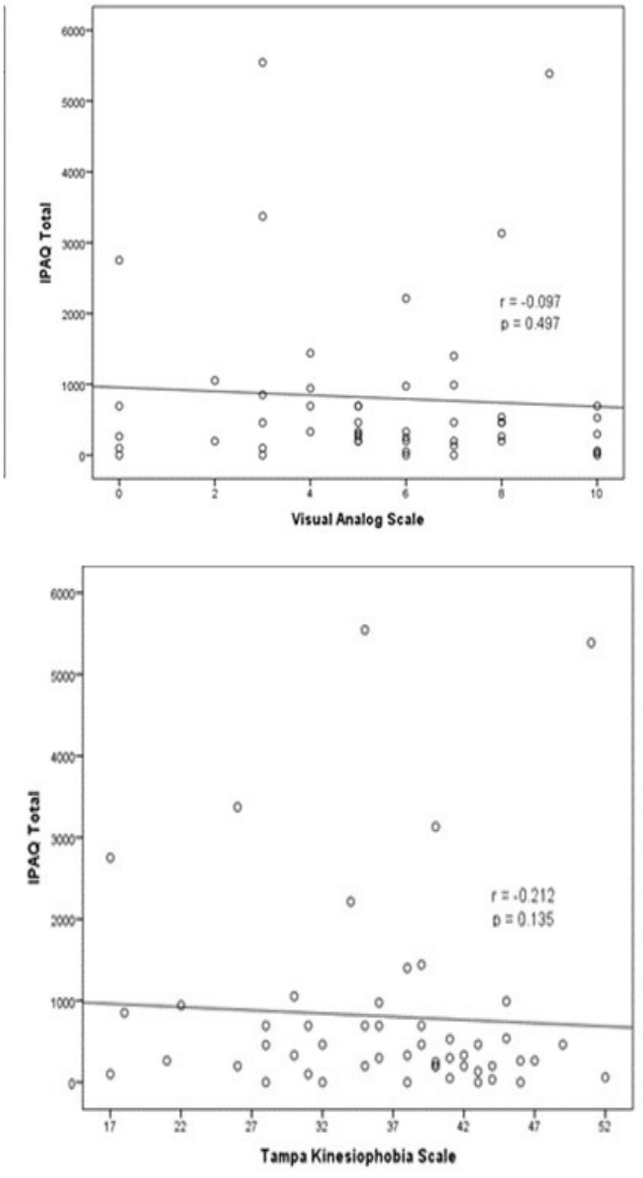


Figure 1. *The relationships between pregnancy-related low back pain and kinesiophobia and physical activity. IPAQ_ International Physical Activity Scale*

4. DISCUSSION

This study is one of the limited studies investigating kinesiophobia caused by pregnancy-related low back pain in the literature. The relationship between pregnancy-related low back pain, kinesiophobia, and physical activity in the third trimester was investigated and a strong positive correlation was found between low back pain and kinesiophobia, while no statistically significant correlation was found between kinesiophobia and physical activity, and between pregnancy-related low back pain and physical activity.

Hormonal, vascular, and biomechanical changes that occur in the body during pregnancy to adapt to the growing fetus play a role in the etiology of low back pain, which is one of the most common types of pain in pregnancy (13). Low back pain during pregnancy causes limitations on the ability to work efficiently, leading to poor quality of life (24,25). Since it is known that the occurrence of low back pain and the symptoms increase as the pregnancy progresses (24), pregnant women in their third trimester were included in our study.

Kinesiophobia is defined as the fear of movement that occurs after a painful injury and limits physical movement (9,19). It has been reported that kinesiophobia reduces the quality of life and increases disability in patients with low back pain (8,15,22). However, while there are a limited number of studies investigating kinesiophobia in pregnancy, most of these studies focus on the postpartum period (2,10,11). In a study conducted by Gutke et al. (11), it was reported that pain severity, quality of life, and kinesiophobia explained the postpartum disability due to lumbopelvic pain and that lumbopelvic pain was observed in one in three postpartum women. In another study investigating disability in the postpartum period due to pregnancy-related low back pain, it was found that kinesiophobia increased in subjects with pregnancy-related low back pain with moderate disability (10). Ebina et al. (2) reported that depressive symptoms increased in late pregnancy and 1 month after delivery in women with pregnancy-related low back pain, and pointed out that approaches to treating kinesiophobia in late pregnancy may reduce the risk of postpartum depressive symptoms. On the other hand, it was demonstrated that kinesiophobia scores decreased in the group treated by a physiotherapist for pregnancy-related low back pain through the third week postpartum (26). In this study, a strong correlation was found between pregnancy-related low back pain and kinesiophobia after the evaluation in the third trimester. Although most of the studies in the literature examine the relationship between kinesiophobia and pregnancy-related low back pain that continues in the postpartum period, we think that it is important to evaluate kinesiophobia during pregnancy to ensure a healthier pregnancy considering the negative effects of kinesiophobia. In addition, pregnancy-related low back pain can be observed from the beginning of pregnancy, although it is more common in the later stages of pregnancy. It would be more helpful for future studies to include all three trimesters of pregnancy.

Physical activity constitutes one of the objectives of multidisciplinary programs for chronic low back pain (27,28). However, the pain was also stated as the main barrier to performing physical activities (28). Marshall et al. (29) showed that the fear of movement significantly mediated the relationship between pain and disability, and the effect of fear on pain-related disability was not related to regular physical activity in patients with chronic low back pain. In another study, no relationship was reported between kinesiophobia and physical activity in patients with low back pain (30). In addition, it has been reported that individuals who exercise do not have work-related kinesiophobia and that they have a lower level of disability, and a low risk of developing low back pain (31). There are a lot of studies in the literature investigating the level of physical activity during

pregnancy and/or during specific periods of pregnancy, and these studies show that physical activity decreases in late pregnancy (12,13,32). It has been reported that sedentary pregnant women are likely to have higher lumbar and pelvic pain intensity than physically active pregnant women (13). In our study, only 4 (7.8%) pregnant women had a high level of physical activity, 12 (23.5%) had moderate, and 35 (68.6%) had a low level of physical activity. Similarly, in a study conducted in Turkey, it was shown that the physical activity levels of pregnant women were low (33). According to a study conducted by Kitiş et al. (34) in Turkey, low physical activity levels of women (regardless of pregnancy) over the age of 20 were evaluated using the short form of IPAQ, and it was reported that 72.5% of these women did not consider exercising. The authors also reported in the same study that most of the women were housewives (67.9%) and had children (82.6%). The method of assessing physical activity and demographic information in our study was similar to the study performed by Kitiş et al. (34). The pre-pregnancy physical activity level of the subjects included in the study was not questioned, so we do not know whether the low physical activity level is due to pregnancy. One reason why physical activity was not associated with kinesiophobia and pregnancy-related low back pain in our study may be explained by the low level of physical activity among women in our country before pregnancy. Another reason may be that more than half of the participants had a low physical activity level, and the sample size of participants with high and moderate physical activity levels was small. The fact that the physical activity levels were not distributed in similar percentages among the participants may have caused the outcome of no statistically significant relationship between physical activity and the other parameters, and the low physical activity levels of the majority (68%) of the patients may have caused the outcome of lack of correlation. More studies should be conducted with a similar percentage of participants from all three levels (low, moderate, and high) of physical activity.

Kinesiophobia due to pregnancy-related low back pain may also increase according to the pain intensity (10). In addition, pregnancy-related low back pain is associated with limitations on the woman's ability to work effectively, leading to reduced quality of life, and as a result, women's productivity in daily life activities decreases (24,25,35). Based on this information, it is important to note that kinesiophobia may affect daily life activities, although no relationship was found between physical activity and kinesiophobia in our study.

Non-pharmacological treatments such as soft tissue mobilization, posture training, stabilization exercises, and electrotherapy approaches, which are generally applied by physiotherapists, have been suggested to be used first in the treatment of low back pain during pregnancy (24,36). In addition, increasing physical activity during pregnancy has many positive effects such as an increase in the quality of life and cardiorespiratory fitness, a decrease in depressive symptoms, the risk of preterm birth, and gestational diabetes (37). For this reason, we think that it is important to identify the pregnant women in need of such treatment with comprehensive evaluations from the early stages of pregnancy to maintain the pregnancy in the best way for both the mother and the baby.

Several limitations may have affected our findings. First, pregnancy-related low back pain was not divided into categories such as lumbal, pelvic, or both. Second, the time and duration of the onset of pain were not questioned. Third, multidimensional scales such as the McGill Melzack Pain Questionnaire, etc. were not used. The NRS is simple and easy for patients to understand, but given the subjectivity of the scale, the complexity of symptoms, and the multifactorial aspect of pain, individuals may have had difficulty isolating the perception of pain from other psycho-cognitive factors. Fourth, the lack of homogeneous physical activity levels among the sample in the study and the fact that the majority of the subjects (68%) had low physical activity levels may have caused the outcome of no correlation. The last and most important limitation is that the pre-pregnancy physical activity levels of the subjects are unknown. Future studies investigating all stages of pregnancy from the beginning should be conducted. We also believe that prospective follow-up studies would be more useful in understanding the process. Studies with a similar sample size with various physical activity levels can provide a better comparison of the effects of kinesiophobia and pregnancy-related low back pain between physical activity levels.

5. CONCLUSION

In this study, although it was noted that pregnancy-related low back pain in the third trimester of pregnancy causes kinesiophobia, no relationship was found between physical activity and pregnancy-related low back pain or between physical activity and kinesiophobia. However, a relationship was found between pregnancy-related low back pain and kinesiophobia. Kinesiophobia may cause limitations of movements, especially daily life activities during pregnancy. Moreover, the results of our study showed that more than half of the pregnant women had low physical activity levels in the third trimester. Considering the negative effects of pregnancy-related low back pain and kinesiophobia during pregnancy, it is important to monitor and evaluate pain, and kinesiophobia during this period by health professionals.

Funding: *The authors received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics committee of Erciyes University (Date: 23/10/2019; Number of approval: 2019/727)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: BKV
Design of the study: BKV, AA, ENT, GF, MS
Acquisition of data for the study: AA, ENT, GF, MS
Analysis of data for the study: BKV, MY
Interpretation of data for the study: BKV, MYG, HA
Drafting the manuscript: BKV
Revising it critically for important intellectual content: BKV, MYG, HA

Final approval of the version to be published: BKV, HA

REFERENCES

[1] Çetin FC, Demirci N, Çalık KY, Akıncı AÇ. Gebelïkte olağan fizïksel yakınmalar. Zeynep Kamil Tıp Bülteni 2017;48(4):135-141. (Turkish)

[2] Ebina A, Sawa R, Kondo Y, Murata S, Takada M, Fuji H, Okuyama Y, Tanikawa Y, Souke K, Ono R. Influence of kinesiophobia with pregnancy-related lumbopelvic pain at late pregnancy on postpartum depressive symptoms. Phys Ther Res. 2020;23(1):92-98.

[3] DiPietro L, Evenson KR, Bloodgood B, Sprow K, Troiano RP, Piersy KL, Vaux-Bjerke A, Powell KE. Benefits of physical activity during pregnancy and postpartum; an umbrella review. Med Sci Sport Exerc. 2016;176(1):100-106.

[4] Morino S, Ishihara M, Umezaki F, Hatanaka H, Iijima H, Yamashita M, Aoyama T, Takahashi M. Low back pain and causative movements in pregnancy: A prospective cohort study. BMC Musculoskelet Disord. 2017;18(1):1-8.

[5] Borg-Stein J, Dugan SA. Musculoskeletal disorders of pregnancy, delivery and postpartum. Phys Med Rehabil Clin N Am. 2007;18(3):459-476.

[6] Larsen PS, Strandberg-Larsen K, Juhl M, Svendsen SW, Bonde JP, Anne-Marie AN. Occupational lifting and pelvic pain during pregnancy: A study within the danish national birth cohort. Scand J Work Environ Heal. 2013;39(1):88-95.

[7] Robinson HS, Mengshoel AM, Bjelland EK, Vøllestad NK. Pelvic girdle pain, clinical tests and disability in late pregnancy. Man Ther. 2010;15(3):280-285.

[8] Picavet HSJ, Vlaeyen JWS, Schouten JSAG. Pain catastrophizing and kinesiophobia: Predictors of chronic low back pain. Am J Epidemiol. 2002;156(11):1028-1034.

[9] Tunca Yılmaz Ö, Yakut Y, Uygur F, Ulu N. Tampa Kinezyofobi Ölçe i'nin Türkçe versiyonu ve test-tekrar test güvenirli i. Fizyoter Rehabil. 2011;22(1):44–49. (Turkish)

[10] Beales D, Lutz A, Thompson J, Wand BM, O'Sullivan P. Disturbed body perception, reduced sleep, and kinesiophobia in subjects with pregnancy-related persistent lumbopelvic pain and moderate levels of disability: An exploratory study. Man Ther. 2016;21:69-75.

[11] Gutke A, Lundberg M, Östgaard HC, Öberg B. Impact of postpartum lumbopelvic pain on disability, pain intensity, health-related quality of life, activity level, kinesiophobia, and depressive symptoms. Eur Spine J. 2011;20(3):440-448.

[12] Román-Gálvez MR, Amezcua-Prieto C, Salcedo-Bellido I, Olmedo-Requena R, Martínez-Galiano JM, Khan KS, Bueno-Cavanillas A. Physical activity before and during pregnancy: A cohort study. Int J Gynecol Obstet. 2021;152(3):374-381.

[13] De Sousa VPS, Cury A, Eufrásio LS, De Sousa SES, Coe CB, De Souza Ramalho Viana E. The influence of gestational trimester, physical activity practice and weight gain on the low back and pelvic pain intensity in low risk pregnant women. J Back Musculoskelet Rehabil. 2019;32(5):671-676.

[14] Park SM, Kim HJ, Jeong H, Kim H, Chang BS, Lee CK, Yeom JS. Longer sitting time and low physical activity are closely associated with chronic low back pain in population over 50 years of age: a cross-sectional study using the sixth Korea National Health and Nutrition Examination Survey. Spine J. 2018;18(11):2051-2058.

[15] Uçurum SG, Kalkan AC. Bel ağrılı hastalarda ağrı, kinezyofobi ve yaşam kalitesi arasındaki ilişki. Ege Tıp Dergisi 2018;57(3):131-135. (Turkish)

[16] Özmen T, Gündüz R, Doğan H, Zoroglu T, Acar D. Relationship between kinesiophobia and quality of life in patients with low back pain. FÜ Sağ Bil Tıp Derg. 2016;30(1):1-4.

[17] Delitto A, George SZ, van Dillen L, Whitman JM, Sowa GA, Shekelle P, Denninger TR, Godges JJ, Beneciuk JM, Bishop MD, Cramer CD, Koch W, Shephard M, Abbott H, Altman RD, Briggs M, Butler D, Farrell JP, Ferland A, Fearon H, Fritz JM, MacDermid J, Matheson JW, McClure P, McGill SM, Torburn L, Werneke M. Low Back Pain: Clinical Practice Guidelines Linked to the International Classification of Functioning, Disability, and Health from the Orthopaedic Section of the American Physical Therapy Association. J Orthop Sports Phys Ther. 2012;42(4):A1-A57.

[18] Williamson A, Hoggart B. Pain: A review of three commonly used pain rating scales. J Clin Nurs. 2005;14(7):798-804.

[19] Lundberg MKE, Styf J, Carlsson SG. A psychometric evaluation of the Tampa Scale for Kinesiophobia – From a physiotherapeutic perspective. Physiother Theory Pract. 2004;20(2):121-133.

[20] Sağlam M, Arıkan H, Savcı S, İnal-İnce D, Boşnak-Güçlü M, Karabulut E, Tokgözoğlu L. International physical activity questionnaire: Reliability and validity of the Turkish version. Percept Mot Skills. 2010;111(1):278-284.

[21] Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P. International physical activity questionnaire: 12-Country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-1395.

[22] Thomas EN, Pers YM, Mercier G, Cambiere JP, Frasson N, Ster F, He´risson C, Blotman F. The importance of fear, beliefs, catastrophizing and kinesiophobia in chronic low back pain rehabilitation. Ann Phys Rehabil Med. 2010;53(1):3-14.

[23] Algina J, Olejnik S. Sample size tables for correlation analysis with applications in partial correlation and multiple regression analysis. Multivariate Behav Res. 2003; 38(3):309-323.

[24] Manyozo SD, Nesto T, Bonongwe P, Muula AS. Low back pain during pregnancy: Prevalence, risk factors and association with daily activities among pregnant women in urban Blantyre, Malawi. Malawi Med J. 2019;31(1):71-76.

[25] Mota MJ, Cardoso M, Carvalho A, Marques A, Sá-Couto P, Demain S. Women's experiences of low back pain during pregnancy. J Back Musculoskelet Rehabil. 2015;28(2):351-357.

[26] Bastiaenen CHG, de Bie RA, Vlaeyen JWS, Goossens MEJB, Leffers P, Wolters PMJC, Bastiaanssen JM, van den Brandt PA, Essed GGM. Long-term effectiveness and costs of a brief self-management intervention in women with pregnancy-related low back pain after delivery. BMC Pregnancy Childbirth 2008;8:1-14.

[27] Chou R, Huffman LH. Clinical guidelines annals of internal medicine nonpharmacologic therapies for acute and chronic low back Pain: a review of the evidence for an American pain society/American college of physicians clinical practice guideline. Ann Intern Med. 2007;147:492-504.

[28] Boutevillain L, Dupeyron A, Rouch C, Richard E, Coudeyre E. Facilitators and barriers to physical activity in people with chronic low back pain : A qualitative study. PloS one. 2017;12(7):e0179826.

[29] Marshall PWM, Schabrun S, Knox MF. Physical activity and the mediating effect of fear, depression, anxiety, and

catastrophizing on pain related disability in people with chronic low back pain. PloS one. 2017;12(7):e0180788.

[30] Sertel M, Aydoğan Arslan S, Tütün Yümin E, Demirci CS, Tarsuslu Şimşek T. Investigation of the relationship between physical activity, kinesiophobia and fear of falling in older adults with chronic pain. Somatosens Mot Res. 2021;38(3):241-247.

[31] Silva BAMD, Gelain GM, Candotti CT. The influence of physical exercise on behavioral habits, kinesiophobia, and disability in people with low back pain: A retrospective cross-sectional study. J Bodyw Mov Ther. 2021;28:348-353.

[32] Mudd LM, Owe KM, Mottola MF, Pivarnik JM. Health benefits of physical activity during pregnancy: An international perspective. Med Sci Sports Exerc. 2013;45(2):268-277.

[33] Daşıkan Z, Güner Ö, Bozkurt T. İkinci ve Üçüncü Trimester Gebelerin Fiziksel Aktivite Düzeyi ve Fiziksel Aktivite Engelleri Adıyaman Üni Sağlık Bilim Derg. 2019;5(3):1731-1745. (Turkish)

[34] Kitiş Y, Gümüş Y. 20 yaş ve üzeri kadinlarin fiziksel aktivite düzeyleri, fiziksel aktiviteye ilişkin inançlari ve davranis aşamalarinin belirlenmesi. Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi 2015;4(3):399-411. (Turkish)

[35] Wang SM, Dezinno P, Maranets I, Berman MR, Caldwell-Andrews AA, Kain ZN. Low back pain during pregnancy: Prevalence, risk factors, and outcomes. Obstet Gynecol. 2004;104(1):65-70.

[36] Katonis P, Kampouroglou A, Aggelopoulos A, Kakavelakis K, Lyoudis S, Makrigiannakis A, Alpantaki K. Pregnancy-related low back pain. Hippokratia 2011;15(3):205-210.

[37] Sayed H, Slayman T, DuChene Thoma K. ACOG Committee Opinion No. 804: Physical Activity and Exercise during Pregnancy and the Postpartum Period. Obs Gynecol. 2021;137:375-376.

How to cite this article: Kepenek Varol B, Aydoğdu A, Temur EN, Fırat G, Selvi M, Yazıcı Gülay M, Aksoy H. The Relationship between Pregnancy-Related Low Back Pain, Kinesiophobia, and Physical Activity in the Third Trimester. Clin Exp Health Sci 2023; 13: 25-31. DOI: 10.33808/clinexphealthsci.984617

Clinical and Experimental Health Sciences

COVID-19-Related Obsessions and Its Predictors: A Community-Based Research in Turkey

Esra Çınar Tanrıverdi[1], Büşra Arslan Cevheroğlu[2], Zulal Özkurt[3], Mine Şahingöz[4]

[1] Atatürk University, Faculty of Medicine, Department of Medical Education, Erzurum, Türkiye.
[2] Erzurum Technical University, Department of Psychology, Erzurum, Türkiye.
[3] Atatürk University, Faculty of Medicine, Department of Infectious Diseases, Erzurum, Türkiye.
[4] Necmettin Erbakan University, Faculty of Meram Medicine, Department of Psychiatry, Konya, Türkiye.

Correspondence Author: Esra Çınar Tanrıverdi
E-mail: esracinart@yahoo.com
Received: 09.09.2021 **Accepted:** 28.10.2022

ABSTRACT

Objective: This study aims to investigate the psychological effects of the COVID-19 pandemic on the Turkish society and identify COVID-19-related obsessions and predictive factors.

Methods: This cross-sectional study was performed with 859 volunteer participants. Data were collected using an online questionnaire between 01 and 08 June 2020. A sociodemographic information form, Depression, Anxiety and Stress Scale-21 (DASS-21), Impact of Event Scale-Revised (IES-R), and Obsession with COVID-19 Scale (OSC), were used as data collection tools. Data were analysed using SPSS 20 statistical software.

Results: The mean age of the participants was 40.41± 13.69 (18-70), 55.3% were women, and %63.7 were married. Cleaning habits increased during the pandemic in 76% of the participants. The prevalence of depression, anxiety, and stress symptoms were 36.9%, 42.3%, and 18.2%, respectively. Depression was severe or very severe in 6.3% of the participants, anxiety in 15.4%, and stress in 4.3%. Post-traumatic stress disorder (PTSD) was determined in 11.3% of the participants, and COVID-19-related obsessions in 17.6%. Obsessions were greater in the variables of eating (r= 0.26, p<0.001), sleep (r= 0.20, p<0.20), cleaning (r= 0.17, p<0.001), television watching habits (r= 0.09, p< 0.05), and family relationships (r= 0.11, p< 0.01) during the pandemic. The most effective predictors among the COVID-19-related obsessions were depression (p<0.001) and anxiety (p<0.001), IES-R scores (p<0.001), and finally age (p< 0.05), gender (p<0.001), and education level (p<0.05).

Conclusion: The COVID-19 pandemic has had severe psychological effects on society, especially in terms of obsessions. Awareness of these must be established, and measures aimed at improving societal mental health must be adopted.

Keywords: COVID-19, pandemic, obsession, impact of event, depression, anxiety, stress

1. INTRODUCTION

COVID-19 is a global pandemic viral infection that has persisted for more than a year. Over 125 million individuals had been affected when this paper was written, with almost three million people losing their lives (1).

Despite the rapid development and approval of the vaccines, the pandemic has persisted worldwide, and public health is under threat. Numerous restrictions were imposed on social life to prevent the transmission of the disease. New regulations and rules eventually reduced the disease incidence and transmission rates. From education to work, numerous areas of life underwent significant changes following the adaptation to the new pandemic regulations. Mask-wearing, handwashing, social distancing, and isolation were the essential rules imposed to prevent the disease. Society had to adjust to this new and restricted social life. Education was provided employing distance learning, and staff began working from home. Information concerning transmission routes and prevention of the disease was delivered continuously by the health authorities for personal and societal protection. However, fear of illness and death resulted in emotional stress and depression, meaning that mental health was also affected by the pandemic (2,3).

Economic changes and job losses due to the new restrictions resulted in severe financial difficulties. Adaptation to the new way of life and the accompanying uncertainties and social changes resulted in stress in many individuals. The elderly and other at-risk groups were particularly anxious due to fear of dying from the disease. Patients and individuals with whom they came into contact also experienced stress due to isolation and stigma, as well as fear of mortality (4). Reactions to the new conditions and individual psychosocial

DOI: 10.33808/clinexphealthsci.993404

durability or vulnerability determined the mental health of each individual and the society as a whole. During the time this paper was written (April 2021), Turkey was experiencing its third and most severe wave of the outbreak. Peak figures in terms of daily case numbers and deaths since the start of the pandemic had recently been reached following a period of controlled normalization (5). Schools remained closed, weekend restrictions were still in place, and a complete lockdown was re-introduced. Uncertainty over when the disease would end, restrictions imposed to prevent its spread, quarantine procedures, and the extended duration of the pandemic have been shown to result in psychological problems (6) while higher than normal levels of depression, anxiety, and worry along with fear, panic, and obsessions have been determined (7-10).

The World Health Organization (WHO) has frequently emphasized the importance of social isolation, handwashing, and hygiene to prevent individuals from being infected. Appropriate handwashing techniques appeared in the media for public information purposes for a long time. Hygiene behaviors increased in all sections of the society during the pandemic (11). The fact that recommendations for protection against COVID-19 involved repetitive behaviors has been described to be increasing the risk of Obsessive-Compulsive Disorder (OCD) (12). Obsessive-compulsive disorder (OCD) is characterized by obsessions (recurring intrusive thoughts or impulses), and compulsions (repetitive behaviors and/or mental acts performed in response to an obsession, or rules that must be applied rigidly) (13). Although OCD is a heterogeneous condition, those related to fears of germs and contamination, along with obligatory washing rituals, are among the most commonly reported obsessions and compulsions (14). Fear of contracting a disease and infecting others may cause these symptoms in some individuals. Hygienic recommendations in the context of the pandemic such as frequent handwashing, being careful with hygiene, and minimizing contact with others can exacerbate or lead to the emergence of OCD symptoms. Indeed, a number of studies have reported that COVID-19 increases the overall symptom severity of OCD as well as contamination-related obsessions and compulsions (15,16).

Furthermore, stressful life events and lifestyle changes associated with the current pandemic may also be an important mechanism underlying the exacerbation of OCD symptoms. Previous studies have shown that stress responses, anxiety and depression, as well as distress in general, are associated with exacerbation of OCD symptoms (16,17). Despite the increase in research on the impact of COVID-19 on OCD symptoms, there are significant gaps in the information currently available and limitations remain in studies to date. Most of the studies in this area are investigating the effect of the pandemic on OCD symptoms in patients with OCD (18). However, it is also important to address subclinical symptoms at the general population level. In other words, further studies are needed to understand the potential role of the current pandemic-related parameters in the exacerbation of OCD symptoms of COVID-19.

Similarly, stockpiling food and cleaning products may lead to hoarding obsessions, and hygiene-related behaviors such as ritualized handwashing with repetitive movements and cleaning and bathing for prolonged periods of time may contribute to obsessions (19). These behaviors occurring during the COVID-19 pandemic not only gave rise to OCD symptoms but also caused a worsening of the symptoms in individuals with pre-existing OCD. Understanding the psychological effects of the pandemic can provide crucial scientific support for measures aimed at preventing and improving mental problems.

The purpose of this study was to determine the psychological effects of the COVID-19 pandemic on the society, using the Depression, Anxiety and Stress Scale-21 (DASS-21) and the Impact of Event Scale-Revised (IES-R). One of the aims of the present study is to provide data on the frequency of depression, anxiety, stress symptoms and obsessions in the Turkish population during the pandemic period which would help to provide important data in understanding how the general mental health of the society is affected by the COVID-19 pandemic.

Another aim of the study is to understand the underlying factors of COVID-19-related obsessions to serve as a reference for studies improving societal mental health. The study focused on the predictive effects of depression, anxiety, stress, and PTSD on COVID-19-related obsessions. In addition to the clinical sample, it is considered important to investigate how societal mental health is affected by the COVID-19 pandemic. As stated earlier, how Covid-19 affects OCD symptoms has been investigated on individuals with OCD patients. However, one of the aims of this research is to investigate factors associated with COVID-19 related obsessions and its predictors in individuals without any previous diagnosis.

2. METHODS

2.1. Ethical Approval

Ethical approval was granted by the Turkish Health Ministry Health Services General Directorate Scientific Research Platform and the Atatürk University Clinical Research Ethical Committee (IRB No.B.30.2.ATA.0.01.00/266, dated 28.05.2020). The study was carried out under the rules of the Declaration of Helsinki.

2.2. Study Process and Population

This cross-sectional study was performed between 01 and 08 June 2020. Printed materials were not employed due to the pandemic, and the data were collected through an online questionnaire prepared by the authors using Google Forms. Since the authors couldn't specify the participants in a digital setting, the convenience sampling method was employed in data collection. The questionnaire was sent through e-mail, WhatsApp, and other social media accounts. The research

was conducted based on voluntary participation. Participants were able to access the questions after consenting to read the informed consent form. Participant consent was thus obtained online. The survey took approximately 15 minutes to complete.

2.3. Participants

The study population consisted of individuals consenting to use the online survey method on a social media platform. Nine hundred and forty-eight individuals were initially contacted, 89 of the participants who failed to complete the scales or were diagnosed with a psychiatric disease were excluded. The research thus continued with 859 participants.

2.4. Data Collection Tools

A questionnaire consisting of four parts was used to collect the data: 1) the form concerning sociodemographic characteristics, 2) DASS-21, 3) IES-R, and 4) OSC.

2.4.1. Sociodemographic Characteristics

Age, gender, marital status, income level, place of residence, daily habits, history of COVID-19, and presence of chronic disease were investigated.

2.4.2. Depression, Anxiety and Stress Scale

This scale was developed by Lovibond and Lovibond (1995) and is completed based on the conditions applying within the previous one week. The scale consists of three sub-dimensions showing depression, anxiety, and stress, and each containing seven items. The dimensions are scored separately possible scores on each sub-dimension range between 0 and 21[19]. DASS-21 was adapted into Turkish by Sarıçam et al. The scale answered according to a 4-point Likert system (from not at all (0), to 'extremely' (4)). Cut-off scores of ≥5, ≥4, and ≥8 represent a positive screening of depression, anxiety, and stress, respectively (20).

2.4.3. The Impact of Event Scale-Revised

IES-R was developed by Weiss and Marmar and is used in social screenings to measure levels of post-traumatic stress. The scale evaluates Post-Traumatic Stress Disorder (PTSD) and consists of 21 items investigating the situation within the previous one week and is scored on a five-point Likert-type basis (from 'not at all (0), to 'extremely' (4)). Total possible scores range from 0 to 88. (21) IES-R was adapted into Turkish by Çorapçıoğlu et al. A score above 33 indicates a high level of stress (22). We determined a Cronbach alpha value of 0.92 for this sample.

2.4.5. Obsession with COVID-19 Scale (OSC)

The OSC was developed by Lee et al. (2020) and is used to screen persistent and disturbed thinking about COVID-19 (23). The scale is completed with a five-point Likert-type system (from 0 (never) to 4 (almost every day)) based on experiences within the previous two weeks. A score of 7 or above indicates dysfunctional thinking associated with coronavirus. The scale was adapted into Turkish by Evren et al. (24). Cronbach alpha value of the scale for this sample was evaluated as .92.

2.6. Statistical Analysis

Data were analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) statistical software. Descriptive statistics were expressed as mean, standard deviation, minimum, and maximum values for continuous variables and number and percentage for categorical data. Pearson's correlation analysis and sequential hierarchical regression analysis were used for data comparisons. Confidence analysis was applied to both scales, and Cronbach alpha coefficients were calculated. The statistical significance level was accepted as $p< 0.05$.

3. RESULTS

3.1. Sociodemographic Characteristics of the Participants

Complete data from 859 participants were evaluated. The mean age of the participants was 40.41±13.69 years (18-70). Four hundred and seventy-five (55.3%) of the participants were women, 547 (63.7%) were married, and 718 (83.6%) were university graduates. Habits such as watching TV had increased in 379 participants (44.1%), religious observances in 299 (34.8%), and hygiene (such as handwashing, bathing, vacuuming the home, and washing clothes) in 653 (76%). Sociodemographic findings are shown in Table 1.

Table 1. *Participants' sociodemographic characteristics and various behaviors during the pandemic*

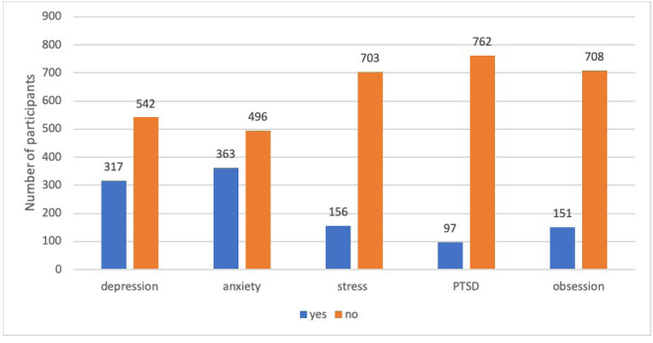
Variable		Frequency (n)	Percentage (%)
Gender (n=859)	Female	475	55.3
	Male	384	44.7
Age (n=859)	18-35	336	39.1
	36-50	291	33.9
	51 or over	232	27
Marital status (n=859)	Married	547	63,7
	Single	282	32,8
	Divorced	30	3,5
Education level (n=859)	Elementary	34	3.8
	High school	107	12.5
	University	714	83.6
	Housewife	62	7.2
	Student	152	17.7
	Not working	39	4.5

Occupation (n=859)	Clerical	289	33.6
	Small trader	35	4.1
	Manual worker	39	4.5
	Retired	96	11.2
	Health worker	115	13.4
	Member of teaching staff	29	3.4
Income (n=859)	Less than 2000 TL	169	19.7
	2000-4000 TL	187	21.8
	More than 4000 TL	503	58.6
Income level during the pandemic (n=859)	Decreased	311	36,2
	Unchanged	548	63,8
Place of residence (n=859)	City	729	84.9
	Small town	110	12.8
	Village	20	2.3
Do you have a chronic disease? (n=859)	Yes No	199 660	23.2 76.8
How would you describe your relationship with your spouse or partner during the pandemic ? (n=568)	Unchanged	424	74.6
	Changed	144	25.4
Has your relationship with your children changed during the pandemic? (n=526)	Unchanged	330	62.7
	Changed	196	37.3
Have your relationships with your family changed during the pandemic? (n=858)	Unchanged Changed	564 290	65.7 33.8
Have your cleaning habits changed during the pandemic (such as handwashing, bathing, vacuuming the home, or washing clothes)? (n=859)	Increased Unchanged	653 206	76 24
Whom do you live with at home? (n=858)	Nuclear family	723	84.2
	Extended family	64	7.5
	Alone	59	6.9
	Housemate	12	1.4
Have you had COVID-19? (n=859)	Yes	6	0.7
	No	853	99.3
Have any of your relatives had COVID-19? (n=859)	Yes	10	1.2
	No	849	98.8
How afraid are you of contracting COVID-19? (n=859)	Not at all	129	15
	Very little	137	15.9
	Not very	320	37.3
	Moderately	142	16.5
	Very	131	15.3
How afraid are you of your relatives contracting COVID-19? (n=859)	Not at all	42	4.9
	Very little	58	6.8
	Not very	170	19.8
	Moderately	254	29.6
	Very	335	39
What happened to your weight during the quarantine period? (n=859)	Changed	474	55.2
	Unchanged	385	44.8
Has there been any change in your TV-watching habits during the pandemic ? (n=859)	Changed	484	56.3
	Unchanged	375	43.7
Has there been any change in your religious observances during the pandemic? (n=859)	Changed	336	39.1
	Unchanged	523	60.9

TL Turkish lira

3.2. Prevalence of Depression, Anxiety, Stress, and PTSD symptoms

The prevalence of depression, anxiety, and stress symptoms in the present study were 36.9%, 42.3%, and 18.2%, respectively. PTSD was detected in 97 participants (11.3%) and COVID-19-related obsessions in (17.6%) (Figure 1). High or very high levels of depression (6.3%, n=54) anxiety (15.4%, n=132), and stress (4.3%, n=37) were observed.



PTSD: Post traumatic stress disorder

Figure 1. *Prevalence of depression, anxiety, PTSD, and obsessions*

3.3. Factors Associated with COVID-19-Related Obsessions

In order to examine the correlations between Covid-19 related obsessions and changing habits of individuals, point

biserial correlation was made. According to the results, a significant positive correlation was determined between changes in eating habits (r_{pb}= 0.26, p< 0.001), sleeping habits (r_{pb}= 0.20, p< 0.001), family relationships (r_{pb}= 0.11, p< 0.01), cleaning habits (r_{pb}= 0.17, p< 0.001) and TV watching habits (r_{pb}= 0.09, p< 0.05) during the pandemic and COVID-19-related obsessions. According to the results, as Covid-19 related obsessions increase, daily routines of individuals change. Factors associated with COVID-19 related obsessions are shown in Table 2.

Table 2. *Point Biserial Correlation Results of Factors correlated with the COVID-19 related obsessions*

		COVID-19 Related Obsession
Have your eating habits changed during the pandemic?	r	0.26***
Have your sleeping habits changed during the pandemic?	r	0.20***
Have your relationships with your family changed during the pandemic?	r	0.11**
Have your cleaning habits changed during the pandemic?	r	0.17***
Have your TV watching habits changed during the pandemic?	r	0.09**

** p<.05, ** p<.01, ***p<.001*

3.4. Predictors of COVID-19-Related Obsessions

Hierarchical regression analysis was performed to determine variables predicting OSC scores. DASS-21 sub-dimensions (depression, anxiety, and stress) were included in the first step of the hierarchical regression analysis, and total IES-R score was included in the second and final step.

According to the results, depression (F_{1-857}= 257.56, p< 0.001) and anxiety (F_{2-856}= 135.14, p< 0.001) made a 24% contribution to total variance. IES-R scores made an 11% contribution to the total variance, the total variance explained, thus rising to 33% (F_{3-855}=141.75, p< 0.001). Variables predicting COVID-19-related obsessions are shown in Table 3.

Table 3. *Hierarchical regression analysis results about predictors of COVID-19-related obsessions*

Variable	Adjusted R^2	B	SE.	βeta	t	F
DASS-Depression	.23	.14	.05	.16	2.78**	257.56***
DASS-Anxiety	.24	.10	.05	.06	1.04*	135.14***
IES-R	.33	.09	.01	.41	10.86***	141.75***

** p<.05, ** p<.01, ***p<.001*

4. DISCUSSION

This study was performed in the third month of the pandemic, investigating the psychological effects of the COVID-19 pandemic on the Turkish society and predicting factors of COVID-19-related obsessions.

It was found that the COVID-19 related obsession rate among the participants was 17.3%, depression rate was 36.9%, anxiety was 42.3%, and PTSD was 11.6%. The prevalence of depression, anxiety, and OCD symptoms in the present study was significantly higher compared to the pre-pandemic period. A study looking at university students in Turkey reported a prevalence of OCD of 4.2% before the pandemic (25). The prevalence of obsession in the present study (17.3%) was significantly higher than that figure. We think that constant hand washing for protection, disinfectant use, and the emphasis on hygiene and social distancing played a significant role in this high figure. Exacerbation of OCD symptoms in children and adolescents has been observed during the pandemic (26). A study from Italy showed a worsening of symptoms in OCD during quarantine (27). Previous studies have also reported significant increases in depression (28) and anxiety (29) rates compared with pre-pandemic values (2-3% and 5%). A previous study from China reported that depression, anxiety, and stress rates of the participants were 27.9%, 31.6%, and 24.4% (30), while another study from India reported 25.1%, 28%, and 11.6% (31). A different study from China reported a depression rate that is similar to the present study (35.1%) but a lower anxiety rate (20.1%) (8). Casagrande et al. reported anxiety symptoms among adults in Italy at a rate of 32.1%, stress rates of 41.8%, and PTSD rates of 7.6% (32). Relatively high rates were also observed in a study from Austria, at 60% for depression, 50% for anxiety, and 64% for stress (33). A study from the USA reported that participants exhibited high levels of depression, with anxiety symptoms being observed in one in four individuals (34). Another Chinese study using the DASS-21 and IES-R reported a depression rate of 16.5%, an anxiety rate of 28.8%, and a stress rate of 8.1%, while the PTSD rate was relatively higher than it is in the present study (53%) (35).

Another result of the present study has shown that there are significant relationships between COVID-19 related obsessions and changing habits of individuals such as eating, sleeping, cleaning, TV-watching and family relationships. It has been observed that people's daily routines change as COVID-19 related obsessions increase. COVID-19 related obsessions in the present research were greater among individuals whose eating habits, sleep habits, family relationships, cleaning habits, and TV-watching had changed during the pandemic. A study from Netherlands reported similar results with our study. Individuals who reported increase in TV-watching and changing in sleeping and daily communication with their loved ones reported that their OCD symptoms worsened (36). Numerous factors, including the daily provision of new information concerning the disease, uncertainty about when the pandemic would come to an end, and the adoption of new measures daily both increased stress and anxiety, and led to the emergence of obsessions about cleanliness, or the worsening of existing obsessions (37). Seventy-six of the participants in the present study reported increased hygiene-related behaviors such as hand washing, clothes washing, and domestic cleaning during the pandemic. With the declaration

of the pandemic by the World Health Organization and the first case in Turkey, an unprecedented wave of information commenced to be distributed through all forms of media. On the one hand, information was given about protective measures and hygiene and the day-to-day global situation. On the other hand, prolonged and extensive knowledge was provided about proper handwashing (including washing the backs of the hands, the fingertips, and the wrists separately and individually) together with consecutive visual presentations that appeared on the television channels, internet, social media, and public information broadcasts. Following this emphasis on the importance of hand hygiene in preventing infection, an increase in hygiene-related behaviors was observed in the general population.

In the current study, hierarchical regression analysis was also conducted to determine the factors affecting COVID-19 related obsessions. According to the results, it was observed that higher anxiety, depression and PTSD scores predicted COVID-19 related obsessions. In other words, people with higher scores for anxiety, depression, and PTSD also have higher COVID-19 related obsessions. Similar to the present research, a study from China performed three months after quarantine reported a prevalence of OCD of 17.9%, and those psychiatric comorbidities were associated with higher obsession rates (38). Studies show that fear and stress result in OCD symptoms (39). We think that factors such as the scales employed, the pandemic period, and the participants' characteristics may account for the discrepancies between these results. Previous research has indicated that the presence of depression may represent a starting point for obsession (40), and depression and obsession have frequently been reported together (41).

Similarly, in a study conducted with adolescents in Turkey during the pandemic, it was observed that depression and anxiety are predictors of OCD symptoms and had a mediating effect between pandemic related fear and OCD symptoms (42). Anxiety emerged as a significant predictor of obsession in this study. In agreement with our results, COVID-19 related anxiety has been linked to obsessions. The two have been found to be associated, with higher anxiety levels being associated with higher levels of obsession (43,44). Srivastava et al. reported an obsession rate of 13%, and there was a positive correlation between OCD, anxiety and obsession (45).

One significant predictor of COVID-19 related obsessions in the present study was IES-R, with obsession being greater in individuals with higher scores. PTSD rates of 40%, depression rates of 36.4%, and OCD and anxiety rates of 15.6% were observed in one study performed 2-4 years after the SARS outbreak (46). These data suggest that the psychological effects of the COVID-19 pandemic will also be severe and long-lasting. According to our knowledge, there are not any specific studies investigating how PTSD symptoms affect COVID-19 related obsessions. However, various studies underline that the pandemic has had a traumatic effect on the society (47,48). In addition, several articles have documented the co-occurrence of PTSD and OCD (49,50). In a study conducted with patients diagnosed with both PTSD and OCD, it was stated that participants had the PTSD onset before the onset of OCD (51) consistent with the current research findings.

4.1. Limitations

There are several limitations to this study. In particular, the study was cross-sectional, and it is difficult to draw causal conclusions from it. Furthermore, since the data were collected online, we could not contact individuals who do not have electronic devices or internet connections, which may have affected the results. Finally, the psychological effects investigated rely on scales and participants' self-evaluations, and face-to-face interviews were not conducted. Bias may therefore be unavoidable.

5. CONCLUSION

The findings of the present study show that the COVID-19 pandemic has had severe psychological impacts on the Turkish society, particularly in terms of depression, anxiety, stress, PTSD, and obsessions. Four out of every ten individuals experience depression or anxiety, while two out of 10 experience PTSD or OCD symptoms. Each of these figures is approximately 5-6 times higher than before the pandemic.

In addition to efforts regarding the physical effects of the pandemic, it is also essential that measures protecting mental health be adopted. It should be remembered that the symptoms of individuals with previous diagnoses of a psychiatric disease may worsen and most importantly new cases may also emerge. The necessary precautions must therefore be adopted and appropriate interventions planned.

Since the study was performed in a relatively early period of the pandemic, large-sample, longitudinal studies should now be performed, and these should become routine matters in preparation for future outbreaks, particularly considering that the findings may be altered over time and that the pandemic may have long-term psychological effects.

Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Atatürk University (Decision date and number: 28.05.2020, IRBNo.B.30.2.ATA.0.01.00/266).*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: ECT, ZO, MS
Design of the study: ECT, ZO,MS
Acquisition of data for the study: ECT, BAC, ZO, MS
Analysis of data for the study: BAC, ECT, ZO
Interpretation of data for the study: ECT, BAC, ZO, MS
Drafting the manuscript: ECT, ZO, MS, BAC
Revising it critically for important intellectual content: ECT, ZO, MS, BAC
Final approval of the version to be published: ECT, ZO, MS, BAC

REFERENCES

[1] Worldometer. COVID-19 Coronavirus Pandemic. 2021. Available at: https://www.worldometers.info/coronavirus/ Accessed 27 March 2021.

[2] Dong L, Bouey J. Public mental health crisis during COVID-19 Pandemic, China. Emerg Infect Dis. 2020;26(7):1616-1618. DOI: 10.3201/eid2607.200407

[3] Miu A, Cao H, Zhang B, Zhang H. Review of Mental Health Response to COVID-19, China. Emerg Infect Dis. 2020;26(10):2482-2484. DOI: 10.3201/eid2610.201113

[4] Brooks SK, Webster RK, Smith LE, Woodland L, Wessely S, Greenberg N, Rubin GJ. The psychological impact of quarantine and how to reduce it: Rapid review of the evidence. The Lancet 2020;395(10227):912-920. DOI: 10.1016/S0140-6736(20)30460-8

[5] TC Ministry of Health. COVID-19 Information Platform. 2021. Available at: https://covid19.saglik.gov.tr. Accessed 23 March 2021.

[6] López-Solà C, Fontenelle LF, Bui M, Hopper JL, Pantelis C, Yücel M, Menchón JM, Alonso P, Harrison BJ. Aetiological overlap between obsessive–compulsive related and anxiety disorder symptoms: multivariate twin study. Br J Psychiatry 2016;208(1):26-33. DOI: 10.1192/bjp.bp.114.156281

[7] Gao J, Zheng P, Jia Y, Chen H, Mao Y, Chen S, Wang Y, Fu H, Dai J. Mental health problems and social media exposure during COVID-19 outbreak. PLoS One 2020;15(4):e0231924 (1-10). DOI: 10.1371/journal.pone.0231924

[8] Huang Y, Zhao N. Generalized anxiety disorder, depressive symptoms and sleep quality during COVID-19 outbreak in China: A web-based cross-sectional survey. Psychiatry Res. 2020;288:112954 (1-6). DOI: 10.1016/j.psychres.2020.112954

[9] Gica S, Kavakli M, Durduran Y, Ak M. The effect of COVID-19 pandemic on psychosomatic complaints and investigation of the mediating role of intolerance to uncertainty, biological rhythm changes and perceived COVID-19 threat in this relationship: A web-based community survey. Psych Clin Psychopharmacol. 2020;30(2):89-96. DOI: 10.5455PCP202.005.14033022

[10] Poyraz BÇ, Poyraz CA, Olgun Y, Gürel Ö, Alkan S, Özdemir YE, Balkan İİ, Karaali R. Psychiatric morbidity and protracted symptoms after COVID-19. Psychiatry Res. 2021;295:113604 (1-9). DOI: 10.1016/j.psychres.2020.113604

[11] Holmes EA, O'Connor RC, Perry VH, Tracey I, Wessely S, Arseneault L, Ballard C, Christensen H, Cohen Silver R, Everall I, Ford T, John A, Kabir T, King K, Madan I, Michie S, Przybylski AK, Shafran R, Sweeney A, Worthman CM, Yardley L, Cowan K, Cope C, Hotopf M, Bullmore E. Multidisciplinary research priorities for the COVID-19 pandemic: A call for action for mental health science. Lancet Psychiatry 2020;7(6):547-560. DOI: 10.1016/S2215-0366(20)30168-1

[12] Mak IWC, Chu CM, Pan PC, Yiu MGC, Chan VL. Long-term psychiatric morbidities among SARS survivors. Gen Hosp Psychiatry 2009;31(4):318-326. DOI: 10.1016/j.genhosppsych.2009.03.001

[13] Asken MJ, Grossman D, Christensen LW. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Arlington, VA: American Psychiatric Publishing, 2013. Archibald, Herbert C., and Read D. Tuddenham."Persistent Stress Reac-tion after Combat: A 20-Year Follow-Up." Arch Gen Psy Therapy 2007;45(10):2317-2325. DOI: 10.1016/j.genhosppsych.2009.03.001

[14] Abramowitz JS, Deacon BJ, Olatunji BO, Wheaton MG, Berman NC, Losardo D, Timpano KR, McGrath PB, Riemann BC, Adams T, Björgvinsson T, Storch EA, Hale LR. Assessment of obsessive-compulsive symptom dimensions: Development and evaluation of the dimensional obsessive-compulsive scale. Psych Assess 2010;22(1):180-198. DOI: 10.1037/a0018260

[15] Benatti B, Albert U, Maina G, Fiorillo A, Celebre L, Girone N, Fineberg N, Bramante S, Rigardetto S, Dell'Osso B. What happened to patients with obsessive compulsive disorder during the COVID-19 pandemic? A multicentre report from tertiary clinics in northern Italy. Front. Psychiatry 2020;720. DOI: 10.3389/fpsyt.2020.00720

[16] Khosravani V, Aardema F, Ardestani SMS, Bastan FS. The impact of the coronavirus pandemic on specific symptom dimensions and severity in OCD: A comparison before and during COVID-19 in the context of stress responses. J Obsessive Compuls Relat Disord. 2021;29:100626 (1-6). DOI: 10.1016/j.jocrd.2021.100626

[17] Wheaton MG, Messner GR, Marks JB. Intolerance of uncertainty as a factor linking obsessive-compulsive symptoms, health anxiety and concerns about the spread of the novel coronavirus (COVID-19) in the United States. J Obsessive Compuls Relat Disord. 2021;28:100605 (1-6). DOI: 10.1016/j.jocrd.2020.100605

[18] Grant JE, Drummond L, Nicholson TR, Fagan H, Baldwin DS, Fineberg NA, Chamberlain SR. Obsessive-compulsive symptoms and the Covid-19 pandemic: A rapid scoping review. Neurosci Biobehav Rev. 2022;132:1086-1098. DOI: 10.1016/j.neubiorev.2021.10.039

[19] Lovibond PF, Lovibond SH. The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. Behav Res Ther. 1995;33(3):335-343. DOI: 10.1016/j.neubiorev.2021.10.039

[20] Sarıçam H. The psychometric properties of Turkish version of Depression Anxiety Stress Scale-21 (DASS-21) in health control and clinical samples. J Cog Behav Psych Res. 2018;7(1):19-30. DOI:10.5455/JCBPR.274847

[21] Weiss D, Marmar C. The Impact of Event Scale Revised. W: Wilson J., Keane T.(red.). Assessing psychological trauma and PTSD: A handbook for practitioners. In: Guildford Press, New York; 1997.

[22] Çorapçıoğlu A, Yargıç İ, Geyran P, Kocabaşoğlu N. Validity and reliability of Turkish version of "impact of event scale-revised"(IES-R). New Symposium 2006;44:14-22.

[23] Lee SA. How much "Thinking" about COVID-19 is clinically dysfunctional? Brain, Behav Immun. 2020;87:97-98. DOI: 10.1016/j.bbi.2020.04.067

[24] Evren C, Evren B, Dalbudak E, Topçu M, Kutlu N. Measuring anxiety related to COVID-19: A Turkish validation study of the Coronavirus Anxiety Scale. Death Stud. 2022; 46(5):1052-1058. DOI: 10.1080/07481.187.2020.1774969

[25] Yoldaşcan E, Özenli Y, Kutlu O, Topal K, Bozkurt AI. Prevalence of obsessive-compulsive disorder in Turkish university students and assessment of associated factors. BMC Psychiatry 2009;9(1):1-8. DOI: 10.1186/1471-244X-9-40

[26] Tanır Y, Karayağmurlu A, Kaya İ, Kaynar TB, Türkmen G, Dambasan BN, Meral Y, Coşkun M. Exacerbation of obsessive compulsive disorder symptoms in children and adolescents during COVID-19 pandemic. Psychiatry Res. 2020;293:113363(1-5). DOI: 10.1016/j.psychres.2020.113363

[27] Davide P, Andrea P, Martina O, Andrea E, Davide D, Mario A. The impact of the COVID-19 pandemic on patients with OCD: Effects of contamination symptoms and remission state before the quarantine in a preliminary naturalistic study. Psychiatry Res. 2020;291:113213(1-5). DOI: 10.1016/j.psychres.2020.113213

[28] Lee S, Tsang A, Huang YQ, He YL, Liu ZR, Zhang MY, Shen YC, Kessler RC. The epidemiology of depression in metropolitan China. Psychol Med. 2009;39(5):735-747. DOI: 10.1017/S003.329.1708004091

[29] Yu W, Singh SS, Calhoun S, Zhang H, Zhao X, Yang F. Generalized anxiety disorder in urban China: prevalence, awareness, and disease burden. J Affective Disord. 2018;234:89-96. DOI: /10.1016/j.jad.2018.02.012

[30] Shi L, Lu ZA, Que JY, Huang XL, Liu L, Ran MS. Prevalence of and risk factors associated with mental health symptoms among the general population in China during the coronavirus disease 2019 pandemic. JAMA Netw Open. 2020;3(7):e2014053-e2014053. DOI: 10.1001/jamanetworkopen.2020.14053

[31] Verma S, Mishra A. Depression, anxiety, and stress and socio-demographic correlates among general Indian public during COVID-19. Int J Soc Psychiatry. 2020;66(8):756-762. DOI: 10.1177/002.076.4020934508

[32] Casagrande M, Favieri F, Tambelli R, Forte G. The enemy who sealed the world: Effects quarantine due to the COVID-19 on sleep quality, anxiety, and psychological distress in the Italian population. Sleep Med. 2020;75:12-20. DOI: 10.1016/j.sleep.2020.05.011

[33] Newby JM, O'Moore K, Tang S, Christensen H, Faasse K. Acute mental health responses during the COVID-19 pandemic in Australia. PloS One. 2020;15(7):e0236562 (1-21). DOI:10.1371/journal.pone.0236562

[34] Fitzpatrick KM, Harris C, Drawve G. Fear of COVID-19 and the mental health consequences in America. Psychol Trauma 2020;12(S1):S17-S21. DOI: 10.1037/tra0000924

[35] Wang C, Pan R, Wan X, Tan Y, Xu L, Ho CS, Ho RC. Immediate psychological responses and associated factors during the initial stage of the 2019 coronavirus disease (COVID-19) epidemic among the general population in China. Int J Environ Res Public Health 2020;17(5):1729 (1-25). DOI: 10.3390/ijerph17051729

[36] Van Ameringen M, Patterson B, Turna J, Lethbridge G, Goldman Bergmann C, Lamberti N, Rahat M, Sideris B, Francisco AP, Fineberg N, Pallanti S, Grassi G, Vismara M, Albert U, Gedanke Shavitt R, Hollander E, Feusner J, Rodriguez CI, Morgado P, Dell'Osso B. Obsessive-compulsive disorder during the COVID-19 pandemic. J Psychiatr Res. 2022;149:114-123. DOI: 10.1016/j.jpsychires.2022.02.001

[37] Yang Y, Li W, Zhang Q, Zhang L, Cheung T, Xiang Y-T. Mental health services for older adults in China during the COVID-19 outbreak. Lancet Psychiatry 2020;7(4):e15-16. DOI: 10.1016/S2215-0366(20)30078-X

[38] Zheng Y, Xiao L, Xie Y, Wang H, Wang G. Prevalence and Characteristics of Obsessive-Compulsive Disorder Among Urban Residents in Wuhan During the Stage of Regular Control of Coronavirus Disease-19 Epidemic. Front Psychiatry 2020;11:1435 (1-7). DOI: 10.3389/fpsyt.2020.594167

[39] O'Neill ML, Nenzel ME, Caldwell W. Intrusive thoughts and psychopathy in a student and incarcerated sample. J Behav Ther Exp. 2009;40(1):147-157. DOI: 10.1016/j.jbtep.2008.07.004

[40] Bellodi L, Sciuto G, Diaferia G, Ronchi P, Smeraldi E. Psychiatric disorders in the families of patients with obsessive-compulsive disorder. Psychiatry Res. 1992;42(2):111-120. DOI: 10.1016/0165-1781(92)90075-E

[41] Millet B, Kochman F, Gallarda T, Krebs MO, Demonfaucon F, Barrot I, Bourdel MC, Olié JP, Loo H, Hantouche EG. Phenomenological and comorbid features associated in obsessive-compulsive disorder: influence of age of onset. J Affect Disord. 2004;79(1-3):241-246. DOI: 10.1016/S0165-0327(02)00351-8

[42] Secer İ, Ulas S. An investigation of the effect of COVID-19 on OCD in youth in the context of emotional reactivity, experiential avoidance, depression and anxiety. Int J Mental Health Addic. 2021;19(6):2306-2319. DOI: 10.1007/s11469.020.00322-z

[43] Choi E, Lee J, Lee SA. Validation of the Korean version of the obsession with COVID-19 scale and the Coronavirus anxiety scale. Death Stud. 2020;46(3):608-614. DOI: 10.1080/07481.187.2020.1833383

[44] Ji G, Wei W, Yue KC, Li H, Shi LJ, Ma JD, He CY, Zhou SS, Zhao Z, Lou T, Cheng J, Yang SC, Hu XZ. Effects of the COVID-19 pandemic on obsessive-compulsive symptoms among university students: Prospective cohort survey study. J Med Internet Res. 2020;22(9):e21915 (1-13). DOI: 10.2196/21915

[45] Srivastava A, Bala R, Srivastava AK, Mishra A, Shamim R, Sinha P. Anxiety, obsession and fear from coronavirus in Indian population: A web-based study using COVID-19 specific scales. Int J Commun Med Public Health 2020;7(11):4570-4577. DOI: 10.18203/2394-6040.ijcmph20204763

[46] Troyer EA, Kohn JN, Hong S. Are we facing a crashing wave of neuropsychiatric sequelae of COVID-19? Neuropsychiatric symptoms and potential immunologic mechanisms. Brain Behav Immun. 2020; 87:34-39. DOI: 10.1016/j.bbi.2020.04.027

[47] Chamaa F, Bahmad HF, Darwish B, Kobeissi JM, Hoballah M, Nassif SB, Ghandour Y, Saliba JP, Lawand N, Abou-Kheir W. PTSD in the COVID-19 Era. Curr Neuropharmacology 2021;19(12):2164-2179. DOI: 10.2174/1570159X196.662.10113152954

[48] Horn M, Wathelet M, Fovet T, Amad A, Vuotto F, Faure K, Astier T, Noël H, Duhem S, Vaiva G, D'Hondt F, Henry M. Is COVID-19 associated with posttraumatic stress disorder? J Clin Psychiatry. 2020;82(1):9886.

[49] Gershuny BS, Baer L, Radomsky AS, Wilson KA, Jenike MA. Connections among symptoms of obsessive–compulsive disorder and posttraumatic stress disorder: A case series. Behav Res Ther. 2003;41(9):1029-1041. DOI: 10.1016/S0005-7967(02)00178-X

[50] Sasson Y, Dekel S, Nacasch N, Chopra M, Zinger Y, Amital D, Zohar J. Posttraumatic obsessive–compulsive disorder: A case series. Psychiatry Res. 2005;135(2):145-152. DOI: 10.1016/j.psychres.2004.05.026

[51] Nacasch N, Fostick L, Zohar J. High prevalence of obsessive–compulsive disorder among posttraumatic stress disorder patients. Eur Neuropsychopharmacology 2011;21(12):876-879. DOI: 10.1016/j.euroneuro.2011.03.007

How to cite this article: Çınar Tanrıverdi E, Arslan Cevheroğlu B, Özkurt Z, Şahingöz M. COVID-19-Related Obsessions and Its Predictors: A Community-Based Research in Turkey. Clin Exp Health Sci 2023; 13: 32-40. DOI: 10.33808/clinexphealthsci.993404

Clinical and Experimental Health Sciences

The Effect of Web-Based Tracheostomy Care Game on Nursing Students' Knowledge Levels and Their Views of the Process

Şule Bıyık Bayram[1], Nurcan Çalışkan[2], Emel Gülnar[3]

[1] Karadeniz Technical University, Faculty of Health Sciences, Department of Nursing, Trabzon, Türkiye.
[2] Gazi University, Faculty of Nursing, Ankara, Türkiye.
[3] Kırıkkale University, Faculty of Health Sciences, Department of Nursing, Kırıkkale, Türkiye.

Correspondence Author: Şule Bıyık Bayram
E-mail: sulebiyik@gmail.com

Received: 10.11.2021 **Accepted:** 22.12.2022

ABSTRACT

Objective: This study investigated nursing students' views of the effect of a web-based tracheostomy care game on their knowledge levels during the COVID-19 pandemic.

Methods: This pretest-posttest single-group quasi-experimental study was conducted between April and July 2021. The study population consisted of all nursing students in Turkey. The sample consisted of 125 students who filled out the pretest and posttest forms. Participation was voluntary. Participants were recruited using snowball sampling method. Participants entered the website "trakeostomibakimi.com" They filled out the demographic characteristics questionnaire and the tracheostomy care knowledge test (TCKT) on the website. They downloaded the virtual tracheostomy care game on their computers. They played the game as much as they wanted and then filled out the posttest and the student opinion forms.

Results: Participants had a higher mean posttest TCKT score than pretest score ($p < .05$). They stated that the game helped them enjoy learning tracheostomy care and remember their prior knowledge and made them feel like they practiced in a real-life clinical setting.

Conclusion: The web-based tracheostomy care game improved nursing students' knowledge levels. There should also be online educational games tailored to other nursing areas.

Keywords: Nursing students, nursing care, tracheostomy, web-based education

1. INTRODUCTION

Technology is transforming nursing education (1). We should integrate technology into nursing education because nursing students are generation Z born into a digital world (2,3). In addition, the COVID-19 pandemic has taken a toll on nursing education, affecting all stakeholders (instructors, students, administrators, etc.) (4). Therefore, countries have taken countermeasures to overcome the adverse effect of the pandemic. The main objective of those measures has been motivating students and enriching course content (5).

Nursing curricula should help students acquire evidence-based knowledge and develop related skills (6). Nursing academics should use innovative methods to turn their students into competent people who can synthesize new information with prior knowledge and put theory into practice (6,7). One of those innovative methods is online games, which transfer real life to digital media by gamification. Online games in nursing education are a type of simulation (8). Virtual simulation allows students to participate in distance learning, access, and information quickly, achieve permanent and meaningful learning, interact with digital interfaces, and put their knowledge into practice in safe settings (6,9,10). Online games support traditional teaching. They focus on making students more motivated and engaged and helping them acquire the knowledge and develop the skills they need in professional life (11,12). Online games allow students to learn by trial and error at their own pace, receive instant feedback, and keep themselves motivated and focused (12-14). They provide them with a safe learning environment in which they can practice their skills without harming patients. They also allow them to repeat as many times as they want anywhere, anytime (9,15). Students play online games on computers, tablets, and smartphones as long as they have an internet connection. In this way, each student can cover course content online before and after class (16). Today, people have been spending more time on digital devices. Therefore, students can access web-based learning tools easily. Students have fewer hours of theoretical and practical classes, cannot visit laboratories to indulge in practical sessions, and cannot perform clinical practice in real-life settings due to the COVID-19 pandemic. However, they can use online games to make up for that (5). Online games can also be used in face-to-face education, where students can

DOI: 10.33808/clinexphealthsci.1021950

practice nursing interventions in simulated laboratories at any time.

Tracheostomy care requires surgical asepsis skills. Nurses should minimize the transmission risk of COVID-19 because tracheostomy care involves aspiration, which causes aerosols to form (17,18). Nurses and nursing students must have adequate knowledge, and skills on tracheostomy care. Therefore, the educational program must focuses on enabling students to deliver effective tracheostomy care by improving knowledge and skills. However, nursing students have not had the opportunity to practice lab skills since the onset of the pandemic. Therefore, this study focused on a web-based game to teach nursing students tracheostomy care. The goal was to provide students with the opportunity to practice tracheostomy care skills online. The study investigated how the game helped nursing students develop tracheostomy care skills and also looked into their thoughts about the process.

Research Hypotheses

This study investigated the effect of a web-based tracheostomy care game on nursing students' knowledge levels and their views of the process.

Research hypotheses

H_0: The game does not increases nursing students' knowledge of tracheostomy care.

H_1: The game increases nursing students' knowledge of tracheostomy care.

2. METHODS

2.1.Research Type

This was a pretest-posttest single-group quasi-experimental study.

2.2. Population and Sample

The study was conducted between April and July 2021. The study population consisted of all Turkish nursing students who have received training in tracheostomy care. The inclusion criteria were 1: agreeing to participate, 2: having played the web-based tracheostomy care game, and 3: having filled out the pretest and posttest forms. Participants were recruited using snowball sampling method. Initially, 496 students participated in the study and completed the pre-test. However, the study was completed with 125 students who completed both the pretest and the posttest.

2.3. Data Collection Tools

Data were collected using a demographic characteristics questionnaire, the Tracheostomy Care Knowledge Test (TCKT), and the Web-Based Tracheostomy Care Game Student Opinion Form.

The demographic characteristics questionnaire was based on a literature review conducted by the researchers (16,19,20). The questionnaire consisted of ten items on age, gender, education, grade level, school type, the location of the school, and tracheostomy care.

The TCKT was developed by Bıyık Bayram and Çalışkan (2019) (20). The instrument consists of 23 multiple-choice questions with five options. This test was evaluated by 76 nursing students in second year. Validity of this test was evaluated with KR21 (0.58) and KR20 (0.63) (Cronbach's Alpha = 0.70). Six questions were removed because they were about anatomy, physiology, and home care. Each correct answer was worth one point, while each wrong answer was given zero points. The total score ranges from 0 to 17. This test was evaluated by 125 nursing students in the study. Validity of this test was evaluated with KR21 (0.53) and KR20 (0.43) (Cronbach's Alpha = 0.53).

The Web-Based Tracheostomy Care Game Student Opinion Form consisted of three open-ended questions about its contribution to learning, its effect on competence and comprehension, and its fidelity. The following are the questions:

- How do you think the game contributed to your learning?
- How did the game affect your competence and comprehension?
- Did you put yourself in the shoes of Melek, the nurse? Did you take up her role? What do you think about it?

2.4. Procedure

The study consisted of two stages. First, the researchers prepared the data collections forms on Google Forms. Then, they created a website (http://trakeostomibakimi.com/) and uploaded the game and the forms there. The game was developed by Bıyık Bayram and Çalışkan (2019a) for their thesis. It is a 10-minute game consisting of six stages, including material preparation for aspiration, material preparation inner cannula cleaning, material preparation peristomal skincare, tracheostomy aspiration, inner cannula cleaning, and peristomal skincare. The player needs to complete each stage to move on to the next one. The game waits with no warning until the player chooses the right equipment and completes the stage. The player needs to know the steps of tracheostomy care to complete all six stages (20).

Second, the researchers sent all participants a link to the website. Participants filled out the demographic characteristics questionnaire and the TCKT (pretest). They then downloaded the game on their computers. They played it as much as they wanted within three months. The researchers then emailed them and invited them to fill out the TCKT (posttest) after they finished the game and whenever they were ready to fill it out. After three months, the researchers uploaded the posttest to the website. Those who finished the game and felt ready filled out the TCKT (posttest) and the student opinion form. The researchers sent a certificate of participation to all those who finished

the game and filled out the pretest and posttest forms. The game does not give feedback on the student's success or failure in the game. The student can progress only when he clicks on the right areas in the game. If he cannot click on the right area, he cannot progress in the game. The student's success in the game is only to finish the game that is, to come to the recording screen.

2.5. Data Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS, 22.0) at a significance level of 0.05. The Kolmogorov Smirnov test was used for normality testing. The results showed that the data were nonnormally distributed. Number, percentage, mean, and median were used for descriptive statistics. The Wilcoxon Signed Rank test was used to assess dependent-group pretest and posttest scores. The Kruskal Wallis and Man Whitney U tests were used to evaluate pretest and posttest scores based on demographic characteristics.

2.6. Ethical Considerations

The study was approved by the Scientific Research Ethics Committee of the Faculty of Medicine of University (Date: February 24, 2021, No: 24237859-190). Permission was obtained from the university. Nursing students were informed about the research purpose, procedure, and confidentiality, and informed consent was obtained from those who agreed to participate. The study was conducted according to the ethical principles outlined by the World Medical Association's Declaration of Helsinki.

2.7. Limitations

The study had three limitations. First, it did not assess skill levels because it was conducted during the COVID-19 pandemic. Second, only one in four students who filled out the pretest form also filled out the posttest form because we reached the students online. Third, the game focused only on tracheostomy care skills.

3. RESULTS

Participants had a mean age of 20.7±2.7 years. The majority of the participants were women (87.2%). More than half the participants had a science high school or Anatolian high school degree (68.8%). Less than half the participants were sophomores (40.8%). More than half the participants lived in the Central Anatolia region of Turkey. Less than a quarter of the participants had provided tracheostomy care on a mannequin in a lab before (17.6%). Eleven participants had had a patient with tracheostomy before (8.8%). Six participants had provided tracheostomy aspiration or care in clinical practice before (4.8%). Twenty-five participants had played games on computers for educational purposes before (20%).

Participants had a median pretest TCKT score of 8.00 (min: 1.00 – max: 15.00). They had a median posttest TCKT score of 10.00 (min: 3.00 – max: 16.00). Participants had a significantly higher posttest TCKT score than pretest score (p= .001). Female participants had a significantly higher pretest TCKT score than male participants (p=0.005). However, there was no significant difference in posttest TCKT scores between male and female participants (p= .250). Participants' pretest TCKT scores did not significantly differ by grade level (p=0.152). However, first-year students had a significantly higher posttest TCKT score than other grade levels (p= .001). It was determined that there was a statistically significant difference between the classes in the posttest scores (p= .003). It was determined that this difference was between first and second (p= .008), second and third year (p= .005) students. It was determined that the scores of the first and second grades were statistically higher than the third grades. In addition, all grade levels had significantly higher posttest TCKT scores than pretest scores (p= .003).

Table 1. *The descriptive characteristics of participants (n=125)*

Characteristics	n	%
Age (Mean±SD)	20.7±2.7 (Min: 18 Max: 36)	
Gender		
Female	109	87.2
Male	16	12.8
Graduated school		
High school of Health	14	11.2
High school	86	68.8
Other*	25	20.0
Class		
1	43	34.4
2	51	40.8
3	15	12.0
4	16	12.8
Area		
Black sea	16	12.8
Central anatolia	77	61.6
East anatolia	13	10.4
Southeast anatolia	2	1.6
Mediterrenian	5	4.0
Aegean	5	4.0
Marmara	7	5.6
The situation of performing tracheostomy care on a model in the laboratory		
Yes	22	17.6
No	103	82.4
Patient follow-up status with tracheostomy		
Yes	11	8.8
No	114	91.2
Status of performing tracheostomy aspiration or maintenance in your clinical practice		
Yes	6	4.8
No	119	95.2
The state of playing games on the computer for educational purposes		
Yes	25	20.0
No	100	80.0

**High school and vocational high school with a foreign language*

Table 2. *Distribution of pretest and posttest mean scores according to some introductory characteristics of students regarding tracheostomy care and total knowledge score*

Introductory features	Pretest score Median (Min-Max)	Posttest score Median (Min-Max)	Statistical evaluation ***
Tracheostomy care knowledge test total score	8.00 (1.00-15.00)	10.00 (3.00-16.00)	Z=-6.575 **p=.000**
Gender			
Female (n=109)	9.00 (1.00-15.00)	10.00 (3.00-16.00)	Z= – 5.888 **p=.000**
Male (n=16)	6.00 (3-.12.00)	9.50 (6.00-13.00)	Z=-2.843 **p=.004**
Statistical evaluation *	Z=-2.812 **p= .005**	Z=-1.150 p= .250	
Class			
1 (n=43)	9.00 (2.00-15.00)	11.00 (3.00-16.00)	Z=-4.725 **p=.000**
2 (n=51)	9.00 (3.00-14.00)	10.00 (5.00-16.00)	Z=-3.346 **p=.001**
3 (n=15)	7.00 (1.00-10.00)	8.00 (5.00-13.00)	Z=-2.024 **p=.043**
4 (n=16)	7.00 (1.00-10.00)	8.00 (5.00-13.00)	Z=-2.913 **p=.004**
Statistical evaluation **	X^2=5.292 p=.152	X^2=14.253 **p=.003**	
Post-Hoc Test		1>3 (p=.005) 2>3 (p=.008)	

**Mann Whitney U test*
***Kruskal Wallis Test*
****Wilcoxon test*

3.1. Participants' Views

The following are our participants' views of the web-based tracheostomy care game:

3.1.1. The Contribution of the Game to Learning

"I got to see the intervention [tracheostomy care] during online education. The game helped me remember the steps of the intervention better. Although it was not as good as doing it in real life, it was better than just reading the book. I think it is a very nice game. I wish there were games for other nursing interventions, too" (S. 2).

"I think it [game] has been very helpful. It helped me keep things in mind. I used to confuse the steps of the intervention. But the game helped me learn them well. It helped me understand the topic better" (S.5).

"I don't think I'll ever confuse the materials because the game showed me what they all were for. Things were catchier because it [game] was all visual." (S.10).

"Although it [game] is all virtual, you get to do the intervention on a patient, which is interesting, and besides it makes things easier to learn. The game helped me see my mistakes." (S.11).

"The game helped me refresh my memory. I felt like I was doing it myself. I kind of pictured it in my mind, even though I have never provided tracheostomy care before." (S.11).

"The game helped me enjoy learning." (S.21).

"I enjoyed learning with the game." (S.37).

"It was nice to play that kind of game because we haven't had the chance to do any lab practice since the pandemic." (P.85)

"The game helped me learn effectively and actively, go over what I've learned before, and remember things that I'd forgotten." (S.67).

3.1.2. The effect of the Game on Competence and Comprehension

"The game helped me realize that I had some gaps in my knowledge, I mean, I thought I learned some things the right way, but apparently, they were wrong. I realized that I didn't know enough about stuff, so the game helped me get a good grasp of things." (S.13).

"The game was effective in terms of the order of the steps. It was all visual, and so I think that it helped me develop skills. The visuals helped me get a good grasp of the topic and the content. The visual context made me feel like I was in a clinical setting." (S.16).

"I remembered the things I'd forgotten. The game helped me get things straight in my mind and refresh my memory." (S.23).

"I think the game helped me do the steps of the intervention correctly and carefully." (S.25).

"It was an easy and fun game, and it made me curious about the next steps. I believe that these kinds of games can get us ready for the patients we're going to care for in real clinical settings. It was an instructive game, and so it made me more interested in the topic and helped me learn more. I was interested in the game because it showed us what to do in that order." (S.27).

"The game taught me what solutions to use for care, what materials to use, and in what order to do things." (P.37).

"The game allowed me to practice like I was in a clinic. It helped me understand the topic better in theory." (S.63).

3.1.3. Putting themselves in the Virtual Nurse's Shoes During Tracheostomy Care

"When I was playing the game, I felt like I was that nurse. I played the game like I was going to hurt the patient if I did something wrong." (S.17).

"I realized that I should be more careful when providing care." (S.52).

"I felt like I was the one providing care to the patient in the game." (S.37).

"I felt like I was really performing those steps." (S.23).

"It felt like I was in a lab providing care to a patient. I felt active and supported." (S.64).

4. DISCUSSION

Nursing education focuses on helping students acquire knowledge and develop skills. Nursing students need cognitive, psychomotor, and affective skills to be able to put theory into practice (20,21). However, they have been studying online since the COVID-19 pandemic, which has caused some problems and urged educators to use different methods (22). Virtual games and simulations have been developed to demonstrate interventions (23). One of those games is the web-based tracheostomy care game. This study investigated the effect of the game on nursing students' knowledge levels and also looked into their views of the process.

Our participants had a significantly higher posttest TCKT score than pretest score ($p < .05$), indicating that the game helped them learn more about tracheostomy care. They stated that it was a fun game that helped them learn the steps of tracheostomy care better, remember the things they forgot, achieve learning retention, and put theory into practice. Students learn tracheostomy care in the theoretical course and put their knowledge into practice in clinical settings. However, six students encounters a patient with tracheostomy in clinical settings or has enough time to practice until perfection. Online games can help students practice clinical skills until perfection and learn how to execute the steps of interventions correctly. Research also shows that web-based games provide nursing students with the opportunity to learn more about nursing interventions (11,19,24-27). Aydın and Dinç (2017) found that a web-based education program improved students' knowledge of medicine dosage calculation (19). Ma et al. (2021) also provided a game-based learning simulation and reported that it improved students' disaster nursing competencies (11). Çakıcı and Çalışkan (2020) presented a web-based animation on nasogastric catheter feeding and found that it improved students' knowledge levels (27). Basit and Korkmaz (2021) also observed that web-based nursing process teaching improved students' knowledge levels (24). Edeer et al. (2019) found that web-based training on care improved students' knowledge levels (28). All in all, research shows that web-based education on different topics improves students' knowledge levels.

Barisone (2019) determined that web-based applications supported traditional education, encouraged students to put theory into practice without being afraid of making mistakes, and helped them develop skills (16). Our participants also noted that the game allowed them to execute the steps of tracheostomy care as if in a real-life clinical setting without being afraid of harming the patient. Ding and Zhang (2018) also reported that web-based applications helped students develop problem-solving skills, made them more motivated, and promoted interpersonal communication (29). Jeon et al. (2021) conducted a focus group after a web-based training on hypovolemic shock. They found that web-based applications appealed to students and helped them enjoy learning (30). Therefore, they concluded that web-based applications were useful for new-generation students to put theory into practice. Our participants also stated that the game allowed them to refresh their memory and learn more about tracheostomy care. Our results suggest that web-based training can complement face-to-face education and help online learners become active and more engaged in their courses.

Female participants had a significantly higher mean pretest TCKT score than male participants. This is probably because the majority of the participants were women (87.2%). Research shows that female students are academically more successful than their male counterparts (31,32). Our first-year participants had a significantly higher mean posttest TCKT score than the other grade levels. In addition, the higher the grade level, the higher the TCKT score. This result showed that the game was suitable for all grade levels. It was beneficial for students who had not had any chance to put their knowledge into practice in clinical settings since the onset of the pandemic. Participants also stated that the game helped them recall their tracheostomy care knowledge. First-year participants had higher TCKT scores than the other grade levels. This may have two reasons. First, first-year students have learned the topic more recently. Second, they were more enthusiastic about doing practice online. This is because they have had distance learning since the pandemic, and therefore, they have developed digital learning skills much more than the other grade levels (33). In addition, the students of Generation Z have more aptitude for online learning and are more interested in web-based applications (34). Our results indicate that the web-based tracheostomy care game provides nursing students with the opportunity to put their theoretical knowledge into practice.

5. CONCLUSION

The web-based tracheostomy care game improved nursing participants' knowledge levels. They also stated that the game helped them enjoy learning and achieve learning retention and made them feel like they were practicing in real-life clinical settings. They also noted that the game jogged their memory and contributed to their learning during the COVID-19 pandemic. Educators should provide nursing students with interactive online applications to help them acquire knowledge and develop skills. Such applications can also support traditional face-to-face learning. In this way, students can prepare for their classes and practice anywhere anytime without harming patients. There should be more applications tailored to nursing.

Acknowledgments: *The authors would like to thank students at Department of Nursing, Faculty of Health Sciences, University.*
Funding: *This study was supported by Gazi University Scientific Research Projects Unit, project no. 47/2017-11.*

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

Ethics Committee Approval: *This study was approved by Ethics committee of the Faculty of Medicine of Karadeniz Technical University (Date: February 24, 2021, No: 24237859-190)*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: ŞBB, EG, NÇ,
Design of the study: ŞBB, EG, NÇ,
Acquisition of data for the study: ŞBB, EG, NÇ,
Analysis of data for the study: ŞBB, EG, NÇ,
Interpretation of data for the study: ŞBB, EG, NÇ,
Drafting the manuscript: ŞBB, EG, NÇ,
Revising it critically for important intellectual content: NÇ
Final approval of the version to be published: ŞBB, EG, NÇ
This study was presented as an Online Oral Presentation at the International Gazi Health Sciences Congress held in Ankara on 15-17 December 2021.

REFERENCES

[1] Şendir M, Şimşekoğlu N, Kaya A, Sümer K. Geleceğin teknolojisinde hemşirelik [Nursing in Future Technology] Sağlık Bilimleri Üniversitesi Hemşirelik Dergisi 2019;1(3):209-214. (Turkish)

[2] Culha I. Active learning methods used in nursing education. Journal of Pedagogical Research 2019;3(2):74-86.

[3] Şenyuva E. Teknolojik gelişmelerin hemşirelik eğitimine yansımaları. Florence Nightingale Journal of Nursing 2019; 27(1):79-90. (Turkish)

[4] Carolan C, Davies CL, Crookes P, McGhee S, Roxburgh M. COVID 19: Disruptive impacts and transformative opportunities in undergraduate nurse education. Nurse Education in Practice 2020;46:102807. DOI:10.1016/j.nepr.2020.102807

[5] Morin KH. Nursing education after COVID-19: Same or different? Journal of Clinical Nursing 2020;29(17-18):3117-3119.

[6] Ning AP, Mok WQ, Chieng YJS, Zhang HM, Ramos P, Mustadi HB, Ying LAU. Effectiveness of virtual reality training in improving knowledge among nursing students: A systematic review, meta-analysis and meta-regression. Nurse Education Today 2020;104655. DOI:10.1016/j.nedt.2020.104655

[7] McEnroe-Petitte D, Farris C. Using gaming as an active teaching strategy in nursing education. Teaching and Learning in Nursing 2020;15(1):61-65.

[8] Ma M, Jain LC, Anderson P. Virtual, augmented reality and serious games for healthcare 1. 1st ed. Heidelberg; Springer Science & Business;2014.

[9] Bayram SB, Caliskan N. Effect of a game-based virtual reality phone application on tracheostomy care education for nursing students: a randomized controlled trial. Nurse Education. Today 2019;79:25-31.

[10] Jarvelainen M, Cooper S, Jones J. Nursing students' educational experience in regional Australia: reflections on acute events. A qualitative review of clinical incidents. Nurse Education Practice 2018;31:188-193.

[11] Ma D, Shi Y, Zhang G, Zhang J. Does theme game-based teaching promote better learning about disaster nursing than scenario simulation: A randomized controlled trial. Nurse Education Today 2021;103:104923. DOI:10.1016/j.nedt.2021.104923

[12] Martí-Parre˜no J, M´endez-Ibanez E, Alonso-Arroyo A. The use of gamification in education: a bibliometric and text mining analysis. J. Comput. Assist. Learn. 2016;32(6):663-676.

[13] Adhikari R, Kydonaki C, Lawrie J, O'Reilly M, Ballantyne, Whitehorn, J, Paterson R. A mixed-methods feasibility study to assess the acceptability and applicability of immersive virtual reality sepsis game as an adjunct to nursing education. Nurse Education Today 2021;103:104944. DOI: 10.1016/j.nedt.2021.104944

[14] Smith SJ, Farra S, Ulrich DL, Hodgson E, Nicely S, Matcham W. Learning and retention using virtual reality in a decontamination simulation. Nurse Education Perspect. 2016;37(4):210-214.

[15] Bracq MS, Michinov E, Arnaldi B, Caillaud B, Gibaud B, Gouranton V, Jannin P. Learning procedural skills with a virtual reality simulator: an acceptability study. Nurse Education Today 2019;79:153-160.

[16] Barisone M, Bagnasco A, Aleo G, Catania G, Bona , Scaglia, SG, Sasso L. The effectiveness of web-based learning in supporting the development of nursing students' practical skills during clinical placements: A qualitative study. Nurse Education in Practice 2019;37:56-61.

[17] Aygencel G, Kemaloğlu YK. COVID-19 Olan yoğun bakım hastalarında trakeostomi uygulamaları. Journal of Ear Nose Throat and Head Neck Surgery 2020;28:84-89. (Turkish)

[18] Swords C, Bergman L, Wilson-Jeffers R, Randall D, Morris LL, Brenner MJ, Arora A. Multidisciplinary tracheostomy quality improvement in the COVID-19 pandemic: Building a global learning community. Annals of Otology, Rhinology & Laryngolog 2020;30(3):262-272.

[19] Aydın AK, Dinç L. Effects of web-based instruction on nursing students' arithmetical and drug dosage calculation skills. CIN: Computers, Informatics, Nursing 2017;35(5):262-269.

[20] Bıyık Bayram Ş, Çalışkan N. Using a game-based virtual reality application in psychomotor skills acquisition: An experience sharing, Journal of Human Sciences 2019;16(1):155-163.

[21] Şendir M, Çelik S, Dişsiz M, Güney R, Açıksöz S, Kolcu M, Bektemür G. Hemşirelik eğitimi ve uygulamasında yeni bir yaklaşım: Hemşirelik eğitimi ve uygulamasının bütünleşmesi [A new approach in nursing education and practice: Integration of nursing education and practice] JAREN. 2018;4(2):92-99. (Turkish)

[22] Yüksekdağ BB. COVID-19 pandemisi döneminde öğrenme ve uzaktan hemşirelik eğitiminde paradigma değişimi [Learning during the COVID-19 pandemic and paradigm change in distance nursing education]. Açık Öğretim Uygulamaları ve Araştırmaları Dergisi 2021;7(1):61-73. (Turkish)

[23] İlaslan N, Demiray A. Koronavirüs 2019 pandemi sürecinde hemşirelik eğitimi: belirsizlikler ve öneriler [Nursing Education During Coronavirus 2019 Pandemic Process: Uncertainties and Suggestions]. E-Journal of Dokuz Eylul University Nursing Faculty 2021;14(2):171-177. (Turkish)

[24] Basit G, Korkmaz F. The effect of web-based nursing process teaching on senior nursing students' care planning skills. International Journal of Nursing Knowledge 2021;32(1):4-19.

[25] Kang J, Seomun G. Evaluating web-based nursing education's effects: a systematic review and meta-analysis. Western Journal of Nursing Research 2018;40(11):1677-1697.

[26] McDonald EW, Boulton JL, Davis JL. E-learning and nursing assessment skills and knowledge–An integrative review. Nurse Education Today 2018;66:166-174.

[27] Çakıcı N, Çalışkan N. The effect of hypermedia-based training method on students learning the application of nasogastric tube feeding: a randomised controlled trial. New Review of Hypermedia and Multimedia 2020;26(3-4):123-137.

[28] Edeer AD, Vural F, Damar HT, Yasak K, Damar M. The Effect of Web-Based Preoperative and Postoperative Patient Care Education on Nursing Students: A Randomized Controlled Study. CIN: Computers, Informatics, Nursing 2019;37(10):541-547.

[29] Ding Y, Zhang P. Practice and effectiveness of web-based problem-based learning approach in a large class-size system: A comparative study. Nurse Education in Practice 2018;31:161-164.

[30] Jeon J, Park S. An exploratory study to develop a virtual reality based simulation training program for hypovolemic shock nursing care: a qualitative study using focus group interview. In Healthcare 2021;9(4):417. DOI: 10.3390/healthcare9040417

[31] Bahar HH, Yıldırım S. İktisadi ve idari bilimler fakültesi öğrencilerinin öğrenme stilleri ile başarılarının cinsiyet, bölüm ve sınıf düzeyine göre incelenmesi. Selçuk Üniversitesi Sosyal Bilimler Enstitüsü Dergisi 2017;38:14-27. (Turkish)

[32] Kantek F, Yeşilbaş H. Türkiye'deki hemşirelik öğrencilerinin problem çözme düzeylerine cinsiyetin etkisi: Bir meta analiz çalışması [The effects of gender on problem solving skills of nursing students in Turkey: a meta-analysis study]. Journal of Human Sciences 2019;6(1):134-142. (Turkish)

[33] Eren DÇ, Korkmaz M, Yıldırım ÖÖ, Avcı İA. COVID-19 pandemi sürecinde hemşirelik öğrencilerinin uzaktan eğitime karşı tutum ve memnuniyet düzeyleri [Investigation of attitude and satisfaction levels of nursing students to distance education during the COVID-19 pandemic process] Journal of Anatolia Nursing and Health Sciences 2021;24(2):246-254. (Turkish)

[34] Raoufı S, Manoochehrı H, Zarıfsanaıey N, Nikravanmofrad M. Developing an E-learning Model for Nursing Education. Journal of Clinical & Diagnostic Research 2020;14(1):11-16.

How to cite this article: Bıyık Bayram Ş, Çalışkan N, Gülnar E. The Effect of Web-Based Tracheostomy Care Game on Nursing Students' Knowledge Levels and Their Views of the Process. Clin Exp Health Sci 2023; 13: *41-47*. DOI: 10.33808/clinexphealthsci.1021950

Clinical and Experimental Health Sciences

Measuring the Knowledge and Behaviors of University Students Toward Rational Use of Herbal Supplement Products in the COVID-19 Pandemic

Sultan Mehtap Büyüker

Üsküdar University, Vocational School of Health Services Pharmacy Services Program, İstanbul, Türkiye.

Correspondence Author: Sultan Mehtap Büyüker
E-mail: sultanmehtap.buyuker@uskudar.edu.tr

Received: 11.11.2021 **Accepted:** 29.08.2022

ABSTRACT

Objective: This study was planned to measure the knowledge and behavior of university students toward the rational use of herbal supplements during the COVID-19 pandemic.

Methods: It was carried out with students from Üsküdar University Faculty of Health Sciences and Health Services Vocational School. The study used a descriptive design and was completed with 640 students who volunteered to participate in the study. The data were collected by using a questionnaire including questions about students' rational use of herbal supplements according to their gender, faculty, the status of having had COVID-19 viral infection, and the status of having received education on pharmacology and medicinal plant products. Counts, mean scores, and percentage values were used in the evaluation of the data.

Results: It was found that 15.5% of the participants were from the Faculty of Health Sciences and their mean age was 20.8±2.84 years and that 84.5% were from the Health Vocational School and their mean age was 21.42±3.87 years. The rate of having had COVID-19 viral infection among students was 22.7%, having received pharmacology education was 75.5%, and having received education on medicinal plant products was 46.3%.

Conclusion: It was found that the pharmacology and medicinal plant products education received by the students studying health significantly affected the rational use of herbal supplements and that students should be given more education on phytotherapy.

Keywords: COVID-19, herbal supplements, students, rational drug use

1. INTRODUCTION

A new coronavirus disease (COVID-19) that broke out in Wuhan, the capital of Hubei province of China, in December 2019 and was defined by the International Virus Taxonomy committee as Severe Acute Respiratory Syndrome Coronavirus 2 (severe acute respiratory syndrome-related coronavirus 2 /SARS-COV-2) has turned into a worldwide pandemic (1). This new coronavirus or severe acute respiratory syndrome coronavirus – 2 (SARS-CoV-2) has a high level of transmissibility from person to person. The World Health Organization (WHO) declared COVID-19 as a pandemic disease on March 11, 2020 (2-4).

With the onset of the COVID-19 pandemic in our country, the interest in herbal supplements has increased, because the COVID-19 viral infection, like all other viral infections, has been frequently on the agenda both on scientific platforms and social media with the news that it affects people with weak immune systems more. As a result, people have turned to herbal supplements to keep their immune systems strong during the pandemic, and they are increasingly using these products. However, as we all know, herbal supplements do not always prove beneficial; they can also cause unwanted side effects (5).

The primary reasons why people use herbal supplements include improving the body condition, treating existing ailments, and avoiding drugs that are considered chemical (6). However, some plants can also show extremely toxic effects and cause unexpected side effects.

However, there is much less data on the interactions of plants used for treatment in chemical drugs with other drugs used. In this case, it is ignored that the preparation used may cause other complications (7). It is also stated that plants produce toxic substances to defend themselves. For this reason, the use of herbal supplements alone or in combination with other drugs may produce unexpected health outcomes.

DOI: 10.33808/clinexphealthsci.1016454

In recent years, individuals have resorted to herbal supplements to cope with the health problems they experience and maintain their general health status or achieve a better level. The news circulating especially on social media channels that some drugs are made from herbal products and that herbal supplements are harmless and even more effective increases the use of herbal supplements (8).

COVID-19 initiates an inflammatory immune response. The release of inflammatory cytokines in a person with COVID-19 disease brings about a cytokine storm and immune impairment, which is followed by acute respiratory distress syndrome and multiple organ dysfunction (9,10). Therefore, it may be helpful to adopt healthy eating habits and using dietary or herbal supplements to support immunity and defend the body against adverse outcomes (11-13). The regulation of the immune function benefit from dietary and herbal supplements, and these supplements control both adaptive and innate immunity in many ways (14).

Dietary or herbal supplements have been stated to show antiviral activity against multiple virus strains, including human immunodeficiency virus, hepatitis B and C, herpes simplex virus, influenza viruses, and earlier coronaviruses, namely, SARS and MERS (15).

The use of herbal and dietary supplements has globally increased significantly during the COVID-19 pandemic. They are usually used alone or in combination with prescription drugs (16-21). In a meta-analysis of seven randomized controlled trials that investigated the effectiveness of herbal remedies in COVID-19 symptoms, such as fever and dry cough, it was reported that the use of herbal remedies and prescription medications in combination shortened the recovery time of these symptoms (16).

2. METHODS

2.1. Study Design and Data Collection

This study used a descriptive design and was carried out in the spring semester of the 2020-2021 academic year. It was conducted with 640 university students from Üsküdar University, Faculty of Health Sciences and Health Vocational Higher School, who agreed to participate in the research. The students who refused to participate in the study or those who submitted an incomplete questionnaire were excluded from the study. The data was solely collected using the Google Forms. The online questionnaire was then distributed over electronic media (WhatsApp, Email) using a snowball sampling method.

A total of 14 questions were asked in the questionnaire applied to the volunteer participants. The questions on the questionnaire were designed to measure the knowledge and behaviors of university students toward rational use of herbal supplement products in the COVID-19 Pandemic. The questionnaire is designed by in the light of our previous studies about Rational Drug Use (22,23). In the questionnaire, the students were asked about their age, the department they are studying in, whether they regularly use prescription drugs, whether they use herbal supplements during their illness, whether they use herbal supplements to protect themselves from diseases during the COVID-19 process, who they get support from when they are sick, herbal supplements, and when they are sick. It was asked whether they benefited from supports or drugs, whether they benefited from herbal support recommendations for protection from COVID-19 in internet publications and social media, and whether they had knowledge about the side effects of herbal supplements. In addition, they were asked to whom they applied first in such a situation. In addition, the participants were asked whether they had received pharmacology training and whether they had taken courses on medicinal herbal products and whether they had COVID-19 infection. Responses collected through Google forms were evaluated statistically.

2.2. Ethical Considerations

Üsküdar University's Non-Interventional Research Ethics Committee approved (Decision No: 61351342/MAY 2021-44) the protocol of the present study and conducted within the framework of the Helsinki Declaration principles. Written and verbal consent of the individuals participating in the study was obtained after informing them about the purpose of the study.

2.3. Statistical Analysis

Within the scope of the research, the knowledge and attitudes of university students towards the rational use of herbal supplements and the use of herbal supplements during the COVID-19 pandemic were investigated. The items on the questionnaire prepared for this purpose were analyzed by using frequency and percentage distributions, as well as bivariate chi-square analysis.

3. RESULTS

Table 1 presents some descriptive characteristics of the students participating in the study. The mean age was 21.33±3.73. It was determined that 15.5% of them were from the Faculty of Health Sciences and 84.5% from the Health Vocational Higher School and that 22.7% of them had had COVID-19 viral infection. Also, 75.5% of these students had taken pharmacology courses, and 46.3% had taken courses related to medicinal plants along with pharmacology.

Table 2 presents the findings of the chi-square analysis conducted to compare the use of herbal supplements and knowledge and attitudes towards using herbal supplements by gender.

The comparison of the use of herbal supplements by gender indicated a significant difference in terms of using them for colds ($\chi2= 30.289$, $p< .05$), before COVID-19 ($\chi2= 7.182$, $p< .05$), and as primary option against diseases ($\chi2= 7.129$, $p<$

.05). When the percentage distributions of these variables with significant differences were examined, it was found that 42.47% of the males and 60.32% of the females used herbal supplements for colds and that 31.51% of the males and 43.93% of the females used them before COVID-19. While the majority of the males (52.74%) used medication against diseases, the majority of the females (59.72%) turned to herbal supplements.

Table 1. *Distribution of the participants' socio-demographic data*

		n	%	Age (Mean ± SD)
Gender	Male	146	22.8	22.10 ± 3.97
	Female	494	77.2	21.10 ± 3.63
School	Faculty of Health Sciences	99	15.5	20.81 ± 2.84
	Health Vocational Higher School	541	84.5	21.42 ± 3.87
Status of having had COVID-19	Yes	145	22.7	21.59 ± 4.12
	No	495	77.3	21.25 ± 3.61
Pharmacology education	Yes	483	75.5	21.29 ± 3.32
	No	157	24.5	21.43 ± 4.78
Medicinal plants education	Yes	296	46.3	21.58 ± 3.96
	No	344	53.8	21.11 ± 3.52
Total		640	100.0	21.33 ± 3.73

SD: standard deviation

Regarding the comparison of knowledge and attitudes toward using herbal supplements by gender, there was a significant difference between genders in terms of sources of information about herbal supplements ($\chi2$= 8.101, p< .05) and having knowledge about the side effects of herbal products ($\chi2$= 18.238, p< .05). When the percentage distributions of these variables with significant differences were examined, it was found that males and females mainly learned about herbal supplements from the Internet, and this rate was 43.84% for males and 34.01% for females. The majority of the males and females knew that herbal products could have side effects, and this rate was 38.36% in males and 48.99% in females. While the females used the Internet less as a source of information compared to males, they had more information about the side effects of herbal products.

Table 4 shows the findings of the chi-square analysis conducted to compare the use of herbal supplements and knowledge and attitudes toward using these supplements according to the status of having had the COVID-19 disease. While there was no significant difference in the knowledge and attitudes of the participants towards using herbal supplements according to the status of having had the COVID-19 disease (p> .05), a significant difference was found according to the variable of using herbal supplements during the COVID-19 process, which is one of the herbal supplement usage variables ($\chi2$=11.170, p< .01). Also, 53.10% of the participants who had had COVID-19 and 37.58% of those who had not had the disease were found to use herbal supplements.

Table 6 presents the findings of the chi-square analysis conducted to compare the use of herbal supplements and the knowledge and attitudes towards using herbal supplements according to the status of having received education on medicinal plants.

According to the status of having received education on medicinal plants, there was a significant difference in the variable of using herbal supplements for colds ($\chi2$= 6.800, p< .05), which is one of the variables of using herbal supplements. When the percentage distributions of these variables, which showed a significant difference, were examined, it was found that the majority of the participants who had and had not received education on medicinal plants used herbal supplements for colds, 23.99% of those who had received education ob medicinal plants and 32.85% of those who had not were found to sometimes use medicinal plants.

According to the status of having received education on medicinal plants, there was a significant difference in the variables of primary sources of information about herbal supplements ($\chi2$= 28.139, p< .001) and primary sources of information on problems related to herbal supplements ($\chi2$= 24.650, p< .001), which are among the variables of knowledge and attitudes towards using herbal supplements. When the percentage distributions of the variables that showed a significant difference were examined, the majority of the participants who had received education on medicinal plants were found to mostly turn to the Internet (34.80%) and pharmacists (34.12%) for using herbal supplements, whereas those who had not received education on medicinal plants mostly got information from the Internet (37.50%) and doctors (36.05%). When the primary sources of information regarding the problems related to herbal supplements were examined, 49.32% of those who had received education on medicinal plants primarily consulted doctors, and the rate of participants primarily consulting a doctor among those who had not received education on medicinal plants was 59.01%.

Table 2. *Comparison of the use of herbal supplements and the knowledge and attitudes towards using herbal supplements by gender*

		Gender				Total (n=640)		χ2	P
		Male (n=146)		Female (n=494)					
		f	%	f	%	f	%		
Status of Using Herbal Supplements									
Using herbal supplements for colds	Yes	62	42.47	298	60.32	360	56.25	30.289	0.000
	Sometimes/ Not sure	42	28.77	142	28.74	184	28.75		
	No	42	28.77	54	10.93	96	15.00		
Using herbal supplements before COVID-19	Yes	46	31.51	217	43.93	263	41.09	7.182	0.007
	No	100	68.49	277	56.07	377	58.91		
Using herbal supplements during COVID-19	Yes	51	34.93	212	42.91	263	41.09	2.967	0.085
	No	95	65.07	282	57.09	377	58.91		
Primary option against diseases	Herbal supplement	69	47.26	295	59.72	364	56.88	7.129	0.008
	Medication	77	52.74	199	40.28	276	43.13		
Knowledge and Attitudes Towards Using Herbal Supplements									
Primary sources of information about herbal supplements	Friends	15	10.27	36	7.29	51	7.97	8.101	0.044
	Doctors	40	27.40	158	31.98	198	30.94		
	Pharmacists	27	18.49	132	26.72	159	24.84		
	Internet	64	43.84	168	34.01	232	36.25		
Relying on herbal products recommended on the Internet and social media to protect against COVID-19	Yes	8	5.48	31	6.28	39	6.09	0.173	0.917
	Sometimes	65	44.52	223	45.14	288	45.00		
	No	73	50.00	240	48.58	313	48.91		
Knowing about the side effects of herbal products	Yes	56	38.36	242	48.99	298	46.56	18.238	0.000
	Somewhat/ Not sure	50	34.25	189	38.26	239	37.34		
	No	40	27.40	63	12.75	103	16.09		
Primary sources of information on problems related to herbal supplements	Family	20	13.70	71	14.37	91	14.22	1.067	0.785
	Doctors	81	55.48	268	54.25	349	54.53		
	Pharmacists	20	13.70	82	16.60	102	15.94		
	Internet	25	17.12	73	14.78	98	15.31		

χ^2: Pearson chi-square value, f: frequency, p: probability value

Table 3. *Comparison of the use of herbal supplements and knowledge and attitudes towards using these products according to students' schools*

		School						χ2	p
		Faculty of Health Sciences (n=99)		Health Vocational Higher School (n=541)		Total (n=640)			
		f	%	f	%	f	%		
Status of Using Herbal Supplements									
Using herbal supplements for colds	Yes	57	57.58	303	56.01	360	56.25	3.619	0.164
	Sometimes/ Not sure	33	33.33	151	27.91	184	28.75		
	No	9	9.09	87	16.08	96	15.00		
Using herbal supplements before COVID-19	Yes	46	46.46	217	40.11	263	41.09	1.396	0.237
	No	53	53.54	324	59.89	377	58.91		
Using herbal supplements during COVID-19	Yes	44	44.44	219	40.48	263	41.09	0.543	0.461
	No	55	55.56	322	59.52	377	58.91		
Primary option against diseases	Herbal supplement	62	62.63	302	55.82	364	56.88	1.579	0.209
	Medication	37	37.37	239	44.18	276	43.13		
Knowledge and Attitudes Towards Using Herbal Supplements									
Primary sources of information about herbal supplements	Friends	13	13.13	38	7.02	51	7.97	5.123	0.163
	Doctors	32	32.32	166	30.68	198	30.94		
	Pharmacists	20	20.20	139	25.69	159	24.84		
	Internet	34	34.34	198	36.60	232	36.25		
Relying on herbal products recommended on the Internet and social media to protect against COVID-19	Yes	2	2.02	37	6.84	39	6.09	3.514	0.173
	Sometimes	48	48.48	240	44.36	288	45.00		
	No	49	49.49	264	48.80	313	48.91		
Knowing about the side effects of herbal products	Yes	49	49.49	249	46.03	298	46.56	3.123	0.210
	Somewhat/ Not sure	40	40.40	199	36.78	239	37.34		
	No	10	10.10	93	17.19	103	16.09		
Primary sources of information on problems related to herbal supplements	Family	19	19.19	72	13.31	91	14.22	5.786	0.123
	Doctors	57	57.58	292	53.97	349	54.53		
	Pharmacists	9	9.09	93	17.19	102	15.94		
	Internet	14	14.14	84	15.53	98	15.31		

χ^2: Pearson chi-square value, f: frequency, p: probability value

Table 4. *Comparison of the use of herbal supplements and knowledge and attitudes towards using these supplements according to the status of having had the COVID-19 disease*

		Status of having had COVID-19				Total (n=640)		χ2	p
		Yes (n=145)		No (n=495)					
		f	%	f	%	f	%		
Status of Using Herbal Supplements									
Using herbal supplements for colds	Yes	90	62.07	270	54.55	360	56.25	4.714	0.095
	Sometimes/ Not sure	41	28.28	143	28.89	184	28.75		
	No	14	9.66	82	16.57	96	15.00		
Using herbal supplements before COVID-19	Yes	64	44.14	199	40.20	263	41.09	0.718	0.397
	No	81	55.86	296	59.80	377	58.91		
Using herbal supplements during COVID-19	Yes	77	53.10	186	37.58	263	41.09	11.170	0.001
	No	68	46.90	309	62.42	377	58.91		
Primary option against diseases	Herbal supplement	84	57.93	280	56.57	364	56.88	0.085	0.770
	Medication	61	42.07	215	43.43	276	43.13		
Knowledge and Attitudes Towards Using Herbal Supplements									
Primary sources of information about herbal supplements	Friends	17	11.72	34	6.87	51	7.97	3.753	0.289
	Doctors	43	29.66	155	31.31	198	30.94		
	Pharmacists	33	22.76	126	25.45	159	24.84		
	Internet	52	35.86	180	36.36	232	36.25		
Relying on herbal products recommended on the Internet and social media to protect against COVID-19	Yes	10	6.90	29	5.86	39	6.09	2.837	0.242
	Sometimes	73	50.34	215	43.43	288	45.00		
	No	62	42.76	251	50.71	313	48.91		
Knowing about the side effects of herbal products	Yes	62	42.76	236	47.68	298	46.56	1.090	0.580
	Somewhat/ Not sure	58	40.00	181	36.57	239	37.34		
	No	25	17.24	78	15.76	103	16.09		
Primary sources of information on problems related to herbal supplements	Family	22	15.17	69	13.94	91	14.22	3.548	0.315
	Doctors	79	54.48	270	54.55	349	54.53		
	Pharmacists	17	11.72	85	17.17	102	15.94		
	Internet	27	18.62	71	14.34	98	15.31		

χ^2: Pearson chi-square value, f: frequency, p: probability value

Table 5. *Comparison of the use of herbal supplements and knowledge and attitudes towards using these supplements according to the status of having received pharmacology education*

		Having received pharmacology education				Total (n=640)		χ2	p
		Yes (n=483)		No (n=157)					
		f	%	f	%	f	%		
Status of Using Herbal Supplements									
Using herbal supplements for colds	Yes	273	56.52	87	55.41	360	56.25	0.830	0.660
	Sometimes/ Not sure	135	27.95	49	31.21	184	28.75		
	No	75	15.53	21	13.38	96	15.00		
Using herbal supplements before COVID-19	Yes	199	41.20	64	40.76	263	41.09	0.009	0.923
	No	284	58.80	93	59.24	377	58.91		
Using herbal supplements during COVID-19	Yes	196	40.58	67	42.68	263	41.09	0.215	0.643
	No	287	59.42	90	57.32	377	58.91		
Primary option against diseases	Herbal supplement	279	57.76	85	54.14	364	56.88	0.634	0.426
	Medication	204	42.24	72	45.86	276	43.13		
Knowledge and Attitudes Towards Using Herbal Supplements									
Primary sources of information about herbal supplements	Friends	35	7.25	16	10.19	51	7.97	9.991	0.019
	Doctors	141	29.19	57	36.31	198	30.94		
	Pharmacists	134	27.74	25	15.92	159	24.84		
	Internet	173	35.82	59	37.58	232	36.25		
Relying on herbal products recommended on the Internet and social media to protect against COVID-19	Yes	28	5.80	11	7.01	39	6.09	0.303	0.859
	Sometimes	218	45.13	70	44.59	288	45.00		
	No	237	49.07	76	48.41	313	48.91		
Knowing about the side effects of herbal products	Yes	233	48.24	65	41.40	298	46.56	2.265	0.322
	Somewhat/ Not sure	174	36.02	65	41.40	239	37.34		
	No	76	15.73	27	17.20	103	16.09		
Primary sources of information on problems related to herbal supplements	Family	64	13.25	27	17.20	91	14.22	9.612	0.022
	Doctors	258	53.42	91	57.96	349	54.53		
	Pharmacists	89	18.43	13	8.28	102	15.94		
	Friends	72	14.91	26	16.56	98	15.31		

χ^2: Pearson chi-square value, f: frequency, p: probability value

Table 6. *Comparison of the use of herbal supplements and the knowledge and attitudes towards using herbal supplements according to the status of having received education on medicinal plants*

		The status of having received education on medicinal plants				Total (n=640)		χ2	P
		Yes (n=296)		No (n=344)					
		f	%	f	%	f	%		
Status of Using Herbal Supplements									
Using herbal supplements for colds	Yes	174	58.78	186	54.07	360	56.25	6.800	0.033
	Sometimes/ Not sure	71	23.99	113	32.85	184	28.75		
	No	51	17.23	45	13.08	96	15.00		
Using herbal supplements before COVID-19	Yes	122	41.22	141	40.99	263	41.09	0.003	0.953
	No	174	58.78	203	59.01	377	58.91		
Using herbal supplements during COVID-19	Yes	116	39.19	147	42.73	263	41.09	0.825	0.364
	No	180	60.81	197	57.27	377	58.91		
Primary option against diseases	Herbal supplement	177	59.80	187	54.36	364	56.88	1.917	0.166
	Medication	119	40.20	157	45.64	276	43.13		
Knowledge and Attitudes Towards Using Herbal Supplements									
Primary sources of information about herbal supplements	Friends	18	6.08	33	9.59	51	7.97	28.139	0.000
	Doctors	74	25.00	124	36.05	198	30.94		
	Pharmacists	101	34.12	58	16.86	159	24.84		
	Internet	103	34.80	129	37.50	232	36.25		
Relying on herbal products recommended on the Internet and social media to protect against COVID-19	Yes	22	7.43	17	4.94	39	6.09	1.802	0.406
	Sometimes	133	44.93	155	45.06	288	45.00		
	No	141	47.64	172	50.00	313	48.91		
Knowing about the side effects of herbal products	Yes	152	51.35	146	42.44	298	46.56	5.083	0.079
	Somewhat/ Not sure	101	34.12	138	40.12	239	37.34		
	No	43	14.53	60	17.44	103	16.09		
Primary sources of information on problems related to herbal supplements	Family	37	12.50	54	15.70	91	14.22	24.650	0.000
	Doctors	146	49.32	203	59.01	349	54.53		
	Pharmacists	70	23.65	32	9.30	102	15.94		
	Friends	43	14.53	55	15.99	98	15.31		

χ^2: Pearson chi-square value, f: frequency, p: probability value

4. DISCUSSION

Herbal supplements are consumed unconsciously because they are not in the prescription drug group. Although people who have not received health education do not have enough information about herbal supplements, their posts on the Internet and social media endanger human health. At the same time, some plants are shown as healing and miraculous plants by herbalists, which increases the demand for herbal supplements. With the onset of the COVID-19 pandemic, the use of herbal supplements has become more widespread with the increase in posts that they have positive effects on the immune system. In our study, we investigated whether university students in the field of health used herbal supplements rationally and their knowledge levels on this subject. A similar study was conducted by Büyüker SM at Üsküdar University Faculty of Health Sciences in 2020 on rational antibiotic use, and as a result of the study, although most of the participants had enough knowledge about rational antibiotic use (RAU), it was found that the majority of them needed more education on rational drug use (RDU) and RAU and that most of them wanted to treat diseases with alternative treatments (22). Also, Büyüker SM et al. (2018) studied the effect of the pharmacology education received by the students of the Pharmacy Services Technician program in the same university on their RDU attitudes and found that the students had enough level of knowledge on RDU (23). In a study conducted by Aldwihi Leen A. et al. in Saudi Arabia, the use of herbal supplement products before and after the COVID-19 pandemic was investigated, and it was observed that the use of herbal supplements increased significantly during the pandemic process (4). The present study was conducted with the participation of health students, and the demographic characteristics of the students, their status of having had COVID-19 viral infection, whether they used herbal supplements during the pandemic process, whether they had received education on pharmacology and medicinal plant products, and the information sources they consulted while using these supplements were investigated. The results of the study indicated that while the majority of the males (52.74%) used medications against diseases, the majority of the females used herbal supplements (59.72%). While the males and females obtained information about herbal supplements from the Internet most, this rate was 43.84% in males and 34.01% in females. However, while the majority of the males and females knew that herbal products might have side effects, this rate was 38.36% in males and 48.99% in females. In other words, females used the Internet less as a source of information compared to males, and they knew more about the side effects of herbal products. Also, 53.10% of the participants who had had COVID-19 and 37.58% of those who had not had COVID-19 used herbal supplements. As the results of the study suggest, having had the COVID-19 infection increased the interest in herbal supplements. When the participants who had and had not received education on pharmacology and medicinal plants were compared, it was determined that those who had received education on these subjects consulted doctors and pharmacists more than those who had not. It was found that the students' confidence in herbal supplements recommended for protection against COVID-19 on the Internet and social media was at a low level. These results show us that it is extremely important for health students to receive pharmacology education on rational use of herbal supplements and medicinal plant supplements.

5. CONCLUSION

In this study, we investigated the status of having had COVID-19 viral infection, use of herbal supplements, the status of having received education on pharmacology and medicinal plant products, the sources of information used before using herbal supplements, and the effects of the Internet and social media on the use of herbal supplements. In conclusion, it was found that the students exhibited a rational approach to herbal supplements since they were studying health sciences and they had received education on pharmacology and medicinal plant products. As a result of the study, it is thought that provision of students with more information on phytotherapy within the targeted pharmacology education and supporting pharmacology courses with seminars to be given in the field of phytotherapy, if necessary, will be effective in the rational use of herbal supplements and prevent health problems that may occur as a result of some disinformation via the Internet and social media.

Since bivariate chi-square analysis allows examining the relationship between two categorical variables, this analysis was used in the examination of the study data. With the chi-square analysis, the knowledge and attitudes of the participants towards the rational use of herbal supplements and the use of herbal supplements were compared according to their gender, school, the status of having had COVID-19, and status of having received education on pharmacology and medicinal plants. The analyses were conducted on the IBM SPSS Statistic 22 software package and the level of significance was determined as $\alpha = 0.05$

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

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

Ethics Committee Approval: *This study was approved by Üsküdar University Non-Interventional Research Ethics Committee was obtained (Decision No: 61351342 /May 2021-44).*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

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

REFERENCES

[1] Dikmen BY, Yağız PAT, Dileköz E, Summak GY, Kul Oğuz, Filaziv A. Pharmacotherapy of COVID-19. Veteriner Farmakoloji ve

Toksikoloji Derneği Bülteni 2020;11(2):80-114. DOI: 10.38137/vetfarmatoksbulten.769889 (Turkish)

[2] Peeri NC, Shrestha N, Rahman MS, Zaki R, Tan Z, Bibi S, Baghbanzadeh M, Aghamohammadi N, Zhang W, Haque U. The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: What lessons have we learned? Int J Epidemiol. 2020;49(3):717-726. DOI: 10.1093/ije/dyaa033

[3] World Health Organization. WHO Director-General's Opening Remarks at the Media Briefing on COVID-19-11 March 2020. Published [11 March 2020]. Accessed [05 April 2021]. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-COVID-19—-11-march-2020

[4] Aldwihi LA, Khan SI, Alamri FF, AlRuthia Y, Alqahtani F, Fantoukh OI, Assiri A, Almohammed OA. Patients' behavior regarding dietary or herbal supplements before and during COVID-19 in Saudi Arabia. International Journal of Environmental Research and Public Health 2021;18(10):5086. DOI: 10.3390/ijerph18105086

[5] Akarsu GD, Akarsu RH. Determination of herbal support status of faculty of health sciences students. YOBU Faculty of Health Sciences Journal 2021;2(1):62-67. DOI: 10.34108/eujhs.1038226

[6] Chan K. Some aspect of toxic contaminants in herbal medicines. Chemosphere 2003;52(9):1361-1371. DOI:10.1016/S0045-6535(03)00471-5

[7] Parlaz EA, Tekgül N, Karademirci E, Öngel K. Adolescence period: Physical growth, psychological and social development process. The Journal of Turkish Family Physician 2012;3(4):10-15. (Turkish)

[8] Erdem S, Eren PA. Adverse effects of herbal medicines and products. Turkish Bulletin of Hygiene and Experimental Biology 2009; 66(3): 133-141.

[9] Hamer M, Kivimäki M, Gale C.R, Batty GD. Lifestyle risk factors, inflammatory mechanisms, and COVID-19 hospitalization: A community-based cohort study of 387,109 adults in UK. Brain Behav Immun. 2020;87:184-187. DOI: 10.1016/j.bbi.2020.05.059

[10] Azkur AK, Akdis M, Azkur D, Sokolowska M, Van de Veen W, Brüggen M-C, O'Mahony L, Gao Y, Nadeau K, Akdis CA. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy 2020;75(7):1564-1581. DOI: 10.1111/all.14364.

[11] Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W, Tian DS. Dysregulation of immune response in patients with Coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis. 2020;71(15):762-768. DOI: 10.1093/cid/ciaa248.

[12] Morais AHA, Aquino JS, da Silva-Maia JK, Vale SHL, Maciel BLL, Passos TS. Nutritional status, diet and viral respiratory infections: perspectives for severe acute respiratory syndrome coronavirus 2. Br J Nutr. 2021;125(8):851-862. DOI: 10.1017/S000.711.4520003311

[13] Briguglio M, Pregliasco FE, Lombardi G, Perazzo P, Banfi G. The malnutritional status of the host as a virulence factor for new Coronavirus SARS-CoV-2. Front Med. 2020;7:146. DOI: 10.3389/fmed.2020.00146

[14] Wang K, Conlon M, Ren W, Chen BB, Bączek T. Natural products as targeted modulators of the immune system. J Immunol Res. 2018; 2018:7862782. DOI: 10.1155/2018/7862782.

[15] Xian Y, Zhang J, Bian Z, Zhou H, Zhang Z, Lin Z, Xu H. Bioactive natural compounds against human coronaviruses: a review and perspective. Acta Pharm Sin B. 2020;10(7):1163-1174. DOI: 10.1016/j.apsb.2020.06.002

[16] Ang L, Song E, Lee HW, Lee MS. Herbal medicine for the treatment of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis of randomized controlled trials. J Clin Med. 2020;9(5):1583. DOI: 10.3390/jcm9051583.

[17] Luo H, Tang QL, Shang YX, Liang SB, Yang M, Robinson N, Liu JP. Can Chinese medicine be used for prevention of corona virus disease 2019 (COVID-19)? A Review of historical classics, research evidence and current prevention programs. Chin J Integr Med. 2020; 26(4):243-250. DOI: 10.1007/s11655.020.3192-6

[18] Panyod S, Ho CT, Sheen LY. Dietary therapy and herbal medicine for COVID-19 prevention: A review and perspective. J Tradit Complement Med. 2020;10(4):420-427. DOI: 10.1016/j.jtcme.2020.05.004

[19] Ang L, Lee HW, Choi JY, Zhang J, Soo Lee M. Herbal medicine and pattern identification for treating COVID-19: A rapid review of guidelines. Integr Med Res. 2020;9(2):100407. DOI: 10.1016/j.imr.2020.100407

[20] El Alami, A, Fattah, A, Abderrahman C. Medicinal plants used for the prevention purposes during the COVID-19 pandemic in Morocco. J Anal Sci Appl Biotechnol. 2020; 2(1):4-11. DOI: 10.48402/IMIST.PRSM/jasab-v2i1.21056

[21] AlNajrany SM, Asiri Y, Sales I, AlRuthia Y. The commonly utilized natural products during the COVID-19 pandemic in Saudi Arabia: A cross-sectional online survey. Int J Environ Res Public Health. 2021;18(9):4688. DOI: 10.3390/ijerph18094688

[22] Büyüker SM. Evaluation of knowledge, attitude and practices of midwifery department students of faculty of health sciences about rational antibiotic use. JAMER. 2020;5(2):11-16. (Turkish)

[23] Büyüker SM, Odabaşı G. Assessment of knowledge and behaviour of pharmacy services students in vocational school of health services for rational drug use. Eastern Journal of Medical Sciences 2018;3(3):28-33.

How to cite this article: Büyüker SM. Measuring the Knowledge and Behaviors of University Students Toward Rational Use of Herbal Supplement Products in the COVID-19 Pandemic. Clin Exp Health Sci 2023; 13: 48-57. DOI: 10.33808/clinexphealthsci.1016454

Clinical and Experimental Health Sciences

Epidemiological Analysis and Management of Patients with Facial Space Infections of Odontogenic Origin: A Retrospective Evaluation of Two Years

Ömer Ekici

Afyonkarahisar Health Sciences University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Afyonkarahisar, Türkiye.

Correspondence Author: Ömer Ekici
E-mail: dromerekici@hotmail.com

Received: 12.09.2022 **Accepted:** 01.11.2022

ABSTRACT

Objective: Odontogenic infections are one of the most common pathologies in the oral and maxillofacial regions. The spread of odontogenic infections after unsuccessful or late treatment can lead to serious complications. The aim of this study is to examine the epidemiological features and treatment management of patients with odontogenic facial abscesses.

Methods: This retrospective study included 88 patients with odontogenic facial area abscesses treated at Afyonkarahisar health sciences university, faculty of dentistry's maxillofacial surgery clinic between 2019-2021. The socio-demographic, socio-economic characteristics and clinical examination findings of the patients and treatment methods for odontogenic abscess were analyzed comprehensively. Data were evaluated using SPSS-20 and the level of significance was set at $p < .05$.

Results: In the two-year period between 2019 and 2021, 88 patients (44 male and 44 female, mean age was 39.72±16.42) were treated for diffuse facial infections of odontogenic origin. The most commonly involved area was the submandibular area (38.6%), the most affected tooth was mandibular 1st molar and mandibular 3rd molars (18.2%), and the most common cause was dental caries (65.90%). Incision and drainage were performed in half of the patients (36.4% intraoral, 13.6% extraoral). The most commonly used drugs were clindamycin (36.4%), amoxicillin-clavulanate, and ornidazole combination (27.3%).

Conclusion: The results of this study confirm that odontogenic abscesses can heal without complications with timely and effective basic interventions such as incision and drainage. In this study, successful results were obtained with the parenteral clindamycin, and a combination of oral amoxicillin-clavulanate and ornidazole in the treatment of odontogenic abscesses.

Keywords: Epidemiological analysis, facial area abscess, odontogenic infection, treatment management.

1. INTRODUCTION

Odontogenic infections are one of the most prevalent pathologies in the oral and maxillofacial regions. Caries or nonvital teeth, postoperative infections, pericoronitis, and periodontal diseases can cause odontogenic infections (1). Odontogenic infections are usually self-limiting and localized, but can sometimes develop into devastating polymicrobial infections that rapidly spread from the facial cavities or deep planes of the neck into the mediastinum, pleural cavities, and pericardium (2,3). Serious complications following odontogenic infection are uncommon, thanks to modern diagnosis and treatment, and only occur where predisposing factors are present (4). Predisposing factors for odontogenic infections include long-term diabetes mellitus, radiation therapy, chemotherapy, Human Immunodeficiency Virus (HIV) infection, immunosuppression drug use, and chronic alcohol abuse (5,6). The spread of odontogenic infection, as well as the patient's decreased immune competence, may be caused by specific virulence and synergistic effects of aerobic and anaerobic microorganisms (7).

Odontogenic infections can vary in severity from minor localized infections to severe, life-threatening infections (8). A mortality rate ranging from 10% to 40% after an odontogenic infection has been recorded in the pre-antibiotic era. The prognosis of odontogenic infections has greatly improved since the advent of antibiotics. However, with the elimination of the odontogenic focus, surgical incision and drainage remain the basis of treatment (9). Most patients recover completely after adequate surgical treatment with appropriate antibiotics administration and removal of the odontogenic focus (10). The spread of odontogenic infections after unsuccessful or late treatment can lead to serious complications such as necrotizing mediastinitis, necrotizing fasciitis, septic shock, multiorgan failure, and death (11).

The treatment of odontogenic abscesses follows a universally accepted protocol that involves the elimination of the reason, abscess drainage, and antibiotic therapy. The treatment of serious odontogenic infections necessitates early detection and an interdisciplinary approach. According to Flynn (12),

DOI: 10.33808/clinexphealthsci.994256

the basic principles in the management of odontogenic abscesses are: 1) determining the severity of the infection, 2) evaluation of the patient's body defense mechanisms, 3) early determination of whether the patient can be treated by a general dentist or to be referred to an oral and maxillofacial surgeon, 4) eliminating the cause of the infection with endodontic treatment or removing the tooth that caused the infection, 5) abscess or in the case of cellulitis, incision and drainage, if necessary culture and antibiotic sensitivity test, 6) medical support of the patient, 7) appropriate antibiotic prescription and appropriate antibiotic management, and 8) frequent evaluation and follow-up of the patient. Early diagnosis, control of the airway, and rapid surgical treatment are very important in the treatment of an odontogenic abscess (13). The aim of this study is to comprehensively examine the epidemiological characteristics and treatment management of patients with odontogenic facial abscesses.

2. METHODS

This retrospective study was carried out on patients with odontogenic facial abscess of odontogenic origin at the department of oral and maxillofacial surgery, faculty of dentistry, Afyonkarahisar health sciences university, between January 1, 2019, and December 31, 2020. The study was approved by the Afyonkarahisar health sciences university, clinical research ethics committee (2020/13 – 504) and was conducted in accordance with the principles of the Helsinki Declaration.

Eighty-eight patients who were evaluated using diagnosis and follow-up forms prepared for patients with odontogenic abscess were included in the study. Clinical, laboratory, and radiological file records of the patients were examined. Thise form included the patient's socio-demographic data, socio-economic data, the patient's medical history, symptoms, clinical examination findings, radiological findings, drug therapy, surgical treatment processes, and follow-up findings (see form example in appendix 1). Patients with non-odontogenic facial area abscesses and patients with incomplete patient file records were excluded from the study.

Statistical analysis of the data was performed using version 20 of the SPSS statistical program (SPSS Inc, Chicago, IL, USA). Mean and standard deviation values were given in the descriptive statistics of continuous data, and number and percentage values were given in nominal data. The normal distribution of data was evaluated using the Kolmogorov Smirnov test. Continuous data that were found to fit normal distribution were analyzed by student's t-test. When comparing categorical variables, Fisher's exact test was employed. A p-value below 0.05 was considered significant.

3. RESULTS

3.1. Sociodemographic and Socioeconomic Characteristics

Between 2019 and 2020, 88 patients, 44 male, and 44 female, were admitted to the oral and maxillofacial surgery clinic due to odontogenic-induced facial area abscesses. The age range of the patients was between 12 and 67 (mean age was 39.72±16.42). The majority of patients (40.9%) were aged 50-59 years (Figure 1). When the participants were examined in terms of educational status, high school graduates were in the majority (36.4%), followed by secondary and primary school graduates. When the occupations of the participants are examined, the largest group is housewives (31.8%) and workers (27.3%). Most of the participants (77.3%) were married. 72.7 % of the participants lived in cities, and in total, 54.5% of the participants had their own homes. Most of the participants (%63.6) had a monthly income between 2.000 and 4.000 TL. When the participants were examined in terms of the number of children, those with two children (40.9%) constituted the largest group (Table 1).

Table 1. *Socio-demographic and socio-economic characteristics*

	n	%
Gender		
Female	44	50
Male	44	50
Education Status		
İlliterate	4	4.5
Primary school	24	27.5
Secondary school	24	27.5
High school	32	36.4
University	4	4.5
Working status		
Student	12	13.6
Housewife	28	31.8
Farmer	4	4.5
Self employment	8	9.1
Worker	24	27.3
Civil servant	12	13.6
Marital status		
Married	68	77.3
Single	20	22.7
Place of residence		
City	64	72.7
Village	24	27.3
Living place		
Own house	48	54.5
House for rent	16	18.2
Country house	24	27.3
Monthly income		
<2000 TL	24	27.3
2.000-4.000 TL	56	63.6
4000-6.000 TL	8	9.1
Number of children		
No children	20	22.7
One child	8	9.1
Two children	36	40.9
Three children	20	22.7
More than three children	4	4.5
Total	88	100

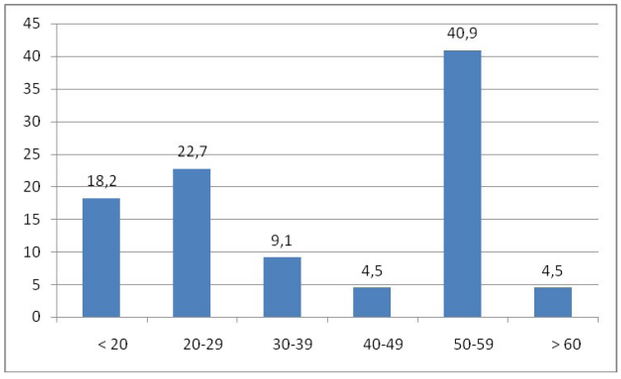


Figure 1. *Distribution of the age ranges of the participants*

Table 2. *Clinical characteristics of the participants*

	n	%
Presence of systemic disease		
Yes	36	40.9
No	52	59.1
Brushing frequency		
< Once / day	28	31.8
Once / day	36	40.9
Twice / day	24	27.3
Smoking		
Yes	36	40.9
No	52	59.1
Duration of symptoms in days		
1 day	4	4,5
2 days	28	31,8
3 days	16	18,2
4 days	4	4,5
5 days	16	18,2
7 days	8	9,1
>10 days	12	13.5
Referral source		
No (Direct application)	12	13.6
Family doctor	12	13.6
Emergency clinic	8	9.1
Dental practitioner	40	45.5
Other clinics	8	9.1
Application to multiple locations	8	9.1
Prognosis		
Acute	64	72.7
Chronic	24	27.3
Underlying dental pathology		
Caries	58	65.9
Post-endodontic	9	10.22
Pericoronitis	16	15.9
Post-extraction	4	4.54
Cysts	2	2.27
Periimplantitis	1	1.13
Total	88	100

3.2. Clinical Signs and Symptoms

The clinical characteristics of the participants were given in Table 2. Forty-point nine percent of the participants had at least one systemic disease in this study. It was observed that the oral hygiene status of the patients was generally poor. Thirty-one point eight percent of the patients did not brush their teeth even once a day and 40.9% of them were smokers. Thirty-one point eight percent of the patients applied on the 2nd day of their symptoms. Only 13.6% of the patients applied directly to the oral and maxillofacial surgery clinic. 72.7% of diffuse facial area abscesses were acute. When the underlying pathology of odontogenic abscesses was examined, dental caries (65.9%), pericoronitis (15.9%), and post-endodontic causes (10.22%) were the top three. The most involved facial spaces were submandibular (38.6%) and fossa canina (22.7%) respectively (Figure 2). When the teeth causing odontogenic abscess were examined, mandibular 1st molar teeth and mandibular 3rd molars were in the first place with a rate of 18.2%, followed by maxillary canine teeth and mandibular 2nd molars with 13.6%. (Figure 3). The most common symptoms in patients with odontogenic abscess were swelling (100%), pain (77.27%), and trismus (63.63%) respectively (Figure 4). In most patients, most of these symptoms occurred together.

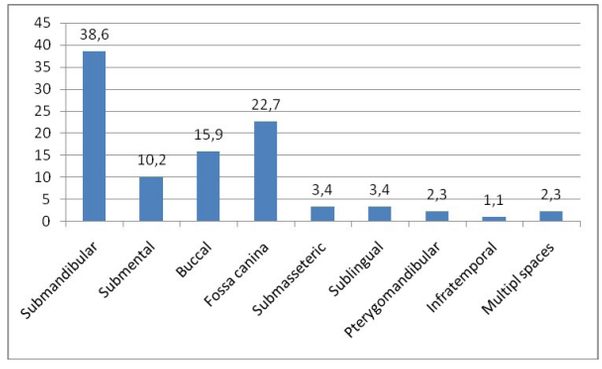


Figure 2. *Percentage of spaces involved in the odontogenic infections*

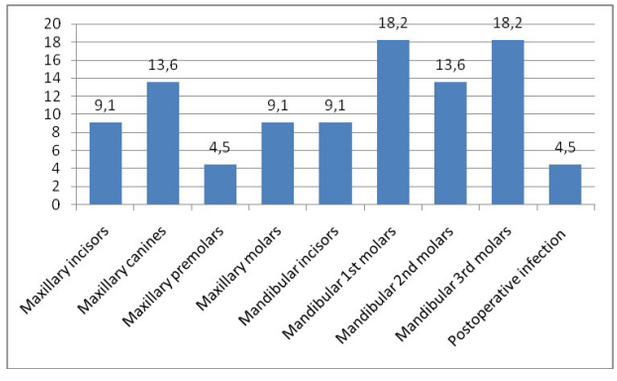


Figure 3. *Percentage of involved teeth in the odontogenic infections*

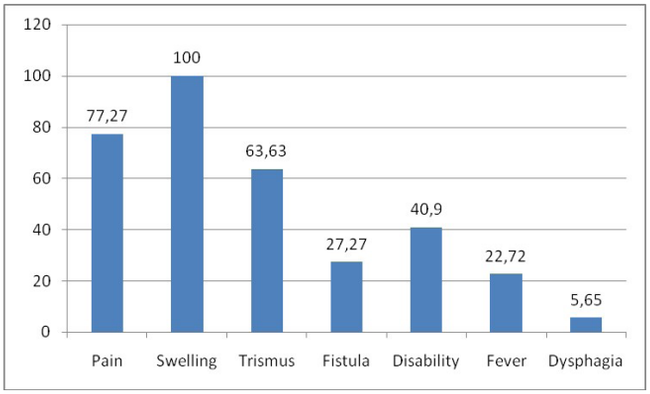


Figure 4. *Percentage distribution of odontogenic abscess signs and symptoms.*

3.3. Treatment Management

Treatment methods applied to patients with odontogenic facial abscesses were given in Table 3. While antibiotic treatment was applied to all patients, surgical treatment consisting of incision and drainage was applied in half. The incisions were performed intraorally in 36.4% of patients, and extra orally in 13.6% of patients. A drain was placed in the majority of patients who underwent drainage by incision, and the drain was removed 48 hours later. In addition to these treatments, the teeth responsible for abscesses were extracted in 63.6% of patients, and root canal treatment was applied to these teeth (especially canine teeth) in 27.3% of patients. After root canal treatment, these teeth were followed for possible apical surgery indication. In 9.1% of the cases, curettage was applied to the alveolar socket.

Patients were treated with broad-spectrum antibiotics, no antibiogram test was performed in any of the patients. Clindamycin was administered parenterally in 36.4% of the patients, while a combination of amoxicillin-clavunate, and ornidazole was administered in 27.3%. In 22.7% of the patients, treatment with clindamycin was started first in the acute period and then continued with a combination of amoxicillin-clavulanate and ornidazole. When analgesic and anti-inflammatory preferences were examined, it was seen that parenterally administered diclofenac was the first choice (40.9%). This was followed by orally administered naproxen sodium (18.2%), dexketoprofen (15.9%), and etodolac (13.6%). Almost all of the patients (96.6%) were treated on an outpatient basis. Infection was more severe in three of the patients, and they were hospitalized to closely monitor the patient's systemic condition. All patients were treated without any problems, and no death or serious complications occurred.

In this study, it was investigated whether there is a relationship between the age and systemic disease status of the patients, the prognosis of odontogenic abscesses, and some clinical findings(fistula and trismus). In the study, it was observed that the age and systemic disease status of the patients were not associated with the prognosis of odontogenic abscess (acute/chronic) (p= .853 and p= .206, respectively). There was no significant relationship between fistula and trismus status and the age of the patients (p= .932 and p= .420, respectively). In addition, no statistically significant relationship was found between fistula and trismus status and systemic disease status in patients (p= .262 and p= .397, respectively).

Table 3. *Treatment methods and drugs used in odontogenic abscesses*

Treatment management	n	%
Medication	88	100
Incision and drainage	44	50
Intraoral incision	32	36.4
Extraoral incision	12	13.6
Other treatments		
Tooth extraction	56	63.6
Endodontic treatment	24	27.3
Curettage	8	9.1
Treatment modality		
Outpatient treatment	85	96.6
Inpatient treatment	3	3.4
Antibiotics and analgesics used in treatment		
Antibiotic drugs		
Amoxicilin-clavulanate	6	6.8
Clindamycin	32	36.4
Sefuroksim	4	4,5
Amoxicilin-clavulanate +ornidazole	24	27.3
Ampcilin-sulbactam	2	2.3
Clindamycin and Amoxicilin-clavulanate +ornidazole	20	22.7
Analgesics and anti-inflammatory drugs		
Deksketoprofen	14	15.9
Diklofenak	36	40.9
Flurbiprofen	8	9.1
Naproksen sodyum	16	18.2
Etodolak	12	13.6
İbuprofen	2	2.3
Total	88	100.0

4. DISCUSSION

Odontogenic abscesses, which are frequently seen in the maxillofacial region, heal without significant complications when treated quickly and correctly. General treatment principles in odontogenic infections include elimination of the cause, drainage of the abscess, and the use of effective antibiotics. When odontogenic infections are not treated in a timely manner, they pose significant health risks for patients and bring serious costs to the health system. In this study, facial area abscesses of odontogenic origin were evaluated retrospectively in terms of general epidemiological features, etiological risk factors, clinical features, and treatment approaches.

Odontogenic abscesses have been observed at mild (14) or significantly (15) higher levels in men than women in previous studies. Sanchez et al. (1) reported that both genders are affected equally. In this study, men and women were equally affected. In the studies of Igoumenakis et al., (16,17) the

mean age of the patients applied for odontogenic infection was found to be 40.8 and 39.1. Similarly, in this study, the average age was found to be 39.72. Poor oral hygiene is linked to an increased risk of odontogenic infections (16). Also, low socioeconomic status is often related to poor oral health (9). The oral hygiene of the patients in this study was poor. More than one-third of the patients stated that they did not brush their teeth, while only about one-third stated that they brushed twice a day. About a third of the patients had an income below the minimum living wage (2.825 TL), while 63.6% had an income just above the minimum wage. The smoking rate was quite high in patients (40.9%). These findings show that patients with odontogenic abscesses have low socio-economic status and poor oral hygiene.

It has been emphasized that systemic diseases, especially diabetes, are predisposing factors for odontogenic infections and may complicate the treatment process (18). Forty point nine percent of the participants had at least one systemic disease in this study. Hypertension, coronary artery disease, diabetes, osteoporosis, and rheumatic joint diseases were among the most common diseases. Four patients were receiving chemotherapy and radiotherapy for malignancy. Two patients reported that they had a penicillin allergy and 3 patients reported using corticosteroid drugs for various reasons. Mathew et al. have shown that diabetes mellitus increased the risk of odontogenic infection (19). A retrospective study conducted in Turkey showed that the presence of the systemic disease may increase the length of hospital stay of patients (20). Consistent with the literature in this study, the duration of treatment in patients with diabetes and those receiving chemotherapy, radiotherapy, and corticosteroids was longer than in other patients.

According to the literature, the most commonly infected region is the submandibular space, followed by the buccal and submental spaces (21). Katoumas et al. (22) reported that 52.82% of cases with odontogenic abscess were seen in the submandibular area, while Sanchez et al. (1) reported it as 30.3%. In this study, the most affected area was the submandibular area (38.6%), while the second most affected area was the fossa canina region (22.7%). This was followed by the buccal and submental area, again similar to the literature. Published reports have shown that mandibular molars are the most commonly included teeth in odontogenic infections (3,23). In another study, lower third molars were the most frequent reason of infection, followed by first and second lower molars (22). Boffano et al. (24) reported that the mandibular posterior teeth were the most commonly included teeth and the submandibular area was the most commonly involved area. Mandibular molars are a well-known dental focus for odontogenic infections. Poor oral hygiene is especially common in this region (1). Periapical infections may spread to the submandibular or neighboring parapharyngeal space since the root tips of the second and third mandibular molars reach the mylohyoid muscle's origin (25). The parapharyngeal space is associated with the neck's major compartments anatomically (26). Inflammation in the submandibular space can spread to the parapharyngeal space, potentially obstructing the airway quickly and severely (27). The most frequently affected teeth, according to Sanchez et al. (1) were those in the lower posterior segments (61.5%), followed by lower molars (26.6 %). The reason for the occurrence of teeth in the posterior segment may be due to the increasing technical difficulty of restorative treatments in this area and less thorough oral hygiene in the posterior regions of the oral cavity. Conversely, other studies have found that lower molars are the most common causal teeth (28,29). In this study, lower 1st molars and 3rd molars were the most frequent causes of infection with a rate of 18.2%, followed by maxillary canine and mandibular 2nd molars with a rate of 13.6%.

The most common cause of odontogenic infections is a necrotic pulp or a deep periodontal pocket (12). Sanchez et al. (1) reported that the most common cause of infection was dental caries (33.8%), followed by post-extraction infectious processes and pericoronitis. Flynn et al. (30) reported decay as the main etiologic factor (65%). Another common cause of orofacial odontogenic infections is pericoronitis. In this study, similar to the study of Flyn et al., dental caries was the main etiological factor with a rate of 65.9%, followed by pericoronitis (15.9%) and post-endodontic problems (10.22%).

Patients with odontogenic infections usually have marked reactive facial swelling; trismus, dyspnea, dysphagia, and are other frequent symptoms (27,30). Sanchez et al. (1) reported that 35.1% of the patients had trismus alone, and 23.2% had trismus due to dysphagia. In this study, the most common symptoms in patients with odontogenic abscesses were swelling (100%), pain (77.27%), and trismus (63.63%). Katoumas et al. (22) reported that only 15.7% of the patients presented within first day, as in the literature, the majority of them presented in the delayed period. In this study, only 4.5% of the patients presented within the first day, while the majority arrived within the second day. While 13.6% of the patients applied directly, the majority were referred by dentists (45.5%) or other medical practitioners. All of the cases were referred to our clinic by another physician or dentist without incision and drainage. Dyspnoea, dysphagia, very high fever (38.3°C and above), and severe trismus for at least four days can be considered basic criteria for hospitalization (8). In this study, almost all of the patients were treated on an outpatient basis, only three patients had feeding problems due to severe trismus, high fever, and dysphagia, so they were treated in the hospital. The patients were closely monitored and all patients recovered smoothly.

Incision and drainage constitute one of the most important major management principles in the management of odontogenic infections. According to the literature, surgical drainage is required in 10-83% of all cases. Sanchez et al. (1) reported that the causative tooth was extracted in 61.8% of the cases, and abscess drainage was performed in 22.9%. Katoumas et al. (22) reported that the incision was made intraorally in 58.9% of the patients, extraoral in 34.3% and combined intraoral and extraoral in 6.8%. They also reported that 85.3% of the responsible teeth were removed without

delay and 3.9% had root canal treatment. In this study, surgical treatment consisting of incision and drainage was applied to half of the patients The incisions were performed intraorally in 36.4% of patients, and extra orally in 13.6% of patients. In addition to these treatments, 63.6% of the patients had the teeth responsible for abscess extracted, while 27.3% received root canal treatment. If endodontic treatment or extraction is not applied and incision and drainage are not applied for the tooth responsible for odontogenic infection, antibiotics cannot prevent the progression of the infection. The penetration of the antibiotic into the infected area is low. Furthermore, evacuating the infection decreases the bacterial burden and changes the anaerobic condition in the affected region created by anaerobic bacteria (8). A commonly adopted but incorrect belief is that extracting a tooth in a acute infection facilitates the dissemination of the infection. Most dentists ignore that antibiotic therapy plays an adjunct role and is not the primary treatment for odontogenic infections (12).

Odontogenic infections are generally polymicrobial and have a mixed bacterial flora in which anaerobes are more than aerobes. Polymicrobial infections are more pathogenic than mono infections due to bacterial synergism (31). Because of the well-known structure of the underlying flora, the first antimicrobial therapy for odontogenic abscesses is empirical (31). Penicillin remains the empirical antibiotic of choice for odontogenic abscesses because of its efficacy, low cost, patient tolerance, and reduced side effects (21,31). It also has broad antimicrobial activity against both aerobic and anaerobic bacteria. Antibiotic resistance, on the other hand, is a major concern in the treatment of orofacial odontogenic infections. Broad-spectrum penicillin regimens containing clavulanate (or other lactamase inhibitors) have broadened the antimicrobial spectrum. Parenteral extended-spectrum cephalosporins, clindamycin and moxifloxacin have also been advised for the treatment of odontogenic abscesses (31). The treatment protocol used in our center includes amoxicillin-clavulanate and ornidazole as the first choice of antibiotic treatment for such diseases. In our clinic, the antibiotic regimen used for odontogenic abscess patients is parenteral clindamycin in cases of cellulitis, while it is a combination of amoxicillin + clavulanate and Ornidazole in the chronic stage. In this study, clindamycin (36.4%) and the combination of amoxicillin + clavulanate and ornidazole (27.3%) were the most preferred antibiotics. A combination of these two was used in 22.7% of the cases. Katoumas et al. (22) reported that 67.5% of patients used sultamicillin (ampicillin + sulbactam) and metronidazole, while 14.3% of them clindamycin. Sanchez et al. (1) reported that clindamycin (33.1%) was the most commonly used antibiotic in odontogenic abscesses, followed by amoxicillin + clavulanate(25.8%) and both combinations (22.5%). Many studies define clindamycin as the treatment of choice among beta-lactam allergic patients or as a second drug option after treatment failure in patients receiving penicillin (32,33).

The use of antibiotics in head and neck infections requires updated protocols based on existing scientific evidence on pathogen profile and resistance. However, the drainage of abscesses is of great importance in achieving rapid success. It is imperative that the relevant teeth be removed immediately; endodontic treatment should be applied in limited periapical lesions. When odontogenic abscesses are treated promptly and adequately, complications are rare. If odontogenic abscesses are not treated in a timely and effective manner, they bring significant costs to the healthcare system. Difficult and complex treatment procedures for severe odontogenic infections prolong the hospital stay of the patient and bring high costs to inpatient units. In a study conducted in Turkey in 2014, the average treatment cost of odontogenic infections in a hospital setting was calculated as 748.35 TL (20). In order to benefit from health services effectively and to reduce unnecessary hospital costs, patients with odontogenic abscesses should be treated in a timely and effective manner. An abscess of odontogenic origin in the maxillofacial region can be easily diagnosed with a routine dental examination. Appropriate antibiotic treatment and referral to an oral and maxillofacial surgeon for drainage in the early period may lead to an early recovery of the patients without complications and decrease the treatment costs by eliminating or reducing the need for hospitalization.

5. CONCLUSION

The results of this study showed that elimination of the cause of infection, drainage of the abscess, and correct antibiotic administration in odontogenic abscesses provide rapid recovery. In this study, it was seen that the empirical use of amoxicillin/clavulanate and clindamycin was effective in odontogenic infections and had no significant side effects. Dentists must have the necessary knowledge and skills to prevent and diagnose odontogenic infections and to refer patients for timely treatment. In addition, continuing education of physicians on the management of odontogenic infections and the development of health programs to raise awareness of patients about odontogenic infections may result in a decrease in serious odontogenic infections requiring hospitalization.

Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by the Clinical Research Ethics committee of Afyonkarahisar Health Sciences University (06.11.2020 dated and 2020/504 issues)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: Ö.E.
Design of the study: Ö.E.
Acquisition of data for the study: Ö.E.
Analysis of data for the study: Ö.E.
Interpretation of data for the study: Ö.E.
Drafting the manuscript: Ö.E.
Revising it critically for important intellectual content: Ö.E.
Final approval of the version to be published: Ö.E.

REFERENCES

[1] Sánchez R, Mirada E, Arias J, Paño JR, Burgueño M. Severe odontogenic infections: Epidemiological, microbiological and therapeutic factors. Med Oral Patol Oral Cir Bucal. 2011;16(5). DOI:10.4317/medoral.16995

[2] Bassiony M, Yang J, Abdel-Monem TM, Elmogy S, Elnagdy M. Exploration of ultrasonography in assessment of fascial space spread of odontogenic infections. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2009;107(6):861-869. DOI:10.1016/j.tripleo.2009.02.016

[3] Akinbami BO, Akadiri O, Gbujie DC. Spread of odontogenic infections in Port Harcourt, Nigeria. J Oral Maxillofac Surg. 2010;68(10):2472-2477. DOI:10.1016/j.joms.2010.01.019

[4] Seppänen L, Lauhio A, Lindqvist C, Suuronen R, Rautemaa R. Analysis of systemic and local odontogenic infection complications requiring hospital care. J Infect. 2008;57(2):116-122. DOI:10.1016/j.jinf.2008.06.002

[5] Juncar M, Popa AR, Baciuț MF. Evolution assessment of head and neck infections in diabetic patients – A case control study. J Cranio-Maxillofacial Surg. 2014;42(5):498-502. DOI:10.1016/j.jcms.2013.06.009

[6] Sandner A, Börgermann J. Update on necrotizing mediastinitis: Causes, approaches to management, and outcomes. Curr Infect Dis Rep. 2011;13(3):278-286. DOI:10.1007/s11908.011.0174-z

[7] Leyva P, Herrero M, Eslava JM, Acero J. Cervical necrotizing fasciitis and diabetic ketoacidosis: Literature review and case report. Int J Oral Maxillofac Surg. 2013;42(12):1592-1595. DOI:10.1016/j.ijom.2013.05.006

[8] Ogle OE. Odontogenic Infections. Dent Clin North Am. 2017;61(2):235-252. DOI:10.1016/j.cden.2016.11.004

[9] [9] Jundt JS, Gutta R. Characteristics and cost impact of severe odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(5):558-566. DOI:10.1016/j.oooo.2011.10.044

[10] Poeschl PW, Spusta L, Russmueller G. Antibiotic susceptibility and resistance of the odontogenic microbiological spectrum and its clinical impact on severe deep space head and neck infections. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2010;110(2):151-156. DOI:10.1016/j.tripleo.2009.12.039

[11] Roccia F, Pecorari GC, Oliaro A. Ten Years of Descending Necrotizing Mediastinitis: Management of 23 Cases. J Oral Maxillofac Surg. 2007;65(9):1716-1724. DOI:10.1016/j.joms.2006.10.060

[12] Flynn TR, Shanti RM, Levi MH, Adamo AK, Kraut RA, Trieger N. Severe Odontogenic Infections, Part 1: Prospective Report. J Oral Maxillofac Surg. 2006;64(7):1093-1103. DOI:10.1016/j.joms.2006.03.015

[13] Agarwal AK, Sethi A, Sethi D, Mrig S, Chopra S. Role of socioeconomic factors in deep neck abscess: A prospective study of 120 patients. Br J Oral Maxillofac Surg. 2007;45(7):553-555. DOI:10.1016/j.bjoms.2007.01.001

[14] Nadig K, Taylor NG. Management of odontogenic infection at a district general hospital. Br Dent J. 2018;224(12):962-966. DOI:10.1038/sj.bdj.2018.445

[15] Bertossi D, Barone A, Iurlaro A. Odontogenic orofacial infections. J Craniofac Surg. 2017;28(1):197-202. DOI:10.1097/SCS.000.000.0000003250

[16] Igoumenakis D, Gkinis G, Kostakis G, Mezitis M, Rallis G. Severe odontogenic infections: Causes of spread and their management. Surg Infect (Larchmt). 2014;15(1):64-68. DOI:10.1089/sur.2012.178

[17] Igoumenakis D, Giannakopoulos NN, Parara E, Mourouzis C, Rallis G. Effect of Causative Tooth Extraction on Clinical and Biological Parameters of Odontogenic Infection: A Prospective Clinical Trial. J Oral Maxillofac Surg. 2015;73(7):1254-1258. DOI:10.1016/j.joms.2015.02.008

[18] Seppänen L, Lauhio A, Lindqvist C, Suuronen R, Rautemaa R. Analysis of systemic and local odontogenic infection complications requiring hospital care. J Infect. 2008;57(2):116-122. DOI:10.1016/J.JINF.2008.06.002

[19] Mathew GC, Ranganathan LK, Gandhi S. Odontogenic maxillofacial space infections at a tertiary care center in North India: a five-year retrospective study. Int J Infect Dis. 2012;16(4). DOI:10.1016/J.IJID.2011.12.014

[20] Çalış AS, Koyuncu BÖ, Öztürk K, Mert A, Bilgen C. General approach to the treatment of odontogenic abscesses and cost analysis. J Istanbul Univ Fac Dent. 2015;49(2):17. DOI:10.17096/JIUFD.90557

[21] Rega AJ, Aziz SR, Ziccardi VB. Microbiology and antibiotic sensitivities of head and neck space infections of odontogenic origin. J Oral Maxillofac Surg. 2006;64(9):1377-1380. DOI:10.1016/j.joms.2006.05.023

[22] Katoumas K, Anterriotis D, Fyrgiola M, Lianou V, Triantafylou D, Dimopoulos I. Epidemiological analysis of management of severe odontogenic infections before referral to the emergency department. J Cranio-Maxillofacial Surg. 2019;47(8):1292-1299. DOI:10.1016/j.jcms.2019.05.002

[23] Daramola OO, Flanagan CE, Maisel RH, Odland RM. Diagnosis and treatment of deep neck space abscesses. Otolaryngol – Head Neck Surg. 2009;141(1):123-130. DOI:10.1016/j.otohns.2009.03.033

[24] Boffano P, Roccia F, Pittoni D, Di Dio D, Forni P, Gallesio C. Management of 112 hospitalized patients with spreading odontogenic infections: Correlation with DMFT and Oral Health Impact Profile 14 indexes. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;113(2):207-213. DOI:10.1016/j.tripleo.2011.02.006

[25] Lorenzini G, Picciotti M, Di Vece L. Cervical necrotizing fasciitis of odontogenic origin involving the temporal region – A case report. J Cranio-Maxillofacial Surg. 2011;39(8):570-573. DOI:10.1016/j.jcms.2010.05.002

[26] Sakamoto H, Aoki T, Kise Y, Watanabe D, Sasaki J. Descending necrotizing mediastinitis due to odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89(4):412-419. DOI:10.1016/S1079-2104(00)70121-1

[27] Ariji Y, Gotoh M, Kimura Y. Odontogenic infection pathway to the submandibular space: Imaging assessment. Int J Oral Maxillofac Surg. 2002;31(2):165-169. DOI:10.1054/ijom.2001.0190

[28] Haug RH, Hoffman MJ, Indresano AT. An epidemiologic and anatomic survey of odontogenic infections. J Oral Maxillofac Surg. 1991;49(9):976-980. DOI:10.1016/0278-2391(91)90063-R

[29] Kuriyama T, Karasawa T, Nakagawa K, Saiki Y, Yamamoto E, Nakamura S. Bacteriologic features and antimicrobial susceptibility in isolates from orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90(5):600-608. DOI:10.1067/moe.2000.109639

[30] Flynn TR, Shanti RM, Hayes C. Severe odontogenic infections, Part 2: Prospective Outcomes Study. J Oral Maxillofac Surg. 2006;64(7):1104-1113. DOI:10.1016/j.joms.2006.03.031

[31] Stefanopoulos PK, Kolokotronis AE. The clinical significance of anaerobic bacteria in acute orofacial odontogenic infections. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2004;98(4):398-408. DOI:10.1016/j.tripleo.2004.01.016

[32] Sato FRL, Hajala FAC, Filho FWVF, Moreira RWF, de Moraes M. Eight-Year retrospective study of odontogenic origin infections in a postgraduation program on oral and maxillofacial surgery. J Oral Maxillofac Surg. 2009;67(5):1092-1097. DOI:10.1016/j.joms.2008.09.008

[33] Warnke PH, Becker ST, Springer ING. Penicillin compared with other advanced broad spectrum antibiotics regarding antibacterial activity against oral pathogens isolated from odontogenic abscesses. J Cranio-Maxillofacial Surg. 2008;36(8):462-467. DOI:10.1016/j.jcms.2008.07.001

Appendix 1: *FOLLOW-UP FORM FOR PATIENTS WITH FACIAL AREA ABSCESS OF ODONTOGENIC ORIGIN*

1. Personal Information

Name Surname: ..... File no: .... Age / Gender: ..... Telephone No: ....

Socio-economic Status of the Family:

1. Monthly/Annual Income: ..... 2. Educational status:..... ..... 3. Profession: .....
4. Place of residence (village, district, city): ..... 5.Does the house belong to him: ....
6.Marital status: ..... 7. Employment status of spouse:..... 8. Number of children: .....

2. Medical CV

Systemic diseases: ....
Drugs used: ....

Oral hygiene status: 0- poor 1-moderate 2-good
Tooth Brushing Status: 0-Cannot Brush 1- Once a day 2- Twice a day 3- 3 times a day
Smoking / Alcohol use: 0- no 1- yes ..... pack/day

3. Clinical / Radiological / laboratory examination

Caused tooth: .....
Cause of abscess: .....
Acute (Cellulitis) or Chronic (Abscess)

The most important complaints: Pain Swelling Difficulty in eating and speaking Fever Other .....
How many days are the complaints? ..... day
Have you applied to another health institution before? Family physician Emergency dep. Dentist Other .....
Did you take any medication? If you took it, who wrote the medicine? .....
Antibiotics used before, if any: ....
Fistula: No yes 1. İntraoral 2. Ekstraoral
Trismus: No yes Max mouth opening :.....mm

Degrre of pain (VAS)
0 1 2 3 4 5 6 7 8 9 10
No Moderate Very severe pain

Local Findings:
1.Calor
2. Rubor
3.Tumor
4. Dolor
5. Loss of function

Systemic Findings
1. Fever
2. Fast pulse
3. Leukocytosis
4. Sediment.
5.Lymphodenop.
6. Chills /shivering
7. Other .....

Odontogenic Abscesses
1. Dentoalveolar abscess
2. Periodontal abscess
3. Submucous abscess
4.Subperiosteal abscess
5. Pericoronal abscess
6.. Palatinal abscess

Facial Area Abscesses

A. primary area	B. secondary area
1. Submandibular abscess	1. Temporal abscess
2. Sublingual abscess	2. Masseteric abscess
3. submental abscess	3. Pterygomandibular abscess
4. Buccal abscess	4. Parapharyngeal abscess
5. Fossa canina abscess	5. Retropharyngeal abscess
6. Infratemporal abscess	6. Peritonsillar abscess

Laboratory Findings: ....
Radiographic Findings: ....
Culture / Antbiogram: .....

4. Treatment

- Antibiotic therapy: 1. Oral ....
  2. Parenteral ....
- Anti-Inflammatory Drugs: ....
- Incision/Drainage: no yes 1. Intraoral drainage 2. Extraoral drainage Drain: yes no
- Other Treatments: 1. Canal drainage 2. Tooth extraction Other: .....
- Hospitalization: yes no

5. Follow up :

Complications
1. Osteomyelitis
2. Septicemia
3. Cavernous sinus thrombosis
4. Vena angularis thrombophlebitis
5. Brain abscess
6. Meningitis
7. Mediastinitis
8. Phlegmonous inf.

How to cite this article: Ekici Ö. Epidemiological Analysis and Management of Patients with Facial Space Infections of Odontogenic Origin: A Retrospective Evaluation of Two Years. Clin Exp Health Sci 2023; 13: 58-66. DOI: 10.33808/clinexphealthsci.994256

Clinical and Experimental Health Sciences

Unmet Health Needs During the COVID-19 Pandemic

Aylin Yalçın Irmak[1], Aslıhan Öztürk Eyimaya[2]

[1] Tekirdağ Namık Kemal University, Faculty of Health Sciences, Department of Nursing, Tekirdağ, Türkiye.
[2] Ankara University, Faculty of Nursing, Department of Nursing, Ankara, Türkiye.

Correspondence Author: Aslıhan Öztürk Eyimaya
E-mail: asliozturk@ankara.edu.tr
Received: 04.12.2021 **Accepted:** 10.03.2022

ABSTRACT

Objective: This study aimed to identify the unmet health needs of adults during the COVID-19 pandemic, the reasons for these needs, solutions sought and socio-demographic determinants.

Methods: The cross-sectional online survey was conducted with 2,074 adult individuals from December 15 to December 31, 2020. Data were collected using Socio-demographic Data Collection Form, Unmet Health Needs Data Collection Form and World Health Organization Quality of Life Scale (WHOQOL).

Results: The percentage of the participants who stated that they had unmet health needs was 66% and the most unmet needs reported were oral and dental treatment (46.3%), eye health and treatment for vision disorders (22.5%), and early diagnosis and annual health screening (11.4%). The reasons with the most impact on the emergence of these needs were fear of being infected with the virus (44.3%), lack of access to health care (42.7%) and not wanting to cause a burden on the health system. There was a significant difference between the groups with and without unmet health needs in terms of gender, economic status, presence of health insurance, presence of chronic disease, perception of health, and WHOQOL total scores.

Conclusion: The findings obtained will benefit policymakers in the rational use of limited resources and making strategic arrangements for needs.

Keywords: Adult, COVID-19, Coronavirus, Unmet Health Needs

1. INTRODUCTION

The world is going through a challenging period due to coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Most of the countries declared a state of emergency and took different measures, including self-isolation, lockdown, and social isolation, to alleviate the spread of disease, protect individuals and disburden health systems (1). Since December 2019, when the first COVID-19 case was recorded, health services throughout the globe have faced increasing pressure, with over 331 million cases and nearly six million deaths (2). Republic of Turkey Ministry of Health declared the first case identified in Turkey on March 11 and the first death from COVID-19 on March 17 (1). Immediately afterwards, many measures were taken to minimize contact and ensure social isolation. These measures included travel restrictions, mandatory quarantine for those with a history of overseas travel, border closures, suspension of education, temporary postponement of activities, closure of common use areas (cafeteria, hairdressers, shopping malls, etc.), and lockdown restrictions. Moreover, during the times when the most stringent measures were taken, the following measures were also implemented with regard to health care services: visitor and attendant restrictions in hospitals, patients with refills being able to buy their medicines from pharmacies without applying to a health care institution, admitting only those with an appointment to hospitals, transformation of many hospitals to pandemic hospitals, suspension or restriction of outpatient clinic examinations, suspension of elective surgeries, and calling people not to come to hospitals unless there is an emergency (3).

During the pandemic, limited health care personnel and resources have been widely directed to the efforts to control and treat COVID-19 rather than routine health service provision. As people still need other routine health services, the necessity of providing services to individuals with and without COVID-19 within the same system has emerged during the pandemic period, in which all efforts have been

DOI: 10.33808/clinexphealthsci.1032287

carried out to control. On the other hand, not only many health care providers have restricted patient visits or closed their practices due to the pandemic, but also both healthy individuals and patients have avoided using health care services (4). In an analysis using the data obtained from more than 50,000 health care providers that are Phreesia clients, outpatient visits between February and April 2020 have been reported to decrease by about 60%. As of March 2020, the number of outpatient visits for children aged three to 17 years has dramatically fallen to below 70% (5). Data from the United States Census Bureau research has shown that up to 36% of adults postponed their health care due to the epidemic and up to 28% did not receive the medical care they needed (6). (4). It would not be wrong to say that the accessibility of health care services for the general population has been interrupted in all developed and developing countries exposed to the COVID-19 outbreak. The epidemic and response to the epidemic have resulted in reduced access to health care services and a significant decrease in its use (4-6). While structural reasons include the fact that many doctors and clinics closed their offices or limited face-to-face visits during the first months of the crisis (7), the followings should be considered among other reasons: job and health insurance losses, difficulties in transportation and childcare, and fear of being infected with or transmitting the virus (8-12).

The impact of the pandemic on public health is not directly limited to the morbidity and mortality caused by COVID-19. The indirect results of the pandemic in the long term and the social response to it may have far-reaching and serious consequences. The most important one of them is unmet or delayed health needs. The concept of unmet health needs is often used to measure the population's accessibility to health services and equity in access to these services. Health services mainly aim to protect, improve and maintain the physical, mental and social health of the individual and society. The realization of this purpose is only possible when people can access health services without encountering any obstacles (13-15). Unmet health care needs occur if health care is not received when required or the health service received is not suitable or adequate for the health conditions of the individual (16). Delayed or nonreceipt of medical care may result in more severe illness for the patient, increased complications, a worse prognosis, and longer hospital stays or treatment period for both healthy individuals or patients (17,18). In the 2014–2015 Ebola epidemic during which more than 28,000 people were infected and over 11,000 people died, significant reductions have been reported in maternity care, malaria admissions, immunization and the use of health care services in affected areas, particularly among women and children. Moreover, the epidemic has prevented the diagnosis and treatment of endemic diseases, increased morbidity and mortality, and reduced life expectancy. Many sources have shown that the increasing number of deaths caused by measles, malaria, HIV/AIDS and tuberculosis have outpaced the number of deaths from Ebola and it has been argued that the consequences of unmet health needs are more severe than the epidemic itself (19-21). The Ebola outbreak provides insight into the points we need to pay attention to and focus on regarding the current COVID-19 pandemic. Furthermore, the World Health Organization suggests that countries should make difficult decisions and engage in strategic planning and coordinated action to respond to the pandemic that creates serious pressure on the health system (21). Determining the health needs of the society may contribute to the rational use of limited resources and prevent the long-term negative impacts of the pandemic (19, 21, 22).

To the best of our knowledge, there is no comprehensive scientific study presenting unmet health needs in the Turkish adult population associated with the COVID-19 pandemic. Therefore, this study aimed to identify the unmet health needs of adults during the COVID-19 pandemic, the reasons for these needs, solutions sought, and socio-demographic determinants. With the results of the present study, it was aimed to emphasize the potential negative long-term impact of the epidemic on the Turkish population. We believe that the results will provide important information to health care workers, policymakers and managers, as well as contributing to efforts to improve access to health services.

2. METHODS

This cross-sectional study was conducted from December 15 to December 31, 2020. In the study, no sampling was performed and a total of 2,074 participants having no mental disability, being a Turkish speaker, living in Turkey, being within the age range of 18-65 years, being able to be reached, and volunteering to participate in the study were included.

2.1. Socio-demographic Data Collection Form

This form consists of questions about age, gender, marital status, economic status, household size, place of residence during the pandemic, employment status, presence of physical disability, health insurance and chronic disease.

2.2. Unmet Health Needs Data Collection Form

The form that evaluates the unmet health needs of the participants during the COVID-19 pandemic was created in line with the literature (23-25). It contains the questions "how has health status changed during the pandemic", "what are unmet or delayed health needs", "what are reasons for not meeting these needs", and "what are the solutions found for unmet health needs". Participants were asked to answer all questions, considering the pandemic starting from March 11, 2020, to the present day. Pilot testing was performed with seven individuals for clarity. Data from seven participants in the pilot study were not included in the main study findings.

2.3. World Health Organization Quality of Life Scale (WHOQOL)

It is a 27-item scale that evaluates how an individual perceives the quality of life. Eser et al. performed the Turkish validity and reliability study of the scale in 1999 and adapted it to the Turkish population. This scale has four domains: physical health, psychological health, social relationships, and environmental health. The questions are of a typical 5-point Likert-type ordinal rating scale type. A maximum of 20 points can be obtained from each sub-dimension. The total score of the scale is 100. Higher scale scores represent higher quality of life (26). In this study, Cronbach's alpha coefficient of the scale was found to be 0.86.

The research data was collected through an online questionnaire. The questionnaire was applied to the individuals in the ninth month following the initiation of COVID-19 control measures in Turkey. Adults were invited to participate in the study through WhatsApp groups for university students, social media, and forums. The questionnaire form used to collect study data was prepared via "Google Forms" to be answered online. Answering the questionnaire took maximum of 20 minutes. A total of 13 questionnaires were excluded from the study due to missing answers.

All statistical analyses were performed using SPSS version 21.0 software (Statistical Packages for Social Sciences). Descriptive statistics were expressed as mean, standard deviation, minimum and maximum for continuous variables and as numbers and percentages for categorical variables. Mann-Whitney U test was used to compare continuous numerical variables for significance statistics. Chi-square test was used to determine the correlations between categorical variables. The statistical significance level was taken in the calculations as 5%. A p value of <0.05 was considered statistically significant and results were evaluated at a 95% confidence interval.

The Republic of Turkey Ministry of Health approved the study. The ethics committee approval was obtained from Ethics Committee of Tekirdağ Namık Kemal University (date: 24.11.2020 and number: 2020.252.11.12). The informed consent form was added to the first page of the online data collection form. Participants answered the data collection form after reading the informed consent form and voluntarily accepting to participate in the study.

3. RESULTS

Among 2,074 participants aged 18-65 years, 66% (n:1368) stated that they had unmet health needs during the pandemic. The mean age of these participants was 26.95±10.61 years and 89% (n:1218) did not have COVID-19, 79.5% (n:1088) were females, 70.5% (n:964) were single, and 55.6% (n:760) stated their economic status as income equal to expenses. The mean household size was 4.12±1.55 and 54.2% (n:742) stated that they were residing in the province during the pandemic, 71.6% (n:980) were unemployed or student, 99.2% (n:1357) had no physical disability, 79% (n:1081) had general health insurance, 84.6% (n:1158) had no chronic disease, and 68.9% (n:942) stated that there was no change in their health during the pandemic. The WHOQOL total score of the participants who stated that they had an unmet health need was 83.34±11.71 (Table 1).

The mean age of 706 participants who stated that they had no unmet health needs was 26.63±10.12 years and 89.4% (n:631) did not have COVID-19, 69.5% (n:491) were females, 70.3% (n:469) were single, and 60.3% (n:426) stated their economic status as income equal to expenses. The mean household size was 4.15±1.44 and 53.4% (n:377) stated that they were residing in the province during the pandemic, 73.8% (n:521) were unemployed or student, 99.6% (n:703) had no physical disability, 73.4% (n:518) had general health insurance, 89.5% (n:632) had no chronic disease, and 83.4% (n:589) stated that there was no change in their health during the pandemic. The WHOQOL total score of the participants who stated that they had no unmet health needs was 89.36±12.41 (Table 1.).

There was a statistically significant difference between the groups with and without unmet health needs during the pandemic in terms of gender (X^2:25.553; p:0.000), economic status (X^2:13.822; p:0.001), health insurance (X^2:10.022; p:0.007), presence of chronic disease (X^2:9.342; p:0.002) and health perception (X^2:71.925; p:0.000). Mann-Whitney U analysis performed to understand the reason for this differentiation showed a significant difference in favor of females, participants who stated their economic status as income less than expense or income equal to expense, those with general health insurance or without health insurance, those with chronic diseases, and participants who perceived that their health was worse and did not change during the pandemic. Furthermore, there was also a significant difference between the two groups with and without unmet health needs in terms of the mean WHOQOL total scores (U:338075.5; p:.0.000) (Table 1.).

The unmet health needs mostly reported by the participants during the pandemic were found to be oral and dental treatment (46.3%), eye health and treatment for vision disorders (22.5%), early diagnosis and annual health screening (11.4%), health care for mental and psychiatric problems (7.2%), and follow-up and monitoring of chronic diseases (6.5%) (Table 2).

The reasons for unmet health needs were found to be the fear of catching the virus from health care personnel or hospital (44.3%), closure of health care institutions due to the pandemic or not being able to reach a specialist (physician/dentist) (42.7%), not wanting to burden the health system (37.1%), not wanting to cause a burden on the health system, believing that the health problem was not very urgent (26.2%), and not wanting to use public transport (21.1%) (Table 2).

When the solutions found for unmet health needs were evaluated, 41.4% of the participants stated that they did nothing whereas 17.6% stated that they applied herbal or alternative medicine techniques, 16.1% used medicines available at home, 11% had followed the recommendations from media such as the internet, television or radio, and 10.4% had received support by calling a health care personnel they knew on the phone.

Table 1. *Socio-demographic and medical characteristics of participants (n=2074).*

	Health Needs			
	Yes % (n) or mean±SD	No % (n) or mean±SD	Test	p
COVID-19 survivors				
Yes	11% (150)	10.6% (75)	X^2: 0.056	0.813
No	89% (1218)	89.4% (631)		
Age	26.95±10.61	26.63±10.12	U:472596; Z:-.801	0.423
Gender				
Female	79.5% (1088)	69.5% (491)	X^2: 25.553	**0.000**
Male	20.5% (280)	30.5% (215)		
Marital status				
Single	70.5% (964)	70.3% (469)		
Married/cohabiting	27.4% (375)	28.2% (199)	X^2: 0.866	0.649
Separated/divorced	2.1% (29)	1.6% (11)		
Economical status				
Income less than expenses	29% (397)	21.5% (152)		
Income equal to expenses	55.6% (760)	60.3% (426)	X^2: 13.822	**0.001**
Income more than expenses	15.4% (211)	18.1% (128)		
Household size	4.12±1.55	4.15±1.44	U:471536; Z:-.909	0.363
Place of residence during the pandemic				
Province center	54.2% (742)	53.4% (377)		
District center	35.1% (480)	35.6% (251)	X^2: 0.150	0.928
Village/town	10.7% (146)	11% (78)		
Wage-earning employment				
Yes	28.4% (388)	26.2% (185)	X^2: 1.085	0.298
No	71.6% (980)	73.8% (521)		
Physical disability status				
Yes	0.8% (11)	0.4% (3)	X^2: 0.999	0.318
No	99.2% (1357)	99.6% (703)		
Health insurance				
No	17.3% (236)	20.7% (146)		
General health insurance	79% (1081)	73.4% (518)	X^2: 10.022	**0.007**
Private health insurance	3.7% (51)	5.9% (42)		
Presence of a chronic disease				
Yes	15.4% (210)	10.5% (74)	X^2: 9.342	**0.002**
No	84.6% (1158)	89.5% (632)		
Perception of health during the COVID-19 pandemic				
Better	3.4% (47)	5.1% (36)		
Worse	27.7% (379)	11.5% (81)	X^2: 71.925	**0.000**
No change	68.9% (942)	83.4% (589)		
WHOQOL total score	83.34±11.71	89.36±12.41	U:338075.5; Z:-11.211	**0.000**

U: Mann-Whitney U, X^2: Chi-Square Test, WHOQOL: World Health Organization Quality of Life Scale

Table 2. *The causes of unmet health needs during the pandemic and the distribution of solutions found (n:1368).*

Unmet health needs	% (n)
Oral and dental treatment	46.3% (960)
Eye health and treatment for vision disorders	22.5% (466)
Early diagnosis and annual health screening	11.4% (238)
Health care for mental and psychiatric problems	7.2% (149)
Follow-up and monitoring of chronic diseases	6.5% (134)
Surgery/surgical procedure	5.0% (104)
Drug/medical equipment/device supply	4.7% (96)
Physiotherapy services	4.7% (97)
Vaccination/immunization services	3.7% (76)
Medical therapy	3.5% (73)
Control examinations	2.9% (60)
Emergency and first aid service	1.3% (26)
Follow-up and monitoring of pregnant/puerperant	0.6% (13)
Health education/consultancy services	0.5% (10)
Family planning/contraception methods	0.0% (1)
Reasons for unmet health needs	
Fear of catching the virus from health care personnel or hospital	44.3% (919)
Health care institutions were closed or it was not possible to reach a specialist	42.7% (887)
Not wanting to cause a burden on the extremely busy health system	37.1% (770)
Believing that the health problem is not so urgent	26.2% (544)
Not wanting to use public transport	21.1% (437)
Appointment scheduled for a date in the distant future	12.2% (252)
Wanting to wait and see if the health problem will go away on its own	7.5% (156)
Having no time due to daily responsibilities	5.2% (108)
Having no trust in the health services provided or believing that it will be inadequate	5.1% (105)
Being unable to spare money for health care expenses	4.8% (99)
Lack of health care institutions at close range	4.6% (95)
Having no health insurance	2.4% (50)
Fear of doctors	1.6% (33)
Not knowing where to go	1.0% (21)
Solution for unmet health needs	
I did not do anything	41.4% (859)
I applied herbal or alternative medicine methods	17.6% (364)
I used medicines available at home	16.1% (334)
I followed the recommendations from the media such as the internet, television, or radio	11% (229)
I received support from a health care personnel I knew over the phone	10.4% (216)
I followed the advice of my friends, neighbours and relatives	4% (83)
I went to healers who were not medical personnel	0.2% (4)

4. DISCUSSION

Discussions about unmet health needs during the pandemic often focus on testing or treatment of COVID-19. This population-based study emphasizes the impact of the pandemic on the health needs and quality of life of the adult population living in Turkey. The social burden of unmet health needs being ignored during the fight against pandemic may go beyond direct health effects and negatively affect the potential productivity of adults in the long term.

In a study by Yetim and Celik, in which dataset gathered from Turkey Health Interview Survey 2016 was used, the level of unmet health needs was reported to be around 13.2% in Turkey (13). However, this does not seem to be the case during the pandemic. Two out of every three adults participating in the study stated that they had unmet health needs. This finding has made it increasingly clear that the pandemic has dramatic negative effects on the availability or accessibility of health services for non-COVID-19 related health needs. Ray et al. reported that more than half of families had unmet health or social service needs in the first month of the home quarantine process (27). The pandemic has been further shown to increase unmet health needs in India. These unmet needs have increased even more in cases where health resources and health care personnel are deployed to manage

the COVID-19 pandemic, disrupting routine and emergency health care services (11, 12).

This study is also important in terms of revealing determinants at the individual scale for unmet health needs during the pandemic period. Within the adult age group; women, those with low economic status, those who have general health insurance or no health insurance, individuals with chronic illnesses, those who perceive their health as worse and unchanged during the pandemic process compared to the past, and people with poor quality of life have been identified as vulnerable or at risk. Wani reported that unmet needs varied according to race, ethnicity, income and length of stay at home (11). Yetim and Celik also found that unmet health needs were higher among women and individuals with lower income levels (13). The majority of the studies have emphasized that women's access to health services is much lower than men and the use of health services varies according to economic inadequacy, lack of health insurance and cultural characteristics (8, 28-30). There is also a close correlation between unmet needs and quality of life (31, 32). Edib et al. stated that unmet needs had an influence on the poor quality of life (33). Compatible with the literature data, unmet health needs for individuals in the vulnerable group also indicates the presence of inequality in the health system. It is important to develop programs or plans for the health needs of people in the risk group during the present and the following pandemic periods.

The most commonly reported unmet health needs during the pandemic have been observed to be in the following areas: oral and dental treatment, eye health and treatment for vision disorders, early diagnosis and annual health screening, health care for mental and psychiatric problems, and follow-up and monitoring of chronic diseases. These unmet health needs may have occurred due to the maintenance of health care services to serve only emergency patients during the pandemic, except for the diagnosis and treatment of COVID-19, in Turkey and calling the society not to use health care services other than emergency health needs. It is remarkable that oral mucosa and eye complaints, which are the firstly-reported transmission route of COVID-19, are the most delayed health needs. Similarly, there are studies reporting that the necessary treatment regimen should be maintained (11) and there has been a great decline in dental care (11), vaccination, screening and mental health services (34-36). In the guidelines published by the World Health Organization to help countries maintain essential health services during the COVID-19 pandemic, it was reported that countries should identify essential services that will be prioritized in their efforts to maintain continuity of service delivery (21). The choice of priorities may vary depending on the health system and local disease burden; however, it is recommended to be made by primarily considering the prevention of infectious diseases, maternal and child morbidity and mortality, and acute exacerbations of chronic conditions by maintaining ongoing treatment regimens, and management of emergencies requiring intervention. Efforts of primary health care institutions in Turkey have had a significant effect in meeting health needs related to primary health care services during this period. Furthermore, the recruitment of an additional 44,000 health care personnel in 2020 has strengthened the health system's capacity to respond to the national crisis (37).

During the pandemic, people may delay or avoid receiving health service for many reasons. In the present study, the most common reason for unmet health needs was observed to be the fear of catching the virus from health care personnel or hospital, followed by the closure of health care institutions due to the pandemic or being unable to reach a specialist (physician/dentist) and thought of not wanting to create a burden on the health system that is extremely busy with the management of COVID-19-positive patients. Although it is a known fact that timely access to health services is essential for optimum physical, mental, and social health, it is clear that such measures as transforming many hospitals into pandemic hospitals during the pandemic process, suspending outpatient clinic examinations, limiting face-to-face examinations, and calling people not to go to hospitals unless there is an emergency are structural barriers to meeting the health needs of the society (7). Similarly, in other studies, the main barriers to health services during the pandemic period have been listed as fear of being infected with COVID-19, lack of services, job and health insurance losses, and difficulties in transportation and looking after children at home (8-12). Furthermore, losing trust in health care institutions and fear have caused communities to deliberately and widely avoid the health system, leading to significant reductions in the use of health facilities. This has been reported in the social impacts of the 2014–2015 Ebola outbreak in West Africa. In the long term, the pandemic has led to the loss of trust, relations between communities and health system, and fear of health care workers, and health care facilities were considered 'plague centres' (20). As the the number of Ebola cases declined, the necessity to address its long-term societal impacts has emerged dramatically.

Considering solutions to unmet health needs, several participants included in the present study stated that they did nothing, while others stated that they used herbal or alternative medicine methods or used medicines available at home. This situation may cause a delay in early diagnosis, intervention, and urgent care and treatment in adults. Au highlighted that accessibility of health care services had become the greatest service gap in the COVID-19 outbreak (38). Management of the non-COVID-19-related health needs of the society by a national telemedicine center to be established during the pandemic or establishment of clinics for unmet health needs is thought to be able to eliminate this gap. In Turkey, there is also an increasing demand for live remote consultations, particularly in private health care institutions. Despite many efforts in this area, there is no regulation regarding the functioning of health services that can be provided via telemedicine (39).

According to the World Health Organization, well-organized and prepared health systems can continue to provide

equitable access to essential service delivery throughout an emergency, limit direct mortality and avoid increased indirect mortality (21). It has been further reported that health systems with a relatively limited COVID-19 caseload should have the capacity to maintain routine service delivery in addition to managing COVID-19 cases. Therefore, measuring the access level of countries to the health services needed by society during the pandemic period is of great importance in terms of policymaking for identifying and solving existing problems (19, 21, 22). Although the health system in Turkey has fought successfully against the COVID-19 pandemic, considering the social impacts of the disease may reduce the negative long-term economic and social effects of the disease.

5. CONCLUSION

It is inevitable that people will be both physiologically and psychosocially affected by serious public health measures taken to control the COVID-19 pandemic. There are many lessons to be learned for the future from the results of the present study, which has clearly demonstrated the impact of the pandemic on the health needs of the adult age group living in Turkey. The results showed that two out of every three adults participating in the study had unmet health needs during the pandemic, particularly for oral and dental treatment, eye health and treatment for vision disorders, early diagnosis and annual health screening, mental and psychiatric disorders, and follow-up and monitoring of chronic diseases. The primary barriers to meeting health needs have been observed to be fear of being infected with the virus, being unable to access health services, and not wanting to create a burden on the health system. As a solution to unmet health needs, participants mostly reported that they did nothing in this regard while some of them stated that they used herbal or alternative medicine methods or used medicines available at home. Socio-demographic determinants for unmet health needs of the adult age group have been found to be as follows: female gender, low economic status, having general health insurance or not having any health insurance, presence of chronic disease, perceiving one's health as poor or unchanged, and poor quality of life. The broader social and socio-economic impacts of unmet health needs, which are an indirect consequence of the pandemic, are likely to affect society. Nevertheless, mortality and morbidity rates reflecting the direct impact of the pandemic are only the tip of the iceberg; the majority of pandemic's impact can be thought of as hidden beneath the surface. We believe that health needs and related factors need to be considered to limit the social and economic consequences of unmet health needs in society before they further deepen and it will be useful to make periodic strategic plans for the rational use of resources.

Acknowledgments: *The authors desire to thank all the participants in the study.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Tekirdağ Namık Kemal University (Decision date and number: 24.11.2020 and 2020.252.11.12)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: AÖE, AYİ
Design of the study: AÖE, AYİ
Acquisition of data for the study: AÖE, AYİ
Analysis of data for the study: AÖE, AYİ
Interpretation of data for the study: AÖE, AYİ
Drafting the manuscript: AÖE, AYİ
Revising it critically for important intellectual content: AÖE, AYİ
Final approval of the version to be published: AÖE, AYİ

REFERENCES

[1] Republic of Turkey Ministry of Health, Koronavirüs alacağımız tedbirlerden güçlü değildir. (cited 2021 February 16). Available from: https://www.saglik.gov.tr/TR,64383/koronavirus-alacagimiz-tedbirlerden-guclu-degildir.html (Turkish)

[2] Coronavirus Update (Live). (cited 2022 January 18). Available from: https://www.worldometers.info/coronavirus/

[3] Avaner T, Barbak A, Fedai R. Covid-19 Pandemisi ve Türkiye. Ankara: TİAV; 2021. (Turkish)

[4] Hamel L, Audrey K, Ashley K, Lunna L, Cailey M, Mollyann B. Impact of coronavirus on personal health, economic and food security, and medicaid. In KFF Health Tracking Poll. San Francisco: Henry J. Kaiser Family Foundation 2020.

[5] Mehrotra A, Chernew M, Linetsky D, Hatch H, Cutler D, Schneider EC. The impact of the COVID-19 pandemic on outpatient care: visits return to prepandemic levels, but not for all providers and patients. (cited 2021 Oct 15) Available from: https://www.commonwealthfund.org/publications/2020/oct/impact-covid-19-pandemic-outpatient-care-visits-return-prepandemic-levels

[6] United States Census Bureau "Household Pulse Survey Data Tables," (cited 2020 September 21). Available from: https://www.census.gov/programs-surveys/household-pulse-survey/data.html.

[7] Corallo B, Tolbert J. Impact of coronavirus on community health centers. San Francisco: Henry J. Kaiser Family Foundation 2020.

[8] Gonzalez D, Stephen Z, Genevieve MK, Michael K. Almost half of adults in families losing work during the pandemic avoided health care because of costs or COVID-19 concerns. Washington, DC: Urban Institute 2020;11.

[9] Kretchy IA, Asiedu-Danso M, Kretchy JP. Medication management and adherence during the COVID-19 pandemic: perspectives and experiences from low-and middle-income countries. Res Social Adm Pharm. 2021;17(1):2023-2026.

[10] Hossain MM, Mazumder H, Tasnim S, Nuzhath T, Sultana A. Geriatric health in bangladesh during COVID-19: Challenges and recommendations. J Gerontol Soc Work. 63(6-7):724-727.

[11] Wani FA, Rather RH, Ahmad M. Self-reported unmet healthcare needs during coronavirus disease-19 pandemic lockdown. Int J Med Public Health 2020;9(9):503-507.

[12] Plachouri KM, Georgiou S. How well prepared are dermatologists redeployed to COVID-19 wards? Int J Dermatol. 2020;59:e247-8.

[13] Yetim B, Çelik Y. Sağlık hizmetlerine erişim: karşılanmamış ihtiyaçlar sorunu. Toplum ve Sosyal Hizmet 2020;31(2):423-440. (Turkish)

[14] Andersen R. Health status indices and access to medical care. Am J Public Health 1978;68(5):458-463.

[15] Allin S, Masseria C. Unmet needs as an indicator of health care access. Eurohealth 2009;15(3):7-9

[16] Casey R. Disability and unmet health care needs in Canada: a longitudinal analysis. Disabil Health J. 2015;8(2):173-181.

[17] Weissman JS, Gatsonis C, Epstein AM. Rates of avoidable hospitalization by insurance status in Massachusetts and Maryland. JAMA 1992;268(17):2388-2394.

[18] Adler NE, Boyce WT, Chesney MA, Folkman S, Syme SL. Socioeconomic inequalities in health: no easy solution. JAMA 1993;269(24):3140-3145.

[19] Elston JWT, Cartwright C, Ndumbi P, Wright J. The health impact of the 2014–15 Ebola outbreak. Public Health 2017;143:60-70.

[20] Parpia AS, Ndeffo-Mbah ML, Wenzel NS, Galvani AP. Effects of response to 2014–2015 Ebola outbreak on deaths from malaria, HIV/AIDS, and tuberculosis, West Africa. Emerg Infect Dis. 2016;22(3):433-441.

[21] World Health Organization. COVID-19: Operational guidance for maintaining core health services during an outbreak. (cited 2020 September 21). Available from https://apps.who.int/iris/bitstream/handle/10665/331561/WHO-2019-nCoV-essential_health_services-2020.1-eng.pdf

[22] Tulchinsky TH, Varavikova EA. Planning and managing health systems in The New Public Health 3rd ed. San Diego: Elsevier Academic Press; 2014. P.613-641.

[23] Allin S, Grignon M, Le Grand J. Subjective unmet need and utilization of health care services in Canada: what are the equity implications? Soc Sci Med. 2010;70(3):465-472.

[24] Cavalieri M. Geographical variation of unmet medical needs in Italy: a multivariate logistic regression analysis. Int J Health Geogr. 2013;12(27):1–11.

[25] Smith S, Connolly S. Re-thinking unmet need for health care: Introducing a dynamic perspective. Health Economics, Policy and Law 2020;15(4):440-457.

[26] Eser E, Fidaner H, Fidaner C, Eser SY, Elbi H, Göker E. Psychometric properties of WHOQOL-100 and WHOQOL-Bref. J Psychiatry Psychol Psychopharmacol. 1999;7(2):23-40.

[27] Ray KN, Ettinger AK, Dwarakanath N, Mistry SV, Bey J, Chaves-Gnecco D, Alston KA, Ripper L, Lavage DR, Landsittel DP, Miller E. Rapid-cycle community assessment of health-related social needs of children and families during COVID-19. Acad Pediatr. 2021;21(4):677-683

[28] Assari S, Hani N. Household income and children's unmet dental care need; Blacks' diminished return. Dent J. 2018;6(2):1-10.

[29] Chaupain-Guillot S, Guillot O. Health system characteristics and unmet care needs in Europe: an analysis based on EU-SILC data. The European Journal of Health Economics 2015;16:781-796.

[30] Karpman M, Dulce G, Genevieve MK. Parents are struggling to provide for their families during the pandemic. Urban Institute (cited 2020). Available from: https://www.urban.org/sites/default/files/publication/102254/parents-are-struggling-to-provide-for-their-families-during-the-pandemic_2.pdf

[31] Wang T, Molassiotis A, Chung BPM, Tan JY. Unmet care needs of advanced cancer patients and their informal caregivers: a systematic review. BMC Palliative Care 2018;17(1):1-29.

[32] Willems RA, Bolman CA, Mesters I, Kanera IM, Beaulen AA, Lechner L. Cancer survivors in the first year after treatment: the prevalence and correlates of unmet needs in different domains. Psycho-Oncology 2016;25(1):51-57.

[33] Edib Z, Kumarasamy V., Binti Abdullah N, Rizal AM, Al-Dubai SA. Most prevalent unmet supportive care needs and quality of life of breast cancer patients in a tertiary hospital in Malaysia. Health Qual Life Outcomes 2016;14(26):1-10

[34] Fact Sheet: Service Use among medicaid and chip beneficiaries age 18 and under during COVID-19, Centers for Medicare & Medicaid Services. (cited 2020 September 5). Available from https://www.cms.gov/newsroom/fact-sheets/fact-sheet-service-use-among-medicaid-chip-beneficiaries-age-18-and-under-during-covid-19.

[35] Kirzinger A, Audrey K, Liz H, Mollyann B. KFF Health Tracking Poll – Early April 2020: The impact of coronavirus on life in America. San Francisco: Henry J. Kaiser Family Foundation 2020.

[36] Panchal N, Kamal R, Orgera K, Cox C, Garfield R, Hamel L. The implications of COVID-19 for mental health and substance use. Kaiser Family Foundation. 21 April 2020. (cited 2022 January 110). Available from: https://www. kff.org/coronavirus-covid-19/issue-brief/the-implications-ofcovid-19-for-mental-health-and-substance-use/

[37] Campbella J, Koca F. Financing and protection for the health and care workforce. Bull World Health Organ 2021;99(1):2.

[38] Au SCL. Revisiting the role of telemedicine under the 2019 novel coronavirus outbreak. Eur J Geriatr Gerontol. 2020;2(1):26-27.

[39] Capacı M, Özkaya S. COVID-19 pandemi döneminde tele-tıp uygulamaları. Anatolian Clinic the Journal of Medical Sciences 2020; 25(Special Issue):260-262. (Turkish)

How to cite this article: Yalçın Irmak A, Öztürk Eyimaya A. Unmet Health Needs During the COVID-19 Pandemic. Clin Exp Health Sci 2023; 13: 67-74. DOI: 10.33808/clinexphealthsci.1032287

Clinical and Experimental Health Sciences

Apocynin Ameliorates Testicular Toxicity in High-Fat Diet-Fed Rats by Regulating Oxidative Stress

İrem Hersek[1, 2], M.Kutay Köroğlu[1, 2], Büşra Coşkunlu[1, 2], Büşra Ertaş[3], Göksel Şener[4], Feriha Ercan[1]

[1] Marmara University, School of Medicine, Department of Histology and Embryology, İstanbul, Türkiye.
[2] Marmara University, Institute of Health Scineces, Department of Histology and Embyology, İstanbul, Türkiye.
[3] Marmara University, Faculty of Pharmacy, Department of Pharmacology, İstanbul, Türkiye.
[4] Fenerbahçe University, Faculty of Pharmacy, Department of Pharmacology, İstanbul, Türkiye.

Correspondence Author: Feriha Ercan
E-mail: fercan@marmara.edu.tr

Received: 10.12.2021 **Accepted:** 15.09.2022

ABSTRACT

Objective: The purpose of this study was to examine the effects of apocynin (APC), an inhibitor of NADPH oxidase (NOX), on high-fat diet (HF)-induced testis cytotoxicity.

Methods: Wistar albino rats were divided into three groups as control, HF and HF+APC groups. Rats in HF and HF+APC groups were fed using HF for 16 weeks and in the last four weeks of this period vehicle solution or APC (25 mg/kg) was administered orally five days a week, respectively. Control group was fed with standart lab chow for 16 weeks. Cholesterol, triglyceride, high-density lipoproteins, leptin, estrogen, testosterone, LH and FSH were estimated in blood serum. Sperm parameters were analysed from the epididymis. Testicular malondialdehyde, 8-hydroxy-2-deoxyguanosine, glutathione, superoxide dismutase and myeloperoxidase levels were estimated biochemically. Testicular morphology, proliferative, apoptotic and NOX2-positive cells were analysed histologically.

Results: HF-induced obesity caused significant alterations in serum lipid and hormone profiles. Testicular malondialdehyde, 8-hydroxy-2-deoxyguanosine, and myeloperoxidase levels increased, glutathione and superoxide dismutase levels decreased in this group. Moreover, altered sperm parameters, increased degenerated seminiferous tubules, apoptotic and NOX2 – positive cells and decreased proliferative cells were observed in the HF group. All these biochemical and histological alterations improved in the HF+APC group.

Conclusion: HF-induced obesity causes altreations in lipid values, sperm parameters and testicular morphology by increasing oxidative stress through NOX2 activity. Apocynin might prevent testis damage via regulating oxidant/antioxidant balance.

Keywords: High fat diet, apocynin, testis, apoptosis, oxidative stress

1. INTRODUCTION

Obesity, one of the main worldwide health problems of the last five decades, is a chronic disease that effects the physiological, economic and psychological quality of individual life, regardless of cultural, financial or ethnic origin (1). Obesity is defined by body mass index (BMI) more than 30 kg/m^2 and it is related to the development of numerous health disturbances, including cardiovascular disorders, type 2 diabetes, insulin resistance, hepatic and renal failure, respiratory disorders, different types of cancer, and infertility (2). High BMI is related to male infertility determined by altered sperm parameters as low sperm concentration, decreased number of motile sperm and spermatozoa with normal morphology (3).

Human and experimental studies have shown the effects of oxidative stress in the pathogenesis of obesity. High fat high carbohydrate food consumption induces intracellular pathways causing oxidative stress by biochemical mechanisms such as superoxide generation from NADPH oxidase (NOX) and oxidative phosphorylation (4). Oxidative stress caused by reactive oxygen species (ROS) have a crucial role in male infertility with DNA damage and testicular germ cell apoptosis in testis, and reduced sperm motility (5) and changes of sperm concentration, motility and morphology (6). In different studies, it was shown that obesity leads to excessive reactive oxygen species (ROS) production which is related to the alterations in sperm parameters, testicular damage with an increase of oxidative stress, inflammation and cell death (7,8).

Activation of NOX initiates the ROS production from oxygen (9). Apocynin (APC, 4´-hydroxy-3´-methoxyacetophenone) is a naturally originating methoxy-substituted catechol taken out from the *Picrorhiza kurroa* (Scrophulariaceae) and *Apocynum cannabinum* (Canadian hemp), known as NOX inhibitor and used in traditional Indian medicine (10). It was shown that

DOI: 10.33808/clinexphealthsci.1035133

APC decreases apoptosis by increasing antioxidant protection in experimental cerebral artery occlusion (11). Moreover, APC protected testicular tissue from cisplatin-induced (12), metotrexate-induced (13), streptozotocin-induced (14) and ischemia-reperfusion-induced (15) testis damage by inhibiting ROS formation and apoptosis.

The protective effects of APC through its antioxidant and antiapoptotic properties have been demonstrated in many testicular injury models (12-16). However, the effects of APC, on testis damage in HF-induced obesity were not examined before. The purpose of this study was to examine the anti-oxidant and anti-apoptotic impacts of APC on testicular cytotoxicity in high-fat diet-fed rats.

2. METHODS

2.1. Animals and Experimental Groups

Wistar albino male rats (250–300 g, eight-week-old) were kept in a laboratory environment with a regular light/dark (12/12 hour) cycle at 22 ±2°C for the duration of the experiment. The animals accessed to water *ad libitum*. This study was appoved by The Animal Care and Ethical Committee for Experimental Animals at Marmara University (68.2017.mar).

The rats were divided into 3 experimental groups as control, HF and HF+APC groups. Rats in the control group (n=8) were fed with standart diet (6% fat). Rats in the HF (n=10) and HF+APC (n=10) groups were fed with high-fat diet (45% fat) for 16 weeks and treated with either vehicle solution (15% dimethyl sulfoxide) or 25 mg/kg APC orally 5 days a week for the last 4-weeks of the experiment, respectively. In a previous study, APC was applied to the control group to observe if it had any adverse effects; but no adverse effect was observed (12). Based on this finding, and also to avoid sacrificing any more animals, an APC treated control group was not created in this study. The solvent agent, dosage and administration of APC were based on previous studies (12, 15). The rats weight was measured every week during the experiments. Rats were decapitated under light ether anesthesia and blood, epididymis and testis samples were obtained at the end of the 16th week. The trunk blood samples were kept at room temperature (30 min), centrifuged at 4000 rpm (15 min) and then separated serum samples were kept at – 20°C for lipid profile and hormone analysis. Testis samples were weighed. Epididymis was dissected for evaluation of sperm parameters. The right testis was processed for histological examinations. The left testis was homogenized in phosphate buffered saline (PBS) and kept at – 80 °C until biochemical evaluation.

2.2. Measurement of Serum Cholesterol, Triglyceride and High-Density Lipoprotein Levels

Total cholesterol, triglyceride and high-density lipoprotein (HDL) levels were estimated with ELISA kits (Elabscience, Wuhan, China). The results were given as mmol/L for cholesterol and ng/ml for triglyceride and HDL.

2.3. Measurement of Serum Leptin, Estrogen, Testosterone, LH and FSH levels

Serum leptin, estrogen, testosterone, LH and FSH levels were estimated with the commercial ELISA kits (Elabscience, Wuhan, China). The results were given as ng/ml for leptin, estrogen and testosterone, and IU/L for LH and FSH.

2.4. Measurement of Malondialdehyde, 8-Hydroxy-Deoxyguanosine, Glutathione, Superoxide Dismutase and Myeloperoxidase Levels in Testis

The malondialdehyde (MDA), 8-hydroxy-deoxyguanosine (8-OHdG), glutathione (GSH), superoxide dismutase (SOD) and myeloperoxidase (MPO) levels were surveyed in testis homogenates by ELISA kits (MyBioSource, Southern California, San Diego USA). The results were given as nmol/g for MDA and GSH, ng/mg for 8-OHdG, % inhibition for SOD, and U/g for MPO.

2.5. Evaluation of Epididymal Sperm Parameters

Caudal epididymis samples were cut into small pieces in Earle's balanced salts solution (5 ml, Sigma, USA) for sedimentation, and supernatants were removed. Using the routine density gradient method, pellets were centrifuged at 1800 rpm (18 min). After removing supernatants, they were diluted with a sperm washing medium (0.3 ml, SAGE, UK) to centrifuge at 2000 rpm (10 min) and supernatants were discharged. Pellets diluted with fertilization medium (0.3 ml, SAGE, UK) were evaluated with Macler Counting Chamber (Sefi Medical Instruments, Haifa, Israel) for the motility rate and sperm counting under a photomicroscope. All of the sperms in 100 squares were counted and multiplied by a million. Smears were stained using the Diff-Quick kit (Medion Diagnostics, Grafelfing, Germany). In each smear slide, 100 sperms were evaluated morphologically at 1000x magnification under the photomicroscope (17).

2.6. Light Microscopic Preparation

The right testes were fixed with 10% formaldehyde solution. Following fixation, the tissues were processed routinely for paraffin embedding. Paraffin sections were stained using hematoxylin and eosin (H&E) for histological analysis. The diameter and epithelial thickness of 20 seminiferous tubules were measured using the Image J (NIH-USA) program. Twenty seminiferous tubules were evaluated in accordance with the modified Johnsen scoring method (17,18). Each tubule was scored from 1 (absence of both germinal and Sertoli cells) to 10 (full spermatogenesis).

2.7. Proliferating Cell Nuclear Antigen Immunohistochemistry

Paraffin sections were treated with 3% hydrogen peroxide (H_2O_2; 30 min) for endogenous enzyme blockade. Sections were incubated with 300 W microwave in citrate buffer for antigen retrival (20 min). The cooled sections were washed with PBS (3x5 min), kept in blocking solution (10 min, Invitrogen) and then kept in rabbit anti-proliferating cell nuclear antigen (PCNA, Novus) primary antibody (1:2000) overnight at 4°C. The sections were washed in PBS (5 min), then kept in biotinylated secondary antibody (30 min, Thermo), washed in PBS, incubated in streptavidin peroxidase (10 min, Invitrogen), washed in PBS, waited in 3,3'-diaminobenzidine (DAB) chromogen (5 min), rinsed in distilled water and then were counterstained with hematoxylin. The proliferation index was estimated by dividing the PCNA – positive cells by the total number of cells into 20 seminiferous tubules in each section.

2.8. Terminal Deoxynucleotidyl Transferase Dutp Nick End Labelling Method

The terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) technique was performed in accordance with the manufacturer's intsruction (ApopTag Plus, In Situ Apoptosis Detection Kit, S7101, Millipore). TUNEL-positive cells were estimated by examining 20 seminiferous tubules in each section. The apoptotic index was calculated by dividing the number of seminiferous tubules with three or more TUNEL-positive cells by the total number of seminiferous tubules.

2.9. NOX2 Immunohistochemistry

Paraffin sections were treated with 3% H_2O_2 solution for endogenous peroxidase blockage and then incubated in microwave in citrate buffer solution (pH 6.0) for antigen retrival. Then the slides were washed in PBS, treated with protein blocking solution (EXPOSE Rabbit specific HRP/DAB Detection IHC Kit, Abcam, Cambridge, UK). The slides were incubated in anti-NOX2/gp91phox primary antibody (1:100 dilution, bs-3889 R, Bioss Inc, Massachusetts, USA) overnight at 4ºC. After washing in PBS, sections were treated with biotinylated secondary antibody (10 min, Ultra Tek Hrp Anti-Polyvalent, ScyTek). Then the slides were washed in PBS, treated with streptavidin peroxidase (10 min), washed in PBS and treated with DAB. The slides were counterstained with hematoxylin. In each section, 20 seminiferous tubules were examined for NOX2-positive cells. Seminiferous tubules with three or more NOX2-positive cells were notified as the percentage of 20 tubules.

All stained sections were evaluated with a photomicroscope (Olympus BX51) and photographed with digital camera (Olympus DP72).

2.10. Statistical Analysis

Biochemical and histological results were estimated using a one-way analysis of variance. Post-hoc testing was completed with Tukey's multiple comparisons test and the data were conveyed as mean ± standard error of the mean (SEM). Significance of differences were used as $p< 0.05$. Analysis was calculated by an instant statistical analysis package (Prism 6.0 GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1. Body and Testis Weight Results

The body weight of rats in the HF ($p< .001$) and HF+APC ($p< .05$) groups significantly increased compared to the rats in the control group at the end of the study (Figure 1). Testis weight was 1.77±0.15 g in control group, 1.78±0.11 g in HF group and 1.83±0.14 g in HF+APC group. This value was not significantly different among the experimental groups.

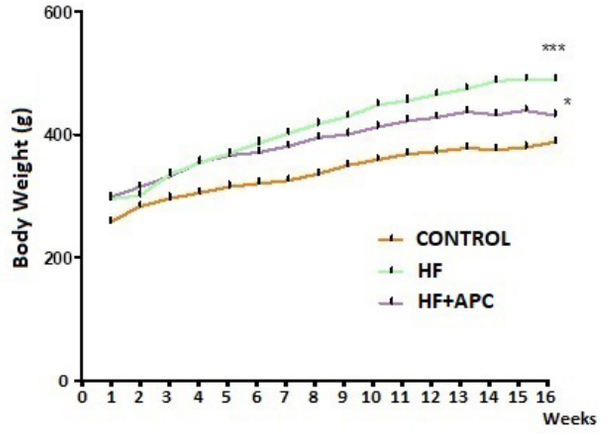


Figure 1. *Body weights of control, HF and HF+APC groups. * P<0.05 and *** P< 0.001 in comparison with control group.*

3.2. Cholesterol, Triglyceride and HDL Values

Serum cholesterol ($p< .001$) and triglyceride ($p< .01$) levels were higher in the HF group than the control group. However, cholesterol ($p< .001$) and triglyceride ($p< .05$) levels decreased in APC treated HF group compared to HF group. Furthermore, HDL level reduced in HF ($p< .001$) and HF+APC ($p< .05$) groups compared to the control group. This value increased in HF+APC group ($P<0.05$) compared to HF group (Figure 2).

3.3. Leptin, Estrogen, Testosterone, LH and FSH Values

Serum leptin ($p< .001$), estrogen ($p< .01$) and testosterone ($p< .001$) levels were higher in the HF group than the control group. These leptin ($p< .001$), estrogen and testosterone ($p< .001$) values were lower in the HF+APC group compared to the HF group. LH level ($p< .01$) decreased in the HF group in comparison with the control group and this value increased

in the HF+APC group (p< .05) in comparison with the HF group. Although the FSH level decreased in the HF group in comparison with control group and increased in comparison with the HF+APC group, these findings were not statistically significant (Figure 3).

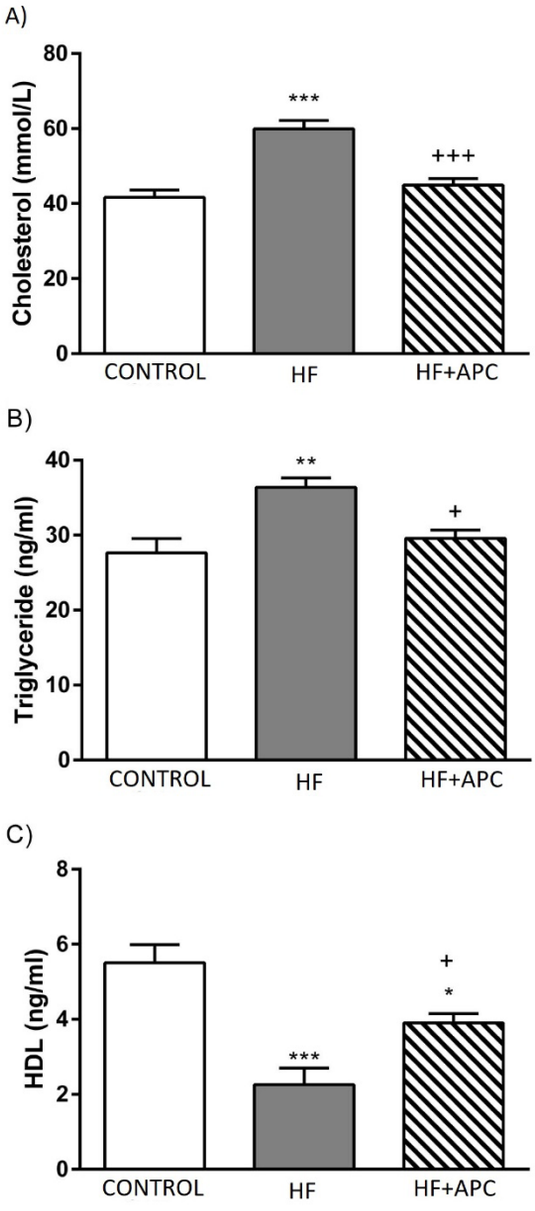


Figure 2. *Cholesterol (A), triglyceride (B) and HDL (C) levels in the control, HF and HF+APC groups. * P<0.05, ** P<0.01 and *** P<0.001 in comparison with control group, + P<0.05 and +++ P<0.001 in comparison with HF group.*

3.4. MDA, 8-OHdG, GSH, SOD and MPO Values

Testicular MDA (p< .001), 8-OHdG (p< .01) and MPO (p< .01) levels increased and GSH (p< .001) and SOD (p< .05) levels decreased in the HF group in comparison with the control group. But, MDA (p< .001), 8-OHdG (p< .05) and MPO (p< .01) levels decreased and GSH level (p< .01) increased in the HF+APC group in comparison with the HF group (Figure 4).

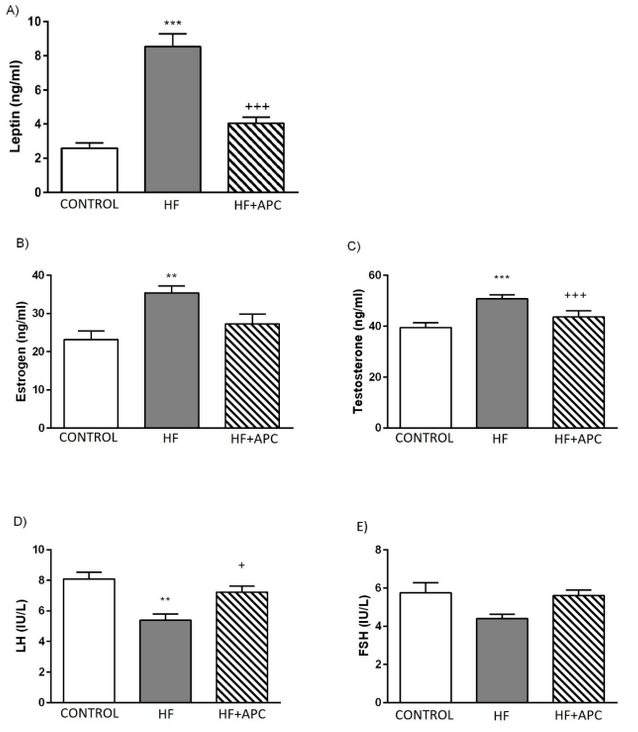


Figure 3. *Leptin (A), estrogen (B), testosterone (C), LH (D) and FSH (E) levels in the control, HF and HF+APC groups. ** P<0.01 and *** P<0.001 in comparison with control group, + P<0.05 and +++ P<0.001 in comparison with HF group.*

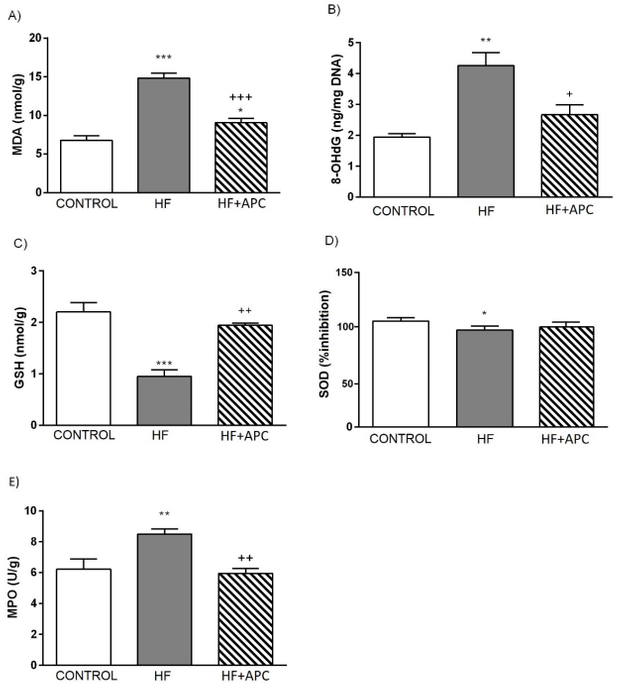


Figure 4. *MDA (A), 8-OHdG (B), GSH (C), SOD (D) and MPO (E) levels in the control, HF and HF+APC groups. * P<0.05, ** P<0.01, *** P<0.001 in comparison with control group. + P<0.05, ++ P<0.01, +++ P<0.001 in comparison with HF group.*

3.5. Sperm Parameter Results

Normal spermatozoa and spermatozoa with tail, neck and head abnormalities were observed in all of the experimental groups. The number of epididymal spermatozoa (p< .01) and sperm motility (p< .05) reduced in the HF group compared to the control group. Although the number of epididymal spermatozoa and sperm motility increased in the HF+APC group in comparison with the HF group, these findings were not statistically significant. Spermatozoa with normal morphology decreased in the HF and HF+APC groups (p< .05), and spermatozoa with tail defects (p< .05) increased in the HF group compared to the control group (Figure 5).

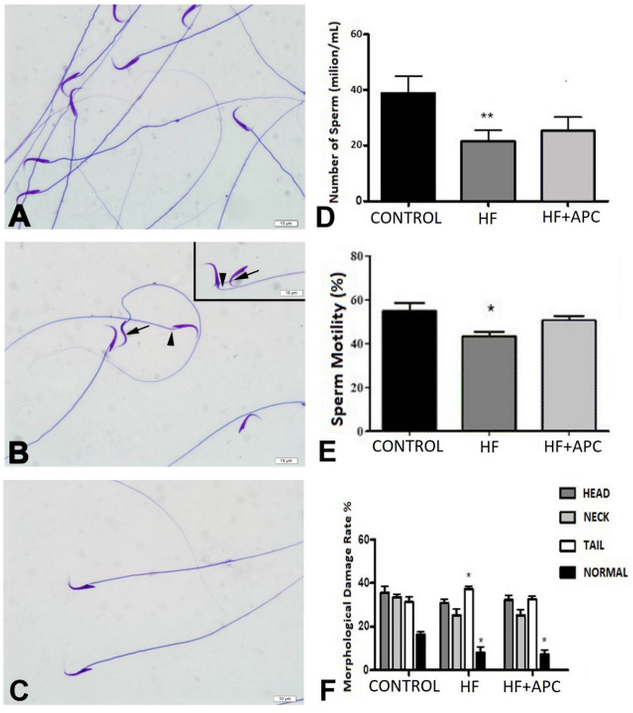


Figure 5. *Representative light micrographs of spermatozoa morphology (A-C), number of spermatozoa (D), sperm motility (E) and sperm morphologic damage ratio (F) in the control, HF and HF+APC groups. Spermatozoa with normal morphology and spermatozoa with head (arrow), neck (arrowhead) and tail abnormalities are seen in the control (A), HF (B) and HF+APC (C) groups. Diff-Quick staining. Original magnification: 1000x. Scale bar: 10µm. * P<0.05, ** P<0.001 in comparison with control group.*

3.6. Histopathological Results

Normal seminiferous tubules morphology were present in the control group. The HF group had many degenerated seminiferous tubules, decreased number of spermatogenic cells, many dilatations among the germinal epithelial cells, and many immature germ cells in the lumen. The HF+APC group had significantly more normal seminiferous tubules, but there were some dilatations among the spermatogenic cells and immature sperms in luminal region. Seminiferous tubule diameter (p< .001), germinal epithelium thickness (p< .001) and histopathologic Johnsen's score (p< .01) decreased in the HF group compared to control group. These seminiferous tubule diameter (p< .05), germinal epithelium thickness (p< .001) and histopathologic Johnsen's score (p< .01) increased in the HF+APC group compared to the HF group (Figure 6).

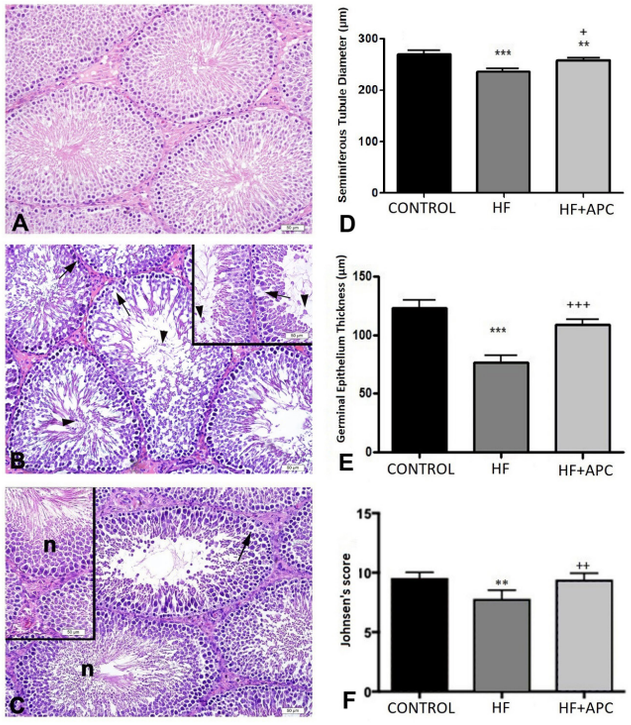


Figure 6. *Representative light micrographs of testis samples (A-C), seminiferous tubule diameter (D), germinal epithelium thickness (E) and Johnsen's histopathological scores (F) of the control, HF and HF+APC groups. Normal germinal epithelium of seminiferous tubules is present in the control group (A). Dilatations (arrow) among the germinal epithelial cells and cellular debris (arrowhead) in lumen are seen in the HF group (B). Normal seminiferous tubules (n) and dilatation of germinal epithelium in some area (arrow) are seen in the HF+APC group (C). Scale bar: 50 µm. ** P<0.01, *** P<0.001 in comparison with control group, $^{+}$P<0.05, $^{++}$ P<0.01 and $^{+++}$ P<0.001 in comparison with HF group.*

3.7. PCNA Immunohistochemistry Results

PCNA-positive cells in the epithelium of seminiferous tubules were seen in all of the experimental groups. Proliferative index was lower in the HF group (p< .01) compared to the control group and this parameter was higher in the HF+APC group (p< .05) compared to the HF group (Figure 7A_1-D_1).

3.8. TUNEL Analysis Results

TUNEL-positive cells were present in all of the experimental groups, but the number of TUNEL-positive cells increased in the HF group compared to the control and HF+APC groups. Apoptotic index was higher in the HF group compared to the control group (p< .01), this parameter was lower in HF+APC group (p< .05) compared to HF group (Figure 7A_2-D_2).

3.9. NOX2 Immunohistochemistry Results

NOX2-positive cells were seen in the interstitial area and germinal epithelium in all of the experimental groups, but the number of NOX2-positive cells increased in the HF group compared to the control and HF+APC groups. NOX2 – positive cells increased in the HF group compared to the control group ($p< .01$) and decreased in the HF+APC group ($p< .05$) compared to the HF group (Figure 7A_3-D_3).

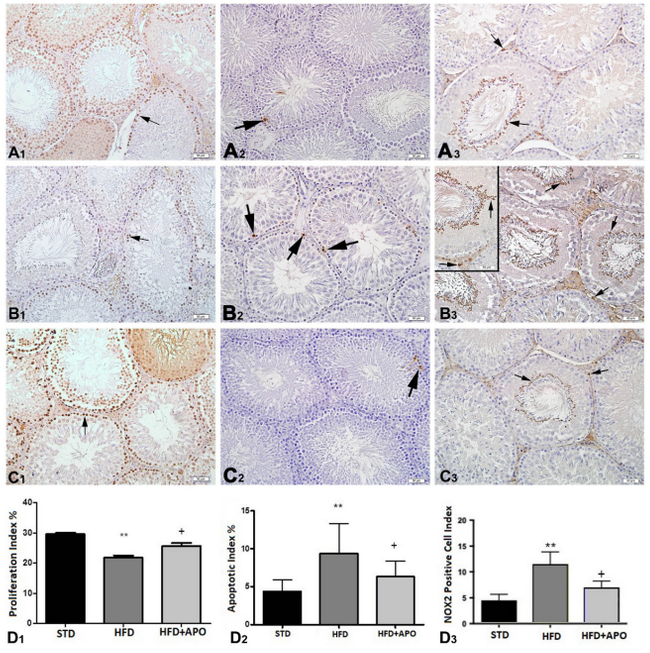


Figure 7. *Representative light micrographs of PCNA immunostained (A_1-C_1), TUNEL-stained (A_2-C_2) and NOX2 immunostained (A_3-C_3) testis samples, proliferation index (D_1), apoptotic index (D_2) and NOX2-positive cell ratio (D_3) in the control, HF and HF+APC groups. PCNA – positive (arrow), TUNEL – positive (arrow) and NOX2 – positive (arrow) cells in seminiferous tubules are seen in the control (A_1-A_3), HF (B_1 – B_3) and HF+APC (C_1 – C_3) groups. Scale bar: 50 µm. ** P<0.01, in comparison with control group, + P<0.05 in comparison with HF group.*

4. DISCUSSION

In this study, increase of body weight, changes in serum lipid and hormone profiles were demostrated in the HF group compared to the control rats. The biochemical results showed increased oxidative stress parameters and decreased endogeneous antioxidant levels in the HF group compared to the control group. Moreover, altered sperm paramaters, increased degenerated seminiferous tubules with apoptotic and NOX2-positive cells and decreased proliferative cells in seminiferous tubules were found in the HF group compared to the control group. All these HF-induced alterations in lipid and hormone profiles, sperm parameters, testicular oxidative stress and histological damage findings were ameliorated by APC administration.

Obesity is related to the increase of white adipose tissue in the body and BMI over 30 kg/m^2. As BMI increases, the rate of infertility increases in men (19). Decrease in semen quality was observed 3 times more in obese men then in men with normal weight (20). Moreover, decrease of testis weight was reported in obese rats (21). Also, decrease of sperm concentration (22) and sperm motility were shown in obese patients (23), and alteration of epididymal sperm parameters (24), teratozoospermia and degeneration of testis morphology (25) were shown in obese animals. Parallel with the previous studies, in the present study, an increase in body weight, the decrease in number, motility and normal sperms were observed in the HF group. Although testis weight was not different amoung the groups, decreased the diameter and epithelial thickness of seminiferous tubules and increased of Johnsen's score was observed in the HF group. APC treatment ameliorated these sperm parameters and testicular morphological damage in the HF-fed rats via its antioxidative activity.

Obesity is related with an increase of blood free fatty acids and the accumulation of fat in the white adipose tissue (1). Increased blood glucose, serum cholesterol, triglyceride, and LDL levels and decreased HDL level were shown in obese animals (8,26,27). An increase in free fatty acid stimulates the activation of NOX in the body cells, ultimately causing oxidation and formation of free radicals (8). Similar to the previous studies, an increase of cholesterol and triglyceride levels and decrease of HDL level were observed in the serum of HF fed rats, and APC treatment ameliorated lipid profiles in the serum of HF-fed rats via its regulatory effects on oxidative stress.

Leptin is one of the important factors affecting weight gain in relation to appetite control. Increased serum leptin level was observed in obese infertile patients in both sexes (28). Increased leptin level and decreased fertility rate was reported in obese mice (29). It was shown that increased leptin level had harmful effects on sperm production in obese male (30). Similarly, Yi et al. (2017) showed an increase in serum leptin, leptin mRNA and protein levels in testis, and sex hormone dysregulation in mice fed with HF (31). Obesity may also adversely affect the male reproductive endocrine system which regulates the reproductive function. It has been reported that obesity alters the sex hormone levels such as increased estrogen level and decreased LH, FSH and testosterone levels (32). Increased estrogen level alters the production of LH, FSH and testosterone levels (33). Similar to the previous studies, increased leptin and estrogen levels and decreased LH and FSH levels were shown in the HF group in this study. Although increased testosterone level was observed in the HF group, degenerated testis morphology and altered sperm parameters were seen in the HF group. Since spermatogenesis is a complex event, it is regulated not only by testosterone level but also from the other hormones and Sertoli cells regulatory function. Therefore, the role of Leydig and Sertoli cells in HF-induced obesity may consider in further studies on this subject. HF-induced obesity in rats might have caused alteration of sexual hormone levels which regulates the spermatogenesis and APC treatment

may regulate the hormone producing cells activity via anti-oxidative activity.

Positive correlation between the oxidative stress, and obesity have been reported previously. Also, increased reactive oxygen and nitrogen species levels and decreased antioxidant defences in obese patients were observed (4). Oxidative stress is quite associated with cumulative injury to the body caused by free radicals that are weakly neutralized by antioxidants, and oxidative damage is worsened by reduction of SOD, catalase (CAT) and glutathione S-transferase activities (34). A decrease in SOD activity and an increase in MDA level induce oxidative stress and lead to cellular damage in testis of obese rats (25). Aditionally, it was noted that, ROS formation causes sperm membrane damage which reduces the motility and ability to fuse with the oocyte and directly sperm DNA damage (1). Plasma MDA values were found higher in obese individuals than non-obese group. It has been shown that the MDA/SOD ratio is higher in obese individuals compared to normal individuals (35). Recent studies have shown the increased MDA level and decreased SOD activity and GSH level in testis of obese rats (8,36). Moreover, increased MDA level and decreased SOD and CAT activities were shown in the testis of obese rats with intake of high-fructose corn syrup (37). In this study, increased MDA and decreased GSH and SOD levels were observed in the testis of HF-fed rats. Increased oxidative stress may cause testicular degereneration with decrease in diameter and germinal epithelium thickness of seminiferous tubules and proliferative cells and increase in NOX2 immunpositive and apoptotic cells. We suggest that APC treatment reversed these oxidative and histopathological parameters in testis samples of HF-fed rats via regulating of oxidative stress.

Obesity causes formation of ROS through NOX activation in the adipose tissue (38). APC, an inhibitor of NOX2, has a role for the inhibition of NOX-induced ROS production. Also, APC stimulates the g-glutamylcysteine synthethase which effects the synthesis of GSH (39). It has been shown that the serum and hepatic MDA levels were higher and hepatic SOD, glutathione-peroxidase and CAT activities and GSH level were lower in the HF-fed mice and APC treatment amelioriated the oxidant/antioxidant parameters in both serum and hepatic tissues in these mice (40). Moreover, it was shown that, administration of 16 mg/kg APC for 4 weeks reduced oxidative stress and apoptotic cells in testicular tissue in diabetic rats (14). Additionally, it was shown that, administration of both 20 mg/kg and 50 mg/kg doses of APC decreased oxidative stress parameters and testicular damage in ischemia/reperfusion induced toxicity (15). Administration of 25 mg/kg APC reduced oxidative stress, testicular damage, apoptosis and sperm parameters in cisplatin induced testis toxicity (12) and administration of both 20 mg/kg and 50 mg/kg doses of APC reduced oxidative stress parameters, testicular damage and apoptosis in methotrexate-induced testis toxicity (13). Parallel to the previous studies, increase of MDA level, apoptotic and NOX2-positive cells and decrease of GSH and SOD level in HF group were observed, while APC (25 mg/kg) administration ameliorated all these oxidative stress parameters, testicular damage and sperm parameters via the inhibiting of NOX activation.

Obesity-induced inflammatory responses and their relation with the generation of free oxygen radicals have been demonstrated previously (4,35). Activation of MPO, a heme protein synthesized by neutrophils, is associated with the obesity-induced inflammation. It was observed that the number of neutrophils in circulatory blood are rised in obese individuals and type 2 diabetic patients. Moreover, studies showed that there is a positive correlation between MPO activation and metabolic disorders and MPO plasma levels are higher in these patients (41,42). Additionally, it has been reported that obesity-induced testicular inflammation causes disruption of spermatogenesis and steroidogenesis (35). Recent studies have shown the anti-inflammatory effects of APC in ischemia/reperfusion – (15), cisplatin – (12) and methotrexate-induced (13) testis damage. In this study, oxidative parameters such as MDA and 8-OHdG levels, NOX2-positive cells as well as MPO activity in HF-fed rats were increased, APC treatment to the HF-fed rats reversed the MPO activity to the control level.

Apoptosis is related to many diseases including obesity. The germ cell population during spermatogenesis is regulated by apoptosis, however increased apoptotic activity may lead to male infertility (43). It was reported that HF caused an increase of apoptotic cells in the testis (8,21). The 8-OHdG measurement is one of the most commonly used method to determine oxidative DNA damage. Increased DNA fragmentation in testis and spermatozoa were reported in obese men (34). Additionally, it was reported that apoptotic cell death and 8-OHdG level were increased in various oxidative testicular damage models (5,8,12-15). Moreover, it has been noted that various antioxidant compounds including APC has neutralizing effect on harmful free radical compounds as well as preventive effect on the apoptotic processes in these oxidative testicular damage models (8,12-15). Parallel to the previous studies, increase of testicular apoptotic cells and 8-OHdG level together with increase of NOX2-positive cells and decrease of proliferative cells were observed in HF-fed rats, APC treatment reversed this apoptotic cell death and oxidative cell damage to the level of standard diet-fed rats.

5. CONCLUSION

In conclusion, HF-induced obesity caused changes of lipid and hormone profiles in serum, changes of sperm parameters, increase of oxidative, inflammatory and apoptotic activity and decrease of proliferative cells and endogeneous antioxidants in rat testis. APC treatment might have ameliorated obesity-induced functional and morphological testis damage by inhibition of oxidative stress, apoptosis, inflammation and regulation of oxidant/antioxidant balance. Additionally, the efficacy of APC as a therapeutic agent was approved in many studies and also in our study, suggesting its testis damage reducing effect in HF-induced obesity. However, limitations of our study were that we did not study the proinflammatory and apoptotic markers, in the testis and oxidative parameters

in the epididymis. Therefore, the molecular mechanisms involved in effects of apocynin on high fat diet-induced testis damage require further studies.

Acknowledgements: *The authors would like to thank Dr. Dilek Akakın for comments of our manuscript.*
Funding: *This study was financially supported by Marmara University Scientific Research Project Committee (SAG-C-YLP-131.217.0655).*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by the Animal Care and Ethical Committee for Experimental Animals at Marmara University.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: F.E.
Design of the study: F.E., G.Ş.
Acquisition of data for the study: İ.E., M.K.K., B.C., B.E., G.Ş., F.E.
Analysis of data for the study: İ.E., M.K.K., B.C., B.E., G.Ş., F.E.
Interpretation of data for the study: F.E., G.Ş.
Drafting the manuscript: F.E, M.K.K., G.Ş.
Revising it critically for important intellectual content: F.E.
Final approval of the version to be published: İ.E., M.K.K., B.C., B.E., G.Ş., F.E.

REFERENCES

[1] Manna P, Jain SK. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: Causes and therapeutic strategies. Metab Syndr Relat Disord. 2015;13(10):423-444.
[2] Jung RT. Obesity as a disease. Br Med Bull. 1997;3(2):307-321.
[3] Hammoud AO, Meikle AW, Reis LO, Gibson M, Peterson CM, Carrell DT. Obesity and male infertility: A practical approach. Semin Reprod Med. 2012;30(6):486-495.
[4] Savini I, Catani MV, Evangelista D, Gasperi V, Aviglian L. Obesity-associated oxidative stress: Strategies finalized to improve redox state. Int J Mol Sci. 2013;14:10497-10538.
[5] Shokoohi M, Shoorei H, Soltani M, Abtahi-Eivari SH, Salimnejad R, Moghimian M. Protective effects of the hydroalcoholic extract of Fumaria parviflora on testicular injury induced by torsion/detorsion in adult rats. Andrologia. 2018;50(7):e13047.
[6] Ribas-Maynou JR, Yeste M. Oxidative stress in male infertility: Causes, effects in assisted reproductive techniques, and protective support of antioxidants. Biology. 2020;9(4):77.
[7] Mortazavi M, Salehi I, Alizadeh Z, Vahabian M, Roushandeh AM. Protective effects of antioxidants on sperm parameters and seminiferous tubules epithelium in high fat-fed rats. J Reprod Infertil. 2014;15(1):22-28.
[8] Suleiman JB, Nna VU, Zakaria Z, Othman ZA, Bakar ABA, Mohamed M. Obesity-induced testicular oxidative stress, inflammation and apoptosis: Protective and therapeutic effects of orlistat. Reprod Toxicol. 2020;95:113-122.
[9] Filho DW, Torres MA, Bordin AL. Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia–reperfusion injury. Mol Aspects Med. 2004;25:199–210.
[10] Stefanska J, Pawliczak R: Apocynin: Molecular aptitudes. Mediators Inflamm. 2008;106507.
[11] Connell BJ, Saleh MC, Khan BV, Saleh TM. Apocynin may limit total cell death following cerebral ischemia and reperfusion by enhancing apoptosis. Food Chem Toxicol. 2011;49:3063–3069.
[12] Köroğlu KM, Çevik Ö, Şener G, Ercan F. Apocynin alleviates cisplatin-induced testicular cytotoxicity by regulating oxidative stress and apoptosis in rats. Andrologia. 2019;51(4):e13227.
[13] Kavram SK, Yardimoğlu YM, Eraldemir FC, Yazir Y. Protective effects of apocynin on damaged testes of rats exposed to methotrexate. Turk J Med Sci. 50(5):20201.409.1420.
[14] Li M, Lıu Z, Zhuan L, Wang T, Guo S, Wang S, Lıu J, Ye Z. Effects of apocynin on oxidative stress and expression of apoptosis-related genes in testes of diabetic rats. Mol Med Rep. 2013;7:47-52.
[15] Şener TE, Yüksel M, Özyılmaz-Yay N, Ercan F, Akbal C, Şimşek F, Şener G. Apocynin attenuates testicular ischemia–reperfusion injury in rats. J Pediatr Surg. 2015:50:1382-1387.
[16] Özbek O, Altıntaş R, Polat A, Vardı N, Parlakpınar H, Sağır M, Duran ZR, Yıldız A. The protective effect of apocynin on testicular ischemia-reperfusion injury. J Urol. 2015:193(4):1417-1422.
[17] Arabacı Tamer S, Yıldırım A, Arabacı Ş, Çiftçi S, Akın S, Sarı E, Köroğlu MK, Ercan F, Yüksel M, Çevik Ö, Yeğen BÇ. Treatment with estrogen receptor agonist ERβ improves torsion-induced oxidative testis injury in rats. Life Sci. 2019;222:203-211.
[18] Johnsen SG. Testicular biopsy score count: A method for registration of spermatogenesis in human testes. Normal values and results of 335 hypogonadal males. Hormones 1970;1:2–25.
[19] Sallmen M, Sandler DP, Hoppin JA, Blair A, Baird DD. Reduced fertility among overweight and obese men. Epidemiology. 2006;17(5):520-523.
[20] Magnusdottir EV, Thorsteinsson T, Thorsteinsdottir S, Heimisdottir M, Olafsdottir K. Persistent organochlorines, sedentary occupation, obesity and human male subfertility. Human Reprod. 2005;20(1):208-215.
[21] Miao XL, Gao GM, Jiang L, Xu R, Wan DP. Asiatic acid attenuates high-fat diet-induced impaired spermatogenesis. Exp Ther Med. 2018;15(3):2397-2403.
[22] Alshahrani S, Ahmed AF, Gabr AH, Abalhassan M, Ahmad G. The impact of body mass index on semen parameters in infertile men. Andrologia. 2016;48(10):1125-1129.
[23] Martini AC, Tissera A, Estofán D, Molina RI, Mangeaud A, Cuneo MF, Ruiz RD. Overweight and seminal quality: A study of 794 patients. Fertil Steril. 2010;94(5):1739-1743.
[24] Açıkel Elmas M, Arbak S, Ercan F. Ameliorating effects of exercise on disrupted epididymal sperm parameters in high fat diet-induced obese rats. Marmara Med J. 2019;32:14-19.
[25] Jia YF, Feng Q, Ge ZY, Guo Y, Zhou F, Zhang KS, Wang XW, Lu WH, Liang XW, Gu YQ. Obesity impairs male fertility through long-term effects on spermatogenesis. BMC Urol. 2018;18(1):42.
[26] Silva JF, Correa IC, Diniz TF, Lima PM, Santos RL, Cortes SF, Coimbra CC, Lemos VS. Obesity, inflammation, and exercise training: Relative contribution of iNOS and eNOS in the modulation of vascular function in the mouse aorta. Front Physiol. 2016;7:386.
[27] Açıkel Elmas M, Çakıcı SE, Dur IR, Kozluca I, Arınç M, Binbuğa B, Bingol Özakpınar O, Kolgazi M, Şener G, Ercan F. Protective effects of exercise on heart and aorta in high-fat diet-induced obese rats. Tissue Cell. 2019;57:57-65.
[28] Farooq R, Lutfullah S, Ahmed M. Serum leptin levels in obese infertile men and women. Pak J Pharm Sci. 2014;27(1):67–71.
[29] Ghanayem BI, Bai R, Kissling GE, Travlos G, Hoffler U. Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. Biol Reprod. 2010;82(1):96-104.

[30] Isidori AM, Caprio M, Strollo F. Leptin and androgens in male obesity: Evidence for leptin contribution to reduced androgen levels. J Clin Endocrinol Metab. 1999;84:3673-3680.

[31] Yi X, Gao H, Chen D, Tang D, Huang W, Li T, Ma T, Chang B. Effects of obesity and exercise on testicular leptin signal transduction and testosterone biosynthesis in male mice. Am J Physiol Regul Integr Comp Physiol. 2017;312(4):R501-R510.

[32] Mintziori G, Nigdelis MP, Mathew H, Mousiolis A, Goulis DG, Mantzoros CS. The effect of excess body fat on female and male reproduction. Metabolism. 2020;107:154193.

[33] Leisegang K, Sengupta P, Agarwal A, Henkel R. Obesity and male infertility: Mechanisms and management. Andrologia. 2021;53(1):e13617.

[34] Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann Bot. 2003;91:179-194.

[35] Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int J Obes (Lond). 2006;30(3):400-418.

[36] Ye J, Luo D, Xu X, Sun M, Su X, Tian Z, Zhang M, Yu C, Guan Q. Metformin improves fertility in obese males by alleviating oxidative stress-induced blood-testis barrier damage. Oxid Med Cell Longev. 2019;10:9151067.

[37] Aslankoç R, Özmen O. The effects of high-fructose corn syrup consumption on testis physiopathology The ameliorative role of melatonin. Andrologia. 2019;51(8):e13327.

[38] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest. 2004;114(12):1752-1761.

[39] Lapperre TS, Jimenez LA, Antonicelli F. Apocynin increases glutathione 421 synthesis and activates AP-1 in alveolar epithelial cells. FEBS Lett. 1999;443:235-239.

[40] Meng R, Zhu D, Bi Y, Yang D, Wang Y. Anti-oxidative effect of apocynin on insulin resistance in high-fat diet mice. Ann Clin Lab Sci. 2011;41(3):236-243.

[41] Herishanu Y, Rogowski O, Polliack A, Marilus R. Leukocytosis in obese individuals: possible link in patients with unexplained persistent neutrophilia. Eur J Haematol. 2006;76:516–520.

[42] Wiersma JJ, Meuwese MC, van Miert JN. Diabetes mellitus type 2 is associated with higher levels of myeloperoxidase. Med Sci Monit. 2008;4(8):CR406–CR410.

[43] Ghosh S, Mukherjee S. Testicular germ cell apoptosis and sperm defects in mice upon long-term high fat diet feding. J Cell Physiol. 2018;233(10):6896–6909.

How to cite this article: Hersek İ, Köroğlu MK, Coşkunlu B, Ertaş B, Şener G, Ercan F. Apocynin Ameliorates Testicular Toxicity in High-Fat Diet-Fed Rats by Regulating Oxidative Stress. Clin Exp Health Sci 2023; 13: 75-83. DOI: 10.33808/clinexphealthsci.1035133

Clinical and Experimental Health Sciences

Oral and Dental Health Knowledge and Attitudes among Parents of Children

Hazal Deniz Köse[1], Betül Şen Yavuz[2], Betül Kargül[2]

[1] Department of Restorative Dentistry, Faculty of Dentistry, Hatay Mustafa Kemal University, Hatay, Türkiye.
[2] Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University, İstanbul, Türkiye.

Correspondence Author: Betül Şen Yavuz
E-mail: dtbetulsen@gmail.com
Received: 11.01.2022 **Accepted:** 22.09.2022

ABSTRACT

Objective: This study aims to evaluate parents' knowledge and attitudes level on oral and dental health.

Methods: One hundred and thirty parents of children aged 6-12 years, were invited to participate in the study. A 33-item questionnaire covering socio-demographic characteristics, dental attitude, dietary practices, tooth eruption, dental caries, and oral hygiene practices, was distributed to parents. The relationship between categorical variables was tested with the Chi-square test.

Results: The sample comprised of 100 parents with a mean age of 37.1 ± 6.4. The knowledge and attitudes towards dental caries and oral health habits among parents did not differ significantly according to the parents' gender, education level, or dental chair experience. There was a statistically significant difference in the responses of the first primary tooth and the first exfoliating primary tooth according to the gender of the parents (p=0.031, p=0.002, respectively). Although the education level of the parents did not affect the answers about the number of primary and permanent teeth, a statistically significant difference was found in the time of first primary tooth eruption (p=0.008).

Conclusion: The study showed that parents do not have enough knowledge and awareness of dental caries and oral healthcare. Oral healthcare should be promoted as a part of general health and the awareness of the public should be increased.

Keywords: Children, Knowledge, Oral health, Parental attitudes

1. INTRODUCTION

Many factors play a role in children's general and oral health. Parents, one of these factors, is of central importance. The perceptions of parents about oral health can affect children's access to preventive dental care and professional dentistry services (1). One of the main factors of oral health awareness in parents is the frequency of going to the dentist (2). The American Academy of Pediatric Dentistry recommends that when children are six months old, their oral health should be evaluated by qualified pediatricians or pediatric dentists (3). The oral health of preschool children is influenced by parental knowledge of oral health, cultural beliefs and baby nutrition and nutrition practices, oral hygiene habits, preventive regular dental visits, and awareness about the care of primary teeth (4). Many behavioral and socioeconomic caries risk factors have been identified; insufficient oral hygiene, lack of preventive treatments such as topical fluoride application, sugary foods/drinks consumption, long-term bottle-feeding, frequent snack consumption, low income, and low-health literacy (5). Additionally, there is also a relationship between these factors and the knowledge level of the parents about oral health (6).

Diet plays a significant role in the emergence and progression of dental caries (7,8). Frequent consumption of unhealthy snacks is associated with a variety of health problems, such as dental caries, increased obesity among humans, and other chronic diseases (9,10). Also, when dental caries occurs in childhood, the child's eating patterns, permanent tooth eruption, and general health are affected (11). In a previous study, it has been determined that parental knowledge about nutrition affects their children's eating habits (12). Public health activities should be developed to address the factors affecting the oral health of children and to provide them with good oral health and a better quality of life (13).

There is a linear relationship between knowledge, attitude, and behavior as external factors such as environmental, social, and family conditions affect human behavior (14). More research on family characteristics and parent-child

DOI: 10.33808/clinexphealthsci.1056574

relationships has been proposed to find factors that promote children's oral health behavior (15). More studies on these issues in developing countries are needed in the literature. Therefore, this study aims to assess the level of knowledge, attitude, and habits on the oral health of parents. The null hypothesis tested was that there were no differences between the knowledge level of parents about dental caries and oral health according to their gender.

2. METHODS

2.1. Study Population and Sampling Method

The study was approved by the ethical committee of Marmara University Institute of Health Science (Protocol no: 47/ date:30.05.2016). The minimum sample size for the study was calculated as 76 with G*power Version 3.1.9.6 by taking impact size 0.840, type 1 error (α) = 0.05, and power (1-β) = 0.95 at a confidence level of 95% (16). A questionnaire was conducted on the parents of children between the ages of 6-12 years who came to the Pediatric Dentistry Clinic of Marmara University between November 2016 and February 2017. Considering the possibility of low participation and low response rate, one hundred and thirty parents were invited to the study. Participation in the research was done voluntarily and the study was performed according to the Declaration of Helsinki (2013). A total of 107 parents agreed to participate in the study. Verbal and written informed consents were obtained from all parents who were included in the study. Seven questionnaires that were not completed were excluded from the study. However, a post hoc power analysis to justify the chosen sample size at least partly was conducted. When the post hoc analysis was examined by taking 62 cases in the mother group and 38 cases in the father group, the power of the test (1-β) was obtained as 98.1% with 95% confidence (1-α) (16).

2.2. The Questionnaire

The questionnaires about oral health knowledge in Turkish were provided to the parents who agreed to participate in the study. The questionnaire questions were composed by reviewing previous studies (1,2,4,5,17-20). The 33-item questionnaire was comprised of six parts. Part 1 consisted of items that pertained to general demographics, such as the age and gender of parents and child's educational backgrounds. Part 2 included questions about dental attitude and behavior, such as the dental care experience and dental fear. Part 3 included questions about the effects of food on teeth, such as the consumption of carbohydrates. Part 4 included questions about tooth eruption, such as the number of teeth and eruption and exfoliating time, Parts 5 and 6 included questions about dental caries and oral health habits, such as the consequences of caries or frequency of tooth brushing.

2.3. Statistical Analysis

Descriptive statistics were given as a number (%) for categorical variables. The relationship between categorical variables was tested with the Chi-square test. The data were analyzed using SPSS (Statistical Package for the Social Sciences) version 22.0. The significance level was assumed at 0.05.

3. RESULTS

The mean age of 100 parents between the ages of 22-55 who participated in the survey was 37.1 ± 6.4 (mean ± standard deviation) years. It was determined that 62 of the parents in the study were mothers, 38 of them were fathers, and the children of them, 47% (n=47) were boys and 53% (n=53) were girls. The age of children was between 6-12 years and their mean age was 7.2 ± 2.5 years. When the educational status of the parents was evaluated, elementary school graduates were 34%, middle school 31%, high school 24%, and university 11%.

The dental attitude and behavior of the parents and their children were summarized in Table 1. According to the data, 55% (n=11) of the parents with no dental care experience (n=20) took their children to the dentist before the study whereas the ratio was 88.8% (n=71) for those with dental care experience (n=80). Most of the mothers and fathers stated that they had gone to the dentist before. The relationship between the answer of parents to the question of whether you went to the dentist and the answer they gave for their children was found to be statistically significant according to fisher's exact test (p=0.001). The majority of parents were not afraid of the dentist. The children of 38.7% (n=24) of the parents without the fear of dentist were afraid of the dentist. The relationship between dental fear of parents and their children was found to be statistically significant according to the chi-square test (p<0.001). The answers about 'when the child's first dental visit should be' showed that 10% of parents had no idea, and 62.2% of those who expressed an opinion also did not know the appropriate time.

The responses of participants regarding the effects of foods on teeth were shown in Table 2. While presenting the data, the 'not sure' option was also considered as an incorrect answer. Besides, when asked which carbohydrates, proteins, fats, and vitamins are beneficial for teeth, 88% stated that proteins and 32% stated that vitamins were beneficial, while 5% stated that carbohydrates were beneficial (Table 3).

Parental knowledge and awareness of the number and eruption of the teeth were shown in Table 4. Parental knowledge and awareness regarding dental caries and oral health habits were shown in Table 5. The parent's fear of dental chairs did not affect their knowledge level of dental caries, and oral health habits at all. Also, there seems no significant effect of the parent's gender, education level, or dental chair experience on their knowledge of dental caries and oral health habits. The answers about the first erupted primary tooth and the first exfoliating primary tooth differ

statistically according to the gender of the parents (p=0.031, p=0.002, respectively). While the education level of the parents did not affect the answers about the number of primary and permanent teeth (p=0.12, p=0.45 respectively), it made a statistically significant difference in the time of first primary tooth eruption (p=0.008). However, in the question about the cause of dental caries, 75% of the parents think that foods and drinks cause dental caries, 4% of them think that all factors were the cause (Table 3). Besides, the parents were asked to indicate their preferred products for oral care. However, no one has chosen all the oral care products. The most preferred products were toothbrush with 71%, toothpaste with 36%, and dental floss with 30%.

Table 1. *Dental attitude and behavior of the parents and their children*

	Mother n (%)	Father n (%)	Total (n=100) (%)
Have you been to the dentist before?			
Yes	51 (82.2%)	29 (76.3%)	80%
No	11 (17.7%)	9 (23.7%)	20%
Did your child go to the dentist before?			
Yes	49 (79%)	33 (86.8%)	82%
No	13 (21%)	5 (13.2%)	18%
Are you afraid of dental treatment?			
Yes	24 (38.7%)	14 (36.8%)	38%
No	38 (61.3%)	24 (63.2%)	62%
Is your child afraid of dental treatment?			
Yes	33 (53.2%)	20 (52.6%)	53%
No	29 (46.8%)	18 (47.4%)	47%
What is the reason for your child's visit to the dentist?			
Caries	21 (33.9%)	17 (44.7%)	38%
Toothache	13 (21%)	13 (34.2%)	26%
Control	18 (29%)	6 (15.8%)	24%
Halitosis	8 (12.9%)	2 (5.3%)	10%
Primary teeth exfoliation	2 (3.2%)	0	2%
Gingival bleeding	0	0	0
When should you take your child to the dentist for the first check-up?			
First primary tooth eruption	25 (40.3%)	9 (23.7%)	34%
First permanent tooth eruption	16 (25.8%)	10 (26.3%)	26%
Tooth problem	12 (19.4%)	12 (31.6%)	24%
At birth	3 (4.8%)	3 (7.9%)	6%
Don't know	6 (9.7%)	4 (10.6%)	10%

Table 2. *Parental knowledge and awareness of the effect of foods on teeth*

	Mother n (%)	Father n (%)	Total (n=100) (%)
How many times a day does your child eat?			
Less than 3	23 (37.1%)	15 (39.5%)	38%
3 and more than 3	39 (62.9%)	23 (60.5%)	62%
How often does your child consume milk, yogurt, and cheese?			
Never	2 (3.2%)	4 (10.5%)	6%
Rarely	7 (11.3%)	5 (13.2%)	12%
Every week	2 (3.2%)	4 (10.5%)	6%
Everyday	36 (58.1%)	24 (63.2%)	60%
Several times a day	15 (24.2%)	1 (2.6%)	16%
How often does your child consume fruit juice and carbonated drinks?			
Never	6 (9.7%)	3 (7.9%)	9%
Rarely	33 (53.2%)	15 (39.5%)	48%
Every week	6 (9.7%)	6 (15.8%)	12%
Everyday	15 (24.2%)	10 (26.3%)	25%
Several times a day	2 (3.2%)	4 (10.5%)	6%
How often does your child consume sugar, sweets, and chocolate?			
Never	0	0	0
Rarely	12 (19.4%)	8 (21.1%)	20%
Every week	17 (27.4%)	10 (26.3%)	27%
Everyday	27 (43.5%)	16 (42.1%)	43%
Several times a day	6 (9.7%)	4 (10.5%)	10%
Does eating between meals increase the risk of caries?			
Yes	42 (67.7%)	21 (55.3%)	63%
No	20 (32.3%)	17 (44.7%)	37%

Table 3. *Multiple answer questions regarding the effect of foods on teeth, dental caries, and oral health habits*

	Total (n=100) (%)
Which food group is beneficial for teeth?†	
Fats	0
Carbohydrates	5%
Vitamins	32%
Proteins	88%
What is the cause of dental caries?†	
Plaque and tartar	11%
Genetic	33%
Bacteria and virus	50%
Food and drinks	75%
Which products do you prefer for oral care?†	
Mouthwash	18%
Toothpick	23%
Dental floss	30%
Toothpaste	36%
Toothbrush	71%

†: *Multiple answer questions*

Table 4. *Parental knowledge and awareness of the number and eruption of the teeth*

	The gender of parents			The educational status of parents					The parents' previous visit to the dentist			Parent's fear of dentist		
	Female n (%)	Male n (%)	p †	Elementary s. n (%)	Middle s. n (%)	High s. n (%)	University n (%)	p†	Yes n (%)	No n (%)	p†	Present n (%)	Absent n (%)	p†
Tooth eruption														
The number of primary teeth														
Correct	16 (25.8)	10 (26.3) (26.3)	0.955	6 (17.6)	6 (19.4)	9 (37.5)	5 (45.5)	0.124	23 (28.8)	3 (15)	0.210	8 (21.1)	18 (29)	0.377
Incorrect	46 (74.2)	28 (73.7)		28 (82.4)	25 (80.6)	15 (62.5)	6 (54.5)		57 (71.3)	17 (85)		30 (78.9)	44 (71)	
The number of permanent teeth														
Correct	21 (33.9)	18 (47.4)	0.179	10 (29.4)	13 (41.9)	10 (41.7)	6 (54.5)	0.455	33 (41.3)	6 (30)	0.356	12 (31.6)	27 (43.5)	0.234
Incorrect	41 (66.1)	20 (52.6)		24 (70.6)	18 (58.1)	14 (58.3)	5 (45.5)		47(58.8)	14 (70)		26 (68.4)	35 (56.5)	
Time of first primary tooth eruption														
Correct	26 (41.9)	14 (36.8)	0.614	17 (50)	6 (19.4)	9 (37.5)	8 (72.7)	**0.008**	30 (37.5)	10 (50)	0.307	13 (34.2)	27 (43.5)	0.355
Incorrect	36 (58.1)	24 (63.2)		17 (50)	25 (80.6)	15 (62.5)	3 (27.3)		50 (62.5)	10 (50)		25 (65.8)	35 (56.5)	
Time of first permanent tooth eruption														
Correct	15 (24.2)	7 (18.4)	0.499	6 (17.6)	7 (22.6)	5 (20.8)	4 (36.4)	0.632	17 (21.3)	5 (25)	0.720	9 (23.7)	13 (21)	0.750
Incorrect	47 875.8)	31 (81.6)		28 (82.4)	24 (77.4)	19 (79.2)	7 (63.6)		63 (78.8)	15 (75)		29 (76.3)	49 (79)	
First primary teeth														
Correct	35 (56.5)	13 (34.2)	**0.031**	20 (58.8)	14 (45.2)	9 (37.5)	5 (45.5)	0.426	42 (52.5)	6 (30)	0.072	15 (39.5)	33 (53.2)	0.182
Incorrect	27 (43.5)	25 (65.8)		14 (41.2)	17 (54.8)	15 (62.5)	6 (54.5)		38 (47.5)	14 (70)		23 (60.5)	29 (46.8)	
First permanent teeth														
Correct	7 (11.3)	1 (2.6)	0.095	1 (2.9)	4 (12.9)	3 (12.5)	0	0.187	8 (10)	0	0.053	2 (5.3)	6 (9.7)	0.417
Incorrect	55 (88.7)	37 (97.4)		33 (97.1)	27 (87.1)	21 (87.5)	11 (100)		72 (90)	20 (100)		36 (94.7)	56 (90.3)	
First exfoliation of primary tooth														
Correct	36 (58.1)	10 (26.3)	**0.002**	17 (50)	16 (51.6)	8 (33.3)	5 (45.5)	0.539	38 (47.5)	8 (40)	0.547	12 (31.6)	34 (54.8)	**0.023**
Incorrect	26 (41.9)	28 (73.7)		17 (50)	15 (48.4)	16 (66.7)	6 (54.5)		42 (52.5)	12 (60)		26 (68.4)	28 (45.2)	

†: Chi-square test, s: school, Bold font: p<0.05

Table 5. *Parental knowledge and awareness regarding dental caries and oral health habits*

	The gender of parents			The educational status of parents					The parents' previous visit to the dentist			Parent's fear of dentist		
	Female n (%)	Male n (%)	p†	Elementary s. n (%)	Middle s. n (%)	High s. n (%)	University n (%)	p†	Yes n (%)	No n (%)	p†	Present n (%)	Absent n (%)	p†
Dental caries														
Is caries a contagious disease?														
Correct	43 (69.4)	25 (65.8)	0.711	20 (58.8)	22 (71)	17 (70.8)	9 (81.8)	0.476	54 (67.5)	14 (70)	0.830	28 (73.7)	40 (64.5)	0.340
Incorrect	19 (30.6)	13 (34.2)		14 (41.2)	9 (29)	7 (29.2)	2 (18.2)		26 (32.5)	6 (30)		10 (26.3)	22 (35.5)	
Can dental caries cause other diseases?														
Correct	45 (72.6)	31 (81.6)	0.306	25 (73.5)	23 (74.2)	19 (79.2)	9 (81.8)	0.918	61 (76.3)	15 (75)	0.907	28 (73.7)	48 (77.4)	0.671
Incorrect	17 (27.4)	7 (18.4)		9 (26.5)	8 (25.8)	5 (20.8)	2 (18.2)		19 (23.8)	5 (25)		10 (26.3)	14 (22.6)	
Does caries present in the primary tooth affect the permanent teeth?														
Correct	36 (58.1)	20 (52.6)	0.595	13 (38.2)	19 (61.3)	17 (70.8)	7 (63.6)	0.068	41 (51.3)	15 (75)	0.056	25 (65.8)	31 (50)	0.123
Incorrect	26 (41.9)	18 (47.4)		21 (61.8)	12 (38.7)	7 (29.2)	4 (36.4)		39 (48.8)	5 (25)		13 (34.2)	31 (50)	
Should they be treated when decayed primary teeth?														
Correct	52 (83.9)	33 (86.8)	0.686	28 (82.4)	24 (77.4)	23 (95.8)	10 (90.9)	0.193	70 (87.5)	15 (75)	0.184	31 (81.6)	54 (87.1)	0.453
Incorrect	10 (16.1)	5 (13.2)		6 (17.6)	7 (22.6)	1 (4.2)	1 (9.1)		10 (12.5)	5 (25)		7 (18.4)	8 (12.9)	
Oral health habits														
How often do toothbrushes need to be changed?														
Correct	23 (37.1)	12 (31.6)	0.574	10 (29.4)	7 (22.6)	12 (50)	6 (54.5)	0.079	27 (33.8)	8 (40)	0.600	14 (36.8)	21 (33.9)	0.762
Incorrect	39 (62.9)	26 (68.4)		24 (70.6)	24 (77.4)	12 (50)	5 (45.5)		53 (66.3)	12 (60)		24 (63.2)	41 (66.1)	
Is brushing teeth sufficient to prevent caries?														
Correct	20 (32.3)	9 (23.7	0.359	10 (29.4)	11 (35.5)	4 (16.7)	4 (36.4)	0.440	21 (26.3)	8 (40)	0.225	14 (36.8)	15 (24.2)	0.176
Incorrect	42 (67.7)	29 (76.3)		24 (70.6)	20 (64.5)	20 (83.3)	7 (63.6)		59 (73.8)	12 (60)		24 (63.2)	47 (75.8)	
From what age should teeth be brushed?														
Correct	29 (46.8)	20 (52.6)	0.570	11 (32.4)	16 (51.6)	14 (58.3)	8 (72.7)	0.067	41 (51.3)	8 (40)	0.368	17 (44.7)	32 (51.6)	0.504
Incorrect	33 (53.2)	18 (47.4)		23 (67.6)	15 (48.4)	10 (41.7)	3 (27.3)		39 (48.8)	12 (60)		21 (55.3)	30 (48.4)	

†: Chi-square test, s: school, Bold font: p<0.05

4. DISCUSSION

Oral health behaviors that begin in childhood affect adulthood. Considering the effects of parents on children, it is important to investigate their attitudes on the factors affecting the oral health of their children. In order to spread the awareness of oral and dental health for the preventive practices and treatments, the parents and dentists are answerable for the patient-dentist relationship. However, more studies are needed to examine these relationships (21). Therefore, this study provides important data on parental knowledge of their children's oral health.

In the study of Sakai et al.(22), 58% of parents and 41% of children had never been to a dentist. In the current study, this parent's rate was found to be 20% and the children's rate was found 18%. However, the fact that 20% of parents and 18% of children never went to a dentist is thought-provoking in terms of public health. Ivica and Galic (23) stated that although the perception of oral health is high, the perception of oral diseases is quite low. This results in fewer dentist visits.

Dental anxiety is a common condition, and this situation may occur due to many factors. Previous studies have reported that there were relationships between parents and their children's dental anxiety (24-26). This was in line with this study. Dental fear causes dental treatments to be delayed, which requires more invasive treatments such as endodontic treatments and tooth extractions (27).

In the study, while most of the parents thought that proteins are useful, no parent thought carbohydrates, proteins, vitamins, and fats were all beneficial. Regarding the frequency of sugar consumption, it was observed that most children ate sweets every week or every day and 10% of children consumed these foods several times a day. Half of the participants who thought that snacks increased the risk of cavities stated that their children often consume snacks such as candy and chocolate. According to Saied-Moallemi et al.(28), most mothers (79-84%) realized the detrimental effect of sweet foods on teeth. Furthermore, almost half of children frequently consumed carbonated drinks and fruit juice. These drinks have a high content of simple carbohydrates and acids, so their frequent consumption should be considered as a risk factor for dental caries and erosion (29).

Parents need to know the factors that cause dental caries and the eruption time of teeth to maintain their children's oral health. The first visit to the pedodontics clinic should be done within six months after the eruption of the first primary tooth for communication and building up a trust (3,30). In this study, the answers to the question 'When should you take your child to the dentist for the first check-up?' were consistent with the previous study (31). The first erupted primary tooth is the lower central incisor (32). In this study, the responses given to question about the first primary tooth differed significantly according to the gender of the parents. Also, the responses to the first exfoliating primary tooth differed significantly according to the gender of the parents. Besides, the education level of the parents made a significant difference in the time of the first primary tooth eruption. After the lower central incisor, the lower lateral incisors and upper central incisors begin to erupt. When the child is approximately 5-6 years old, the lower central primary incisor begins to loosen. Under normal circumstances, the first permanent tooth that is molar begins to erupt around the age of six. Usually, 20 primary teeth are completed at the age of 2.5-3 and 28 permanent teeth except wisdom teeth are completed approximately at 12-13 ages (32). In the study of Jain et al. (31), 29% of the parents did not know the total number of primary teeth, 36% gave the correct answer. In this study, 74% of the participants did not know the total number of primary teeth. However, the rate of participants who know the total number of permanent teeth was higher. Only eight participants were aware of the eruption times of both primary teeth and permanent teeth. Additionally, eight participants knew both the first erupted primary tooth and the first permanent tooth. Almost half of the parents gave the correct answer to the first primary tooth question.

Oral health problems change metabolic habits such as eating, and sleeping patterns, which can cause impairment of the general health (33). In the present study, 76% of participants thought that dental caries would cause another disease. This rate was lower than the study of Wyne et al. (34) Moreover, dental caries is associated with an increased risk of serious diseases such as cardiovascular and respiratory diseases. Therefore, everyone should know the causes of dental caries and take precautions (35). The answer of the parents regarding the cause of dental caries revealed a lack of knowledge on this subject. The number of participants who knew all the factors causing caries was very low. In a study published in 2015, 19% of the participants knew all factors (36). While 18% of the participants thought that caries was not contagious and would not cause other diseases, 26% supported the opposite of this view. In the study, it was observed that parents had a lack of knowledge about dental caries. However, caries is currently defined as a dynamic, non-communicable disease in the light of current knowledge (37, 38) and 68% of parents knew this. In the study of Saied-Moallemi et al. (28), approximately 75% of mothers answered those primary teeth as important. The rate was lower in other studies (1,31). In the current study, most participants stated that primary teeth should be treated if necessary.

After the first tooth erupted, tooth brushing should begin. In the study of Jain et al., the rate of 'after first primary tooth eruption' answer was 21% (31). In another study, this rate was almost all of the participants (39). This rate of the answer in this study was only half. The incorrect answer of half of the participants may be a serious oral health concern. Most of those who use 'only' toothbrushes stated that they know the fact that toothbrush is not enough to maintain oral health. In the study of Jain et al. (31), the percentage of parents (52%) who knew that it was necessary to change the toothbrush every three months was higher than in this study (35%). The responses of parents to dental caries and oral health habits did not show a statistically significant difference according to gender, education level, dental chair fear, or previous

visit to the dentist of parents. Therefore, the null hypothesis of the study was accepted. While there are a few studies investigating the oral and dental health knowledge and attitudes of mothers in the literature, the data on fathers are very limited. Manohar et al. (40) reported that the knowledge level of the fathers was significantly lower than that of the mothers, but the level of attitude did not differ between the genders of the parents. In a study evaluating the knowledge levels of mothers, half of the mothers stated that dental caries affected general health, but caries of primary teeth did not affect permanent teeth. In this study, both mothers and fathers gave correct answers to these questions at a higher rate (4).

The study had some limitations. One limitation was the small sample size. One of the limitations was the low sociodemographic diversity since the study was conducted in a single hospital. The rate of university graduate parents in the study was only 11%. Another limitation is the relatively high average age of the children and the wide age range. More studies are needed to better understand the relationship between parents' questionnaire responses and their children's ages.

To date, and to the best of our knowledge, the education level of parents, dental chair experience, eruption times of teeth, eating habits, and oral health habits have not all been presented in a single study. Further studies are needed to assess the association of studied variables with actual parameters of oral health in children. According to the results of the studies conducted in this field, the importance of general health is explained clearly with various programs to be used based on the variables affecting the oral and dental health parameters, and the public is made aware of this issue, the success rates of the methods used in caries prevention may increase. By repeating training could give more effective results in increasing the continuity of knowledge and improving the attitudes of future generations about dental health. The hanging of information posters in pediatric dentistry clinics or/and information brochures for children to take to their parents in the fluor varnish application programs carried out by the Ministry of Health in schools can be given as examples of these strategies. In addition, as another suggestion, dentistry can be included in primary healthcare services. Thus, considering the population of the country, individual meetings with parents can provide complementary information to parents on issues they lack, thanks to the family dentistry practice.

5. CONCLUSION

The result of the study showed that the parents did not have enough knowledge and awareness about dental caries and oral health. It has been understood that informative training on the necessity of oral and dental healthcare, tooth eruption, dental caries, and healthy foods should be expanded.

REFERENCES

[1] Prabhu A, Rao AP, Reddy V, Ahamed SS, Muhammad S, Thayumanavan S. Parental knowledge of pre-school child oral health. J Community Health. 2013;38(5):880-884. DOI:10.1007/s10900.013.9693-x

[2] Gurunathan D, Moses J, Arunachalam SK. Knowledge, attitude, and practice of mothers regarding oral hygiene of primary school children in Chennai, Tamil Nadu, India. Int J Clin Pediatr Dent. 2018;11(4):338. DOI:10.5005/jp-journals-10005-1535

[3] American Academy of Pediatric Dentistry, Clinical Affairs Committee, Infant Oral Health Subcommittee. Guideline on infant oral health care. Pediatr Dent. 2012;34(5):e148-152.

[4] Chhabra N, Chhabra A. Parental knowledge, attitudes and cultural beliefs regarding oral health and dental care of preschool. Eur Arch Paediatr Dent. 2012;13(2):76-82. DOI:10.1007/BF03262848

[5] Tsang C, Sokal-Gutierrez K, Patel P, Lewis B, Huang D, Ronsin K, Baral A, Bhatta A, Khadka N, Gurung S. Early childhood oral health and nutrition in urban and rural Nepal. Int J Environ Res Public Health. 2019;16(14):2456. DOI:10.3390/ijerph16142456

[6] Dagon N, Ratson T, Peretz B, Blumer S. Maternal knowledge of oral health of children aged 1–4 years. J Clin Pediatr Dent. 2019;43(2):116-120. DOI:10.17796/1053-4625-43.2.8

[7] Garg N, Garg A. Dental caries. Ravi Kapur, editor. Textbook of Operative Dentistry. New Delhi: Jaypee Brothers, Medical Publishers Ltd; 2010.p.40-79.

[8] Kavitha D, Varghese A, Prabath S, Sreeram R, Asha J, Ravi AB. Prevalence of dental caries in adult South Indian population in association with dietary pattern: a comparative study. J Pharm Bioallied Sci. 2020;12(5):546. DOI:10.4103/jpbs.JPBS_155_20

[9] Ma X. Assessing school effects on dental hygiene and nutrition behaviors of Canadian adolescents. Educ Rev. 2007;59(1):37-54. DOI:10.1080/001.319.10600796876

[10] Scardina G, Messina P. Good oral health and diet. J Biomed Biotechnol. 2012;2012. DOI:10.1155/2012/720692

[11] Du MQ, Li Z, Jiang H, Wang X, Feng XP, Hu DY, Lin HC, Wang B, Si Y, Wang CX, Zheng SG, Liu XN, Rong WS, Wang WJ, Tai BJ. Dental caries status and its associated factors among 3-to 5-year-old children in China: a national survey. Chin J Dent Res. 2018;21(3):167-179. 167-79. DOI:10.3290/j.cjdr.a41076

[12] Scaglioni S, De Cosmi V, Ciappolino V, Parazzini F, Brambilla P, Agostoni C. Factors influencing children's eating behaviours. Nutrients 2018;10(6):706. DOI:10.3390/nu10060706

[13] De Castilho ARF, Mialhe FL, de Souza Barbosa T, Puppin-Rontani R. Influence of family environment on children's oral health: a systematic review. J Pediatr (Rio J). 2013;89(2):116-123. DOI:10.1016/j.jped.2013.03.014

[14] Cirakoglu N, Gokcek M. The impact of socioeconomic factors and oral health behaviour on knowledge levels of the dental health and procedures: the questionnaire based research. Clin Exp Health Sci. 2021;11:105-112. DOI:10.33808/clinexphealthsci.789597

[15] Christensen P. The health-promoting family: a conceptual framework for future research. Soc Sci Med. 2004;59(2):377-387. DOI:10.1016/j.socscimed.2003.10.021

[16] Naidu R, Nunn J, Irwin JD. The effect of motivational interviewing on oral healthcare knowledge, attitudes and behaviour of parents and caregivers of preschool children: an

exploratory cluster randomised controlled study. BMC Oral Health. 2015;15(1):1-5. DOI: 10.1186/s12903.015.0068-9

[17] Al-Omiri MK, Al-Wahadni AM, Saeed KN. Oral health attitudes, knowledge, and behavior among school children in North Jordan. J Dent Educ. 2006;70(2):179-187.

[18] Mesgarzadeh AH, Shahamfar M, Hefzollesan A. Evaluating knowledge and attitudes of elementary school teachers on emergency management of traumatic dental injuries: a study in an Iranian urban area. Oral Health Prev Dent. 2009;7(3):297-308.

[19] Vishwanathaiah S. Knowledge, attitudes, and oral health practices of school children in Davangere. Int J Clin Pediatr Dent. 2016 Apr;9(2):172. DOI:10.5005/jp-journals-10005-1358

[20] Ashkanani F, Al-Sane M. Knowledge, attitudes and practices of caregivers in relation to oral health of preschool children. Med Princ Pract. 2013;22(2):167-172. DOI: doi.org/10.1159/000341764

[21] Blumer S, Ratson T, Peretz B, Dagon N. Parents' attitude towards the use of fluorides and fissure sealants and its effect on their children's oral health. J Clin Pediatr Dent. 2018;42(1):6-10. DOI:10.17796/1053-4628-42.1.2

[22] Sakai VT, Oliveira TM, Silva TC, Moretti ABS, Geller-Palti D, Biella VA, Machado MAMM. Knowledge and attitude of parents or caretakers regarding transmissibility of caries disease. J Appl Oral Sci. 2008;16(2):150-154. DOI:10.1590/S1678.775.7200800.020.0013

[23] Ivica A, Galić N. Attitude towards oral health at various colleges of the University of Zagreb: a pilot study. Acta Stomatol Croat. 2014;48(2):140-146. DOI:10.15644/asc48/2.140

[24] Assuncao CM, Losso EM, Andreatini R, de Menezes JVNB. The relationship between dental anxiety in children, adolescents and their parents at dental environment. J Indian Soc Pedod Prev Dent. 2013;31(3):175-179. DOI:10.4103/0970-4388.117977

[25] Peretz B, Nazarian Y, Bimstein E. Dental anxiety in a students' paediatric dental clinic: children, parents and students. Int J Paediatr Dent. 2004;14(3):192-198. DOI: 10.1111/j.1365-263X.2004.00545.x

[26] Tickle M, Jones C, Buchannan K, Milsom KM, Blinkhorn AS, Humphris GM. A prospective study of dental anxiety in a cohort of children followed from 5 to 9 years of age. Int J Paediatr Dent. 2009;19(4):225-232. DOI:10.1111/j.1365-263X.2009.00976.x

[27] Armfield JM, Stewart JF, Spencer AJ. The vicious cycle of dental fear: exploring the interplay between oral health, service utilization and dental fear. BMC Oral Health. 2007;7(1):1. DOI: 10.1186/1472-6831-7-1

[28] Saied-Moallemi Z, Virtanen J, Ghofranipour F, Murtomaa H. Influence of mothers' oral health knowledge and attitudes on their children's dental health. Eur Arch Paediatr Dent. 2008;9(2):79-83. DOI:10.1007/BF03262614

[29] Søvik JB, Skudutyte-Rysstad R, Tveit AB, Sandvik L, Mulic A. Sour sweets and acidic beverage consumption are risk indicators for dental erosion. Caries Res. 2015;49(3):243-250. DOI:10.1159/000371896

[30] Mileva SP, Kondeva VK. Age at and reasons for the first dental visit. Folia Medica. 2010;52(4):56-61. DOI:10.2478/v10153.010.0018-x

[31] Jain R, Oswal KC, Chitguppi R. Knowledge, attitude and practices of mothers toward their children's oral health: a questionnaire survey among subpopulation in Mumbai (India). J Dent Res Sci Develop. 2014;1(2):40-45. DOI:10.4103/2348-3407.135073

[32] Dean JA. Growth of the face and dental arches. Ferguson DJ, Dean JA, editors. McDonald and Avery's Dentistry for the Child and Adolescent. Philadelphia: Mosby Elsevier; 2015.p.349-374.

[33] Sheiham A. Oral health, general health and quality of life. Bull World Health Organ. 2005;83(9):644.

[34] Wyne AH, Chohan AN, Al-Rowily FH, Mubarkal-Shehri B. Oral health knowledge, attitude and practices by parents of the children attending KSUCD clinics. Pak Oral Dent J. 2004;24(2):145-148.

[35] Yadav K, Prakash S. Dental caries: a microbiological approach. J Clin Infect Dis Pract. 2017;2(1):1-15. DOI:10.4172/2476-213X.1000118

[36] Murshid EZ. Dental knowledge of educators and healthcare providers working with children with autism spectrum disorders. Saudi Med J. 2015;36(12):1477-1485. DOI:10.15537/smj.2015.12.12622

[37] Splieth CH, Banerjee A, Bottenberg P, Breschi L, Campus G, Ekstrand KR, Giacaman RA, Haak R, Hannig M, Hickel R, Juric H. How to intervene in the caries process in children: A Joint ORCA and EFCD Expert Delphi Consensus Statement. Caries Res. 2020;54(4):297-305. DOI:10.1159/000507692

[38] Machiulskiene V, Campus G, Carvalho JC, Dige I, Ekstrand KR, Jablonski-Momeni A, Maltz M, Manton DJ, Martignon S, Martinez-Mier EA, Pitts NB. Terminology of dental caries and dental caries management: consensus report of a workshop organized by ORCA and Cariology Research Group of IADR. Caries Res. 2020;54(1):7-14. DOI:10.1159/000503309

[39] Gussy MG, Waters E, Riggs E, Lo SK, Kilpatrick N. Parental knowledge, beliefs and behaviors for oral health of toddlers residing in rural Victoria. Aust Dent J. 2008;53(1):52-60. DOI:10.1111/j.1834-7819.2007.00010.x

[40] Ashkanani F, Al-Sane M. Knowledge, attitudes and practices of caregivers in relation to oral health of preschool children. Med Princ Pract. 2013;22(2):167-172. DOI:10.1159/000341764

How to cite this article: Köse HD, Şen Yavuz B, Kargül B. Oral and Dental Health Knowledge and Attitudes among Parents of Children. Clin Exp Health Sci 2023; 13: 84-91. DOI: 10.33808/clinexphealthsci.1056574

Clinical and Experimental Health Sciences

The Effects of Office Bleaching Techniques on Nanoceramic Composite Resin

Esin Ülper[1], Cafer Türkmen[1], Zühre Hale Cimilli[2]

[1] Department of Restorative Dentistry, Faculty of Dentistry, Marmara University, İstanbul, Türkiye.
[2] Department of Endodontics, Faculty of Dentistry, Marmara University, İstanbul, Türkiye.

Correspondence Author: Esin Ülper
E-mail: esinulper@hotmail.com
Received: 14.01.2022 **Accepted:** 14.09.2022

ABSTRACT

Objective: The aim was to evaluate the effect of activated bleaching agents on the nanoceramic composite resins based on microhardness and surface roughness measurements.

Methods: Opalescence Boost (Ultradent Products, USA) and Whiteness HP Blue Calcium (FGM Dental Products, Brazil) bleaching agents and a nanoceramic composite resin Ceram.X® SphereTEC™ One (DENTSPLY, Germany) were used in the study. Fifty composite samples in disc form (8x2mm) were prepared. The samples were polished with discs (Sof-Lex, 3M Dental Products, St.Paul, USA) and incubated in the dark in distilled water. Samples were divided into 5 main groups (n=10). Control group (C), Opalescence Boost/ without light (OB), Opalescence Boost/ with light (OBL), Whiteness HP Blue/ without light (WB), and Whiteness HP Blue/ with light (WBL). Surface roughness (Ra) and Vickers microhardness (VHN) measurements were conducted after the bleaching process. Statistical analyses were performed using the Kruskal Wallis – H test and the Mann Whitney – U test; a p value of < 0.05 was considered statistically significant.

Result: The VHN was significantly different among groups in terms of the application of bleaching agents and light (p = 0.008). The Ra was not significantly different among groups with respect to the application of bleaching agents or lighting conditions (p = 0.144).

Conclusion: Within the conditions of this study, after bleaching procedure the microhardness values of the nanoceramic composite increased, however bleaching did not show any effect on surface roughness.

Keywords: Bleaching, composite resin, microhardness, surface roughness.

1. INTRODUCTION

Nowadays aesthetic dentistry has become more popular and patients often asked to their dentist for whiter and smoother teeth. The most preferred office bleaching agent in clinical use is hydrogen peroxide (HP) in a gel form at 25–40% concentration (1, 2). HP application may cause discoloration because of changes in surface roughness on restorative materials. The HP oxidation reaction accelerates the hydrolytic degradation of polymer chains in the resin matrix, thus changing the surface properties of restorative materials (3). The bleaching effects are evaluated in terms of the surface roughness, microhardness, and color change.

The effects of bleaching agents on composite resins depend on the resin matrix, filler content, bleaching gel and application time of the gel (4).

Ceram.X SphereTEC One used in the present study, contains inorganic fillers that consists of barium, aluminum, borosilicate glass, and ytterbium fluoride. In general, the total amount of inorganic filler is 72–73% by weight and 48–50% by volume; it contains structurally modified ceramic nanoparticles and nanofillers combined with approximately 1 µm standard glass fillers. Hybrid composite filling technology and nanotechnology are used to manufacture the nanoceramics for aesthetic dentistry applications.

The use of light sources has become popular in office bleaching processes. HP can be activated with or without a light source (5,6). In the past halogen lamps, plasma arc and ultraviolet light were used as light sources to activate the bleaching process, however in recent years light-emitting diodes (LEDs) and diode lasers are commonly used (7,8).

There have been inconsistent results regarding the effects of bleaching on the surface roughness and microhardness of composite resins (4,9-16). Thus, the purpose of our study

DOI: 10.33808/clinexphealthsci.1057225

was to analyses the effects of activated bleaching agents on the physical properties of nanoceramic composite resins, with or without a light source, based on SEM images, as well as microhardness and surface roughness measurements.

The hypothesis of the study was,

1. There is no significant difference in microhardness values of nanoceramic composite resin with/without light bleaching techniques.

2. There is no significant difference in surface roughness values of nanoceramic composite resin with/without light bleaching techniques.

2. METHODS

Two bleaching agents were used on the nanoceramic composite resin in this study: Opalescence Boost (Ultradent Products, Inc. South Jordan, Utah, USA) containing 40% HP and Whiteness HP Blue (FGM Dental Products, Joinville, SC, Brazil) containing 35% HP. The materials used in the study are listed in Table 1.

Table 1. *Compositions and manufacturing details of the tested composite resins and bleaching agents*

Material	Type	Content	Producer
Ceram.X SphereTEC One	Nanoceramic composite	Matrix: Polyurethane methacrylate, Bis-EMA*, TEGDMA* Fillers: Prepolymerized spherical fillers (15 µm), 0.6-µm ytterbium fluoride, 0.6-µm barium glass filler and silicon dioxide nanofillers (10 nm); 77–79% by weight and 59–61% by volume	Dentsply DeTrey GmbH, Konstanz, Germany
Opalescence Boost PF	Office-type vital bleaching agent	Water, Carbopol, propylene glycol, glycerin, 40% HP*, potassium hydroxide, 1.1% sodium fluoride, 3% potassium nitrate	Ultradent, South Jordan, UT, USA
Whiteness HP BLUE	Office-type vital bleaching agent	Active ingredients: 20% or 35% HP (after mixing of the phases) Inactive ingredients: thickeners, inert violet pigment (35% HP blue) or inert blue pigment (20% HP blue), glycol, calcium gluconate, neutralizing agent and deionized water	FGM Dental Products, Joinville SC, Brazil

**HP: Hydrogen Peroxide, Bis-EMA: Bisfenol A Etoksile Dimetakrilat, TEGDMA: Trietilen Glikol Dimetakrilat*

2.1. Preparation of the Composite Resin Samples

Fifty disc-shape composite samples (8x2mm) were prepared using pleximolds. After the restorative materials were placed in the molds microscopic lam were applied on the materials, pressed with it and then polymerized with a Demi Ultra LED (Demi™ Ultra, Kerr, USA) light device for 20sec (Figure 1). The top surfaces of the samples were polished with medium, fine and super fine grained discs (Sof Lex, 3M ESPE, St. Paul, MN, USA). All samples were incubated in distilled water at 37°C for 24 h in the dark. Composite resin samples were then randomly divided into 5 main groups (n = 10).

Group 1 – Control group (C)

Group 2 – Opalescence Boost/ without light (OB)

Group 3 – Opalescence Boost/ with light (OBL)

Group 4 – Whiteness HP Blue/ without light (WB)

Group 5 – Whiteness HP Blue/ with light (WBL)

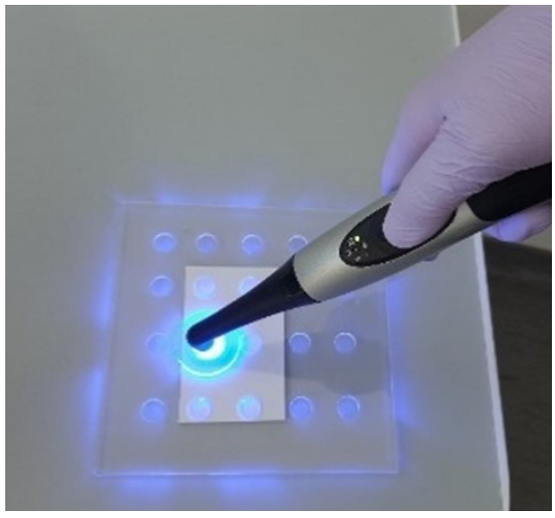


Figure 1. *Preparation of the composite resin samples*

2.2. Application of the Bleaching Process

The samples were subjected to bleaching with and without light conditions, in accordance with the manufacturer's advises (Table 2). A week later, the same procedures were repeated in the second session. Between two sessions, the samples were kept in distilled water. The applications of the bleaching agents and bleaching agents are shown in Figures 2 and 3.

Beyond™ Whitening Accelerator (BEYOND™ Technology Corp., China) Bleaching system was used as a light source in groups OBL and WBL.

2.3. Surface Roughness Test

A mechanical profilometer device (Perthometer M2, Mahr GmbH, Göttingen, Germany) was used to evaluate the surface roughness of the samples. A measurement length (tracing length) of 1.75 mm was used; the cut-off value was 0.25. The mean surface roughness value (Ra), which expresses the arithmetic mean of the absolute sum of all surface

irregularities (height and depth) at a certain distance, of each sample was calculated in µm. Calibration was performed using five measurements. The mean of three measurements of the polished surface of each sample was presented.

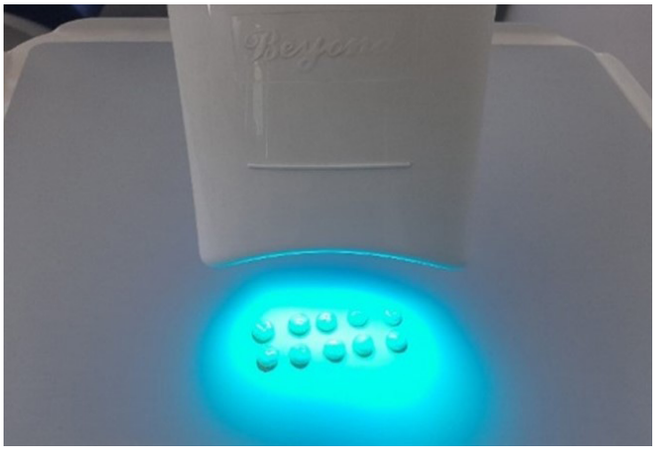


Figure 2. *Application of bleaching agents with light conditions.*

Figure 3. *Application of bleaching agents without light conditions.*

2.4. Vickers Microhardness Test

Microhardness measurements (DURALINE-M, Metkon, Turkey) were performed by applying a force of 200 g (1.96 N) to the surfaces of the samples for 10 seconds. Vickers hardness (VHN) values were calculated as the mean of three measurements from each sample.

$VHN = 1{,}8544\ (F/\ D^2)$

F: Force (kgf)

D^2: track area (mm^2)

2.5. SEM Analysis

One sample from five groups were sputter-coated with Au-Pd alloy and the surface alterations were evaluated in Scanning Electron Microscopy (SEM) (Zeiss EVO MA10; Carl Zeiss, Oberkochen, Germany). Photographs of the images were collected at ×1000 and ×3000 magnifications.

2.6. Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) for Windows 22.0 software. Means and standard deviations were used as illustrative statistics. The Mann Whitney – U test was performed to compare scaler continual data between two free groups, and the Kruskal–Wallis H test was applied to compare scaler constant data more than independent two groups. In addition, the Mann Whitney – U test was used as a supplemental evaluation to define the differences, after the Kruskal Wallis – H test ($p < 0.05$).

Table 2. *Application procedures of the bleaching agents*

Group	Bleaching agent	Application procedure	Condition
C	Control group	-	-
OB	Opalescence Boost	3 × 20 min 2 sessions	Without Light
OBL			With Light
WB	Whiteness HP Blue	1 × 40 min 2 sessions	Without Light
WBL			With Light

3. RESULTS

3.1. Microhardness Results

The microhardness values were significantly different among groups in terms of the application of bleaching agents and light ($p = 0.008$) (Table 3). The highest microhardness values were obtained in groups WBL (61.410) and OB (61.403), while the lowest values were recorded in group OBL (58.130). In groups which Opalescence Boost was applied (OB and OBL), the mean microhardness value decreased significantly when light was used ($p=0.023$) (Table 4). However, in groups which Whiteness HP Blue was applied, the mean microhardness value increased significantly when light was used ($p=0.049$). When comparing the bleaching agents, Opalescence Boost showed superior results than Whiteness HP Blue when light was not applied ($p=0.023$) (Table 4). On the other hand, light application, led to higher results in Whiteness HP Blue than Opalescence Boost ($p=0.029$) (Table 4).

3.2. Surface Roughness Results

The surface roughness values were not significantly different among groups with respect to the application of bleaching agents or lighting conditions ($p = 0.144$) (Table 5). While the highest mean Ra value was observed in WB (0.112), the lowest Ra values were observed in WBL (0.093). There was no significant difference between the two bleaching agents when light was applied ($p= 0.739$) and when light was not applied ($p= 0.684$) (Table 6).

Table 3. *Vickers Microhardness (VHN) Values*

	Group	Mean	SD (±)	KW	p
VHN	C	58.240[a]	2.110	13.767	0.008
	OB	61.403[b]	1.815		
	OBL	58.130[c]	5.786		
	WB	59.073[d]	3.151		
	WBL	61.410[e]	2.126		

KW: Kruskal Wallis-H Testi

C: Control group; OB: Opalescence Boost (without light); OBL: Opalescence Boost (with light) WB: Whiteness HP (without light); WBL: Whiteness HP (with light)

Table 4. *Bleaching effects on composite microhardness values with/ without light*

VHN	Bleach		Bleach+ Light		MW	p
	Mean	SD (±)	Mean	SD (±)		
Opalescence Boost	61.403	1.815	58.130	5.786	20.000	0.023
Whiteness HP	59.073	3.151	61.410	2.126	24.000	0.049
MW	20.00		0.029			
p	0.023		0.029			

MW: Man Whitney-U Testi

Table 5. *Surface roughness (Ra) values*

	Group	Mean	SD (±)	KW	p
Ra	C	0.090	0.011	6.853	0.144
	OB	0.108	0.028		
	OBL	0.095	0.015		
	WB	0.112	0.023		
	WBL	0.093	0.018		

KW: Kruskal Wallis-H Testi

C: Control group; OB: Opalescence Boost (without light); OBL: Opalescence Boost (with light) WB: Whiteness HP (without light); WBL: Whiteness HP (with light)

Tablo 6. *Bleaching effects on composite surface roughness values with/without light*

Ra	Bleach		Bleach+ Light		MW	p
	Mean	SD	Mean	SD		
Opalescence Boost	0.108	0.028	0.095	0.015	36.500	0.307
Whiteness HP	0.112	0.023	0.093	0.018	26.000	0.069
MW	44.000		45.500			
p	0.684		0.739			

MW:Man Whitney-U Testi

3.3. SEM Imaging Results

In SEM images, the application of Opalescence Boost and Whiteness HP bleaching agents with and without light caused no changes on the nanoceramic composite surface. In SEM evaluation, no change was observed in the resin structure in accordance with the surface roughness values. SEM images of the control, Opalescence Boost PF, and Whiteness HP Blue samples after bleaching with/without light conditions at ×1000 and ×3000 magnifications are shown in Figures 4–6.

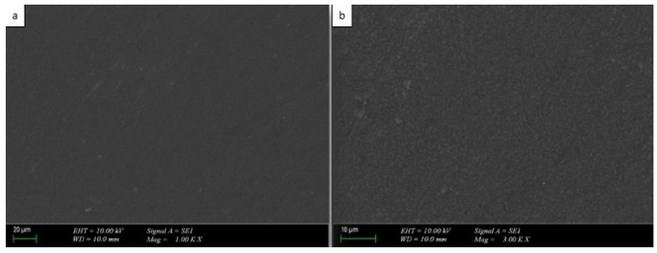


Figure 4. *SEM images of the Control group a. ×1000 and b. ×3000 magnification*

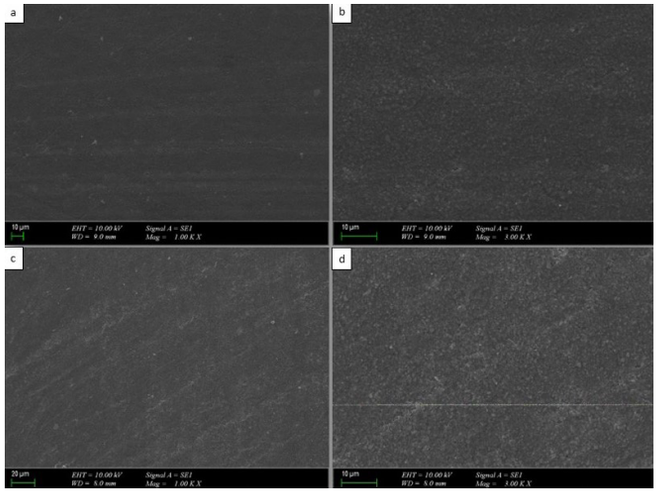


Figure 5. *Opalescence Boost SEM images a. OB ×1000, b. OB ×3000, c. OBL ×1000, d. OBL ×3000 (OB: Opalescence Boost/ without light, OBL: Opalescence Boost/ with light)*

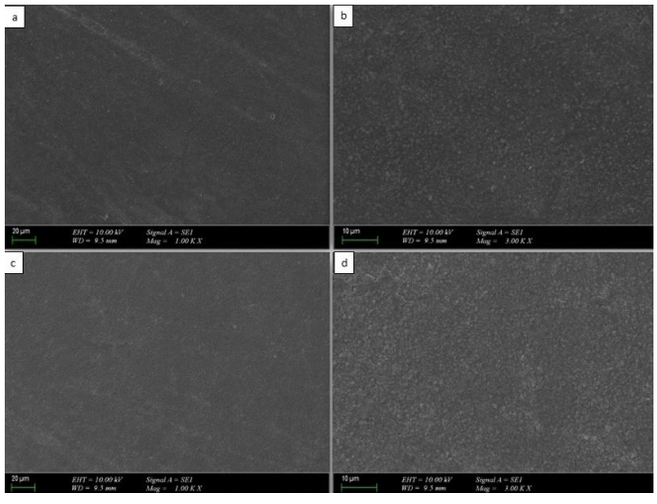


Figure 6. *Whiteness HP SEM images* ***a.*** *WB ×1000,* ***b.*** *WB ×3000,* ***c.*** *WBL ×1000,* ***d.*** *WBL ×3000 (****WB:*** *Whiteness HP/ without light,* ***WBL:*** *Whiteness HP/ with light)*

4. DISCUSSION

There is no significant difference in surface roughness values of nanoceramic composite resin with/without light bleaching techniques. Therefore, the second hypothesis of the present study was accepted.

In clinical practice, teeth restored with composite are often affected by bleaching (17). Therefore, the effects of bleaching agents on restorative materials must be considered (18). In this study, the effects of activated bleaching agents on the physical properties of nanoceramic composite resin, with/without light for activation, using SEM and measurements of microhardness and surface roughness were evaluated.

Light curing units induce HP decomposition, thus accelerating chemical reactions during bleaching. The free radical perhydroxyl produced by the breakdown of HP affects restorative materials. HP has a high oxidizing potential that can affect both the pigment macromolecules and the resin matrix. It also induces oxidative break down of polymer chains of peroxides, leading to bond failure between the resin matrix and the inorganic fillers.

Some research results have been inconsistent regarding the effects of bleaching on the surface roughness and microhardness of resin composites (19-27).

Leal et al., applied 10% CP home and 35% HP office bleaching agents to nanofilled and nanohybrid composites. Composite resin surfaces were evaluated for microhardness and surface roughness. In nanohybrid composites, microhardness values were higher in the application with 35% HP than in the home bleached group. No significant differences in surface roughness were found (19). These results are consistent with our findings, whereby light source did not affect the surface roughness with respect to Opalescence Boost or Whiteness HP products ($p=0.307$; $p=0.069$); however, the microhardness of the resin was decreased by light source in Opalescence Boost application ($p=0.023$), and increased in Whiteness HP application ($p=0.049$).

Yikilgan et al., evaluated the effects of various polishing methods and bleaching agents on the surface hardness and roughness of nanohybrid composite resins. Bleaching agent groups containing 10% CP and 38% HP showed significant differences between before and after treatment hardness values ($p < 0.05$). However, no statistically significant differences between before and after bleaching surface roughness measurements were found in any group ($p>0.05$) (20). In this study, similarly the bleaching application technique significantly increased the microhardness values; however, bleaching did not affect the surface roughness (20).

Cengiz et al., applied 10% HP and 10% CP bleaching agents to a micro-hybrid, an ormocer-based nano-hybrid, and three nano-hybrid composites. Surface changes were evaluated using profilometry and SEM. Ra values were significantly higher in the nanohybrid composite resin group (Ceram.X Mono) than in the distilled water (control) group when both bleaching agents were applied ($p < 0.05$) (21).

Mohammadi et al., determined the effects of light duration and bleaching agents on the surface microhardness of microhybrid composite resins. Office type application with 40% HP on microhybrid composite resin surfaces reduced on microhardness values comparable to home types with 15% CP (22).

In the in vitro study conducted by Özyılmaz et al., the microhardness values of six restorative materials were evaluated after office bleaching using a blue LED and a diode laser. The use of 35% and 46% HP in nanofilled, nanohybrid, hybrid polymer, nanofilled, and microfilled ceramic restorative materials caused significant decreases in the microhardness values of these materials. Additionally, the nanohybrid composite resin showed the lowest microhardness value among the materials examined (23).

Maran et al. compared with/without light bleaching applications in terms of bleaching effectiveness and tooth sensitivity. It was found that the light application did not increase the office bleaching efficiency, regardless of the HP concentration (24,25).

In a study conducted by Yazıcı et al., the surface roughness effects of office bleaching applied with a laser to three composite resins were evaluated, using a 35% HP gel and a diode laser. Laser bleaching significantly increased the surface roughness of a nanoceramic composite Ceram-X Mono ($p < 0.05$). In our study, the application of bleaching agent did not affect the surface roughness (26).

Cengiz et al., used SEM to evaluate the changes in nanohybrid composite surface morphology after the application of 10% HP and 10% CP bleaching gel. No important changes were examined in the composite surface (21).

Qasim et al. used SEM to appraise the effects of office bleaching agents (Opalescence Boost 40% HP and Whiteness HP Blue 35% HP) on the surface roughness values of dental materials (ceramic, nanohybrid, nanofilled resin composite restorations). There were no differences between the bleaching agents. Subjective evaluations of SEM images of dental composites before and after bleaching were consistent with the surface roughness analysis. (27). According to SEM evaluation no significant change was observed in the present study.

Limitations of this study may be included such as using a single type of composite, low sensitivity due to the mechanical nature of the profilometer device, absence of saliva. In this study color change and temperature change were not examined, the number of applications of the bleaching agent might have been increased. Therefore, the results of this study were different from some previous studies due to these limitations.

5. CONCLUSION

Within the conditions of this study, after bleaching procedure the microhardness values of the nanoceramic composite increased, however bleaching did not show any effect on

surface roughness. In SEM images, there is no change in the structure and surface properties of the nanoceramic composite. More studies on this material are needed. The results of this study showed that the application of bleaching agents with/ without light caused a change in the physical properties of the evaluated nanoceramic composite resin.

Funding

There was no grant support or research funding for this article.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] Briso ALF, Rahal V, Azevedo FA, Gallinari MO, Gonçalves RS, Frascino SMB, Dos Santos PH, Cintra LTA. Neurosensory analysis of tooth sensitivity during at-home dental bleaching: a randomized clinical trial. J Appl Oral Sci. 2018;26:e20170284. doi:10.1590/1678-7757-2017-0284

[2] Esmaeili B, Abolghasemzadeh F, Gholampor A, Daryakenari G. The effect of home bleaching carbamide peroxide concentration on the microhardness of dental composite resins. Gen Dent. 2018;66(1):40-44. doi:10.1007/s10640.013.9664-9

[3] de Alexandre RS, Sundfeld RH, Briso AL, Bedran-Russo AK, Valentino TA, Sundefeld ML. Effect of 10% carbamide peroxide dental bleaching on microhardness of filled and unfilled sealant materials. J Esthet Restor Dent. 2006;18(5):273-278. doi:10.1111/j.1708-8240.2006.00037.x

[4] Wang L, Francisconi LF, Atta MT, Dos Santos JR, Del Padre NC, Gonini A, Jr., Fernandes KBP. Effect of bleaching gels on surface roughness of nanofilled composite resins. Eur J Dent. 2011;5(2):173-179. doi:10.1055/s-0039.169.8876

[5] Joiner A. The bleaching of teeth: a review of the literature. J Dent. 2006;34(7):412-9. doi:10.1016/j.jdent.2006.02.002

[6] Sulieman M, MacDonald E, Rees JS, Addy M. Comparison of three in-office bleaching systems based on 35% hydrogen peroxide with different light activators. Am J Dent. 2005;18(3):194-197.

[7] Nguyen C, Augros C, Rocca JP, Lagori G, Fornaini C. KTP and Er:YAG laser dental bleaching comparison: a spectrophotometric, thermal and morphologic analysis. Lasers Med Sci. 2015;30(8):2157-2164. doi:10.1007/s10103.015.1778-6

[8] Shahabi S, Assadian H, Mahmoudi Nahavandi A, Nokhbatolfoghahaei H. Comparison of Tooth Color Change After Bleaching With Conventional and Different Light-Activated Methods. J Lasers Med Sci. 2018;9(1):27-31. doi:10.15171/jlms.2018.07

[9] El-Murr J, Ruel D, St-Georges AJ. Effects of external bleaching on restorative materials: a review. J Can Dent Assoc. 2011;77:b59.

[10] Hafez R, Ahmed D, Yousry M, El-Badrawy W, El-Mowafy O. Effect of in-office bleaching on color and surface roughness of composite restoratives. Eur J Dent. 2010;4(2):118-127. doi:10.1055/s-0039.169.7819

[11] Rattacaso RM, da Fonseca Roberti Garcia L, Aguilar FG, Consani S, de Carvalho Panzeri Pires-de-Souza F. Bleaching agent action on color stability, surface roughness and microhardness of composites submitted to accelerated artificial aging. Eur J Dent. 2011;5(2):143-149. doi:10.1055/s-0039.169.8871

[12] Silva Costa SX, Becker AB, de Souza Rastelli AN, de Castro Monteiro Loffredo L, de Andrade MF, Bagnato VS. Effect of four bleaching regimens on color changes and microhardness of dental nanofilled composite. Int J Dent. 2009;2009:313845. doi:10.1155/2009/313845

[13] Hannig C, Duong S, Becker K, Brunner E, Kahler E, Attin T. Effect of bleaching on subsurface micro-hardness of composite and a polyacid modified composite. Dent Mater. 2007;23(2):198-203. doi:10.1016/j.dental.2006.01.008

[14] Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations—a systematic review. Dent Mater. 2004;20(9):852-861. doi:10.1016/j.dental.2004.04.002

[15] Yap AU, Wattanapayungkul P. Effects of in-office tooth whiteners on hardness of tooth-colored restoratives. Oper Dent. 2002;27(2):137-141.

[16] de ASMF, Davies RM, Stewart B, DeVizio W, Tonholo J, da Silva Júnior JG, Pretty IA. Effect of whitening gels on the surface roughness of restorative materials in situ. Dent Mater. 2006;22(10):919-924. doi:10.1016/j.dental.2005.11.029.

[17] Polydorou O, Mönting JS, Hellwig E, Auschill TM. Effect of in-office tooth bleaching on the microhardness of six dental esthetic restorative materials. Dent Mater. 2007;23(2):153-158. doi:10.1016/j.dental.2006.01.004.

[18] Yu H, Li Q, Hussain M, Wang Y. Effects of bleaching gels on the surface microhardness of tooth-colored restorative materials in situ. J Dent. 2008;36(4):261-267. doi:10.1016/j.jdent.2008.01.008

[19] Leal A, Paula A, Ramalho A, Esteves M, Ferreira MM, Carrilho E. Roughness and microhardness of composites after different bleaching techniques. J Appl Biomater Funct Mater. 2015;13(4):e381-388. doi:10.5301/jabfm.5000239.

[20] Yikilgan İ, Kamak H, Akgul S, Ozcan S, Bala O. Effects of three different bleaching agents on microhardness and roughness of composite sample surfaces finished with different polishing techniques. J Clin Exp Dent. 2017;9(3):e460-e465. doi:10.4317/jced.53136.

[21] Cengiz E, Kurtulmus-Yilmaz S, Ulusoy N, Deniz ST, Yuksel-Devrim E. The effect of home bleaching agents on the surface roughness of five different composite resins: A SEM evaluation. Scanning. 2016;38(3):277-283. doi:10.1002/sca.21307.

[22] Mohammadi N, Alavi FN, Rikhtehgaran S, Chaharom MEE, Salari A, Kimyai S, Bahari M. Effect of Bleaching Method and Curing Time on the Surface Microhardness of Microhybrid Composite Resin. Maedica (Bucur). 2020;15(3):359-364. doi:10.26574/maedica.2020.15.3.359

[23] Özyılmaz ÖY, Alptekin T, Aykent F, Kara HB. Effect of two light activated in-office bleaching agents on microhardness of different esthetic restorative materials. Selcuk Dental Journal. 2018;5(2):142-149. doi:10.15311/selcukdentj.344313

[24] Maran BM, Burey A, de Paris Matos T, Loguercio AD, Reis A. In-office dental bleaching with light vs. without light: A systematic review and meta-analysis. J Dent. 2018;70:1-13. doi:10.1016/j.jdent.2017.11.007

[25] Maran BM, Ziegelmann PK, Burey A, de Paris Matos T, Loguercio AD, Reis A. Different light-activation systems associated with dental bleaching: a systematic review and a network meta-analysis. Clin Oral Investig. 2019;23(4):1499-1512. doi:10.1007/s00784.019.02835-x.

[26] Yazıcı A. R, Atalay C, Bektaş B. The effect of laser-assisted in-office bleaching on surface roughness of different types of

restorative resins. Clinical Dentistry and Research. 2016; 40(1), 3 – 10. doi:10.21608/edj.2020.26509.1084

[27] Qasim S, Ramakrishnaiah R, Alkheriaf AA, Zafar MS. Influence of various bleaching regimes on surface roughness of resin composite and ceramic dental biomaterials. Technol Health Care. 2016;24(2):153-161. doi:10.3233/THC-151101.

How to cite this article: Ülper E, Türkmen C, Cimilli ZH. The Effects of Office Bleaching Techniques on Nanoceramic Composite Resin. Clin Exp Health Sci 2023; 13: 92-98. DOI: 10.33808/clinexphealthsci.1057225

Clinical and Experimental Health Sciences

Intracellular Levels of IL-10 and STAT3 in Patients with Chronic Lymphocytic Leukemia

Özden Özcan[1,2], Metin Yusuf Gelmez[2], Suzan Çınar[2], Günnur Deniz[2], Melih Aktan[3]

[1] İstanbul University, Graduate School of Health Sciences, İstanbul, Türkiye.
[2] İstanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, İstanbul, Türkiye.
[3] İstanbul University, İstanbul Medical Faculty, Internal Medicine, Division of Hematology, İstanbul, Türkiye.

Correspondence Author: Özden Özcan
E-mail: ozdenozcann@gmail.com

Received: 14.01.2015 **Accepted:** 09.06.2015

ABSTRACT

Objective: Chronic Lymphocytic Leukemia (CLL) is characterized by the accumulation of $CD5^+CD19^+$ B cells in the bone marrow and peripheral blood. Recent studies indicated that expression of *IL-10*, AID and *mir-155* which are regulated by STAT3 are increased in CLL patients. $CD5^+CD19^+$ regulator B (B_{reg}) cells secrete IL-10 and suppress the immune system. While the CLL cells show similar immunophenotypic properties to Breg cells, they are also thought to be functionally similar. In this study, STAT3 and IL-10 levels of CLL patients were investigated.

Methods: Peripheral blood samples obtained from patients (n:24) and healthy controls (n:14). Peripheral blood mononuclear cells were cultured for 48 hours in the presence and absence of CpG for IL-10 expression and cultured with and without PMA for STAT3 expression. IL-10 and STAT3 expression were analyzed with anti-CD5, anti-CD19, anti-CD38, anti-STAT3 and anti-IL-10 monoclonal antibodies by using flow cytometry.

Results: Compared to healthy subjects, increased $IL\text{-}10^+$, $IL\text{-}10^+CD19^+$, $STAT3^+CD19^+$ were obtained in lymphocyte population of patients. Increased IL-10 was showed $CD19^+$ B cells of CLL patients. Our results showed that IL-10 levels had no significant difference between $CD5^+CD19^+$ cells, whereas STAT3 levels were found lower in patient compared to healthy controls.

Conclusion: These results made us thought that the levels of IL-10 and STAT3 expression in CLL B cells is clearly different from normal B lymphocytes might have a role in the biology of CLL. It is believed that the presented data will contribute to the studies that scrutinize the similarity of CLL cells to Breg cells.

Keywords: CLL, IL-10, STAT3, Breg, flow cytometry

1. INTRODUCTION

Chronic lymphocytic leukemia **(**CLL) is the most common leukemia in the western countries, and is characterized by the accumulation of $CD5^+CD19^+$ B cells in the bone marrow, peripheral blood and lymphoid organs (1). The clinical course of patients with CLL is highly variable and therefore difficult to predict (2)

Regulator B (B_{reg}) cells secrete IL-10 and suppress the immune system (3). There is no unique surface marker to identify B_{reg}, $CD19^+CD24^{hi}CD38^{hi}$ and $CD19^+CD5^+$ have been used B_{reg} marker in different studies (4). IL-10 is immunosuppressant and anti-inflammatory cytokine (5). Majority of IL-10 are produced by monocytes, remaining are done by lymphocytes (6). IL-10 inhibits proinflammatory responses of both innate and adaptive immune cells, and proinflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8 (3). IL-10 have a role to maintain the integrity and homeostasis of the epithelial layers of tissues (6).

The signal transducer and transcription activator 3 (STAT3) is a signal transduction molecule that is activated as a result of the binding of many cytokines, growth factors and hormones to their receptors in the cell membrane (7). Cytokine signal transduction is usually mediated by the JAK/STAT signaling pathway (8). After STAT3s are phosphorylated by JAKs, it becomes dimerized into active form (9). The activated STAT3s regulated the expression of their target genes which play a role in regulation of cell proliferation, angiogenesis and apoptosis (10, 11).

Recent studies indicated that expression of *IL-10* mRNA, *AID* mRNA and *mir-155* expressions which are regulated by *STAT3* are increased in CLL patients. IL-10 is an anti-inflammatory cytokine. In recent studies, it has been shown that IL-10 is also secreted in B cells. B_{reg} cells expressed CD5 and CD19, and suppress the immune system. B-CLL cells expressed CD5 surface molecule like B_{reg} cells. While the CLL cells show similar immunophenotypic properties to B_{reg} cells, they might

DOI: 10.33808/clinexphealthsci.1056727

also be thought to be functionally similar (10). In this study, it was aimed to determine the intracellular IL-10 and STAT3 levels of CLL cells by flow cytometry.

In this study, STAT3 and IL-10 expressions of CLL patients were investigated.

2. MATERIAL AND METHODS

2.1. Study Population

CLL patients who were newly diagnosed or being followed up in the outpatient clinic of Hematology Division were included in the study. Patients receiving treatment were excluded from the study. Twenty-four patients (17 males, 7 females) and 14 healthy individuals (8 males, 6 females) were included in the study. The mean age of the 24 patients in the study was 65 (53-83 years), while the mean age of the healthy control group was 57 (52-68 years). The clinical features of the patients are shown in the Table-1. The clinic data of the patients was collected retrospectively.

All patients met the National Cancer Institute (NCI) diagnostic criteria for CLL, and all samples showed the characteristic immunophenotype: cells expressed CD5 and CD19. Written informed consent was obtained according to the Declaration of Helsinki and the study was approved by the local ethics committee (Ethical Number:1135/09.06.2015).

Table 1. *The clinical features of the patients*

Patient No	Age	Gender	Cytogenetic Status	BINET	RAI	WBC (10^9/L)	Lymphocyte (10^3/µL)	CD38
1	63	M	no mutation	A	0	56	52.00	N
2	68	M	no mutation	C	1	34.6	30.01	N
3	48	M	no mutation	A	0	15.9	9.00	N
4	54	M	no data	A	1	25.4	21.59	N
5	56	M	no mutation	B	2	44.2	37.80	N
6	77	M	tri12	C	3	55	46.60	N
7	68	M	no mutation	A	2	47.83	43.77	N
8	65	F	del11q	B	2	54.5	46.90	P
9	73	M	tri12	A	0	28.2	19.60	N
10	60	F	no mutation	C	2	15.6	12.30	N
11	64	M	del13q	A	1	9.7	4.60	N
12	63	F	no data	A	1	63	52.20	N
13	68	F	no mutation	C	1	226.3	186.70	N
14	83	M	del13q	A	1	44.9	36.50	N
15	62	F	tri12	A	0	36.3	30.00	N
16	65	F	no mutation	C	1	13.7	8.50	N
17	86	F	tri12	C	4	149.2	137.10	N
18	70	M	no mutation	C	4	89.9	86.30	N
19	70	M	no data	A	1	37.2	30.20	N
20	53	M	del13q+del11q	C	4	20.8	17.68	P
21	62	M	del13q14	B	3	225.5	207.20	N
22	56	M	no mutation	B	2	111.8	87.10	N
23	66	M	del13q+del11q	B	2	196.2	178.70	N
24	55	M	no mutation	B	1	100.5	87.10	N

%30 ≥ CD38 positive (P); %30 < CD38 negative (N); M: Male; F: Female

2.2. Cell Cultures and Stimulation

2.2.1. IL-10 Expression

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples by density gradient centrifugation using Ficoll-Paque (Histopaque-1077; Biochrom, Cambridge, UK) at 2100×rpm for 30 min. The acquired PBMCs were washed twice with PBS and suspended in a complete cell culture medium RPMI containing 10% Fetal Bovine Serum (Sigma Chem. Co., Hamburg, Germany), 1% L-glutamine (Sigma Chem. Co., Hamburg, Germany), 1% anti-micotic and antibiotic solutions (Sigma Chem. Co., Hamburg, Germany). Viability was determined by trypan blue dye exclusion. PBMCs ($1x10^6$) were stimulated with CpG (1 ug/ml) at 48 hours and PMA (50 ng/ml-final concentrations, Sigma Chem. Co, Hamburg, Germany) and Ionomycine (1 µg/

ml-final concentration, Sigma Chem. Co, Hamburg, Germany) and Brefeldin A (BFA; Sigma Chem. Co., Hamburg, Germany) (final concentration of 10 µg/mL) was added for 4 hours to promote intracytoplasmic cytokine accumulation.

PBMCs were washed, then were labeled with anti-human CD19-APC (HIB19 clone), anti-human CD5-PE.Cy7 (UCHT2), anti-human CD38-FITC (HIT2 clone) and incubated for 20 min at room temperature. PBMCs were washed with PBS; fixed and permeabilized using Cytofix&Cytoperm Kit, (Nordic, MUbio, Netherlands), and then stained with anti-human IL-10 PE (JES3-9D7 clone) (all from Biolegend, San Diego,USA) mAbs for 20 min at room temperature and finally washed and data collected by FACS Calibur. Analyses were carried out using CellQUEST Software (Becton Dickinson, San Jose, USA) on FACS Calibur cytometer Becton Dickinson San Jose, USA.

Lymphocytes were gated on SSC (side scatter)/FSC (forward scatter) dot-plot per tube, then $CD19^+$ cells and $CD5^+CD19^+$ were gated in SSC/CD19 dot-plot (Figure 1). IL-10 expression were analyzed in lymphocytes, $CD19^+$ and $CD5^+CD19^+$ cells.

Table 2. *IL-10+CD19+, IL-10-CD19+, STAT3+CD19+ and STAT3-CD19+ levels*

	$IL\text{-}10^+CD19^+$ % in Lymphocyte Median (min-max)	$IL\text{-}10^+$ % in $CD19^+$ cells Median (min-max)	$IL\text{-}10^+$ % in $CD5^+CD19^+$ cells Median (min-max)	$STAT3^+CD19^+$ % in Lymphocyte Median (min-max)	STAT3% in $CD19^+$ cells Median (min-max)	STAT3% in $CD5^+CD19^+$ cells Median (min-max)
Heathy Control	0.39 (0.07-1.60)	2.76 (0.54-3.36)	6.97 (0.00-18.47)	0.16 (0.01-1.15)	5.14 (0.71-9.57)	6.77 (1.82-25.00)
CLL	6.73 (1.06-33.68)	7.67 (1.75, 38.09)	8.82 (1.08-38.09)	1.86 (0.37-12.64)	2.28 (0.48-21.59)	2.25 (0.41-29.38)
CD38 Negative	9.02 (1.06 – 33.68)	9.91 (1.75-38.09)	9.42 (1.08-38.09)	2.89 (0.37-12.64)	4.98 (0.48-21.59)	4.96 (0.41-29.38)
CD38 Positive	21.28 (14.43 – 27.85)	22.90 (13.66-32.13)	23.32 (14.49-32.02)	5.24 (0.48-11.58)	6.54 (0.93-16.00)	7.58 (1.78-14.41)
Deletion Negative	8.96 (1.23-19.63)	12.16 (1.70-24.70)	10.84 (1.08-25.26)	3.08 (0.37-12.64)	3.93 (0.48-13.32)	3.61 (0.41-13.88)
Deletion Positive	9.46 (1.06 – 27.85)	10.87 (2.01-32.13)	11.01 (2.01-32.02)	2.44 (0.48-11.58)	3.52 (0.89-16.00)	3.22 (0.81-14.41)
Rai 0	10.87 (1.03-29.80)	14.35 (2.01-32.36)	14.14 (2.01 – 35.33)	4.51 (0.84-11.58)	6.73 (2.00-16.00)	6.19 (1.91-14.41)
Rai I	6.88 (1.23-33.68)	5.62 (1.75-38.09)	4.97 (1-08-38.09)	2.50 (0.48-5.78)	4.10 (0.98-11.22)	3.99 (0.97-11.09)
Rai II	15.69 (9.54-21.5)	18.22 (10.33 – 24.70)	17.56 (10.66-25.26)	4.81 (0.37-12.64)	5.66 (0.48-13.32)	5.30 (0.41-13.88)
Rai III+ Rai IV	10.77 (2.67-27.85)	12.71 (2.82 – 32.13)	12.30 (2.86-32.02)	1.25 (0.89-1.89)	1.43 (0.89-2.32)	1.32 (0.81-2.38)
Binet A	10.78 (1.06-33.68)	10.38 (2.01-38.09)	9.58 (2.01-38.09)	4.74 (0.48-12.64)	6.60 (1.85-16.00)	6.13 (1.78-14.41)
Binet B	13.29 (6.73-21.57)	15.33 (4.54-24.70)	15. 59 (3.48-25.26)	1.39 (0.37-3.55)	2.06 (0.48-6.73)	2.02 (0.41-6.81)
Binet C	8.23 (1.23-27.85)	10.23 (1.75-32.13)	9.84 (1.08-32.02)	3.49 (0.95-11.40)	4.00 (0.98-11.61)	3.81 (0.94-11.74)

2.2.2. STAT3 Expression

PBMCs ($1x10^6$ cell/ml) were cultured for STAT3 expression with and without PMA (20 ng/ml) (Sigma Chem. Co, Hamburg, Germany) at 15 min. After washing step, cells were stained with anti-CD5-PE-Cy7 (UCHT2 clone), anti-CD19-APC (HIB19 clone) and anti-CD38-FITC (HIT2 clone) (Biolegend, San Diego,USA) for 15 min at room temperature. Cells were fixed with fix-perm kit (Nordic MUbio, Susteren, Netherlands) for 20 min after washing, the cells were permeabilized with the Perm Buffer solution (Nordic MUbio, Susteren, Nederland) and anti-STAT3 PE (Biolegend, San Diego, USA) for 15 min and finally washed and data collected by FACS Calibur. Analyses were carried out using CellQUEST Software (Becton Dickinson, San Jose, USA) on FACS Calibur cytometer Becton Dickinson San Jose USA.

2.3. Statistics

The Mann–Whitney U-test was used to analyze for the measurement variables when the numbers within the groups were low and not normally distributed. P-value less than 0.05 was considered statistically significant. All calculations were performed using GraphPad InStat version 5.03 (GraphPad Software Inc., San Diego, CA).

3. RESULTS

3.1. Clinical Features of Patients

Twenty-four patients with CLL were evaluated and 70% of them were male. The patients' clinical features are summarized in Table 1. All patients were currently not under treatment and not taking any medicine at the time of blood sampling. None of the patients had any finding of acute or chronic infection. According to Binet stage; 41% of patients had Binet A (n: 10), 25% of patients had Binet B (n: 6), and 34% of patients had Binet C (n: 8) disease. Four (16%), 9 (38%), 6 (25%), 2 (8%) and 3 (13%) patients had Rai stage 0, I, II, III and IV disease, respectively.

3.2. Increased IL-10 levels in lymphocyte and CD19+ cells but not in CD5+CD19+ cells were detected in CLL patients

Increased $IL\text{-}10^+CD19^+$ levels were detected in lymphocyte of CLL patients compared to healthy controls ($p<0.0001$). There is a significant difference in $IL\text{-}10^+CD19^+$ of CD38 negative and positive ($p=0.009$, $p=0.0001$, respectively), chromosomal deletion negative and positive ($p=0.004$ and $p=0.005$, respectively) patient groups when compared to healthy

controls. CD38 positive patient groups have high IL-10^{+}CD19^{+} levels than CD38 negative patient group (p=0.04). High IL-10^{+}CD19^{+} levels were found in Rai 0, I, II, III+IV stage of CLL patients than healthy controls (p=0.006, p<0.0001, p=0.0006, p=0.009, respectively). Similarly, high IL-10^{+}CD19^{+} levels were found in Binet A, B, C stage of CLL patients (p<0.0001, p=0.0006, p=0.0002, respectively). On the other hand, there were no differences in IL-10^{+}CD19^{+} cells for deletion status, Rai or Binet staging system within the CLL cohort (Figure 1).

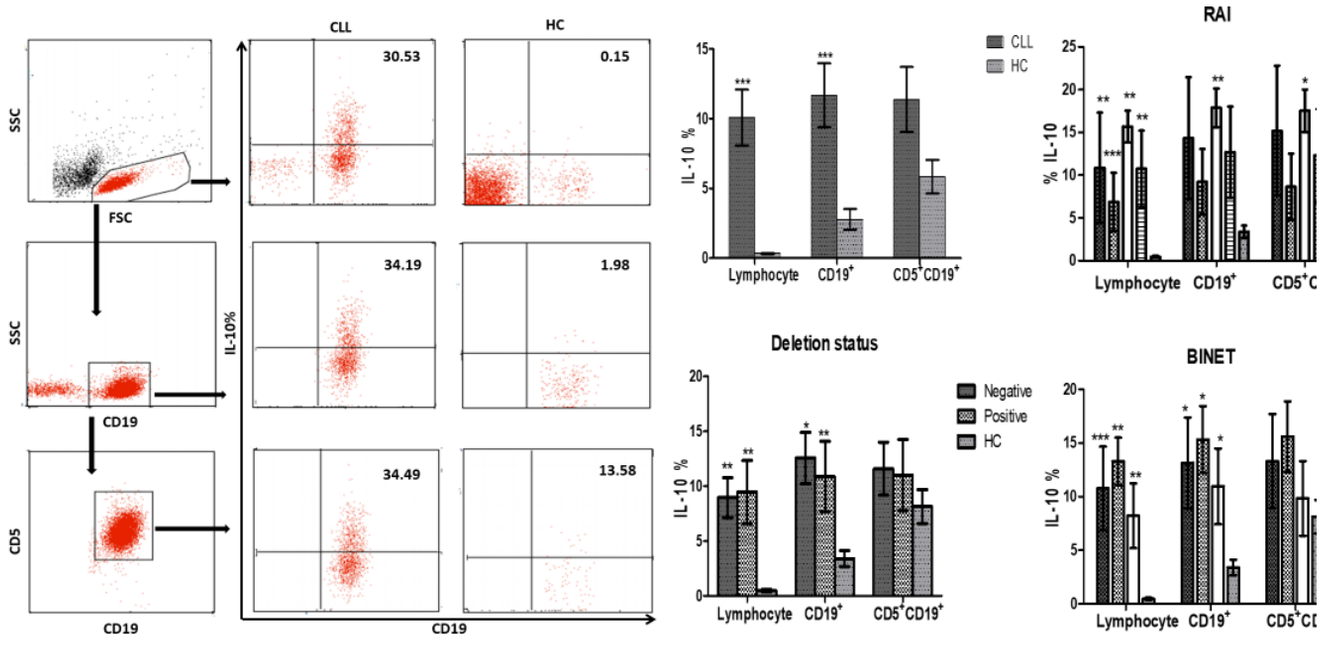


Figure 1. *IL-10+CD19+ levels in CLL patients and healthy controls obtained from lymphocytes (A), CD19+ B (B), CD5+CD19+ cells (C). Stim: Stimule-CpG (48-h), PMA (4 hours min.)*

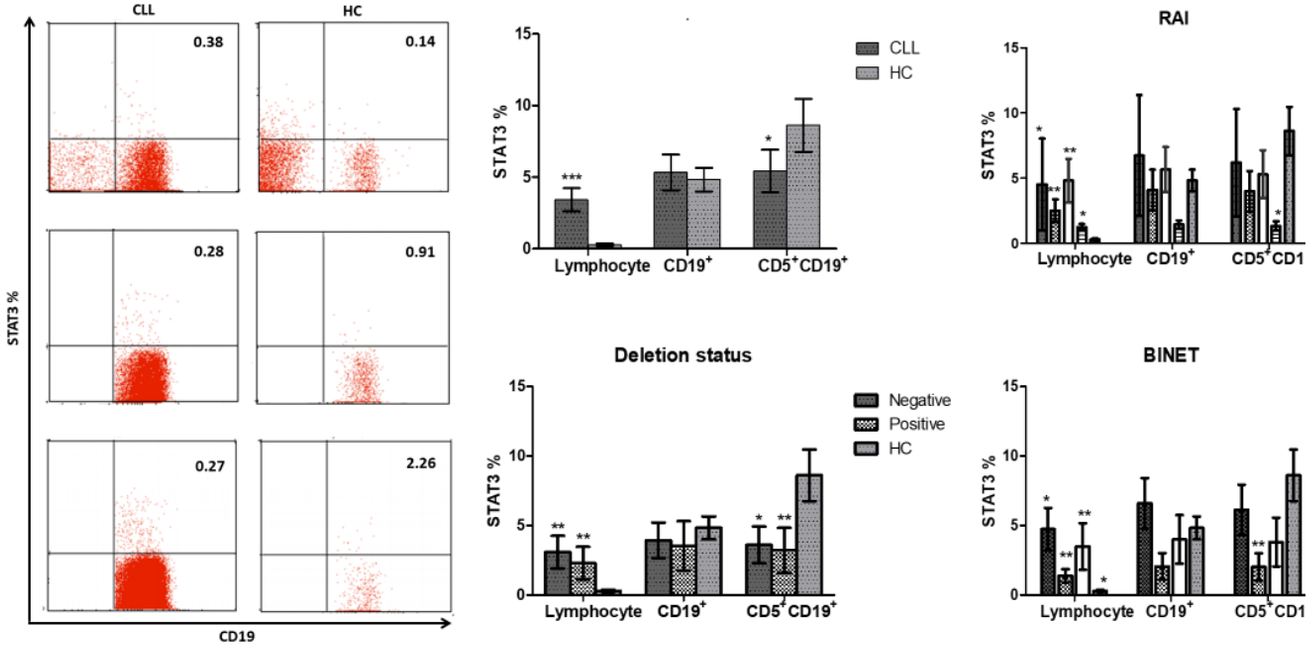


Figure 2. *STAT3+CD19+ levels in CLL patients and healthy controls obtained from lymphocytes (A), CD19+ B (B), CD5+CD19+ cells (C).*

According to IL-10$^+$ levels in CD19$^+$ gate, increased IL-10$^+$ levels were detected in CLL patients compared to healthy controls ($p<0.0001$). There is a significant difference in IL-10 level of CD38 negative and positive ($p=0.009$ and $p=0.02$, respectively), deletion negative and positive ($p=0.02$ and $p=0.002$, respectively), when compared to healthy controls. According to Rai staging system, there were no significant differences to healthy controls, except Rai II ($p=0.0006$). High IL-10 levels were determined in all Binet stage compared to healthy control, (Binet A; $p=0.03$, Binet B; $p=0.002$ and Binet C; $p=0.02$). There were no differences in IL-10 levels of CD19$^+$ cells for chromosomal deletions, CD38 expression status.

In the CD5$^+$CD19$^+$ cells, there were no difference in IL-10 levels between CLL patient and healthy controls. Similarly, there were no differences in IL-10 levels of CD5$^+$CD19$^+$ cells for chromosomal deletion or CD38 expression status. However, increased IL-10 level of CD5$^+$CD19$^+$ in CD38 positive patients compared to healthy control ($p=0.01$). According to Rai and Binet staging systems, there were no significant differences between CLL patients and healthy controls, except Rai II ($p=0.02$).

3.3. STAT3+CD19+ levels in lymphocyte of CLL patients

High STAT3$^+$CD19$^+$ cells in lymphocyte were found in CLL patients compared to healthy controls ($p<0.0001$). There is a significant difference in STAT3$^+$CD19$^+$ cells of CD38 negative and positive ($p<0.0001$ and $p=0.004$, respectively), chromosomal deletion negative and positive ($p=0.0003$ and $p=0.0007$, respectively) patient groups when compared to healthy controls. According to Rai and Binet staging systems, increased STAT3$^+$CD19$^+$ cells were detected in all stage of patients, except Rai III compared to healthy controls (Rai 0; $p=0.01$, Rai I; $p=0.0008$, Rai II; $p=0.0006$, Rai III+IV; $p=0.01$, Binet A; $p=0.0003$, Binet B; $p=0.003$, and Binet C; $p=0.001$, respectively). On the other hand, there were no differences in STAT3$^+$CD19$^+$ cells for deletion or CD38 expression status and staging system within the CLL cohort.

In the CD19$^+$ cells, there were no differences in STAT3 levels between CLL patients and healthy controls. Similarly, we did not found any differences in STAT3 level for deletions or CD38 expression status, Rai and Binet staging system within the CLL cohort.

In CD5$^+$CD19$^+$ cells, decreased STAT3 levels were found in CLL patients compared to health controls ($p<0.04$). There is a significant difference in STAT3$^+$ levels in CD5$^+$CD19$^+$ cells of CD38 negative ($p=0.01$), chromosomal deletion negative and positive ($p=0.01$ and $p=0.008$, respectively) when compared to healthy controls. Except decreased STAT3 level in Rai III+IV ($p=0.01$) and Binet B ($p=0.007$), there were no differences in STAT3 levels in CD5$^+$CD19$^+$ cells for Rai and Binet staging system. On the other hand, there were no differences in STAT3 levels for deletion or CD38 expression status and staging system within the CLL cohort (Figure 2).

4. DISCUSSION

CLL is a chronic lymphoproliferative disease characterized by monoclonal B cell accumulation and it is the most common type of leukemia in adults in western countries (13). Most of the CLL cells have low proliferative capacity and most of them are in G_0 phase of cell cycle (14). Since the mechanism of apoptosis in CLL is disrupted, the tumor cells accumulate in the lymph nodes and bone marrow. Rai and Binet staging systems have been used in CLL patients for about 40 years to predict the clinical outcome (15).

STAT3 is a transcription factor and activate many genes like cytokines, growth factors etc. Receptor-ligand interaction was induced phosphorylation to activate the STAT3. It was shown that, *STAT3* expression were increased in many solid tumor cells or hematologic malignancies (16, 17). Anti-inflmatory cytokine IL-10 is regulated by STAT3 (5).

In 2014, Antosz et al. investigated mRNA transcript levels of *IL-6*, *IL-10*, *c-jun* and *STAT3* in CLL patients and, found that high *IL-10* mRNA expression in normal lymphocytes than in CLL cells in the non-stimulated condition, but after stimulation with LPS for 30 min increased *IL-10* mRNA expression were found in patients with CLL compared to healthy controls (18). Dilillo et al. showed that CD5$^+$CD19$^+$ B_{reg} cells suppress the immune system by secreting IL-10 and these cells are similar in both immunophenotipically and functionally as a B cell suppressant (12).

In our study, high IL-10$^+$ levels were detected in lymphocytes and CD19$^+$ cells of patients with CLL after 48 hours of CpG stimulation than healthy controls, but in the CD5$^+$CD19$^+$ cells, there is no difference were found in expressing IL-10 cells. These findings might suggest that CLL cells can be functionally similar, in addition to immunophenotypic properties similar to those of B_{reg} cells. Gary-Gouy et al. reported that an increase in IL-10 expression may cause an excessive increase in CD5 expression in CLL and that CD5 enhances IL-10 production as an immunosuppressive cytokine (19).

IL-10 signals through the Janus kinase (Jak)/signal transducer and activator of transcription (STAT) signaling pathway (6). Antosz et al. found increased *STAT3* mRNA expression in CLL patients (18). In our study, STAT3$^+$CD19$^+$ levels in lymphocytes were higher in patients with CLL than healthy controls and our data were consistent with this study. However, no significant difference was observed in STAT3$^+$ levels in CD19$^+$ B cells, whereas in CD5$^+$CD19$^+$ cells, STAT3$^+$ levels of patients with CLL after stimulation were found to be lower than healthy individuals. In patients with CLL, absolute number of B cell are increased due to disease. However, B cell number in healthy subjects is low in normal physiological conditions. Increased number of B cell may cause enhanced ratio of STAT3 cells in CLL patients compared to healthy subjects. In our study analysis of STAT3 were carried out by flow cytometry, but that of Antosz et al. were made by real time PCR in the RNA isolation obtained in total cells (18). Therefore, Antosz et al. was found *STAT3* mRNA expression to be high due to the high B cells in the patients (18).

These results made us thought that the levels of IL-10 and STAT3 expression in CLL B cells is clearly different from healthy controls B lymphocytes and might have a role in the biology of CLL. It is though that the presented data will contribute to the studies that scrutinize the similarity of CLL cells to B_{reg} cells.

Conflicts Of Interest/Disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

Funding: *This work was supported by Scientific Research Projects Coordination Unit of Istanbul University, Project number: 55010 and TSA-2018-27695.*

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

Ethics Committee Approval: *This study was approved by Ethics Committee of Istanbul University (Decision date and number: 09.06.2015, 1135)*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Interpretation of data for the study: OO, MYG, SC, GD, MA.
Drafting the manuscript: OO, MYG, SC, GD, MA.
Revising it critically for important intellectual content: OO, MYG, SC, GD, MA.
Final approval of the version to be published: OO, MYG, SC, GD, MA.
Research idea: OO, MYG, MA.
Design of the study: OO, MYG, GD, MA.
Acquisition of data for the study: OO, SC, MYG.
Analysis of data for the study: OO, SC, MYG.

REFERENCES

[1] Hallek M and Al-Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. Am J Hematol. 2021;96:1679–1705. DOI:10.1002/ajh.26367.

[2] Maliha K, Rabbia S, Philip AT. Approach to Richter transformation of chronic lymphocytic leukemia in the era of novel therapies. Ann Hematol. 2018; 97(1):1-15. DOI: 10.1007/s00277.017.3149-9.

[3] Catalán D. Mansilla MA, Ferrier A, Soto L, Oleinika K, Aguillon JC and Aravena O. Immunosuppressive mechanisms of regulatory B cells. Front Immunol. 2021;12:611795. DOI:10.3389/fimmu.2021.611795.

[4] Zhao Q and Jung LK. Frequency of $CD19^{+}$ $CD24^{hi}CD38^{hi}$ regulatory B cells is decreased in peripheral blood and synovial fluid of patients with juvenile idiopathic arthritis: a preliminary study. Pediatric Rheumatol Online J. 2018;16(1):44. DOI:10.1186/s12969.018.0262-9.

[5] Saulep-Easton D, Vincent FB, Quah PS, Wei A, Ting SB, Croce CM. The BAFF receptor TACI controls IL-10 production by regulatory B cells and CLL B-cells. Leukemia 2016; 30(1):163-172. DOI: 10.1038/leu.2015.174.

[6] Rutz S, Ouyang W. Regulation of interleukin-10 expression. Adv Exp Med Bio. 2016; 941:89-116. DOI: 10.1007/978-94-024-0921-5_5.

[7] Juliane L, Victor S, Johannes H, Christoph G. Jak-Stat signaling induced by interleukin-6 family cytokines in hepatocellular carcinoma. Cancers (Basel). 2019;11(11): 1704. DOI: 10.3390/cancers11111704.

[8] Liqun Y, Pengfei S, Gaichao, Zhao G, Xu J, Peng W, Zhang J, Zhang G,Wang X, Dong Z, Chen F and Cui H. Targeting cancer stem cell pathways for cancer therapy. Signal Transduct Target Ther. 2020; 5: 8. DOI:10.1038/s41392.020.0110-5.

[9] Wook J. Role of JAK/STAT3 Signaling in the regulation of metastasis, the transition of cancer stem cells, and chemoresistance of cancer by epithelial–mesenchymal transition. Cells 2020; 9(1): 217. DOI:10.3390/cells9010217.

[10] Chikuma S, Kanamori M, Mise-Omata S, Yoshimura A. Suppressors of cytokine signaling: potential immune checkpoint molecules for cancer immunotherapy. Cancer 2017; 108:574–580. DOI:10.1111/cas.13194.

[11] Daniel EJ, Rachel A. O'K, Jennifer RG. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol. 2018; 15(4): 234–248. DOI:10.1038/nrclinonc.2018.8.

[12] DiLillo DJ, Weinberg JB, Yoshizaki A, Horikawa M, Bryant JM, Iwata Y. Chronic lymphocytic leukemia and regulatory B cells share IL-10 competence and immunosuppressive function. Leukemia 2013; 27(1):170-182. DOI: 10.1038/leu.2012.165.

[13] Bewarder M, Stilgenbauer S, Thurner L, Kaddu-Mulindwa D. Current treatment options in CLL. Cancers (Basel). 2021; 13(10): 2468. DOI:10.3390/cancers13102468.

[14] Alkan S. Kronik lenfositik lösemi (KLL): patogenezi ve biyolojisi. Türk Hematoloji Derneği. http://www.thd.org.tr/thdData/userfiles/file/kroniklenfositik.pdf7. 2006. (Turkish)

[15] Gupta R, Garg N. and Singh A. Anti-globulin test positivity indicates advanced disease in Indian CLL patients. Am J Blood Res. 2021; 11(2): 157–162.

[16] Liu Y. Liao S, Bennett S, Tang H, Song D, Wood D, Zhan X, and Xu J. STAT3 and its targeting inhibitors in osteosarcoma. Cell Prolif. 2021; 54(2): e12974. DOI: 10.1111/cpr.12974.

[17] Zhao J, FenQi Y, RongYu Y. STAT3: A key regulator in liver fibrosis. Annals of Hepatology 2021;100224. DOI:10.1016/j.aohep.2020.06.010.

[18] Antosz H, Wojciechowska K, Sajewicz J, Choroszynska D, Marzec-Kotarska B, Osiak M. IL-6, IL-10, c-Jun and STAT3 expression in B-CLL. Blood Cells Mol Dis. 2015; 54(3):258-265. DOI:10.1016/j.bcmd.2014.11.006.

[19] Gary-Gouy H, Harriague J, Bismuth G, Platze, C, Schmitt C, Dalloul AH. Human CD5 promotes B-cell survival through stimulation of autocrine IL-10 production. Blood 2002; 100:4537-4543. DOI:10.1182/blood-2002-05-1525.

How to cite this article: Özcan Ö, Gelmez YM, Çınar S, Deniz G, Aktan M. Intracellular Levels of IL-10 and STAT3 in Patients with Chronic Lymphocytic Leukemia. Clin Exp Health Sci 2023; 13: 99-104. DOI: 10.33808/clinexphealthsci.1056727

Clinical and Experimental Health Sciences

Investigation of the Radiological Status of First Permanent Molars in a Turkish Subpopulation

Ali Altındağ, Fatma Yüce, Güldane Magat

Necmettin Erbakan University, Faculty of Dentistry, Department of Dentomaxillofacial Radiology, Konya, Türkiye.

Correspondence Author: Ali Altındağ
E-mail: aaltindag@erbakan.edu.tr
Received: 17.01.2022 **Accepted:** 29.04.2022

ABSTRACT

Objective: Missing or filled first permanent molars (FPMs) affects occlusal status and oral health. This study aims to determine the prevalence of missing, restored FPMs (filling, root canal treatment, prosthetic restoration), and dental implants in the first molar region.

Methods: In our study, panoramic radiographs of 1765 female and 1230 male patients were evaluated. Patients were divided into three age groups: 15-34 years old, 35-54 years old, and >55 years old. The maxillary and mandibular FPMs status is divided into two groups presence (caries, filled, root treatment, prosthetic restoration, root treatment + prosthetic restoration, and healthy) or absence (empty, radix relicta, dental implant, and dental bridge).

Results: In the evaluated images, 36.92% of maxillary FPMs and 27.39% of mandibular FPMs were healthy. FPMs on the right or left side of the jaws did not affect their clinical status, while their presence on the lower or upper jaw affected their clinical status.

Conclusion: Most of the FPMs (67.85%) were with caries or restorations. The number of healthy FPMs decreases with increasing age. The study indicates an important result regarding protecting FPMs and preventive dentistry.

Keywords: First permanent molar, panoramic radiography, health status

1. INTRODUCTION

As in all countries around the world, oral and dental diseases are one of the leading health problems in our country. However, the health service potential of countries can not meet all the needs of the society. Therefore, the World Health Organization (WHO) stated that preventive dentistry practices, therapeutic practices, and sometimes preventive services should be prioritized and emphasized to reduce the prevalence of oral and dental diseases (1-3). Tooth decay, one of the oral and dental diseases, is now considered the second most common disease after the common cold (1, 4).

Many factors must come together for tooth decay to occur. Although factors such as diet, microorganisms, tooth morphology, and saliva are influential in forming dental caries, it has been stated that environmental, cultural, and social factors are also effective (5-7). The presence of first permanent molars (FPMs) in the mouth is significant as they are more effective in chewing with their large occlusal surface that plays a crucial role in occlusion and coordinates the horizontal, anterior-posterior, and transverse growth of both jaws, facial development and facial height (6, 7).

FPMs are more prone to dental caries and the most frequently extracted teeth due to caries (8, 9). Being the first permanent tooth to erupt in the mouth, being adjacent to primary teeth for a long time, having recesses, pits, and fissures in its morphological structure, and insufficient cleaning of the posterior teeth in childhood cause a high prevalence of caries in FPMs (6, 10, 11).

Panoramic radiography is a technique that provides a single image of the maxillary and mandibular arches and their supporting facial structures and is widely used to evaluate oral health. The main advantage of panoramic radiography is less exposure time to radiation than cone-beam computed tomography (CBCT). The reliability of panoramic radiography has been evaluated based on CBCT images by several authors, who suggest that panoramic radiography is an invaluable tool with the best cost-information ratio (12, 13). In addition, CBCT may not be available in clinics, despite its

DOI: 10.33808/clinexphealthsci.1058955

advantages such as providing 3D imaging, high resolution, and no magnification (14).

It is known that patients' dentist visits are mainly for emergencies and pain relief, not for preventive medicine. Therefore, this study aims to emphasize the importance of preventive medicine by radiologically determining the prevalence of FPMs in a Turkish subpopulation in terms of missing, dental implants, and restoration (filling, root canal treatment, prosthetic rehabilitation).

2. METHODS

The Research Ethics Committee of Necmettin Erbakan University approved this study(approval date and number: 29.04.2021; 2021/04-50) which was conducted according to the guidelines of the Declaration of Helsinki. All patients signed informed consent.

This retrospective study evaluated the panoramic radiographs of patients who applied to Necmettin Erbakan University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology with various dental problems between January 2020 and March 2021. A total of 9850 FPMs (2610 upper right FPMs; 2538 upper left FPMs; 2368 lower left FPMs; 2334 lower right FPMs) in 2995 panoramic radiographs were examined.

Within the scope of the study, panoramic radiographs acquired from individuals without systemic disease were included. In the evaluated radiographs, attention was paid to the fact that the root apex of the teeth was closed, there was no pathology associated with the teeth, and the image was clear and free of artifacts. First, FPMs were classified as present or absent. If the FPMs were present, it was categorized as healthy, decayed, filled, root treatment (RT), prosthetic restoration (PR), root treatment, and prosthetic restoration (RT+PR) (**Figure 1**). Missing FPM cases were grouped as empty, radix relicta, dental implant, and pontic.

All the panoramic images were obtained with the Morita Veraviewepocs 3D R100-P (J Morita MFG Corp., Kyoto, Japan). The acquisition protocol was chosen following the manufacturer's instructions and was determined as 6 mA, 66 kVp, and 7.5 sec. Images were analyzed by two examiners (maxillofacial radiologists with 2 and 7 years of experience) on an LCD monitor under ambient light. After an inter-observer consensus, the final classification was recorded.

Statistical Package for the Social Science (SPSS) (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, IBM Corp., Armonk, NY, USA) software was used for statistical analysis. Descriptive statistics were calculated for all parameters in the study. The chi-square test was used to determine the relationships between categorical variables, and it was considered significant at the $p < .05$ level.

3. RESULTS

A total of 2995 patients (1765 female and 1230 male) between the ages of 15 to 81 (34.42 ± 12.44) were evaluated in this study. Regarding gender, the prevalence of no missing FPMs was 33.9% for females and 22% for males (Table 1). In the 31-55 age group, the number of tooth loss was higher than in other groups ($p < .05$). However, no statistically significant difference was found between the gender and missing FPM ($p > .05$) (Table 1).

Of the total study sample, 13.4% of patients had endodontically and prosthodontically restored upper right FPMs (316 RT, 84 RT + PR), this rate was 13.3%, 14.1%, and 14% for upper left, lower left, and lower right FPMs, respectively (Table 2).

For teeth #16, #26, #36, and #46, healthy teeth prevalence rates in patients aged 15-34 were 43.30%, 40.64%, 30.04% and 30.35%, respectively (Table 3). When evaluated according to age groups, there was a decrease in the frequency of healthy teeth in the advanced age group ($p<.05$). (Table 3). The prevelance of patients with healthy teeth in the 55+ age range was 27.03%, 28.75%, 22.31%, and 21.45% for teeth 16, 26, 36, and 46, respectively.

The missing teeth in the lower jaw were found to be 20.56% (16.60% single tooth, 3.96% two teeth) in women and 27.72% (20.57% single tooth, 7.15% two teeth) in men. In the upper jaw, it was found to be 32.80% (21.81% single tooth, 10.99% two teeth) in women and 32.19% (22.52% single tooth, 9.66% two teeth) in men (Table 4). The difference in missing teeth in the upper jaw by gender was not statistically significant ($p > .05$), this difference was statistically significant in the lower jaw ($p < .001$). Missing teeth rate increases with age in both jaws and a statistically significant difference was found between age groups ($p < .05$) (Table 4).

According to the results of our study, an increase in the prevalence of missing teeth, dental implants, or dental bridges was observed in advanced age groups compared to other age groups ($p < .01$) (Table 5).

32.65% of the right maxillary and mandibular teeth were healthy, and 31.66% of the left maxillary and mandibular teeth were found to be healthy. There was no statistically significant relationship between radiological tooth conditions and the right-left position of the tooth ($p > .05$) (Table 6). 36.92% of maxillary FPMs were healthy, and 27.39% of mandibular FPMs were found to be healthy. There is a statistically significant difference between the presence of permanent first molars in the maxilla or mandible and its radiological status ($p < .001$) (Table 6).

Table 1. *Frequency of teeth missing according to age-groups and gender*

		No Missing	One Missing	Two Missing	Three Missing	Four Missing	Total	p value
Gender								
Female	n	1016	389	265	93	2	1765	.293
	%	33.9	13	8.8	3.1	0.1	58.9	
Male	n	659	295	200	75	1	1230	
	%	22	9.8	6.7	2.5	0.01	41.1	
Total	n	1675	684	465	168	3	2995	
	%	55.9	22.8	15.5	5.6	0.1	10000%	
Age								
15-34	n	1206	293	94	21	0	1614	**.000****
	%	40.3	9.8	3.1	0.7	0%	53.9	
35-54	n	428	340	287	91	2	1148	
	%	14.3	11.4	9.6	3	0.1	38.3	
55+	n	41	51	84	56	1	233	
	%	1.4	1.7	2.8	1.9	0	7.8	
Total	n	1675	684	465	168	3	2995	
	%	55.9	22.8	15.5	5.6	0.1	10000%	

*n: Number, %: Percent, **: p<.01*

Table 2. *Radiological situations for first permanent molars according to the gender*

Tooth Number	Gender		Caries	Filled	Root Treatment (RT)	Porcelain Restoration (PR)	RT + PR	Healthy	Empty	Total	p value
16	Female	n	209	389	171	65	52	687	192	1765	**.000****
		%	7	13	5.7	2.2	1.7	22.9	6.4	58.9	
	Male	n	161	208	145	51	32	440	193	1230	
		%	5.4	6.9	4.8	1.7	1.1	14.7	6.4	41.1	
	Total	n	370	597	316	116	84	1127	385	2995	
		%	12.4	19.9	10.6	3.9	2.8	37.6	12.9	100	
26	Female	n	205	364	190	58	51	656	241	1765	**.017***
		%	6.8	12.2	6.3	1.9	1.7	22.9	6.4	58.9	
	Male	n	169	216	119	42	39	429	216	1230	
		%	5.6	7.2	4	1.4	1.3	14.3	7.2	41.1	
	Total	n	374	580	309	100	90	1085	457	2995	
		%	12.5	19.4	10.3	3.3	3	36.2	15.3	100	
36	Female	n	242	419	203	36	46	445	374	1765	**.016***
		%	8.1	14	6.8	1.2	1.5	14.9	12.5	58.9	
	Male	n	167	240	128	31	44	367	253	1230	
		%	5.6	8	4.3	1	1.5	12.3	8.4	41.1	
	Total	n	409	659	331	67	90	812	627	2995	
		%	13.7	22	11.1	2.2	3	27.1	20.9	100	
46	Female	n	210	382	203	54	57	460	399	1765	**.02***
		%	7	12.8	6.8	1.8	1.9	15.4	13.3	58.9	
	Male	n	181	228	126	32	32	369	262	1230	
		%	6	7.6	4.2	1.1	1.1	12.3	8.7	41.1	
	Total	n	391	610	329	86	89	829	661	2995	
		%	13.1	20.4	11	2.2	3	27.7	22.1	100	

*n: Number, %: Percent, **: p<0.01,*

16: Right Superior First Molar Tooth, 26: Left Superior First Molar Tooth, 36: Left Inferior First Molar Tooth, 46: Right Inferior First Molar Tooth

Table 3. *Radiological situations for first permanent molars according to the age groups*

Tooth Number	Age Groups		Caries	Filled	Root Treatment (RT)	Porcelain Restoration (PR)	RT + PR	Healthy	Empty	Total	p value
16	15-34	n	255	353	184	17	31	699	75	1614	.000**
		%	8.5	11.8	6.1	0.6	0.1	23.3	2.5	53.9	
	35-54	n	102	226	119	69	47	365	220	1148	
		%	3.4	7.5	4	2.3	1.6	12.2	7.3	38.3	
	55+	n	13	18	13	30	6	63	90	233	
		%	0.4	0.6	0.4	0.1	0.2	2.1	3	7.8	
	Total	n	370	597	316	116	84	1127	385	2995	
		%	12.4	19.9	10.6	3.9	2.8	37.6	12.9	100	
26	15-34	n	276	325	182	13	28	680	110	1614	.000**
		%	9.2	10.9	6.1	0.4	0.9	22.7	3.7	53.9	
	35-54	n	83	234	121	65	51	338	256	1148	
		%	2.8	7.85	4	2.2	1.7	11.3	8.5	38.3	
	55+	n	15	21	6	22	11	67	91	233	
		%	0.5	0.7	0.2	0.7	0.4	2.2	3	7.8	
	Total	n	374	580	309	100	90	1085	457	2995	
		%	12.5	19.4	10.3	3.3	3	36.2	15.3	100	
36	15-34	n	289	417	210	8	30	485	175	1614	.000**
		%	9.6	13.9	7	0.3	1	16.2	5.8	53.9	
	35-54	n	101	222	110	39	50	275	351	1148	
		%	3.4	7.4	3.7	1.3	1.7	9.2	11.7	38.3	
	55+	n	19	20	11	20	10	52	101	233	
		%	0.6	0.7	0.4	0.7	0.3	1.7	3.4	7.8	
	Total	n	409	659	331	67	90	812	627	2995	
		%	13.7	22	11.1	2.2	3	27.1	20.9	100	
46	15-34	n	311	385	201	9	34	490	184	1614	.000**
		%	10.4	12.9	6.7	0.3	1	16.4	6.1	53.9	
	35-54	n	68	206	114	57	46	289	368	1148	
		%	2.3	6.9	3.8	1.9	1.5	9.6	12.3	38.3	
	55+	n	12	19	14	20	9	50	109	233	
		%	0.4	0.6	0.5	0.7	0.3	1.7	3.6	7.8	
	Total	n	391	610	329	86	89	829	661	2995	
		%	13.1	20.4	11	2.2	3	27.7	22.1	100	

*n: Number, %: Percent, **: p<0.01,*

16: Right Superior First Molar Tooth, 26: Left Superior First Molar Tooth, 36: Left Inferior First Molar Tooth, 46: Right Inferior First Molar Tooth

Table 4. *Distribution of tooth loss in maxilla and mandible by gender and age*

			No Missing	One Missing	Two Missing	Total	p value
Maxilla	**Gender**						
	Female	n	1186	385	194	1765	.495
		%	39.6	12.9	6.5	58.9	
	Male	n	834	277	119	1230	
		%	28	9.2	4	41.1	
	Total	n	2020	662	313	2995	
		%	67.4	22.1	10.5	100.0	
	Age						
	15-34	n	1315	239	60	1614	**.000****
		%	43.9	8	2	53.9	
	35-54	n	618	341	189	1148	
		%	20.6	11.4	6.3	38.3	
	55+	n	87	82	64	233	
		%	2.9	2.7	2.1	7.8	
	Total	n	2020	662	313	2995	
		%	67.4	22.1	10.5	100.0	
Mandibula	**Gender**						
	Female	n	1402	293	70	1765	**.000****
		%	46.8	9.8	2.3	58.9	
	Male	n	889	253	78	1230	
		%	30	8.4	2.6	41.1	
	Total	n	2301	546	148	2995	
		%	76.8	18.2	4.9	100.0	
	Age						
	15-34	n	1449	146	19	1614	**.000****
		%	48.4	4.9	0.7	53.9	
	35-54	n	759	301	88	1148	
		%	25.3	10.1	2.9	38.3	
	55+	n	93	99	41	233	
		%	3.1	3.3	1.4	7.8	
	Total	n	2301	546	148	2995	
		%	76.8	18.2	4.9	100.0	

*n: Number, %: Percent, **: p<.01*

Table 5. *Cross-table of radiological situations in missing teeth by gender and age groups*

		No Missing	Empty	Radix Relicta	Dental Implant	Dental Bridge	Total	p value
Gender								
Female	n	5854	736	22	49	399	7060	
	%	82.9	10.4	0.3	0.7	5.7	100.0	
Male	n	3996	582	32	32	278	4920	**.008****
	%	81.2	11.1	0.7	0.7	5.7	100.0	
Total	n	9850	1318	54	81	677	11980	
	%	82.2	11.0	0.5	0.7	5.7	100.0	
Age								
15-34	n	5912	442	31	9	62	6456	
	%	91.6	6.8	0.5	0.1	1.0	100.0	
35-54	n	3397	716	19	39	421	4592	
	%	74.0	15.6	0.4	0.8	9.2	100.0	**.000****
55+	n	541	160	4	33	194	932	
	%	58.0	17.2	0.4	3.5	20.8	100.0	
Total	n	9850	1318	54	81	677	11980	
	%	82.2	11.0	0.5	0.7	5.7	100.0	
*n: Number, %: Percent, **: p<.01*								

Table 6. *Cross table of first molars for jaws and right-left sides*

		Radiological Status							
		Caries	Filled	Root Treatment (RT)	Porcelain Restoration (PR)	RT + PR	Healthy	Empty	Total
Maxilla	Right	370	597	316	116	84	1127	385	2995
	Left	374	580	309	100	90	1085	457	2995
Mandible	Right	391	610	329	86	89	829	661	2995
	Left	409	659	331	67	90	812	627	2995
Total		1544	2446	1285	369	353	3853	2130	11980
Right-Left Jaw p=.536									
*Maxilla-mandible p=.00007***									
RT+PR: Root Treatment + Porcelain Restoration									

4. DISCUSSION

This retrospective study, which was carried out using the data of Necmettin Erbakan University Faculty of Dentistry, especially focused on FPMs. Because these teeth play an important role in the dental and general health of the individual. The FPMs are the first permanent teeth to erupt in the mouth and have an important role in the alignment of both anterior and posterior teeth that will erupt later, as the other teeth are positioned relative to the previously erupted first molar and come into occlusion. The FPMs are also the largest tooth in the oral cavity and bear the greatest occlusal load. It affects the vertical distance, occlusal height, and aesthetic ratios between the maxilla and mandible. Considering these, an evaluation of the health status of these teeth provides us with sufficient information about the oral health of a population (15).

It is important to protect these teeth, especially since the early loss of FPMs causes many problems such as asymmetry of teeth, deterioration of occlusal relations, and lack of space in the jaws. In addition, determining the number of patients who lost their teeth early in the community will be useful in determining treatment plans and health policies (16). In the population studied, at least one FPM deficiency was found to be 57.56% in females and 53.57% in males. In the 15-34 age group, at least one missing tooth was determined as 25.27%. This rate was determined by Rezaie et al (5) found to be 40% in their study, while İncebeyaz et al (7) similar to our study, it was found it to be 55% in both women and men. Özmen (16),

on the other hand, found that at least one FPM deficiency was 9.70% in women and 6.37% in men, in a study performed on a population aged 7-17 years. The differences between the results may be due to the different sample numbers and age groups, and the differences in protective measures based on countries. In addition, the lack of FPM in this study was evaluated in a wide age range and a large sample size.

Dental implants are a very common treatment option for partial or complete edentulism for a long time (15). In the 55+ age group, 3.5% of the FPM tooth loss was treated with an implant, while 20.8% was treated with fixed prosthetic restoration. The rate of those who did not receive any treatment was found to be 17.2%. In the 35-54 age group, the rate of treatment with implants is 0.8%, while the rate of fixed restoration is 9.2%. In the study of Aksoy et al (15) on the preference for implant restorations in the Turkish population, covering all oral regions, the rate of patients over the age of 60 was found to be 23%. Considering the results of this study and other studies in the literature (7,8), it was observed that the incidence of those who provided treatment for missing teeth was low. Considering the possible consequences of missing teeth, patients should be informed about the complication of missing teeth and should be referred for any prosthetic treatment.

Root canal treatment aims to prevent the spread of the disease in the pulpal tissues to the periapical tissues and to treat the existing periapical disease. When endodontic treatment is applied to teeth that can be restored in terms of tissue loss, the relevant tooth does not need to be extracted, and aesthetic and functional loss is prevented by keeping the tooth in the mouth (17). In the present study, the rate of root canal treatment in FPMs was 13.4%, 13.3%, 14.1%, and 14% for teeth 16, 26, 36, and 46, respectively. In the study of Çobankara et al (18), in which they examined the incidence of root canal treatment according to age groups, they found that 35% of FPMs had root canal-treated teeth. While the permanent teeth in the upper jaw constitute 17% of this rate, the permanent teeth in the lower jaw constitute 18%. In an epidemiological study conducted in Taiwan (19), endodontic treatment was detected in 27% of all molars. There is a limited number of studies in the literature on this subject. However, the results of these two studies show that the rates of root canal treatment are also low. Therefore, it is important to increase the patient population with teeth in the mouth over time and to raise awareness of the patients about keeping their teeth in the mouth instead of the prosthetic approach.

In the literature, it has been stated that the incidence of caries is higher in these teeth because the depth of the sulci in the occlusal morphology of FPMs is suitable for food retention and the eruption times coincide with the primary dentition period. Another factor is the earlier eruption of FPMs compared to maxillary molars, which has been argued to be effective in higher tooth loss (7). İncebeyaz et al (7) found an FPMs deficiency with a rate of 38.6% in the upper jaw and 43.9% in the lower jaw. In the present study, contrary to their study, a higher rate of missing teeth was found in the upper jaw (32.55% in the upper jaw, 23.17% in the lower jaw). The difference between these studies may be due to the different age ranges evaluated and the number of samples.

In the literature, the frequency of dental caries is from most to low; lower and upper FPMs, lower and upper second molars, upper second premolars, lower second premolars, upper first premolars, lower first premolars, upper central and lateral teeth, upper and lower canines and lower incisors have been reported (20). FPMs being the first permanent teeth, early exposure to caries attacks and fissure morphology are the predisposing and important factors for caries formation (4). Although only the FPMs were examined in the present study, only 32.15% of the detected teeth were healthy. The lower rate (67.85%) of actively caries or processed teeth compared to other studies (21, 22) (78.3% and 74.4%) can be thought to be due to social differences, examination of FPMs only, and initial caries not showing on radiography.

In the present study, a statistically significant relationship was found between gender and dental status. Both decayed and filled or root canal-treated teeth are relatively more common in women. In previous studies, it has been reported that more caries are seen in women than in men, which is consistent with our study (20-22). It was thought that the early onset of permanent dentition in women, the effect of hormonal changes on the oral state, and the early onset of hormonal changes in women and biological differences were effective in the high incidence of caries (22-24).

According to the study's results, the presence of FPMs on the right-left side is not a significant variable. In contrast, their presence in the maxilla or mandible is an important parameter affecting the status of the teeth. In studies conducted with different age groups, it has been reported that the right or left side of the teeth is not effective in the formation of caries (20-25). Manji and Fejerskov (26) reported that mandibular molars are the most affected teeth in all teeth, being affected more frequently than upper molars. However, in the study by Demirci et al (27) and Zemaitene et al (22), it was reported that maxillary teeth develop more caries than mandibular teeth. Although there is no consensus among researchers, in the present study, it was determined that mandibular FPMs were affected and processed (filled, root treatment, porcelain treatment, extracted) more than the FPMs in the opposite jaw.

The present study found that mandibular FPMs showed a higher caries prevalence than maxillary FPMs. These findings were consistent with the previous studies (28,29). This can be attributed to various factors such as more complex pit and fissure morphology of mandibular molars, earlier eruption, and different salivary effects (30).

Duman et al. (31) evaluated the status of 5996 molar teeth in the radiology records of 1499 pediatric patients. It was determined that 45.7% of the FPMs were healthy, and 54.3% required treatment. Consistent with the results of our study, it was determined that the rate of healthy teeth decreased with age ($p < .001$). Similar to the present study's results,

boys were found to have more healthy FPMs than girls in their study. In the literature, studies conducted in similar age groups indicate that the FPMs of girls are more affected, and this is because girls enter puberty before boys, and FPMs erupt earlier in girls than boys (32). In line with the results of the present study, it was observed that the FPMs in the maxilla were less treated than the FPMs in the mandible.

Gjermo et al. (33) and Halıcıoğlu et al. (34) evaluated FPMs extractions. Contrary to the results of the present study, it was observed that the number of teeth extracted was higher in the mandible than in the maxilla and that the number of teeth was higher in boys than in girls. These differences may be due to the samples's different ethnic origins and different age distribution.

The present study was carried out retrospectively on panoramic images. To evaluate the health status of the tooth holistically, it should also be evaluated clinically. Therefore, future studies should be conducted on a larger sample size by evaluating radiological and clinical findings together.

5. CONCLUSION

The following conclusions were reached with the findings of the study:

- 67.85% of permanent first molars consist of decayed or treated teeth.
- The incidence of healthy teeth appearance in permanent first molars decreases with age.
- The presence of teeth on the right or left side does not affect the clinical status of the FPMs, however, their presence in the maxilla or mandible affects their clinical status.

This study reveals that preventive practices and oral hygiene motivations are important in reducing the need for restoration, extraction, dental implants, and dental bridges, and ensuring the continuity of stomatognathic systems, especially in elderly individuals.

REFERENCES

[1] Balkaya B, Aydemir H. Birinci büyük azı dişlerin çürük, eksiklik ve dolgu dağılımı. Atatürk Univ Diş Hek Fak Derg. 2000;10(1):17-20. (Turkish) https://dergipark.org.tr/tr/download/article-file/27776

[2] Hescot P, Bourgeois D, Doury J. Oral health in 35–44 year old adults in France. Int Dent J. 1997;47(2):94-99. DOI: 10.1111/j.1875-595X.1997.tb00682.x

[3] Yılmaz AB, Ceylan G, Yanıkoğlu N, Akgül M. Atatürk Üniversitesi Diş Hekimliği Fakültesi öğrencilerinde ağız sağlığı çalışması ve diş hekimliği eğitiminin ağız sağlığına etkisinin araştırılması. Atatürk Üniv Diş Hek Fak Derg 1997;7(1):36-39. (Turkish) https://dergipark.org.tr/en/download/article-file/27902

[4] Bulucu B, İnan AGU. Daimi birinci molar dişin çürük deneyimi, çürük risk tahmin modeli olabilir mi? Atatürk Üniv Diş Hek Fak Derg. 2005;15(3):5-10. (Turkish) https://dergipark.org.tr/en/download/article-file/27564

[5] Shyam R, Manjunath BC, Kumar A, Narang R, Goyal A, Piplani A. Assessment of dental caries spectrum among 11 to 14-year-old school going children in India. J Clin Diagn Res. 2017;11(6): ZC78. DOI: 10.7860/JCDR/2017/27254.10107

[6] Al-Hadi Hamasha A, Sasa I, Al Qudah M. Risk indicators associated with tooth loss in Jordanian adults. Community Dent Oral Epidemiol. 2000;28(1):67-72. DOI: 10.1034/j.1600-0528.2000.280109.x

[7] İncebeyaz B, Deniz HA, Polat E, Kolsuz ME. Birinci daimi molar dişlerin yaş ve cinsiyete göre klinik durumlarinin değerlendirilmesi. Ankara Üniv Diş Hek Fak Derg. 2019;46(3):137-144. (Turkish) https://dergipark.org.tr/en/download/article-file/1590337

[8] Cheng R-B, Tao W, Zhang Y, Cheng M, Li Y. Analysis of the first permanent molar caries epidemiological investigation in area of northeast China. Hua Xi Kou Qiang Yi Xue Za Zhi (West China J Stomatol). 2008;26(1):73-76. PMID: 18357890

[9] Maden DEA, Altun C. Çocuk dişhekimliğinde kötü prognozlu daimi birinci molarların çekim endikasyonları ve klinik değerlendirmeleri. Atatürk Üniv Diş Hek Fak Derg 2013;23(3):408-413. (Turkish) https://dergipark.org.tr/en/download/article-file/27139

[10] Gülhan A. Pedodonti. İstanbul, İstanbul Üniversitesi Rektörlüğü Basımevi ve Film Merkezi;1994. (Turkish)

[11] Karabekiroğlu S, Ünlü N. Yüksek Çürük Riskli Genç Yetişkinlerde Diş Çürüğüne Ait Temel Parametrelerin Değerlendirilmesi. Ege Üniv Diş Hek Fak Derg. 2014;35(2):26-31. (Turkish) DOI: 10.5505/eudfd.2014.03274

[12] Nakayama K, Nonoyama M, Takaki Y, Kagawa T, Yuasa K, Izumi K, Ozeki S, Ikebe T. Assessment of the relationship between impacted mandibular third molars and inferior alveolar nerve with dental 3-dimensional computed tomography. J Oral Maxillofac Surg. 2009;67(12):2587-2591. DOI: 10.1016/j.joms.2009.07.017

[13] Opydo-Szymaczek J, Gerreth K. Developmental enamel defects of the permanent first molars and incisors and their association with dental caries in the region of Wielkopolska, Western Poland. Oral Health Prev Dent. 2015;13(5):461-469. DOI: 10.3290/j.ohpd.a33088

[14] Fuentes R, Farfán C, Astete N, Navarro P, Arias A. Distal root curvatures in mandibular molars: analysis using digital panoramic X-rays. Folia Morphol. 2018;77(1):131-137. DOI: 10.5603/FM.a2017.0066

[15] Aksoy MC, Kocer G, Kucukesmen HC, Eroglu E, Senturk MF. Distribution and pattern of implant therapy in a part of the Turkish population. J Pak Med Assoc. 2016;66(10):1277-1280. PMID: 27686303

[16] Ozmen B. Evaluation of permanent first molar tooth loss in young population from North Turkey. Balk J Dent Med. 2019;23(1):20-23. DOI: 10.2478/bjdm-2019-0004

[17] Salehrabi R, Rotstein I. Endodontic treatment outcomes in a large patient population in the USA: an epidemiological study. J Endod. 2004;30(12):846-850. DOI: 10.1097/01.don.000.014.5031.04236.ca

[18] Cobankara F, Orucoglu H. Kök kanal tedavisi insidansının yaş, cinsiyet ve diş gruplarına göre incelenmesi. Cumhuriyet Üniv Diş Hek Fak Derg 2004;7:20-24.

[19] Chen SC, Chueh LH, Hsiao CK, Tsai MY, Ho SC, Chiang CP. An epidemiologic study of tooth retention after nonsurgical endodontic treatment in a large population in Taiwan. J Endod. 2007;33(3):226-229. DOI: 10.1016/j.joen.2006.11.022

[20] Macek MD, Beltran-Aguilar ED, Lockwood SA, Malvitz DM. Updated comparison of the caries susceptibility of various

morphological types of permanent teeth. J Public Health Dent. 2003;63(3):174-182. DOI: 10.1111/j.1752-7325.2003.tb03496.x

[21] García-Cortés JO, Medina-Solís CE, Loyola-Rodriguez JP, Mejía-Cruz JA, Medina-Cerda E, Patiño-Marín N, Pontigo-Loyola AP. Dental caries' experience, prevalence and severity in Mexican adolescents and young adults. Rev Salud Publica. 2009;11(1):82-91. PMID: 19721982

[22] Žemaitienė M, Grigalauskienė R, Vasiliauskienė I, Saldūnaitė K, Razmienė J, Slabšinskienė E. Prevalence and severity of dental caries among 18-year-old Lithuanian adolescents. Medicina. 2016;52(1):54-60. DOI: 10.1016/j.medici.2016.01.006

[23] Dodds MW, Johnson DA, Yeh CK. Health benefits of saliva: a review. J Dent. 2005;33(3):223-233. DOI: 10.1016/j.jdent.2004.10.009

[24] Lukacs JR, Largaespada LL. Explaining sex differences in dental caries prevalence: Saliva, hormones, and "life-history" etiologies. Am J Hum Biol. 2006;18(4):540-555. DOI: 10.1002/ajhb.20530

[25] Wyne AH. The bilateral occurrence of dental caries among 12-13 and 15-19 year old school children. J Contemp Dent Pract. 2004;5(1):42-52. PMID: 14973559

[26] Manji F, Fejerskov O. An epidemiological approach to dental caries; in Thylstrup A, Fejerskov O (eds): Textbook of Clinical Cariology. Copenhagen: Munksgaard; 1994. p. 167-168.

[27] Demirci M, Tuncer S, Yuceokur AA. Prevalence of caries on individual tooth surfaces and its distribution by age and gender in university clinic patients. Eur J Dent. 2010;4(03):270-279. DOI: 10.1055/s-0039.169.7839

[28] Sanchez-Perez L, Irigoyen-Camacho ME, Molina-Frechero N, Zepeda-Zepeda M. Fissure depth and caries ıncidence in first permanent molars: A five-year follow-up study in schoolchildren. Int J Environ Res Public Health. 2019;16(19):3550. DOI: 10.3390/ijerph16193550

[29] Shaffer JR, Wang X, Desensi RS, Wendell S, Weyant RJ, Cuenco KT, Court R, McNeil DW, Marazita ML. Genetic susceptibility to dental caries on pit and fissure and smooth surfaces. Caries Res 2012;46(1):38-46. DOI: 10.1159/000335099

[30] Demirbuga S, Tuncay O, Cantekin K, Cayabatmaz M, Dincer AS, Kılınç HI, Sekerci AE. Frequency and distribution of early tooth loss and endodontic treatment needs of permanent first molars in a Turkish pediatric population. Eur J Dent. 2013;7(1):99-104. DOI: 10.4103/1305-7456.119085

[31] Duman S, Duruk G. The evaluation of the clinical and radiographic records of the first molar teeth in pediatric patients. Ann Med Res. 2019;26(10):2333-2339. DOI: 10.5455/annalsmedres.2019.08.490

[32] Poureslami P, Pouradeli S, Poureslami H, Shahrokhi E. Evaluation of health status of first permanent molar teeth among 12-year-old students in rural areas of south of Kerman, Iran, 2016. J Oral Health Oral Epidemiol 2018;7(1):33-38. DOI: 10.22122/johoe.v7il.391

[33] Gjermo P, Beldi MI, Bellini HT, Martins CR. Study of tooth loss in an adolescent Brazilian population. Community Dent Oral Epidemiol. 1983;11(6):371-374. DOI: 10.1111/j.1600-0528.1983.tb01394.x

[34] Halicioglu K, Toptas O, Akkas I, Celikoglu M. Permanent first molar extraction in adolescents and young adults and its effect on the development of third molar. Clin Oral Invest. 2014;18(5):1489-1494. DOI: 10.1007/s00784.013.1121-1

How to cite this article: Altındağ A, Yüce F, Magat G. Investigation of the Radiological Status of Permanent First Molars in a Turkish Subpopulation. Clin Exp Health Sci 2023; 13: 105-113. DOI: 10.33808/clinexphealthsci.1058955

Clinical and Experimental Health Sciences

Knowledge and Attitudes of Pregnant Women with and without Children about Fluoride and Herbal Toothpastes

Zeynep Ceren Çelik[1], Çiğdem Elbek Çubukçu[2], Halil Çelik[3], Gül Dinç Ata[1]

[1] Department of Restorative Dentistry, Faculty of Dentistry, Bursa Uludag University, Bursa, Türkiye.
[2] Department of Pedodontics, Faculty of Dentistry, Bursa Uludag University, Bursa, Türkiye.
[3] Department of Periodontology, Faculty of Dentistry, Istinye University, İstanbul, Türkiye.

Correspondence Author: Zeynep Ceren Çelik
E-mail: zeynepceren@uludag.edu.tr
Received: 18.01.2022 **Accepted:** 01.09.2022

ABSTRACT

Objective: Objective: Pregnant women may be vulnerable to dental caries due to their inability to fully implement oral hygiene practices. Toothpastes are main component of oral hygiene and the most important tool for the primary prevention of caries. The study aimed to examine the knowledge and attitudes of pregnant women with children (PC) and without children (PNC) about fluoride and herbal toothpastes.

Methods: A self-administered and validated 20-item questionnaire was completed by a total of 219 pregnant participants, 85 PC and 134 PNC. Statistical analyses were performed using the SciPy v1.2.3. program.

Results: Most of the PC (57.65%; 69.41%) and PNC (72.39%; 47.76%) participants responded with "no idea" when asked about fluoride sources and the optimal amount of fluoride added to tap water by local health authorities (p= .006). The majority of the PC (62.4%) and the PNC (47.0%) had no preference for herbal toothpastes during pregnancy (p= .03). In addition, 86.6% of the PNC showed low knowledge about the non-fluoride content of herbal toothpastes (p= .023). While 51.5% of the PNC responded with "no idea" about a preference for herbal toothpastes for their children, 56.47% of the PC stated that they might not prefer using herbal toothpastes for their children's routine oral hygiene.

Conclusion: The findings show that both PC and PNC participants had little knowledge of toothpastes and their contents. Considering that toothpastes are the most common self-applied oral hygiene tools, knowledge and awareness of fluoride and herbal toothpastes should be raised via antenatal programs.

Keywords: pregnant women, toothpastes, knowledge, fluorides, herbal

1. INTRODUCTION

Dental caries is a disease of the hard tissues of the teeth that affects all age groups. It is caused by an imbalance in the interactions between cariogenic bacteria in dental plaques and fermentable carbohydrates over time (1-3). Pregnant women may be more susceptible to dental caries (4, 5) due to nausea (6), changes in dietary habits (7), and delayed treatment (8) impacting their ability to practice oral hygiene. Regular and frequent tooth brushing with fluoride-containing toothpaste is considered the principal primary prevention method in caries control (9).

While there is substantial literature on the caries-preventing and remineralizing effects of fluoride (10-12), questions remain about its potentially unfavorable impact on the pineal gland, dental fluorosis, and mental retardation in children (13, 14). However, these side effects usually occur after systemic and excessive fluoride intake (13).

Toothpastes are the most common topical fluoride sources, and a review (15) found that no definite conclusions could be drawn about side effects that may arise from the application of topical fluoride. Nevertheless, people who are concerned about the amount of fluoride they are exposed to from other sources may prefer using alternatives to fluoride-containing toothpastes, such as fluoride-free herbal toothpastes that are widely available.

It has been reported that many women prefer to use herbal products during pregnancy (16). Pregnancy is a new experience full of uncertainty, especially for first-time pregnant women (17, 18). Fetal development and the use of everyday products that contain chemicals during pregnancy is a topic of interest, and many pregnant women seek a source of information to help them deal with their doubts

DOI: 10.33808/clinexphealthsci.1059505

and to navigate their decisions (18). Study groups have been designed to consider this phenomenon.

There are antenatal education programs and websites designed to support positive pregnancy experiences where pregnant women can obtain up-to-date information on wellbeing topics, such as nutrition, smoking, alcohol use, physical activity, maternal mental health, and use of everyday self-care products (including oral hygiene products). Hence, it is important to understand the level of knowledge and perspectives of pregnant women regarding self-care when developing pregnancy care guidelines and antenatal program curricula (19, 20). It is also worth noting that a pregnant woman's level of knowledge and vigilance toward the use of everyday self-care products may vary based on whether she has had prior pregnancies or not.

To our knowledge, to date, no study has investigated the level of knowledge of pregnant women about fluoride and herbal toothpastes. Therefore, the current study aimed to evaluate the knowledge and attitudes of pregnant women with and without children about the use of fluoride and herbal toothpastes.

2. METHODS

2.1. Ethical Approval

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Faculty of Medicine-Clinical Researches, Istinye University (Date: 29.06.2021 / Protocol #: 21-62). All participants provided verbal consent for their participation and data use.

2.2. Participants

This study was conducted in the Gynecology and Oral and Dental Care department in VM Medicalpark Bursa Hospital, Turkey, from July to August 2021. HC interviewed pregnant women with and without prior pregnancies. The eligibility criteria for this study were (i) Turkish nationality, (ii) Turkish language literacy, and (iii) aged 18 years or older. The preliminary group of participants received a background information sheet and verbal explanation of the study. Participants who were unwilling to participate or who had not met any of the above-mentioned eligibility criteria were excluded.

2.3. Study Design

A 20-item self-administered and validated questionnaire was designed and prepared. The 20-item questionnaire was designed to collect information on two topics: the demographic characteristics of the participants, and the participants' knowledge of fluoride and non-fluoride (herbal) toothpastes. The participants were divided into two study groups. Group 1 consisted of pregnant women with children (PC), and Group 2 consisted of pregnant women without a prior pregnancy (PNC). The questionnaires were completed by the participants while they were waiting for their regular appointments in the obstetrics and gynecology outpatient clinic.

2.4. Validation Procedure

The following procedure was used to validate the questionnaire. First, the questionnaire was checked for common errors, including intrinsically leading, confusing, and repeated questions. Then, a pilot test of the questionnaire was conducted with a small group of pregnant women (n=24) to remove irrelevant and weak questions. The data collected during the pilot study were transferred to a spreadsheet, and the scores of individuals on positively phrased questions and negatively phrased questions were compared to check the consistency. Entering the collected responses from the questionnaire into a spreadsheet allowed cleaning of the data. ZCC read the values aloud and HC entered the data into the spreadsheet; this greatly reduced the risk of error and helped detect inconsistent answers. Cronbach's alpha coefficient was used to ensure the consistency of the survey answers and the reliability of the survey questions. As Cronbach's alpha coefficient in this study was 0.7, modifications were made to the questions when forming the final version of the questionnaire.

2.5. Statistical Analysis

The data obtained from the completed questionnaires were recorded for statistical analysis using the SciPy v1.2.3. program with χ^2 and Fisher's exact tests used for the multivariate frequency distribution of the variables.

3. RESULTS

Of the 255 pregnant women approached to participate in this study, 219 agreed to participate and met the inclusion criteria (PC: n=85; PNC: n=134). Table 1 shows the demographic characteristics of all the participants. The majority of participants were aged 25–28 years (n=161; 73.5%). In terms of the education level of the participants, the majority of the PC (69.4%) and of the PNC (57.6%) had completed primary school.

Of all participants, 36.5% perceived their oral health status as fair. Less than half of the participants in each group utilized professional dental care (PC= 41.1% and PNC= 38.8%) and only when they had pain. They did not specify that they utilized dental services at regular intervals.

There were significant differences ($p< .05$) between the PNC and PC groups in their answers to questions Q1, Q5, Q14, Q16, and Q18 (Tables 2 and 3).

Most of the PC (57.65%) and PNC (72.39%) responded with "no idea" to Q1 ($p = .047$), which asked about their daily-life awareness of fluoride as an additive. The mean ages of these "no idea" responders for Q1 were similar, with the mean age of the PC respondents being 26.8 ± 1.97 years

and the mean age of the PNC respondents being 27.0 ± 2.67 years.

A considerable proportion of both PC (69.41%) and PNC (47.76%) responded with "no idea" to Q5 (p = .006), which asked about their knowledge of the addition of the optimal amount of fluoride to tap water by local health authorities.

In terms of preference for herbal toothpastes during pregnancy, the majority of the PC (62.4%) and PNC (47.0%) responded with "no idea" (p= .03). In addition, 86.6% of the PNC showed low knowledge of the non-fluoride content of herbal toothpastes (sum of the "false" plus "no idea" answers to Q16; p= .023). While 51.5% of the PNC participants responded with "no idea" about a preference for herbal toothpaste for their children, the majority of the PC (56.47%) participants stated that they might not prefer using herbal toothpaste for their children's routine oral hygiene.

Table 1. *Descriptives variables of study groups*

	PC n (%)	PNC n (%)	Total n (%)
Age			
20-24	10 (5.9)	11 (8.2)	21 (9.5)
25-28	52 (73.5)	101 (75.3)	153 (69.9)
29-32	14 (13.8)	16 (11.9)	30 (13.7)
33-37	9 (6.8)	6 (4.6)	15 (6.9)
Education			
Primary School	59 (69.4)	80 (57.6)	139 (63.5)
High School / University	26 (30.6)	54 (42.4)	80 (36.5)
Trimester			
1st	26 (30.6)	33 (24.6)	59 (26.9)
2nd	36 (42.4)	61 (45.5)	97 (44.3)
3rd	23 (27.0)	40 (29.9)	63 (28.8)
Self-reported oral status			
Poor	23 (27.1)	27 (20.2)	50 (22.8)
Fair	53 (52.3)	79 (58.9)	132 (60.2)
Good	9 (10.6)	28 (20.9)	37 (17.0)
Dental visit frequency			
6 months	20 (23.5)	24 (17.9)	44 (20.1)
12 months	15 (17.7)	39 (29.1)	54 (26.6)
12-18 months	15 (17.7)	19 (14.1)	34 (15.5)
In case of pain	35 (41.1)	52 (38.8)	87 (39.7)
Toothpaste recommendation prescription by dental/health professional			
Yes	29 (34.1)	55 (41.0)	84 (38.3)
No	56 (65.9)	79 (59.0)	135 (61.7)

Table 2. *Frequency distribution of study groups based on fluoride part of an questionnaire*

	PC N=85 n (%)	PNC N=134 n (%)	p
Q1. Fluoride is found in nature in water sources, some foods and beverages.			
True	4 (4.71)	2 (1.49)	
False	32 (37.65)	35 (26.12)	.047
No idea	49 (57.65)	97 (72.39)	
Q2. Fluoride can be found in pet bottled water.			
True	17 (20)	22 (16.42)	
False	21 (24.71)	47 (35.07)	.267
No idea	47 (55.29)	65 (48.51)	
Q3. Black tea (especially when it brewed) contains high amounts of fluoride.			
True	17 (20)	26 (19.4)	
False	23 (27.06)	45 (33.58)	.579
No idea	45 (52.94)	63 (47.01)	
Q4. Fluoride can be added to tap water to strengthen tooth enamel.			
True	15 (17.65)	22 (16.42)	
False	26 (30.59)	40 (29.85)	.954
No idea	44 (51.76)	72 (53.73)	
Q5. Addition of optimal amounts of fluoride to tap water by local health authorities is harmful for health.			
True	11 (12.94)	26 (19.4)	
False	15 (17.65)	44 (32.84)	.006
No idea	59 (69.41)	64 (47.76)	
Q6. Fluoride prevents or delays the formation of tooth decay by strengthening tooth enamel.			
True	14 (16.47)	31 (23.13)	
False	32 (37.65)	42 (31.34)	.420
No idea	39 (45.88)	61 (45.52)	
Q7. Fluoride is found in toothpastes.			
True	16 (18.82)	22 (16.42)	
False	27 (31.76)	42 (31.34)	.879
No idea	42 (49.41)	70 (52.24)	
Q8. Fluoride in toothpastes causes pituitary tumors.			
True	13 (15.29)	29 (21.64)	
False	23 (27.06)	50 (37.31)	.073
No idea	49 (57.65)	55 (41.04)	
Q9. Dental fillings contain fluoride.			
True	14 (16.47)	23 (17.16)	
False	28 (32.94)	32 (23.88)	.328
No idea	43 (50.59)	79 (58.96)	
Q10. The use of fluoride toothpaste during pregnancy allows the baby to have stronger teeth.			
True	15 (17.65)	20 (14.93)	
False	25 (29.41)	33 (24.63)	.549
No idea	45 (52.94)	81 (60.45)	
Q11. Fluoride tablets should be used during pregnancy.			
True	14 (16.47)	26 (19.4)	
False	23 (27.06)	44 (32.84)	.453
No idea	48 (56.47)	64 (47.76)	
Q12. Fluoride reaches the baby through the baby cord.			
True	15 (17.65)	26 (19.4)	
False	23 (27.06)	43 (32.09)	.609
No idea	47 (55.29)	65 (48.51)	

Table 3. *Frequency distribution of study groups based on herbal part of an questionnaire*

	PC N=85 n (%)	PNC N=134 n (%)	p
Q13. Herbal toothpastes are sold in pharmacies.			
True	16 (18.82)	27 (20.15)	
False	27 (31.76)	46 (34.33)	.854
No idea	42 (49.41)	61 (45.52)	
Q14.I prefer to use herbal toothpaste during my pregnancy.			
True	13 (15.29)	25 (18.66)	
False	19 (22.35)	46 (34.33)	.03
No idea	53 (62.35)	63 (47.01)	
Q15. I think herbal toothpastes are healthier than fluoride toothpastes.			
True	14 (16.47)	25 (18.66)	
False	19 (22.35)	41 (30.6)	.292
No idea	52 (61.18)	68 (50.75)	
Q16. Herbal toothpastes do not contain fluoride.			
True	17 (20.0)	18 (13.43)	
False	14 (16.47)	44 (32.84)	.023
No idea	54 (63.53)	72 (53.73)	
Q17. Herbal dental products are better for gingival bleeding.			
True	12 (14.12)	26 (19.4)	
False	27 (31.76)	45 (33.58)	.492
No idea	46 (54.12)	63 (47.01)	
Q18. I prefer herbal toothpaste for my child.			
True	14 (16.47)	27 (20.15)	
False	48 (56.47)	38 (28.36)	.046
No idea	23 (27.06)	69 (51.49)	
Q19. I think herbal toothpastes are more expensive than fluoride toothpastes.			
True	19 (22.35)	27 (20.15)	
False	26 (30.59)	46 (34.33)	.832
No idea	40 (47.06)	61 (45.52)	
Q20. Herbal toothpastes prevent tooth decay as well as fluoride toothpastes.			
True	14 (16.47)	23 (17.16)	
False	22 (25.88)	42 (31.34)	.634
No idea	49 (57.65)	69 (51.49)	

4. DISCUSSION

A good understanding of the knowledge, attitudes, and perspectives that pregnant women have about oral and dental health will enable the establishment of preventive dentistry modules in antenatal programs (21, 22). One of the most important functions of preventive dentistry is to help individuals develop correct and adequate oral hygiene habits so that they can maintain their oral and dental health (23).

The toothbrush and toothpaste are the most basic tools used to establish oral hygiene (24). Therefore, the present study investigated the knowledge and attitudes of pregnant women with and without children about fluoride and herbal toothpastes.

In Turkey, in 2020, the highest fertility rate was recorded in the 25–29 years age group (25). Consistent with this data, in this study, pregnant women aged 25–28 years made up 73.5% and 75.3% of the PC and PNC groups, respectively.

In terms of self-reported oral health status, 60.2% of the participants (n = 132) expressed that their oral and dental health was “fair,” which is in accordance with the findings of Lakshmi et al. (26) and Gaszyńska et al. (27). Education level is regarded as one of the main factors that influences oral health knowledge and attitudes (26, 28). A significant proportion of the present study cohort (63.5%) were primary school graduates. Therefore, the low education level of the participants may explain why the response “no idea” was given to most of the questions, as shown in Tables 2 and 3.

In a recent study (29), first-time pregnant women with no children were found to be the most active participants (72.5%) in antenatal programs in Turkey. This led us to

hypothesize that PNC may have up-to-date information about maternal health issues than PC. However, the fact that both PNC and PC answered with "no idea" to 90% (18/20) of the questions indicates that antenatal programs may not be providing adequate information about oral and dental health care tools.

Despite the wide availability of dental health services in Turkey, most of the participants' dental visits (41.1% and 38.8%) were due to the participants experiencing symptoms. Dental visits play a significant role in improving the oral hygiene status of the individual and increasing their knowledge of oral health. Therefore, it is crucial that antenatal programs emphasize the need to schedule regular dental visits, even in the absence of symptoms.

The best indicator of dental health is being caries-free, and fluoride is added to toothpastes in optimal doses due to its caries-preventing effect. Even though fluoride is found in some foods, water, and almost all commercial toothpastes (30), only 4.71% of the PC and 1.49% of the PNC participants answered Q1 correctly (Table 2). It has been almost 80 years since fluoride was introduced to the market (31), yet the pregnant women in our study had little knowledge of the use of fluoride in dental products.

In addition to including fluoride in self-applied topical products (e.g., toothpastes, mouth rinses, and gels), systemic water fluoridation is practiced as a preventive measure and managed by local authorities (30, 32). The optimal approved fluoride level in water is 0.7–1.1 parts per million (ppm), and it is set according to the climate (32, 33). More than 100 health organizations, including the USA Centers for Disease Control and Prevention, the American Medical Association, the World Health Organization, and the Turkish Dental Association, recognize the benefits of water fluoridation in caries prevention. In addition, delivering appropriately fluoridated water to a large number of people may alleviate the economic burden of countries in terms of avoiding expensive dental treatments. The majority of the PNC in this study stated that the optimal dose of fluoridated water was harmful. The reason for their lack of knowledge on this subject needs to be identified. A probable reason for their lack of knowledge may be the fact that water fluoridation is not a current preventive dentistry measure applied in Turkey.

The use of herbs (34), including medicinal herbs, as botanical drugs, teas, and dietary supplements has increased significantly over the past 20 years (35, 36). Women constitute a large share of herbal product consumers. In fact, studies have shown that the preference for herbal products during pregnancy ranges from 6.4% to 67.9% (37, 38).

Herbal toothpastes are an alternative to traditional fluoride-containing toothpastes; they alleviate the symptoms of pregnancy gingivitis (39, 40) and show the same remineralizing and caries-preventing effects as fluoride-containing toothpastes (41). However, there is concern about the use of herbal products by children because little information is available about the benefits and risks in this population (42). It has previously been stated that women are the major (67%) primary source of information about children's oral hygiene habits (43). In the present study, the PNC did not express any preference for herbal toothpastes for their children, while the PC stated that they might reject herbal toothpastes for their children's routine oral hygiene. The lack of interest in using herbal toothpastes and the safety concerns expressed by the pregnant women in this study highlight the need for education on herbal dental products.

5. CONCLUSION

The data and findings of the present study were consistent with those of previous studies. The findings show that all the pregnant women who participated in this study had little to no knowledge of the vast majority of the evaluated parameters, regardless of their past pregnancy status. Considering the significant impact that pregnant women have on their future children's dental health, their knowledge and awareness of fluoride-containing and herbal toothpastes should be increased.

REFERENCES

[1] Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev. 1986;50(4):353–380. DOI: 10.1128/mr.50.4.353.380.1986.

[2] Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, Ismail A. Dental caries. Nat Rev Dis Primers. 2017;3(1):17030. DOI: 10.1038/nrdp.2017.30.

[3] Anil S, Anand PS. Early childhood caries: Prevalence, risk factors, and prevention. Front Pediatr. 2017;5:157. DOI: 10.3389/fped.2017.00157.

[4] Vergnes JN, Kaminski M, Lelong N, Musset AM, Sixou M, Nabet C. Frequency and risk indicators of tooth decay among pregnant women in France: A cross-sectional analysis. PLoS One. 2012;7(5):e33296. DOI: 10.1371/journal.pone.0033296.

[5] Steinberg BJ, Hilton IV, Iida H, Samelson R. Oral health and dental care during pregnancy. Dent Clin North Am. 2013;57(2):195–210. DOI: 10.1016/j.cden.2013.01.002.

[6] Enabulele J, Ibhawoh L. Resident obstetricians' awareness of the oral health component in management of nausea and vomiting in pregnancy. BMC Pregnancy Childbirth. 2014;14:388. DOI: 10.1186/s12884.014.0388-9.

[7] Orloff NC, Flammer A, Hartnett J, Liquorman S, Samelson R, Hormes JM. Food cravings in pregnancy: Preliminary evidence for a role in excess gestational weight gain. Appetite. 2016;105:259–265. DOI: 10.1016/j.appet.2016.04.040.

[8] Tadakamadla SK, Agarwal P, Jain P, Balasubramanyam G, Duraiswamy P, Kulkarni S. Dental status and its socio-demographic influences among pregnant women attending a maternity hospital in India. Rev Clín Pesq Odontol. 2007;3:183–192.

[9] Holmes RD. Tooth brushing frequency and risk of new carious lesions. Evid Based Dent. 2016;17(4):98–99. DOI: 10.1038/sj.ebd.6401196.

[10] König KG. Role of fluoride toothpastes in a caries-preventive strategy. Caries Res. 1993;27(1):23–28. DOI: 10.1159/000261598.

[11] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, Lingström P, Mejàre I, Nordenram G, Norlund A, Petersson LG, Söder B. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand. 2003;61(6):347–355. DOI: 10.1080/000.163.50310007590.

[12] Hatipoğlu Z, Özbay Yavlal G, Kargül B. Effects of different fluoride-containing toothpastes on in vitro enamel remineralization. Bezmialem Science. 2019;7(1):12–17. DOI: 10.14235/bas.galenos.2018.1810.

[13] Abanto Alvarez J, Rezende KM, Marocho SM, Alves FB, Celiberti P, Ciamponi AL. Dental fluorosis: Exposure, prevention and management. Med Oral Patol Oral Cir Bucal. 2009;14(2):E103–107.

[14] Unde MP, Patil RU, Dastoor PP. The untold story of fluoridation: Revisiting the changing perspectives. Indian J Occup Environ Med. 2018;22(3):121–127. DOI: 10.4103/ijoem.IJOEM_124_18.

[15] Marinho VC, Higgins JP, Sheiham A, Logan S. One topical fluoride (toothpastes, or mouthrinses, or gels, or varnishes) versus another for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2004;2004(1):CD002780. DOI: 10.1002/14651858.CD002780.pub2.

[16] Cuzzolin L, Francini-Pesenti F, Verlato G, Joppi M, Baldelli P, Benoni G. Use of herbal products among 392 Italian pregnant women: focus on pregnancy outcome. Pharmacoepidemiol Drug Saf. 2010;19(11):1151–1158. DOI: 10.1002/pds.2040.

[17] Cronin C, McCarthy G. First-time pregnant women – identifying their needs, perceptions and experiences. J Clin Nurs. 2003;12(2):260–267. DOI: 10.1046/j.1365-2702.2003.00684.x.

[18] Wilkins C. A qualitative study exploring the support needs of first-time pregnant women on their journey towards intuitive parenting. Midwifery. 2006;22(2):169–180. DOI: 10.1016/j.midw.2005.07.001.

[19] Feng XL, Wen C. Evaluation of a pilot program that integrated prenatal screening into routine antenatal care in western rural China: An interrupted time-series study. Lancet Reg Health West Pac. 2020;6:100075. DOI: 10.1016/j.lanwpc.2020.100075.

[20] Novick G. Women's experience of prenatal care: An integrative review. J Midwifery Womens Health. 2009;54(3):226–237. DOI:10.1016/j.jmwh.2009.02.003.

[21] Artieta-Pinedo I, Paz-Pascual C, Grandes G, Espinosa M. Framework for the establishment of a feasible, tailored and effective perinatal education programme. BMC Pregnancy Childbirth. 2017;17(1):58. DOI: 10.1016/j.jmwh.2009.02.003.

[22] Afaya A, Azongo TB, Dzomeku VM, Afaya RA, Salia SM, Adatara P, Kaba Alhassan R, Amponsah AK, Atakro CA, Adadem D, Asiedu EO, Amuna P, Amogre Ayanore M. Women's knowledge and its associated factors regarding optimum utilisation of antenatal care in rural Ghana: A cross-sectional study. PLoS One. 2020;15(7):e0234575. DOI: 10.1371/journal.pone.0234575.

[23] Glassman P, Miller CE. Effect of preventive dentistry training program for caregivers in community facilities on caregiver and client behavior and client oral hygiene. NY State Dent J. 2006;72(2):38–46.

[24] Hitz-Lindenmüller I, Lambrecht JT. Oral care. Curr Probl Dermatol. 2011;40:107–115. DOI: 10.1159/000321060. Epub 2011 Feb 10.

[25] TURKSTAT, TÜİK. https://data.tuik.gov.tr/Bulten/Index?p=Birth-Statistics-2020-37229&dil=2 (Available: 18.01.2022).

[26] Lakshmi SV, Srilatha A, Satyanarayana D, Reddy LS, Chalapathi SB, Meenakshi S. Oral health knowledge among a cohort of pregnant women in south India: A questionnaire survey. J Family Med Prim Care. 2020;9(6):3015–3019. DOI: 10.4103/jfmpc.jfmpc_329_20.

[27] Gaszyńska E, Klepacz-Szewczyk J, Trafalska E, Garus-Pakowska A, Szatko F. Dental awareness and oral health of pregnant women in Poland. Int J Occup Med Environ Health. 2015;28(3):603–611. DOI: 10.13075/ijomeh.1896.00183.

[28] Pentapati KC, Acharya S, Bhat M, Rao SK, Singh S. Knowledge of dental decay and associated factors among pregnant women: A study from rural India. Oral Health Prev Dent. 2013;11(2):161–168. DOI: 10.3290/j.ohpd.a29734.

[29] Turgut N, Güldür A, Çakmakçı H, Şerbetçi G, Yıldırım F, Ender Yumru A, Bebek A, Gülova SS. Gebe okulunda eğitim alan gebelerin bilgi düzeyleri üzerine bir araştırma (A study about knowledge level of pregnants that educated in pregnancy school). JAREN. 2017;3(1):1–8. (Turkish)

[30] Edmunds W, Smedley PL. Fluoride in natural waters. Selinus O, Alloway B, Centeno JA, Finkelman RB, Fuge R, Lindh U, Smedley PL. Essentials of Medical Geology. Springer; 2013.p.311–336. DOI:10.1007/978-94-007-4375-5_13.

[31] O'Mullane DM, Baez RJ, Jones S, Lennon MA, Petersen PE, Rugg-Gunn AJ, Whelton H, Whitford GM. Fluoride and oral health. Community Dent Health. 2016;33(2):69–99. DOI:10.1922/CDH_3707O'Mullane31

[32] Yeung CA. A systematic review of the efficacy and safety of fluoridation. Evid Based Dent. 2008;9(2):39–43. DOI: 10.1038/sj.ebd.6400578.

[33] Iheozor-Ejiofor Z, Worthington HV, Walsh T, O'Malley L, Clarkson JE, Macey R, Alam R, Tugwell P, Welch V, Glenny AM. Water fluoridation for the prevention of dental caries. Cochrane Database Syst Rev. 2015;2015(6):CD010856. DOI: 10.1002/14651858.CD010856.pub2.

[34] World Health Organization. WHO global report on traditional and complementary medicine Geneva: World Health Organization: 2019 Licence: CC BY-NC-SA 3.0 IGO.

[35] Barnes J, McLachlan AJ, Sherwin CM, Enioutina EY. Herbal medicines: Challenges in the modern world. Part 1. Australia and New Zealand. Expert Rev Clin Pharmacol. 2016;9(7):905–915. DOI: 10.1586/17512.433.2016.1171712.

[36] Teng L, Zu Q, Li G, Yu T, Job KM, Yang X, Di L, Sherwin CM, Enioutina EY. Herbal medicines: Challenges in the modern world. Part 3. China and Japan. Expert Rev Clin Pharmacol. 2016;9(9):1225–1233. DOI: 10.1080/17512.433.2016.1195263. Epub 2016 Jun 8.

[37] Louik C, Gardiner P, Kelley K, Mitchell AA. Use of herbal treatments in pregnancy. Am J Obstet Gynecol. 2010;202(5):439.e1–439.e10. DOI: 10.1016/j.ajog.2010.01.055.

[38] Abdollahi F, Yazdani Chareti J. The relationship between women's characteristics and herbal medicines use during pregnancy. Women Health. 2019;59(6):579–590. DOI: 10.1080/03630.242.2017.1421285. Epub 2019 Mar 27.

[39] George J, Hegde S, Rajesh KS, Kumar A. The efficacy of a herbal-based toothpaste in the control of plaque and gingivitis: A

clinico-biochemical study. Indian J Dent Res. 2009;20(4):480–482. DOI: 10.4103/0970-9290.59460.

[40] Laleman I, Teughels W. Novel natural product-based oral topical rinses and toothpastes to prevent periodontal diseases. Periodontol 2000. 2020;84(1):102–123. DOI: 10.1111/prd.12339.

[41] Celik ZC, Yavlal GO, Yanıkoglu F, Kargul B, Tagtekin D, Stookey GK, Peker S, Hayran O. Do ginger extract, natural honey and bitter chocolate remineralize enamel surface as fluoride toothpastes? An in-vitro study. Niger J Clin Pract. 2021;24(9):1283–1288. DOI: 10.4103/njcp.njcp_683_20.

[42] Tomassoni AJ, Simone K. Herbal medicines for children: An illusion of safety? Curr Opin Pediatr. 2001;13(2):162–169. DOI: 10.1097/00008.480.200104000-00014.

[43] Wapniarska K, Buła K, Hilt A. Parent's pro-health awareness concerning oral health of their children in the light of survey research. Przegl Epidemiol. 2016;70(1):59–63,137–140.

How to cite this article: Çelik ZC, Elbek Çubukçu C, Çelik H, Dinç Ata G. Knowledge and Attitudes of Pregnant Women with and without Children about Fluoride and Herbal Toothpastes. Clin Exp Health Sci 2023; 13: 114-121. DOI: 10.33808/clinexphealthsci.1059505

Clinical and Experimental Health Sciences

A Curriculum Development Project for a School Nursing Certification Program in Turkey: A Delphi Study

İrem Nur Özdemir[1], Hasibe Kadıoğlu[2]

[1] Bakırköy Dr. Sadi Konuk Training and Research Hospital, Department of Training and R&D, İstanbul, Türkiye.
[2] Marmara University, Faculty of Health Science, Department of Public Health Nursing, Maltepe, Türkiye.

Correspondence Author: İrem Nur Özdemir
E-mail: iremozdemir_92@hotmail.com; iremnur.ozdemir@saglik.gov.tr
Received: 19.01.2022 **Accepted:** 12.05.2022

ABSTRACT

Objective: School nursing education has not been standardized in Turkey so that it was decided the Delphi technique would be used to develop a curriculum for a program of education in school nursing.

Methods: The data were collected from the expert panel via the Delphi technique by means of e-mail. Data were collected in three consecutive Delphi rounds. The population of the study comprised academic nurses and school nurses working in the area of school nursing in Turkey. A total of 83 experts were invited to participate in the study. The data were evaluated in terms of Quartile 1, Quartile 3, medians, interquartile ranges. Interquartile range refers to the difference between Quartile 3 and 1. Consensus was considered as less than the interquartile range of 1.2.

Results: At the end of Round-1, 177 learning outcomes, nine main topics, 262 sub-topics were obtained. At the end of Round-2, 167 learning outcomes, nine main and 255 sub-topics were gathered, while at the end of Round-3, agreement was reached regarding nine main topics, 255 sub-topics, 167 learning outcomes, thus ending the Delphi rounds.

Conclusions: The curriculum on which agreement was reached will be treated as a guide to school nursing education and contribute to the standardization of this instruction.

Keywords: Certification, curriculum, delphi technique, school nurse education

1. INTRODUCTION

School nursing is a specialized branch of nursing that contributes to students' health, academic achievement, and life-long wellbeing. Toward this aim, school nurses help students to adapt to their normal process of development, work to improve the health and safety of both students and the community, intervene in actual and potential health issues, engage in case management, help students and their families develop their capacities for self-management and learning, while at the same time working with other employees in active collaboration (1).

School nurses are required to have the skills of analyzing data for diagnosis, determining nursing diagnoses and results, identifying outcomes, planning, implementation, coordinating care, providing health education and improving health, consultation, progressing toward attaining results and evaluating the quality of nursing practices, gaining skills and competences, assessing professional practices, contributing to the development of their colleagues and school personnel, and developing competences and standards in the areas of cooperation, ethics, research, in using resources, engaging in leadership and program management (2). In Turkey, the Ministry of Health has outlined the duties, powers and responsibilities of school nurses in its "Regulation No. 27910 on Amending Nursing Regulations" that was published in 2011. In line with this regulation, this branch of nursing includes a widely comprehensive range of duties related to matters such as defining school health needs, defining powers, performing periodical physical examinations, immunization, health screening, health education and planning (3).

In school nursing applications around the world, there are differences in the approach to the role of the school nurse, in percentages of nursing students, care supervision, proposed nursing education as well as in local needs. Systems of education and services are shaped differently where there is no standard model for school health services or implementing school nursing practices. Because of this, the effectiveness of the school nurse and the quality of school health services are directly related to the leadership, management, cooperative

DOI: 10.33808/clinexphealthsci.1060107

effort and advocacy skills with which nurses can meet students' needs (4).

In Turkey, school nursing education is provided within the scope of basic nurses' training as part of the public health nursing curriculum, whereas in developed countries, this is defined as a separate field of specialization (5, 6). The American Nurses Association (ANA) requires a minimum bachelor's degree for performing professional nursing practices (7). ANA and the National Association of School Nurses (NASN) requires school nurses to pass a program of education in order to be prepared for their roles in this capacity. It has been stated that this training needs to be conducted at the level of a national certification program (8). In Turkey, school nursing is taught as an elective course in School nursing in Nursing Undergraduate programs, as a course as part of the Public Health Nursing Graduate Program, as a Graduate program in School nursing, and as a School Nursing Certification program.

The lack of a standard in school nursing education in Turkey, the fact that school nurses are predominantly employed in private schools and boarding institutions, the lack of agreement on what school nursing education programs should entail are among the problem experienced in this area. Nurses must attend a course of education after graduation in order to be able to work in the field of school health. Well-known international organizations such as the NASN and the American Speech-Language-Hearing Association (ASHA) recommend that school nurses attend a certification program (9, 10, 11). Setting up certification programs and making graduate education in the area of school nursing more widespread is of the greatest importance in terms of educating qualified school nurses and improving school health in general (12). School nursing is only newly developing in Turkey and yet, no certification is required to become a school nurse. A national standard for knowledge and practices must be adopted if certification programs and graduate education are to succeed. In the light of the lack of standardization in Turkey for school nursing, we made the decision to use the Delphi technique to develop a national school nursing curriculum in order to answer the need to achieve consensus among experts as to curriculum content in this branch of nursing.

The Delphi technique is a means of identifying consensus. The technique was first developed by Helmer and Dalkey in the 1950s for an evaluation of military topics. The goal of the Delphi technique is to achieve consensus by collecting expert judgments. It is a fact that in political interaction especially and in other settings where there are differences of status, individuals may be reluctant to freely express their views. This technique allows persons to be comfortable with expressing their views, leading to possible consensus. In general, the Delphi technique has three characteristics: 1. Confidentiality of participation, 2. Statistical analysis of group reaction, 3. Controlled feedback. In the implementation of the technique, a sequence of questionnaires might be sent out to the experts. Each feedback is reported to the participants. This procedure continues until consensus has been reached (13, 14).

An examination of studies in the international nursing literature that have used the Delphi technique reveals that this method is commonly employed in determining research priorities, introducing a particular method, carrying out scale development processes, identifying nursing branch competences, developing protocols, setting up care protocols, and in determining nursing program curriculums (15,16).

Because of the wide use of the Delphi technique in nursing, we aimed in this study to identify the content of a certification program for school nurses by reaching a consensus through the collection of views from school nurses and academics.

2. METHODS

This study was conducted by using the Delphi consensus technique over the period 24 September 2020 – 8 July 2021.

2.1. Data Collection

The data were collected from the expert panel via the Delphi technique by means of email. Data were collected in three consecutive Delphi rounds.

2.2. Steps in the Delphi Technique

Step 1: Identifying the Experts

In this study, academic nurses (with the status of Professor, Associate Professor, Assistant Professor and Research Assistant) and School Nurses working in the area of School Nursing in Turkey were identified as the panel of experts.

Step 2: Creating the First Delphi Questionnaire

At the end of a full-day workshop in which the authors shared experiences with a panel of 10 experts, the main headings, sub-headings, learning outcomes and application terms for the certification program that could be included in a school nursing certification program were identified. The first Delphi questionnaire was thus created. Besides the topic headings and the learning outcomes, the experts were also asked for their views regarding the prerequisites for a school nursing certification program application, the period of time needed for theoretical and practical instruction in the program, and the term of validity of the proposed certification.

Step 3: Delivery of the First Delphi Questionnaire to the Experts

The first Delphi questionnaire that contained the information put together by the researchers addressing the was sent to experts by email. At this stage, the experts were asked to make additions to the curriculum that had been drawn up.

Step 4: Reviewing the First Delphi Questionnaire and Creating the Second

The first Delphi questionnaires returned by the experts were revised by the research team, and the second Delphi questionnaire was thus created. At this stage, repeating views were combined and grouped. An evaluation scale was added to each learning outcome and topic on the second questionnaire. This scale was a Likert-type of scale with points between 1-7 (1: must definitely not be in the curriculum; 7: must definitely be in the curriculum).

Step 5: Delivery of the Second Delphi Questionnaire

The second Delphi Questionnaire was re-sent to the participating experts. At this stage, the experts were asked to use the 7-point Likert scale to evaluate each learning outcome, main topic and sub-topic.

Step 6: Analysis of the Second Delphi Questionnaire and Creating the Third

The data of the second Delphi questionnaire evaluated by the experts were analyzed. In this analysis, Quartile 1, Quartile 3, interquartile range and median calculations were made; the results of the analysis were added to the second Delphi questionnaire, thus creating the third Delphi questionnaire.

Step 7: Delivery of the Third Delphi Questionnaire to the Experts

The third Delphi questionnaire was sent to the experts once again for evaluation. At this stage, the experts were asked to review the topics on which they did not agree and score these.

Step 8: Analysis of the Third Delphi Questionnaire and Ending the Rounds

At this stage, another analysis was made of the third Delphi Questionnaire. Since there were no changes in the views, the rounds were ended (Figure 1).

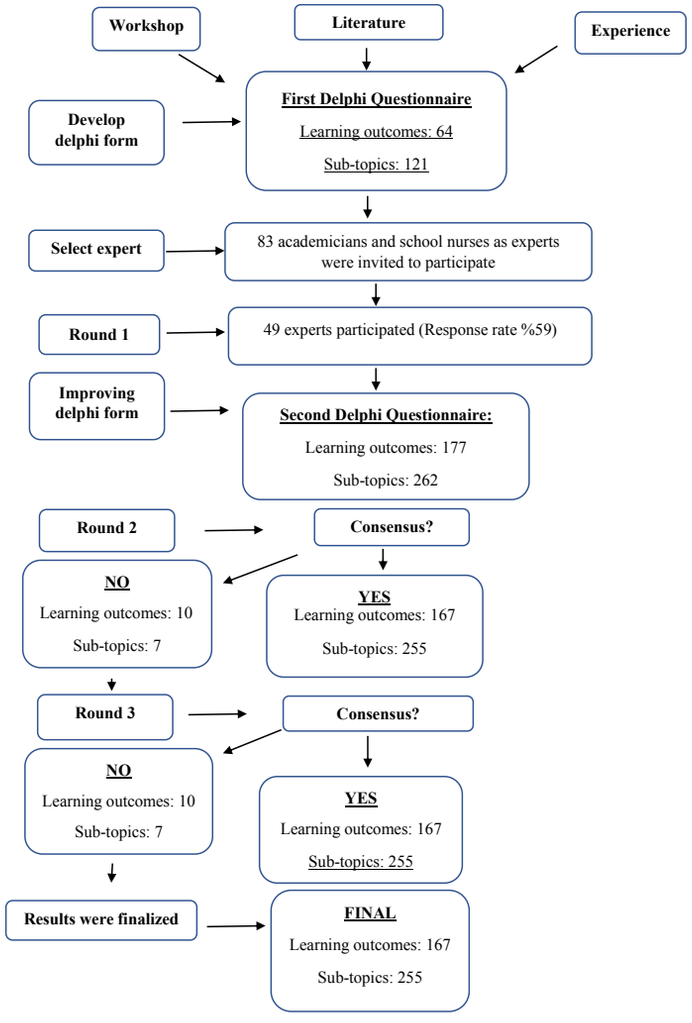


Figure 1. *Three round Delphi process*

2.3. Study Population

The population of the study comprised academic nurses (with the status of Professor, Associate Professor, Assistant Professor and Research Assistant) and School Nurses working in the area of School Nursing in Turkey. Eighty three experts were invited to participate in the study.

2.4. Ethical Considerations

Approval for the study was obtained from the Ethics Board of Marmara University Health Sciences Institute (19.12.2019-222). The faculty members and school nurses were invited to the study through the delivery to them of the data collection tool, consent form, and information sheet.

2.5. Analysis of the Data

The data were analyzed on the SPSS 26 package program. The data were evaluated in terms of Quartile 1, Quartile 3, medians and interquartile ranges. Interquartile range refers to the difference between Quartile 3 and Quartile 1. A low difference indicates agreement; a higher value means that consensus has not been reached. It is recommended that the interquartile range should be 1.2 or less for consensus (13).

3. RESULTS

First Delphi Questionnaire was sent via email to the 83 experts invited to participate in the study (Table 1). In Round 1, 49 of the 83 experts participated (participation rate of 59%), contributing their views. In Round 1, 177 learning outcomes, 9 main topics and 262 sub-topics were created. In Round 2, 43 out of the 49 experts participated; the consensus was achieved on 167 learning outcomes, 9 main topics and 255 sub-topics. The agreement could not be reached regarding 7 sub-topics, 10 learning outcomes and the prerequisites for application to a school nursing certification program, the theoretical and practical duration of such a program, exemption from practical work, and the term of validity of a certificate. Forty-two out of 43 experts participated in Round 3. Consensus could still not be achieved regarding the 7 sub-topics and 10 learning outcomes on which agreement could not be reached previously. Therefore, the Delphi rounds were ended at the end of Round 3, with a total of 9 main topics, 255 sub-topics and 167 learning outcomes. At the end of the Delphi rounds, agreement had been reached on the following main topics: School nursing, Identifying the school community, Health evaluation and nursing diagnosis, Improving health and disease prevention, Health issues and nursing management, School environment and safety, Disaster and emergency management, Sensitive/vulnerable/disadvantaged groups, risk evaluation and nursing interventions, School health services management and evidence-based practices (Table 2). Besides the topic headings and the learning outcomes, the experts were also asked for their views regarding the prerequisites for applying for a school nursing certification program, the period of time needed for theoretical and practical instruction in the program and the term of validity of the proposed certification. All of the experts were in agreement that nurses would have to hold an undergraduate degree as a prerequisite for application to a school nursing program. Among the experts, 64.3% stated that the applicant's 4-year grade average would have to be at least 2.5 on a scale of 4. Among the experts, 78.6% believed that prerequisites for a certificate should be a health report obtained from a fully qualified hospital, while 88.1% said that basic life support certification or a certificate earned during the program would be necessary. A majority (92.9%) of the experts said that clinical experience would be a requirement. On the other hand, the experts' views on how long a period of clinical experience was necessary varied from between at least 6 months to 5 years, leading to a failure to achieve consensus on this point. A group of experts representing 85.7% stated that there should be no exemption from practical work, and that each participant in the program should be required to complete the practical instruction. On the matter of how long the theoretical part of the certification program should take suggestions for a period of 20 hours-240 hours were offered in Round 1, but at the end of the Delphi rounds, durations of between a minimum of 80 hours-240 hours were suggested. No agreement could be reached on any of the suggestions, however. In the matter of the duration of practical work in the certification program, all of the experts agreed that the program should include practice sessions. No agreement could be reached, however, on the duration of these practice sessions. The experts offered the view that practice sessions should be held for a period of 40-300 hours. The highest percentage in this context was 38.1%, seen in the suggestion that half of the program be devoted to apply teaching. Concerning the matter of the term of validity of the certification program, the experts formed a consensus on establishing a refresher course every 5 years.

Table 1. *Response rates of professionals*

Professionals	Round 1	Round 2	Round 3
	n	n	n
Professor	7 of 22	7 of 7	6 of 7
Associate Professor	9 of 15	7 of 9	7 of 7
Assistant Professor	21 of 32	20 of 21	20 of 20
Research Assistant	4 of 6	3 of 4	3 of 3
School Nurse	8 of 8	6 of 6	6 of 6
Total	49 of 83	43 of 49	42 of 43

Table 2. *Number of learning outcomes and sub-topics meeting consensus from the three round Delphi process*

Main topics	Learning Outcomes			Sub-topics		
	Number of learning outcomes at baseline	Number of learning outcomes meeting consensus	Percentage of learning outcomes meeting consensus	Number of sub-topics at baseline	Number of sub-topics meeting consensus	Percentage of sub-topics meeting consensus
School nursing	25	22	88	21	20	95.2
Identifying the school community	12	11	91.6	7	6	85.7
Health evaluation and nursing diagnosis	18	17	94.4	15	14	93.3
Improving health/disease prevention	25	22	88	53	50	94.3
Health issues and nursing management	15	14	93.3	90	90	100
School environment and safety	25	24	96	25	24	96
Disaster and emergency management	9	9	100	12	12	100
Sensitive/vulnerable/disadvantaged groups, risk evaluation and nursing interventions	24	24	100	21	21	100
School health services management and evidence-based practices	24	24	100	18	18	100
Total	177	167	94.3	262	255	97.3

4. DISCUSSION

In this study, we looked into the learning outcomes, curriculum and prerequisites of a school nursing certification program by employing the Delphi technique to seek consensus among a panel of 42 experts comprising academic nurses and school nurses working in the field of school nursing in Turkey. We encountered no study in the literature that dealt with the curriculum of school nursing education. The lack of literature in this particular area makes this study unique. At the moment, school programs, including undergraduate programs, are in flux and every university program does not belong to a single prototype. School nursing in Turkey is taught at several levels–in the form of a course in School nursing as part of the Nursing Undergraduate program or the Graduate Nursing program, as a graduate program in School nursing, as well as in the form of a School Nursing Certification program. As can be understood from the various levels at which this discipline is taught in Turkey, it could be seen that there is no consensus on what the curriculum of a school nursing education program should consist of.

An examination of the topics on which consensus was achieved in this study shows that these are generally consistent with what is reported in the school nursing literature. It is evident that the experts reached consensus on matters such as the evaluation of health, system-related health issues and nursing management, acute and emergency situations, chronic health problems, infectious diseases, groups with special health needs, health education, and professionalism in school nursing, similar to those topics set forth by the National Board for Certification of School Nurses (17). Similar topics are also contained in school nursing certification programs at the university level in Turkey (18, 19) (Available from: https://selcuksem.selcuk.edu.tr/Educations/uzaktan-egitimler/17; https://sayem.subu.edu.tr/egitimler/uzaktan-egitim/saglik-egitimleri/okul-sagligi-hemsireligi-sertifika-programi Accessed Date: 10.09.2021).

A study in Turkey that also used the Delphi consensus technique to create a curriculum for public health nursing included similar subjects in the school nursing division of the department (20).

All of the experts in our study were in agreement that nurses would have to hold an undergraduate degree as a prerequisite for application to a school nursing program. In the same way, NASN has required that nurses be graduates with a B.A. degree from an accredited college or university and be registered on a board of nurses. These requirements constitute the minimum preparation for the skills needed for entry-level school nursing (9). Furthermore, NASN supports the granting of school nursing certification on a state level and also certifies school nurses through the NBCSN. The Nursing and Midwifery Council (NMC) is the regulatory authority in the U.K. This council requires school nursing certification to be granted on an undergraduate or graduate level and that application for this certification be restricted to registered midwives or nurses (21, 22).

In our study, 88.1% of the experts stated that applicants for certification must have basic life support certification or a certificate earned during the program. The U.S. New Jersey State School Nurses Association states that nurses to apply for school nursing certification must hold Cardiopulmonary Resuscitation or Automatic External Defibrillator Certification from authorized organizations (23).

Almost all of the experts in the study set forth the view that clinical experience was necessary. The NBCSN requires

at least 1000 hours of clinical practice before entering the certification examination. This period of time is equivalent to about a full working year of a full-time school nurse who works at least 6 hours a day in a 180-day school year (17). Similarly, in our study, the experts reached a consensus on the view that candidates should have at least 6 months of clinical experience. According to the New Jersey State School Nurses Association, the requirement for becoming a school nurse is to have completed at least 21 semesters worth of credits, at least 6 semesters of credit hours being derived from clinical practice in a school nurse's office (23).

As to the theoretical training in the certification program, this was deemed to be a minimum of 80 and a maximum 240 hours of instruction. No agreement could be reached on any of the suggestions, however. It is to be noted that certification programs at the university level in Turkey amount to 150 hours. The accredited certification and graduate programs in the U.K. require a minimum 52 weeks–1 full year–of a 120-180-credit program of education. In the matter of the duration of practice sessions in the certification program, all of the experts agreed that the program should include applied sessions. As to the matter of the duration of the program, 38.1% of the experts expressed the view that half of the program should be devoted to practical instruction. Certification programs conducted in the U.K. require that half of the program be devoted to clinical practice. In certification programs conducted in different states around the U.S., the duration of practical instruction varies from 75 to 300 hours.

Concerning the matter of the term of validity of a certification program, the experts agreed that a refresher course should be conducted every 5 years. Similarly, NASN defines a five-year validity period for school nursing certification (17).

The limitation of this research is that the number of experts at the beginning of the first Delphi round is gradually decreasing in the ongoing Delphi rounds and seven experts have left the study.

5. CONCLUSION

A three-stage Delphi process was used to reach consensus on the curriculum of a nationwide school nursing certification program. The result of our study was that the main topics and learning outcomes of a school nursing certification program, as well as the prerequisites for application to the program were defined. There is a need for a certification program in Turkey for school nursing and for standardization of education in this branch of nursing. The curriculum created is expected to be treated as a guide to school nursing education, becoming a framework for its implementation. We believe that our findings will provide a resource for eliminating differences in school nursing education.

Acknowledgements: *We are grateful for the contributions from all the participants.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Marmara University, Institute Health Sciences, Non-Invasive Clinical Trial Ethics Committee (Decision date and number: 19.12.2019-222).*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: İNÖ, HK
Design of the study: İNÖ, HK
Acquisition of data for the study: İNÖ, HK
Analysis of data for the study: İNÖ, HK
Interpretation of data for the study: İNÖ, HK
Drafting the manuscript: İNÖ, HK
Revising it critically for important intellectual content: İNÖ, HK
Final approval of the version to be published: İNÖ, HK

REFERENCES

[1] National Association of School Nurses. Definition of school nursing. Accesed [03 Nov 2019] https://www.nasn.org/about-nasn/about

[2] National Association of School Nurses. Framework for 21st century school nursing practice. NASN Sch Nurse. 2016; 31(1): 45-53.

[3] Official Gazette of Republic of Turkey. (2011). Hemsirelik Yonetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. The Law number: 27910. 19.04.2011 (Turkish)

[4] Wolfe LC. The profession of school nursing. J. Selekman, editor. School Nursing: A Comprehensive Text, 2nd ed. Philadelphia: F.A. Davis Company; 2013.p. 25-47

[5] Bahar Z. Okul sağlığı hemşireliği. Dokuz Eylül Üniversitesi Hemşirelik Yüksekokulu Elektronik Dergisi 2010;3(4):195-200 (Turkish)

[6] Schainker E, Obrien MJ, Fox D, Bauchner H. School nursing services: Use in an urban public school system. Arch Pediatr Adolesc Med. 2005;159(1):83-87. DOİ:10.1001/archpedi.159.1.83

[7] American Nurses Association (ANA). Nursing education. Accessed [03 Nov 2019]. http://www.nursingworld.org/MainMenuCategories/PolicyAdvocacy/State/Legislative-Agenda-Reports/NursingEducation.

[8] American Nurses Association & National Association of School Nurses. School nursing: Scope & standards of practice. 3rd edition. Silver Spring:2017

[9] National Association of School Nurses. Education, licensure, and certification of school nurses. Accessed [03 Nov 2019]. https://www.nasn.org/nasn/advocacy/professionalpracticedocuments/position-statements/ps-education.

[10] Cluskey M, Schwend K. The Role of the school nurse in special education. Advances in Special Education 2015; 30B:1-18. DOI: 10.1108/S0270.401.3201500.000.30008

[11] Loschiavo J. Fast Facts for the School Nurse: School Nursing in a Nutshell. 2nd ed. New York: Springer Publishing Company; 2015

[12] Ulutaşdemir N, Balsak H, Çopur EÖ, Demiroğlu N. Halk sağlığı hemşireliğinin bir dalı: Okul sağlığı hemşireliği. Turkiye Klinikleri J Public Health Nurs-Special Topics 2016;2(1):121-124 (Turkish)

[13] Şahin AE. Eğitim araştırmalarında Delphi tekniği ve kullanımı. Hacettepe Üniversitesi Eğitim Fakültesi Dergisi 2001; 20:215-220.

[14] Powell C. The Delphi technique: Myths and realities. J Adv Nurs. 2003; 41(4), 376–382. DOI: 10.1046/j.1365-2648.2003.02537.x

[15] Shariff NJ. Utilizing the Delphi survey approach: A review. J Nurs Care. 2015; 4(3): 1000246. DOI:10.4172/2167-1168.100.0246

[16] Sim J, Crookes P, Walsh K, Halcomb E. Measuring the outcomes of nursing practice: A Delphi study. J Clin Nurs. 2018; 27 (1-2):368-378. DOI: 10.1111/jocn.13971

[17] National Board for Certification of School Nurses. Certification Examination for School Nurses Handbook for Candidates. Updated [9 March 2021]. Accessed [16.08.2021]. https://www.nbcsn.org/wp-content/uploads/2021/11/nbcsn-handbook-2021a.pdf

[18] Selçuk University. Okul Hemşireliği Eğitimi. Accessed [10 Sep 2021]. https://selcuksem.selcuk.edu.tr/Educations/uzaktan-egitimler/17 (Turkish)

[19] Sakarya University of Applied Sciences. Okul Sağlığı Hemşireliği Sertifika Programı Accessed [10 Sep 2021]. https://sayem.subu.edu.tr/egitimler/uzaktan-egitim/saglik-egitimleri/okul-sagligi-hemsireligi-sertifika-programi (Turkish)

[20] Kadıoğlu H, Albayrak S, Ergun A, Yurt S, Gur K, Kader M, Erol S, Esin MN. Achieving consensus on the undergraduate curriculum of public health nursing in Turkey. Public Health Nurs. 2018; 36(2): 238–244. DOI: 10.1111/phn.12576

[21] PHD-PHN/32420. Maximising the school nursing team contribution to the public health of school-aged children. Published [April 2014]. Accessed [15 Sep 2021]. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/303769/Service_specifications.pdf.

[22] The Queen's Nursing Institute. Transition to the school nursing service. Accessed [15 Sep 2021]. https://www.qni.org.uk/wp-content/uploads/2017/01/Transition-to-School-Nursing.pdf

[23] New Jersey State School Nurses Association. New Jersey Requirements for School Nurse Certification. Accessed [10 Sep 2021] https://www.njssna.org/resources/certification

How to cite this article: Özdemir İN, Kadıoğlu H. A Curriculum Development Project for a School Nursing Certification Program in Turkey: A Delphi Study. Clin Exp Health Sci 2023; 13: 122-128. DOI: 10.33808/clinexphealthsci.1060107

Clinical and Experimental Health Sciences

Effects of Scorzonera Cinerea on Immune System and Hematological Parameters in Short-Term Hyperglycemia

Mehmet Ali Temiz

Karamanoğlu Mehmetbey University, Vocational School of Technical Sciences, Programme of Medicinal and Aromatic Plants, Karaman, Türkiye.

Correspondence Author: Mehmet Ali Temiz
E-mail: matemiz@kmu.edu.tr

Received: 21.01.2022 **Accepted:** 07.12.2022

ABSTRACT

Objective: Objective: Medicinal herbs offer natural remedies for various ailments because of their comprehensive effects. *Scorzonera cinerea* L. (Sc) is an edible wild plant and is used in traditional medicine against various diseases. The purpose of this study was to investigate the immune system effects of *S. cinerea* radical leaf extract in diabetic rats.

Methods: The 5 groups were formed — Control, Diabetic, Sc-Dried, Sc-Frozen, and Acarbose. Adenosine deaminase (ADA), xanthine oxidase (XO), and myeloperoxidase (MPO) activities in liver and kidney tissues were analyzed. Hematological parameters were also evaluated.

Results: ADA, XO, and MPO activities in both tissues significantly increased in diabetic conditions. However, Sc treatments significantly decreased liver ADA, liver and kidney XO, and liver and kidney MPO activities. There was no significant change in red blood cell (RBC) parameters. Although platelet (PLT) count and MPV were raised in the diabetic group, these parameters were reduced with Sc-Dried and Sc-Frozen treatment. While lymphocyte (LYM%) significantly decreased in defense cells in the diabetic group, neutrophil (NEU%), monocyte (MO%), and eosinophil (EOS%) elevated. However, a significant decrease was observed in NEU% and EOS% with Sc treatment.

Conclusion: *S. cinerea* treatment can exert a potential immunoregulatory effect in diabetes. Thus, *S. cinerea* can be considered as an adjuvant to augment immune system on diabetes.

Keywords: Adenosine deaminase, Hematology, Immunity, myeloperoxidase, *Scorzonera cinerea*, xanthine oxidase

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic, heterogeneous, and progressive disease that is characterized by hyperglycemia due to deficiency of insulin and/or absence. Diabetes related many diseases that among these are heart, eyes, nerves, and kidney diseases, skin complications, and immune system disorders (1). It has been supported by many researchers that there is also a serious relationship between diabetes and oxidative stress (2,3). Increased free radical production causes the activation of some major pathways such as the polyol pathway, protein kinase C, an increase in the formation of advanced glycation end-products (AGE), and overactivity of the hexosamine pathway. At the same time, these pathways lead to the production of free radicals again and have a role in the pathogenesis of complications (4). Diabetes has significant adverse effects on the immune system. High blood glucose levels increase the activity of immune cells. These cells eventually become depleted and less sensitive; as a result, their effectiveness against invading pathogens is reduced. Protein kinase C is activated by hyperglycemia, thereby inhibiting neutrophil migration, phagocytosis, superoxide production, and microbial killing. Hyperglycemia can also reduce the formation of neutrophil extracellular traps (5). The chronic inflammatory state in diabetics may play a role in impaired immune function, thus increasing susceptibility to infections. On the other hand, regulation of the activities of some enzymes responsible for immunity, such as ADA, MPO, and XO, is important to the proper functioning of the immune system.

Medicinal herbs offer exhaustive properties because of their multiple effects. Natural compounds such as polyphenols have the ability to modulate ADA enzyme activity as well as reverse oxidative damage (6). A fall in ADA levels was greater in diabetic patients taking metformin with garlic than in patients taking only metformin (7). It is known that MPO has a critical role in the onset and progression of acute and chronic inflammatory diseases. Moreover, as the XO pathway is considered an important pathway for the production of reactive oxygen species (ROS) and the high oxidative stress has a significant impact on immunity, XO pathway may have

DOI: 10.33808/clinexphealthsci.1061083

a critical role in modulating immunity (8). Studies with plants show promise in reducing inflammation and regulating immunity. A previous study demonstrated that *Withania coagulans* corrects reduced the proinflammatory markers in kidneys (9). Another plant study, *Combretum molle* treatment elicited a decline in MPO and XO activity in diabetic rats (10). Additionally, grape seed extract attenuated both XO and ADA activities in the diabetic rats (11). Previously, *Mesona procumbens* extract downregulated streptozotocin-induced liver XO activity, and it restored renal organic anion transporter 1 and urate transporter expression (12). Recent study, *Rumex crispus* root exhibited potent XO inhibitory activity in *in vitro* assays (13).

The genus Scorzonera (Asteraceae) is widely spread in Europe, Asia and Africa. They are edible wild plants and are used up generally raw in spring because of their nutritional and dietary value (3). Scorzonera genera are used in traditional medicine in Europe, China, Tibet, Mongolia, Libya, and Turkey to treat gastrointestinal disorders, colds, fever, pulmonary diseases, and parasitic diseases. Moreover, these genera are used as galactagogues and appetizers as well as for rheumatic disorders, renal failure, hepatic pains, abscesses, and diabetes mellitus (3). To the best of our knowledge, there are limited experimental studies on *Scorzonera cinerea* (Sc) in the literature. This study aims to investigate the immune system effects of *S. cinerea* radical leaf extract in diabetic rat.

2. METHODS

2.1. Plant Materials and Extraction

S. cinerea was collected from Van, Turkey, in April 2017. The taxonomic identification of the plant was performed and a specimen is kept in the herbarium of Van Yüzüncü Yıl University. Parts of *S. cinerea* radical leaves were dried outdoors approx. 18°C and 55% humidity for 4 days and then powdered. The other parts of them were frozen at −22°C for 3-month storage. The dried radical leaves were extracted with ethanol (75%) at 50°C for 3 h. The frozen radical leaves were first gradually thawed and then crumbled and extracted with ethanol (75%) at 50°C for 3 h. The extractions were filtered through filter paper and then concentrated under reduced pressure at 40°C (IKA RV3 V, Germany).

2.2. L-Ascorbic Acid Determination

The amount of ascorbic acid was determined by the method of Lee and Coates (14) with some modifications. The plant samples were homogenized with ice-cold metaphosphoric acid (4%) in ice bath. The homogenate was centrifuged at 10000×g for 5 min at 4°C. The upper clear liquid was filtered and immediately injected into the HPLC system. The system consists of a Rheodyne 7725i injector, an LC-20 AD gradient pump, a CTO-10AS VP column furnace, and an SPD-M20A diode array detector. Hypersil Gold aQ C18 column was used with a mobile phase (H_2O:H_2SO_4 98:2 v/v, pH 2.54). Flow rate of 0.7 mL/min and the injection volume of 20 µL were adjusted at 25°C. The measurement was performed at 244 nm. Ascorbic acid was identified and calculated according to retention times and by comparison with the external standard ($y = 86755x + 3611.6$, $r^2 = 0.9992$).

2.3. Animals

Experiment was performed using 40 healthy *Wistar albino* male rats (200–300 g; 2–3 months of age) which obtained from Van Yüzüncü Yıl University Experimental Application Center. The rats were housed under standard conditions (22±2°C, 50% humidity, and under a 12-h light/dark cycles). They were provided standard chow and tap water *ad libitum*. Ethics Committee of Van Yüzüncü Yıl University approved the study protocol (Date:2019, decision no: 2019/02).

2.4. Experimental Design

Experimental diabetes was induced by a single dose intraperitoneally (i.p) injection of streptozotocin (STZ) (45 mg/kg body weight (bw) in citrate buffer). Rats were allowed to develop diabetes for 3 days. Thereafter, rats with fasting blood glucose levels over than 200 mg/dL were considered diabetic. The rats were divided randomly into 5 groups (n=8) as follows:

Control group (CG) received single dose of 1 mL citrate buffer i.p and 1 mL physiological saline p.o for 21 days; Diabetic group (DG) – diabetic rats who received 1 mL physiological saline orally (p.o) for 21 days after DM induction; Sc-Dried group – diabetic rats who were administered 100 mg/kg bw dried Scorzonera extract p.o for 21 days after DM induction; Sc-Frozen group – rats who were administered 100 mg/kg bw frozen Scorzonera extract p.o for 21 days after DM induction; Acarbose group – rats who were administered 50 mg/kg bw acarbose p.o for 21 days after DM induction. After 21 days, the rats were anesthetized, and then blood and tissue samples were taken.

2.5. Biochemical Analyses

Rat tissues were homogenized with ultrasonic homogenizers in an ice-cold phosphate-buffered solution (pH 7.4) and centrifuged at 8570×g for 30 min at +4°C. The obtained supernatants were used to evaluate ADA, MPO, and XO activities. Protein quantification was measured by modifying the Lowry method (15).

2.6. Measurement of ADA Activity

ADA was measured using the method of Giusti (16). The method is based on the generation of ammonia, which is directly proportional to the extinction of indophenol as a final product. The ammonia reacts with hypochlorite and phenol in an alkaline solution thereby formation of an intense blue color which is measured at 630 nm.

2.7. Measurement of XO Activity

XO was determined using the method of Prajda and Weber (17). The XO method is based on formation of uric acid from xanthine at 37°C. XO activity was measured at 293 nm and calculated in mmol uric acid produced per min.

2.8. Measurement of MPO Activity

MPO was analyzed by the method of Bradley *et al*. (18). MPO catalyzes the conversion of hydrogen peroxide (H_2O_2) and chloride (Cl^-) to highly toxic hypochlorous acid ($HOCl^-$). The produced oxygen radical (O^-) reacts with o-dianisidine dihydrochloride to form a colored compound which is measured spectrophotometrically at 460 nm.

2.9. Hematological Parameters

The complete blood count was evaluated using an autoanalyzer (Abaxis Vetscan HM2, Allied analytic, USA).

2.10. Statistical Analyses

All the data were statistically analyzed by one-way ANOVA and Tukey post hoc test. The findings were presented as mean±SD. Value of $p< .05$ was considered as significantly different.

3. RESULTS

The amount of ascorbic acid in *S. cinerea* is presented in Table 1. Although ascorbic acid in dried Sc was found, it could not be detected in frozen Sc.

Table 1. *The amount of ascorbic acid in Scorzonera cinerea*

Analysis	Dried	Frozen
Ascorbic acid (mg/kg)	36.67	n.d

n.d, not detected.

ADA activities of liver and kidney tissues were increased in the diabetic group compared to the control group ($p< .05$) (Figure 1). Sc-Dried, Sc-Frozen, and acarbose administration significantly decreased ADA activity in comparison with the diabetic group in the liver. Although kidney ADA activity was low in the Sc groups, it was not significant. Liver and kidney XO activity elevated significantly in the diabetic group than those in the control group (Figure 2). However, XO activity was found to be significantly lower in both tissues in the Sc-Dried, Sc-Frozen, and acarbose administered groups in comparison with the diabetic group. MPO activity in both tissues of the diabetic group was remarkably higher than those in the control group (Figure 3). Notably, Sc-Dried and Sc-Frozen supplementation were decreased MPO activity in the liver and kidney tissues compared to the diabetic group ($p< .05$). Besides, administration of acarbose also significantly reduced MPO activity in both tissues ($p< .05$).

The hematological values of the rats are shown in Table 2. There was no significant difference between the groups in RBC and mean corpuscular hemoglobin (MCH) parameters. However, hemoglobin (Hb) was found significantly higher in the Sc-Dried and Sc-Frozen groups than those in the diabetic group. A significant decrease was observed in mean corpuscular volume (MCV) and hematocrit (HCT) in the diabetic group compared to the control. On the other hand, Sc-Dried administration increased HCT and Sc-Frozen administration increased MCV in comparison with the diabetic group. The significant increase in the PLT count and mean platelet volume (MPV) observed in the diabetic group drastically reduced to near normal level following the administration of Sc-Dried and Sc-Frozen. Moreover, acarbose treatment decreased the PLT count in comparison with the diabetic group. As regards to the white blood cell and fragments, while white blood cells (WBC) level was not significantly affected by diabetes, there was a significant decrease in Sc treated groups compared to those in the diabetic group. Whilst the percentage of LYM significantly decreased, NEU%, MO%, and EOS% raised in the diabetic group in comparison with the control group. However, a significant decrease was found in NEU%, MO%, and EOS% in the Sc-Dried group in comparison with the diabetic group. Similarly, the Sc-Frozen group also had a significant decline for NEU% and EOS%.

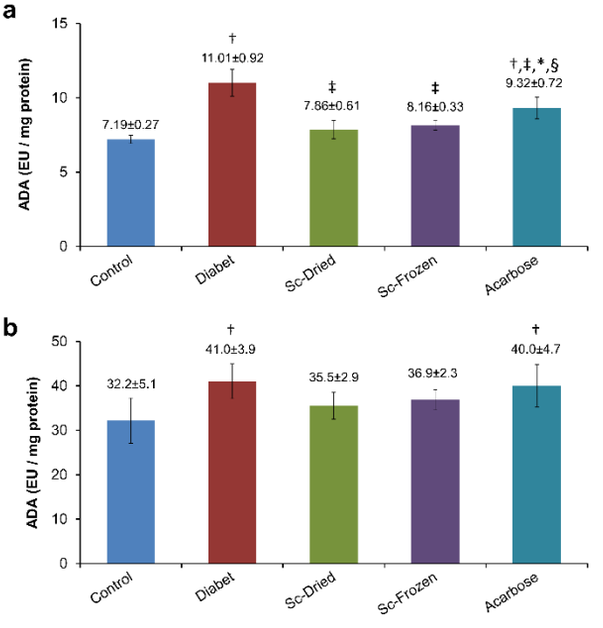


Figure 1. *Liver (A) and Kidney (B) ADA activities of rats.*
*†: It was significantly different from control group ($p< .05$), ‡: It was significantly different from Diabetic group ($p< .05$), *: It was significantly different from Sc-Dried group ($p< .05$), §: It was significantly different from Sc-Frozen group ($p< .05$).*

Table 2. *Hemogram parameters of rats*

Blood	Control	Diabetic	Sc-Dried	Sc-Frozen	Acarbose
RBC (10^{12}/L)	9.16±0.39	8.53±0.27	9.15±0.62	9.08±0.50	8.87±0.32
Hb (g/dL)	16.30±0.50	15.50±1.02	17.57±0.66†,‡	17.40±0.97‡	16.20±0.77§
MCV (fL)	57.26±1.16	54.49±0.98†	56.81±2.97	56.29±0.96‡	57.19±1.26‡
HCT (%)	52.472.83	46.51±4.11†	52.03±2.94‡	51.10±2.90	50.73±2.14
MCH (pg)	17.79±1.40	18.17±0.61	19.21±1.28	19.16±0.85	18.26±0.95
MCHC (g/dL)	31.09±1.20	33.34±1.13†	33.77±1.09†	34.04±1.20†	31.93±1.02*,§
PLT (10^9/L)	413±57	608±31†	479±31‡	407±65‡	488±62‡
MPV (fL)	6.44±0.19	7.30±0.31†	6.39±0.34‡	6.49±0.55‡	6.96±0.21*
WBC (10^9/L)	5.87±0.73	7.11±1.06	5.26±1.05‡	4.87±0.99‡	5.64±1.27
LYM (%)	82.17±2.04	70.40±2.05†	76.87±4.87‡	74.96±4.93†	73.27±4.44†
NEU (%)	11.79±1.54	18.00±2.32†	14.74±1.25‡	13.99±1.75‡	17.63±2.48†,§
MO (%)	6.04±0.66	9.70±1.56†	7.43±1.01‡	7.87±0.56	9.24±1.70†
EOS (%)	0.11±0.04	0.33±0.16†	0.14±0.05‡	0.17±0.08‡	0.21±0.01

†: It was significantly different from control group (p< .05).
‡: It was significantly different from Diabetic group (p< .05).
**: It was significantly different from Sc-Dried group (p< .05).*
§: It was significantly different from Sc-Frozen group (p< .05).

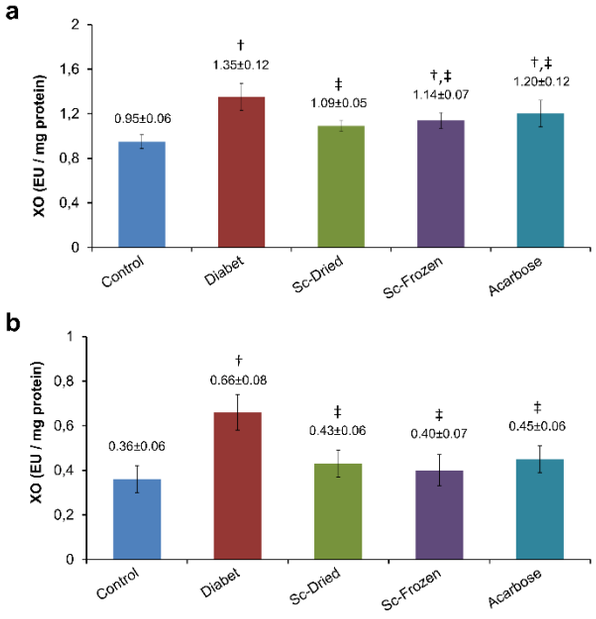
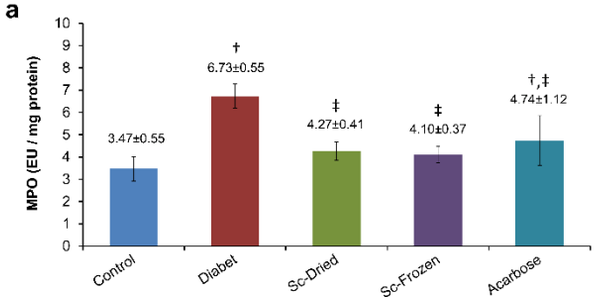


Figure 2. *Liver (A) and Kidney (B) XO activities of rats.*
†: It was significantly different from control group (p< .05), ‡: It was significantly different from Diabetic group (p< .05).



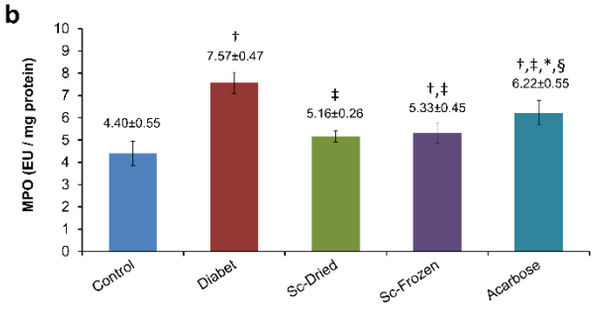


Figure 3. *Liver (A) and Kidney (B) MPO activities of rats.*
*†: It was significantly different from control group (p< .05), ‡: It was significantly different from Diabetic group (p< .05), *: It was significantly different from Sc-Dried group (p< .05), §: It was significantly different from Sc-Frozen group (p< .05).*

4. DISCUSSION

The immune system is affected by many conditions such as smoking and excessive use of alcohol, sedentary lifestyle and irregular sleep, irregular and wrong eating habits or some diseases, one of which is DM (19). Due to the inability to control the spread of invading pathogens in diabetics, hyperglycemia is thought to cause dysfunction of the immune response. For this reason, it is known that diabetics are more susceptible to infections (20). The immune system is a complex and multi-layered system that protects the body against infections and other diseases. So, strengthening the immune system is very important in this sense. Phytochemicals such as polyphenols and vitamins found in medicinal and wild edible plants may have beneficial therapeutic effects on immune system disorders in DM. Polyphenols may provide protection indirectly through the activation of endogenous defense systems and through the modulation of cellular signaling processes such as NF-kB

activation, glutathione synthesis, MAPK pathway, and PI3/Akt pathway (21). Moreover, polyphenols can enhance the level of IL-21 and decrease the release of IL-1β and IL-6 (22). It is also well known that vitamins especially ascorbic acid is responsible for the immune system.

In the current study, although ascorbic acid in dried Sc was found, it could not be detected in frozen Sc (Table 1). The frozen and thawing processes resulted in the complete loss of ascorbic acid after 3-month storage. It has been stated that thawing at 4°C, which is a common practice, resulted in pigment and ascorbic acid losses (23). This was assumed to result from oxidation by polyphenol oxidases and ascorbate oxidase activities of anthocyanins and ascorbic acid, respectively. Accordingly, it was determined that after thawing, ascorbic acid was almost completely depleted after 48 hours at 20°C (23). Moreover, it has been informed that ascorbic acid continues to degrade during the storage process of frozen products and losses is about 20–50% for fruits and vegetables stored at −18 to −20 °C (24). Since ascorbic acid is a water-soluble vitamin, cellular compartments can be disorganized in the freezing process and can be quickly depleted by ascorbate oxidase in the thawing process (23). On the other hand, it is normal to have high component concentration in the dry sample, but the ascorbic acid in the wet sample may be below the detectable limit due to the high-water content. Undetected may be due to this. Dried *S. cinerea* has 36.67 mg/kg of ascorbic acid. Dietary reference intake (DRI) of ascorbic acid is 75 and 90 mg/d for females and males, respectively (25). Therefore, the amount of ascorbic acid in dried *S. cinerea* can supply the DRI of 40.7 – 48.9 %.

ADA deaminates adenosine to inosine, and deoxyadenosine to deoxyinosine irreversibly. In the ensuing reaction hypoxanthine is formed. It is regarded as a marker in evaluating cell-mediated immune response (26). It is also suggested that ADA plays an important role in modulating the bioactivity of insulin. In cases of oxidative stress and cell membrane damage, serum ADA is increased (27). ADA activity increased in both tissues of the diabetic group (Figure 1). Current findings are supported by many studies that were found a significant elevation in ADA activity in tissues of STZ-induced diabetic rats (28-30). It has been stated that in type 2 diabetes, ADA activities are mainly raised which has a positive correlation with glycemic parameters (26). In our recent study, it was shown that glycemic parameters such as blood glucose and Hb_{A1c} were high in the diabetic group (3). The liver ADA activity significantly decreased with *S. cinerea* treatment. In studies of various plant extracts, for example, *Beta vulgaris* leaves and *Syzygium cumini* seed extracts decreased ADA activity in diabetic conditions through purinergic signaling inhibition (28,29). Bitencourt *et al*. (29) reported that chlorogenic acid and rutin are the most abundant phenolic compounds in *Syzygium cumini*. In a previous study, rutin administration reduced the elevated ADA level of STZ-induced diabetic rats (31). *S. cinerea* contains many phenolic compounds such as chlorogenic acid, gallic acid, ellagic acid, and rutin (3). Therefore, *S. cinerea* may have been effective in reducing the activity by modulating ADA through the individual phenolics or their synergistic effects.

Hypoxanthine formed ensuing ADA reactions is oxidized to xanthine by XO, and xanthine is also oxidized to uric acid by XO. Namely, an increment in ADA activity causes an increase in XO activity. The reactions catalyzed by XO also produce ROS. The current study demonstrated that diabetes resulted in about 2-fold XO activity than that of control groups in both tissues (Figure 2). The present findings are in line with earlier reports in which diabetes led to ROS generation in tissues through increasing XO activity (32). Fortunately, Sc and acarbose treatments were effective in reducing XO activity. In a previous study, grape seed extract supplementation reduced the plasma XO activity of STZ-induced diabetic rats (11). Many plant extracts and isolated compounds showed XO inhibitory effect such as *Citrullus colocynthis* leaf extract (33), apigenin and hispidulin isolated from *Centaurea virgata* aerial parts (34). It was stated that some isolated compounds from *Rumex crispus* root showed strong XO and α-glucosidase inhibitory effect (13). Recently, it was reported that dried *S. cinerea* and frozen *S. cinerea* possess high α-amylase and α-glucosidase inhibitory effects (3). The dried and frozen *S. cinerea* have α-amylase inhibitory effect 0.037±0.000 and 0.053±0.005 mg/mL, respectively. Besides, α-glucosidase inhibitory effect of dried and frozen *S. cinerea* are 0.074±0.002 and 0.067±0.000 mg/mL, respectively. However, acarbose has shown α-amylase and α-glucosidase inhibitory effect 0.380±0.019 and 0.420±0.010 mg/mL, respectively (3). ADA and XO activity may have diminished due to both antidiabetic and antioxidant as well as purinergic enzymes inhibitory effects of *S. cinerea*.

MPO is an activated neutrophil-specific enzyme that regulates modulation of immunological responses and inflammation, inhibition of nitric oxide generation, and modification of lipoprotein function (35). MPO catalyzes the reaction of chloride and H_2O_2 to produce $HOCl^-$. MPO also mediates oxidative stress by inducing the generation of ROS and reactive nitrogen species. Therefore, increased stress conditions in diabetes can exacerbate MPO activity. In a previous study compatible with the present study, Gezginci-Oktayoglu *et al*. found a significant elevation in liver MPO activity in diabetic rats induced with STZ. In the same study, it has been reported that administration of *Beta vulgaris* leaves extract remarkably restored liver MPO activity in diabetic rats (28). It is estimated that MPO is a marker of an increased inflammatory state and leukocyte count in diabetics. The inflammatory biomarkers such as WBC, NEU%, MO%, and EOS% elevated in the diabetic group (Table 2). Considering that diabetes plays a role in many pathogeneses such as the activation of leukocytes, it can be said that increases in inflammation also contribute to the increase in MPO (36,37). It has been shown that rutin and curcumin pretreatments are effective in preventing hyperlipidemia-induced immune cell activation and inflammation by reducing MPO activities (38). It is known that phenolic compounds alleviate oxidative stress under favour of having antioxidant effects. Therefore,

MPO may be diminished by suppressing stress conditions due to the remarkable phenolic compounds of *S. cinerea*.

Patients with DM have infections more often than those without DM as diabetes negatively influences blood parameters. In the present study, overall significant differences in cellular elements of the blood were observed between the control and diabetic groups (Table 2). Although Hb decreased relatively in the diabetic group, a significant increase was seen in the Sc-treatment groups. More recently, we found that Sc possesses high mineral content (3). Considering the DRI of iron for adult (25), *S. cinerea* can provide with the DRI of 228 % of iron (3). Therefore, Hb may be higher in Sc groups due to high iron content. Although PLT and MPV increased in the diabetic group, Sc administrations effectively reduced these parameters. Acarbose also reduced PLT. However, in previous study, while PLT was not significantly different in diabetics, it has been shown that MPV was independently associated with the presence of diabetes (39). In the current study leukocytes, such as NEU%, MO%, and EOS%, were increased but that of LYM% was decreased in the diabetic group. Diabetes induces apoptosis in lymphocytes, which may explain low LYM% due to impaired immune function in the diabetic states (40). Our findings are consistent with Mahmoud (41), who reported while LYM% decreased in the diabetic group, hesperidin or naringin administration increased LYM%. On the other hand, it can be said that dried Sc provides more support to the immune system due to its vitamin C content and provides a relative relief in defense cells, such as WBC and LYM%. Moreover, dried Sc administration alleviated NEU%, MO%, and EOS%. Similarly, frozen Sc showed a significant decrease for NEU% and EOS%. Mahmoud (41) stated that hesperidin or naringin administration to diabetic rats was effective to reduce NEU% and MO%. Activation of the NF-κB signaling pathway is associated with regulation of inflammatory response. Hyperglycemia is an important mediator of neutrophil dysfunction in DM, as it upregulates the receptor for AGEs on the neutrophil cell surface. AGEs induce oxidative stress and pro-inflammatory gene expression (NF-κB) in multiple cell types, including neutrophils. Activation of NF-κB can be induced in the diabetic state, thereby triggering the initiation of inflammation by neutrophils (42). Anti-inflammatory feature of phenolic compounds is to inhibit neutrophil degranulation, which is a direct way to decrease the release of arachidonic acid by neutrophils and other immune cells. Using an *in vitro* TNF-α and IL-1 production inhibition assay, the anti-inflammatory potential of some Scorzonera extracts, including *S. cinerea*, was confirmed through the inhibition of NF-κB activation (43). Administration of *S. cinerea* may have attenuated the activities of immune cells by regulating the inflammatory response.

CONCLUSIONS

Increased ADA, MPO, and XO activities and some hematological parameters may be an important indicator in the immunopathogenesis of diabetes mellitus. This study demonstrated that *S. cinerea* exhibited modulatory effects on immune system. *S. cinerea* can regulate immune function by reducing oxidative stress with its efficient compounds. Naturally, no herb should be preferred to be used as an isolated form of therapy, but it can be used as an adjuvant to regulate immunity.

Acknowledgments: *We would like to thank the editor and reviewers for their contributions during the review and evaluation phase of the article.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *Ethics Committee of Van Yüzüncü Yıl University approved the study protocol (Date:2019, decision no: 2019/02).*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: MAT
Design of the study: MAT
Acquisition of data for the study: MAT
Analysis of data for the study: MAT
Interpretation of data for the study: MAT
Drafting the manuscript: MAT
Revising it critically for important intellectual content: MAT
Final approval of the version to be published: MAT

REFERENCES

[1] World Health Organization. Global report on diabetes. Geneva: WHO press; 2016.
[2] Rochette L, Zeller M, Cottin Y, Vergely C. Diabetes, oxidative stress and therapeutic strategies. Biochim Biophys Acta. 2014;1840(9):2709–2729.
[3] Temiz MA. Antioxidant and antihyperglycemic activities of *Scorzonera cinerea* radical leaves in streptozocin-induced diabetic rats. Acta Pharm. 2021;71:603–617.
[4] Zhang P, Li T, Wu X, Nice EC, Huang C, Zhang Y. Oxidative stress and diabetes: Antioxidative strategies. Front Med. 2020;14(5):583–600.
[5] Jafar N, Edriss H, Nugent K. The effect of short-term hyperglycemia on the innate immune system. Am J Med Sci. 2016;351(2):201–211.
[6] Reichert KP, Schetinger MRC, Gutierres JM, Pelinson LP, Stefanello P, Dalenogare DP, Baldissarelli J, Lopes TF, Morsch VM. Lingonberry extract provides neuroprotection by regulating the purinergic system and reducing oxidative stress in diabetic rats. Mol Nutr Food Res. 2018;62(1800050):1–7.
[7] Kumar R, Chhatwal S, Arora S, Sharma S, Singh J, Singh N, Bhandari V, Khurana A. Antihyperglycemic, antihyperlipidemic, anti-inflammatory and adenosine deaminase–lowering effects of garlic in patients with type 2 diabetes mellitus with obesity. Diabetes Metab Syndr Obes: Targets Ther. 2013;6:49–56.
[8] Ahmad AA, Merkhan MM, Abdulrazzaq GM. Effect of xanthine oxidase inhibition on immunity modulation in human. Eur J Mol Clin Med. 2020;7(10):1037–1316.
[9] Ojha S, Alkaabi J, Amir N, Sheikh A, Agil A, Fahim MA, Adem A. *Withania coagulans* fruit extract reduces oxidative stress and inflammation in kidneys of streptozotocin-induced diabetic rats. Oxid Med Cell Longev. 2014;201436:1–9.

[10] Hamza RZ, Al-Baqami NM, Khojah E, Mansour AMA, Al-Motaani SE, Al-Salmi FA, El-Megharbel SM. Possible antioxidant and antidiabetic effects of *Combretum molle* extract in a diabetes mellitus experimental model in male rats. Nat Prod Commun. 2021;16(10):1–10.

[11] Belviranli M, Gökbel H, Okudan N, Büyükbaş S. Effects of grape seed extract on oxidative stress and antioxidant defense markers in streptozotocin-induced diabetic rats. Turk J Med Sci. 2015;45:489–495.

[12] Jhang, JJ, Ong JW, Lu CC, Hsu CL, Lin JH, Liao JW, Yen GC. Hypouricemic effects of *Mesona procumbens* Hemsl. through modulating xanthine oxidase activity in vitro and in vivo. Food & Function. 2016;7(10):4239–4246.

[13] Minh TN, Van TM, Andriana Y, Vinh LT, Hau DV, Duyen DH, Guzman-Gelani C. Antioxidant, Xanthine Oxidase, α-Amylase and α-Glucosidase inhibitory activities of bioactive compounds from *Rumex crispus* L. root. Molecules. 2019;24(3899):1–12.

[14] Lee HS, Coates GA. Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: A storage study. Food Chem. 1999;65(2):165–168.

[15] Lowry H, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-275.

[16] Giusti G. Adenosine deaminase. Bergmeyer HU, editors. Methods of Enzymatic Analysis. New York: Academic Press; 1974.p.1092–1099.

[17] Prajda N, Weber G. Malignant transformation-linked imbalance: Decreased xanthine oxidase activity in hepatomas. FEBS Lett. 1975;59(2):245–249.

[18] Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Investig Dermatol. 1982;78:206–209.

[19] Nizami NS, Uddin CSM. Strong Immunity – A major weapon to fight against Covid-19. IOSR J Pharm Biol Sci. 2020;15(3):22–29.

[20] Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R. Type 2 diabetes and its impact on the immune system. Curr Diabetes Rev. 2020;16:442–449.

[21] Badescu M, Badulescu O, Badescu L, Ciocoiu M. Effects of *Sambucus nigra* and *Aronia melanocarpa* extracts on immune system disorders within diabetes mellitus. Pharm Biol. 2015;53(4):533–539.

[22] Ding S, Jiang H, Fang J. Regulation of immune function by polyphenols. J Immunol Res. 2018;(1264074):1–8.

[23] Holzwarth M, Korhummel S, Carle R, Kammerer DR. Evaluation of the effects of different freezing and thawing methods on color, polyphenol and ascorbic acid retention in strawberries (*Fragaria×ananassa* Duch.). Food Res Int. 2012;48:241–248.

[24] Rickman JC, Barrett DM, Bruhn CM. Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds. J Sci Food. Agric 2007;87:930–944.

[25] Institute of Medicine. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Washington DC: The National Academies Press; 2006.p.286–402.

[26] Sapkota LB, Thapa S, Subedi N. Correlation study of adenosine deaminase and its isoenzymes in type 2 diabetes mellitus. BMJ Open Diabetes Res Care. 2017;5(e000357):1–6.

[27] Niraula N, Thapa S, Kunwar S, Lamsal M, Baral N, Maskey R. Adenosine deaminase activity in type 2 diabetes mellitus: Does it have any role? BMC Endocr Disord. 2018;18(58):1–5.

[28] Gezginci-Oktayoglu S, Saçan O, Bolkent S, İpci Y, Kabasakal L, Şener G, Yanardağ R. Chard (*Beta vulgaris* L.var. cicla) extract ameliorates hyperglycemia by increasing GLUT2 through Akt2 and antioxidant defense in the liver of rats. Acta Histochem. 2014;116:32–39.

[29] Bitencourt PER, De Bona KS, Cargnelutti LO, Bonfanti G, Pigatto A, Boligon A, Athayde ML, Pierezan F, Zanette RA, Moretto MB. *Syzygium cumini* seed extract ameliorates adenosine deaminase activity and biochemical parameters but does not alter insulin sensitivity and pancreas architecture in a short-term model of diabetes. J Complem Integr Med. 2015;12(3):187–193.

[30] Saçan O, Türkyılmaz IB, Bayrak BB, Mutlu O, Akev N, Yanardağ R. Protective role of zinc in liver damage in experimental diabetes demonstrated via different biochemical parameters. J Biochem Mol Toxicol. 2020;35(e22617):1–6.

[31] Chielle EO, Bonfanti G, De Bona KS, Cargnelutti LO, Bitencourt PER, Da Silva PS, Campos, MMA, Moretto MB. Rutin restores adenosine deaminase activity in serum and the liver and improves biochemical parameters in streptozotocin-induced diabetic rats. Rev Bras de Plantas Medicinais. 2016;18(1):273–278.

[32] Liu J, Wang C, Liu F, Lu Y, Cheng J. Metabonomics revealed xanthine oxidase-induced oxidative stress and inflammation in the pathogenesis of diabetic nephropathy. Anal Bioanal Chem. 2015;407:2569–2579.

[33] Nessa F, Khan SA. Evaluation of antioxidant and xanthine oxidase inhibitory activity of different solvent extracts of leaves of *Citrullus colocynthis*. Pharmacog Res. 2014;6(3):218–226.

[34] Tüzün BS, Hajdú Z, Orbán-Gyapai O, Zomborszki ZP, Jedlinszki N, Forgo P, Kıvçak B, Hohmann J. Isolation of chemical constituents of *Centaurea virgata* Lam. and xanthine oxidase inhibitory activity of the plant extract and compounds. Med Chem. 2017;13:498–502.

[35] Davies MJ, Hawkins CL. The role of myeloperoxidase in biomolecule modification, chronic inflammation, and disease. Antioxid Redox Signal. 2020;32(13):957–982.

[36] Tong PC, Lee KF, So WY, Ng MH, Chan WB, Lo MK, Chan NN, Chan JC. White blood cell count is associated with macro – and microvascular complications in Chinese patients with type 2 diabetes. Diabetes Care. 2004;27:216–222.

[37] Tsai JC, Sheu SH, Chiu HC, Chung FM, Chang DM, Chen MP, Shin SJ, Lee YJ. Association of peripheral total and differential leukocyte counts with metabolic syndrome and risk of ischemic cardiovascular diseases in patients with type 2 diabetes mellitus. Diabetes Metab Res Rev. 2007;23:111–118.

[38] Manzoni AG, Passos DF, da Silva JLG, Bernardes VM, Bremm JM, Jantsch MH, de Oliveir JS, Mann TR, de Andrade JM, Leal DBR. Rutin and curcumin reduce inflammation, triglyceride levels and ADA activity in serum and immune cells in a model of hyperlipidemia. Blood Cells Mol Dis. 2019;76:13–21.

[39] Chen X, Fang L, Lin H, Shen P, Zhang T, Li H, Li X, Yu M, Xu C, Zhang J, Lu F, Du X, Hu R, Zhong J. The relationship between type 2 diabetes and platelet indicators. Iran J Public Health. 2017;46(9):1211–1216.

[40] Otton R, Soriano FG, Verlengia R, Curi R. Diabetes induces apoptosis in lymphocytes. J Endocrinol. 2004;182(1):145–156.

[41] Mahmoud AM. Hematological alterations in diabetic rats – role of adipocytokines and effect of citrus flavonoids. EXCLI J. 2013;12:647–657.

[42] Dowey R, Iqbal A, Heller SR, Sabroe I, Prince LR. A Bittersweet response to infection in diabetes; targeting neutrophils to modify inflammation and improve host immunity. Front Immunol. 2021;12:1–21.

[43] Bahadır Açıkara Ö, Hošek J, Babula P, Cvačka J, Budešínský M, Dračinský M, Saltan-İşcan G, Kadlecová D, Ballová L, Šmejkal K. Turkish Scorzonera species extracts attenuate cytokine secretion via inhibition of NF-κB activation, showing anti-inflammatory effect in vitro. Molecules. 2016;21(1):E43.

How to cite this article: Temiz MA. Effects of Scorzonera Cinerea on Immune System and Hematological Parameters in Short-Term Hyperglycemia. Clin Exp Health Sci 2023; 13: 129-136. DOI: 10.33808/clinexphealthsci.1061083

Clinical and Experimental Health Sciences

The Effects of Fulvic Acid Against Water Avoidance Stress-Induced Damage of Rat Colon Mucosa

Esra Çikler[1], İbrahim Söğüt[2], Sezen Gizem Aydoğan[3],Seda Kırmızıkan[1], Canan Hürdağ[3]

[1] University of Health Science, Hamidiye Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye.
[2] Demiroğlu Bilim University, Faculty of Medicine, Department of Biochemistry, İstanbul, Türkiye.
[3] Demiroğlu Bilim University, Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye.

Correspondence Author: Esra Çikler
E-mail: esracikler@gmail.com
Received: 29.01.2022 **Accepted:** 19.01.2023

ABSTRACT

Objective: Chronic stress plays an important role in the etiology of many inflammatory diseases. Reactive oxygen species (ROS), a source of free radicals, act as signaling molecules in the progression of stress-related inflammatory diseases. Oxidative stress occurs as a result of an increase in free radicals in the tissues. The damage caused by oxidative stress can be reduced by antioxidant replacement. In our study, the effect of fulvic acid, a powerful antioxidant, on the damage caused by the water avoidance stress model in the rat colon was investigated morphologically and biochemically.

Methods: Experimental groups (n=6, Sprague-Dawley male rats, 300 g): control (C), water avoidance stress (WAS), and water avoidance stress+fulvic acid (WAS+FA). Rats in the WAS + FA group were given a single dose of FA (150 mg/kg i.p.) immediately after exposure to water avoidance stress. The colons were stained with hematoxylin-eosin and toluidine blue. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were analyzed biochemically.

Results: Compared to the C group, the WAS group showed epithelial damage, a few empty goblet cells, inflammatory cell infiltration, and many active mast cells in the connective tissue. Mucosal integrity, the number of goblet cells, and mast cell activity improved in the WAS+FA group as compared to the WAS group. Biochemically, as compared to the C group, TAS levels decreased, and TOS and OSI levels increased in the WAS group. In the WAS+FA group, TAS levels increased, and TOS and OSI levels decreased with respect to those in the WAS group.

Conclusion: Our findings indicated that fulvic acid reduced the damage caused by chronic oxidative stress in the colon.

Keywords: Fulvic acid; water avoidance stress; oxidative stress; inflammation; colon

1. INTRODUCTION

Stress is an impaired homeostasis that must be balanced by the adaptive stress response (1). When an organism is exposed to internal and external stressors, it develops molecular, cellular, psychological, and behavioral adaptations to maintain homeostasis (2). Stress, which can have physical and emotional types, is a global problem in the modern age. Various experimental animal models have been developed to study the stress-related effects. Chronic water avoidance stress exposure in rodents mimics the experience of stress in humans (3). In experimental animals exposed to chronic water avoidance stress, inflammation in their gastrointestinal systems, damage to epithelial cells, and an increase in oxidative damage in their tissues have been reported (4).

Stress, which is frequently experienced in daily life, stimulates inflammatory responses in multiple bodily systems and causes and aggravates diseases of unknown etiology (5,6). In cases of chronic stress, the formation of reactive oxygen species (ROS) increases in tissues (7). Under normal conditions, ROS is compensated for by endogenous antioxidants, whereas under stress conditions, antioxidants are insufficient against increased levels of oxidants, and oxidative stress occurs in the tissues. Oxidative stress first causes damage to the lipid and protein components of the cell membranes, then to DNA, and ultimately to the entire cell (8).

Gastrointestinal system disorders are in the foreground of diseases triggered by stressors, such as economic and social pressure, that intensely affect daily life of adults (2,9). The gastrointestinal barrier consists of intestinal epithelial cells, subepithelial immune cells, and mucus layer. Under conditions that increase oxidative stress, such as chronic stress, pro-inflammatory cytokines secreted by mucosal mast cells disrupt this barrier (10). Cell death and tissue damage resulting in oxidative stress in the inflammation area are caused by insufficient endogenous antioxidants against the increased levels of oxidants. When the body's antioxidant

DOI: 10.33808/clinexphealthsci.1036048

system is inadequate, supplementation with external antioxidants is favored.

Humic substances, which have antioxidant and anti-inflammatory properties, have been used as supplements against inflammatory diseases in conventional medicine for more than 3000 years (11). Humic substances are divided into two forms, humic acid and fulvic acid, depending on their solubility in acids and bases (12). Because the average molecular weight of fulvic acid is 500–5000 Da, it can cross all morphological barriers. Because it has an organic structure, the body does not perceive fulvic acid as an antigen and can easily enter the targeted area (13). In this study, we investigated the protective effects of fulvic acid against the oxidative damage caused by daily stress in the colon.

2. METHODS

2.1. Animals

Eighteen male Wistar albino rats weighing 250–300 g were used in this study. Animals were housed in standard cages with a 12 hours light/dark cycle at 22°C and 55% humidity during the experiment. They were fed standard pellets and tap water (ad libitum). Ethics committee approval was obtained from İstanbul Medeniyet University (Decision date and number: 20/08/2020-42), and experimental studies were conducted at this institution.

2.2. Fulvic Acid Preparation

Fulvic acid ("Pahokee Peat Fulvic Acid Standard II") (International Humic Substance Society, Denver, CO, USA; cat no:2S103F) was homogeneously dissolved in distilled water at a ratio of 150 mg/kg. The fulvic acid solution was maintained at room temperature (14).

2.3. Water Avoidance Stress Protocol

Plexiglas pools of dimensions 50 cm × 50 cm × 50 cm were used for chronic water avoidance stress. The pools had a platform of size 4 cm × 6 cm in the center. They were filled with warm water, up to 1 cm below the platform. The rats were left on the platform for 1 h a day. The stress protocol was repeated for 10 consecutive days and was applied to all rats between 08.00–10.00 am (15). On the last day of the stress protocol, the rats were sedated with 4% isoflurane. The colons were obtained and fixed in Bouin's solution for histological examination. Colons reserved for biochemical analysis were stored at – 80 °C until analysis.

2.4. Experimental Design

The rats were divided into three groups of six animals each: control (C), chronic stress (WAS), and chronic stress+fulvic acid (WAS+FA). The C group did not receive any treatment for 10 days, the WAS group was subjected to the water avoidance stress protocol for 10 days, and the WAS+FA group was subjected to water avoidance stress followed by intraperitoneal (i.p.) injection of 150 mg/kg fulvic acid (International Humic Substance Society, Denver, CO; cat no: 2S103F) for 10 days.

2.5. Histological Analysis

In the colon, the general tissue morphology was examined with hematoxylin-eosin (H&E), and the morphology of the goblet cells was evaluated by applying the periodic acid-Schiff (PAS) reaction. In addition, morphometric evaluation of the amount and morphology of mast cells in the mucosa was performed using toluidine blue staining (TB).

The colon tissues obtained for histological examination were fixed in Bouin's solution, dehydrated in an alcohol series starting from 70% to 100%, and cleared in xylene. Tissues were kept in paraffin overnight in an incubator at 60°C and embedded in paraffin blocks at room temperature. For histological evaluations, 5 µm-thick sections were prepared from paraffin blocks with a microtome (Leica RM, IL, USA) and stained with H&E, PAS, and TB. For the morphometric evaluation of mast cells, a total of 10 sections were taken, one in every five sections, and mast cell counts were performed in five different areas of each section using a light microscope (Zeiss PrimoStar, Oberkochen, Germany). After counting the granulated and degranulated mast cells at x400 magnification, the microscope field was adjusted to x100 magnification and the section was photographed.

2.6. Biochemical Analysis

For biochemical analysis, the total antioxidant status (TAS) and total oxidant status (TOS) were determined in tissues stored at – 80 °C, and the oxidative stress index (OSI) was determined as the ratio of TOS to TAS (16).

For biochemical studies, colons were homogenized in 0.15 N potassium chloride (KCl) solution using Ultra Turrax T10 (IKA, Wilmington, NC, USA). TAS and TOS levels were determined in the supernatant obtained after centrifugation of the colon homogenates using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey). The oxidative stress index (OSI) was calculated as the ratio of TOS to TAS (16).

2.7. Statistical Analysis

GraphPad Prism 5.03 (GraphPad Software Inc.) program was used for statistical analysis. One-way ANOVA and post-hoc Tukey test were performed for data suitable for normal distribution. Kruskall-Wallis test and post-hoc Dunn's comparison test were used for data not suitable for normal distribution. Results were given as mean ± standard error (SE) or mean (minimum-maximum). A p value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Histological Results

In the C group, H&E staining of the colonic mucosa exhibited a normal morphology. PAS staining in the C group revealed that the glycocalyx continued uninterrupted; the goblet cells were generally filled with mucus and they showed intense PAS (+) staining. Few mast cells were found in the submucosal layer of some of the TB-stained colons (Figure 1 a, b, and c).

In the WAS group, H&E staining revealed that there were losses of goblet cells and enterocytes in the epithelial tissues and openings in the connective tissue of the colon. In addition, extensive inflammatory cell migration was observed in the connective tissues. The superficial mucus layer of the colon sections stained with PAS was thin, and the PAS staining was weak in the goblet cells. In the sections stained with TB, no mast cells were found in the mucosa, whereas an increased number of granulated and degranulated mast cells was observed in the submucosa (Figure 1 d, e, and f).

In the WAS+FA group, the staining with H&E showed reduced cellular loss in epithelial tissues, and the morphology was intact in most areas, similar to the C group. In addition, similar to the C group, the continuity of the glycocalyx layer was preserved in the sections stained with PAS and goblet cells showed intense PAS (+) staining. Few granular mast cells were observed in the submucosa of TB-stained colon sections, but no mast cells were found in the mucosa (Figure 1 g, h, and i).

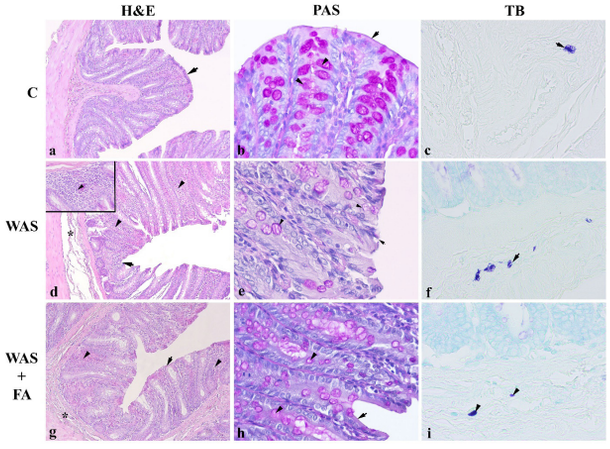


Figure 1. *C: Intact colonic epithelium of the control group (arrow), (a); Goblet cells (arrowhead) and intact glycocalyx (arrow), (b); Few mast cells in connective tissue (arrow), (c). WAS: Damaged epithelium (arrow), increased inflammatory cells in the lamina propria (arrowhead), and detachments in submucosa (asterix), (d); Discontinued glycocalyx (arrow), few and low-intensity PAS (+) reacted goblet cells (arrowhead), (e); Numerous active, degranulated mast cells (arrow) in connective tissue, (f). WAS+FA: Intact colonic epithelium (arrow), inflammatory cells persisting in the lamina propria (arrowhead), integrity in connective tissue organization (asterix), (g); Intact glycocalyx (arrow), numerous PAS (+) reacted goblet cells (arrow head), (h); Few granular mast cells in connective tissue (arrow), (i). H&E staining, x100 microscope magnification (a, d, g) and H&E staining, x400 microscope magnification (d, insert); PAS reaction, x400 microscope magnification (b, e, h); TB staining, x400 microscope magnification (c, f, i).*

3.2. Biochemical Findings

TAS (mmol Trolox/L) levels in the colon samples were lower in the WAS group than in the C group and higher in the WAS+FA group than in the WAS group. However, these changes in the TAS levels were not statistically significant (n.s.); (Table 1). TOS (µmol H_2O_2 eq/L) and OSI (arbitrary units) levels increased significantly in the WAS group compared to the C group and decreased significantly in the WAS+FA group compared to the WAS group ($p < .05$) (Table 1).

Table 1. *Comparison of TOS, TAS and OSI values of C, WAS and WAS+FA groups.*

Groups → Values ↓	C	WAS	WAS+FA	p
TOS	O.469 ±0.13	0.929 ±0.22	0.537 ±0.13	C-WAS* WAS-WAS+FA*
TAS	3.165 (2.43-3.99)	2.491 (2.00-2.99)	3.918 (3.20-4.83)	ns
OSI	17.05 ±7.90	39.37 ±13.28	14.56 ±6.45	C-WAS* WAS-WAS+FA*

*Data are means ± SE; n=6. C, control; WAS, water avoidance stress; WAS+FA, water avoidance stress+fulvic acid; TOS, total oxidant status; TAS, total antioxidant status; OSI, oxidative stress index. *p < 0.05, ns: not statistically significant.*

4. DISCUSSION

In this study, it was shown that prolonged water avoidance stress, which mimics daily life stress, increases ROS production in colon tissues, leading to oxidative damage. Stress causes the formation of reactive oxygen species (ROS) in tissues of several systems (7). In this study, we hypothesized that the harmful effects of oxidative stress can be minimized by supplementation with fulvic acid, a powerful antioxidant and anti-inflammatory agent. Chronic stress is a cognitive, behavioral, physiological, and psychological response to long-term internal and external stressors. Chronic stress leads to the deterioration of homeostasis in organisms (17). Anxiety and depression, which increase under the influence of chronic stress, significantly reduce the quality of life of individuals and even cause an increase in mortality (18). Animal models developed to mimic life stress, which causes depression and anxiety in humans, have shown that stress is an important risk factor for the occurrence or accumulation of diseases in various organ systems. (5,19). The organism develops cognitive, behavioral, physiological, and psychological adaptations to adapt to chronic stress conditions; however, under repetitive or long-term stress conditions, homeostasis is impaired and cellular damage and inflammation occur due to increased oxidative stress in tissues. The gastrointestinal tract is highly sensitive to stress factors (2). Irritable bowel syndrome (IBS) is an example of a gastrointestinal tract disease that is associated with stress-induced symptoms such as fatigue, anxiety, and depression, and significantly reduces the quality of life of individuals (20). Studies have reported that, similar to the clinical findings of IBS, under chronic stress conditions, mucosal barrier

permeability increases in the colon, the mucus layer becomes thinner, epithelial cell hyperplasia and neutrophil and mast cell infiltration into connective tissue increases (21,22).

The water avoidance stress model, which experimentally mimics physiological and psychological stress, mimics life stress when applied for ten days (19). This stress model stimulates stress-related mechanisms in the brains of rats and mice. In the presence of chronic stress, rats exhibit behaviors such as anxiety and major depression (23). In this study, it was observed that compared with the other groups, the animals in the WAS group showed more grooming and aggression. In addition to the behavioral responses of the animals in the WAS group, epithelial damage in the colon tissues and an increase in inflammatory cells in the connective tissue were observed. At the same time, an increase in the number and activity of mast cells was observed in the colon tissues of the WAS group compared with the control group. The increase in the number and activity of mast cells, which play a key role in the pathophysiology of stress, causes increased permeability of the colon tissue (24).

Mast cells play a critical role in the pathogenesis of inflammatory diseases by secreting mediators that stimulate inflammatory cell migration from the blood to connective tissue (6). In our study, we observed that histological damage in the colon tissues of the WAS group increased mast cell activation, inflammation, and oxidative stress. In our study, we observed that the total oxidant (TOS) amount and oxidative stress index (OSI) increased as a result of damage to the colon tissues of rats in the chronic stress group. The increase in the amount of TOS and OSI in the colon tissues of the WAS group and the decrease in the amount of TAS indicated that oxidative stress in the colon tissues increased and the endogenous antioxidants in the body were depleted. This suggests that pro-inflammatory cytokines secreted by mast cells migrate from the blood to connective tissue against oxidative stress, increase mucosal permeability, and stimulate inflammation. Similar to our results, Sun et al. reported morphological changes in the intestinal mucosa and increased inflammatory cell infiltration in experimental animals exposed to a 10-day chronic water avoidance stress (25). Zeybek et al. reported that a 5-day chronic water avoidance stress caused mucosal degeneration, inflammatory cell infiltration, and mast cell activation in the rat gastrointestinal tract (26). In our study, it was observed that the number of goblet cells in the colon tissues in the WAS group decreased compared to that in the C group and showed a weaker PAS (+) staining. The decrease in the number of goblet cells and mucus secretion under stress conditions suggests that goblet cells are also damaged by oxidative stress like other cells, correspondingly mucus secretion is reduced. Similar to our findings, Söderholm et al. reported that the number of goblet cells containing mucus in the ileum and colon tissues decreased as a result of a 10-day WAS (21).

Supplementation with exogenous antioxidants can minimize the effects of oxidative stress caused by long-term stress in tissues (27). It has been reported that inflammation induced by oxidative damage caused by ethanol in the gastrointestinal tract can be reversed with natural compounds with antioxidant effects (28,29). It is known that substances such as peat, sapropel, and shilajit containing fulvic acid as well as several agents such as antioxidant compounds, vitamins A and E, melatonin, and alpha-lipoic acid have been used in traditional medicine for more than 300 years (11). Due to its antioxidant, anti-inflammatory, anti-allergic, and anti-apoptotic properties, fulvic acid is an important agent that has been studied for many years in the field of medicine (11).

A study by Goel et al. in rats reported that treatment with 100 mg/kg/d fulvic acid played an antiulcerogenic and anti-inflammatory role (30). Bahçıvan et al. concluded that 150 mg/kg fulvic acid could be used as a therapeutic agent against testicular damage caused by chronic stress (15). In our study, fulvic acid was administered to rats via intraperitoneal (i.p.) injection immediately after the chronic stress protocol. The epithelial cell morphology of the group administered fulvic acid after chronic stress was similar to that of the control group. The number of goblet cells and the level of PAS (+) staining increased. In the same group, it was observed that the number and activation of mast cells in the connective tissue decreased compared to that in the chronic stress group. In parallel with the findings on mast cells, the significant decrease in leukocyte infiltration in the connective tissue in the WAS+FA group as compared to the stress group suggests that fulvic acid contributes to anti-inflammatory mechanisms and may have a protective effect. Fulvic acid may have decreased the mast cell activation via antiallergic activities mediated by the biphenyl-type hydrocarbons present in it (11). According to the biochemical results obtained from the group administered fulvic acid after stress, TOS and OSI values decreased compared to those in the stress group, while TAS values increased. According to these findings, since fulvic acid has an antioxidant effect, the TAS ratio in the colon tissues increased compared with that in the chronically stressed group, and the TOS and OSI ratios decreased. As a result of the reduction in oxidative stress, the damage caused by stress in tissues, mast cells, and inflammation were also reduced. Our biochemical results, which are compatible with our morphological findings, support our hypothesis that fulvic acid supplementation may be beneficial for restoring the oxidant-antioxidant balance that is disrupted under oxidative stress conditions.

5. CONCLUSION

In conclusion, fulvic acid can be used as an alternative preventative agent against stress-induced colonic damage owing to its antioxidant and anti-inflammatory properties. The findings of this study may serve as the basis for future experimental studies.

Acknowledgement: *The authors would like to thank Prof. Dr. Ertuğrul Kılıç, Msci. Vet. Ekrem Musa Özdemir, and Msci. Eng. Ali Şenbahçe.*
Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by İstanbul Medeniyet University Ethical Committee for Animal Research (Decision date and number:20/08/2020-42)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: E.Ç., S.G.A.
Design of the study: E.Ç.
Acquisition of data for the study: İ.S., S.G.A., S.K.
Analysis of data for the study: E.Ç., İ.S., S.G.A, S.K.
Interpretation of data for the study: E.Ç., İ.S., C.H.
Drafting the manuscript: E.Ç., İ.S., S.G.A.
Revising it critically for important intellectual content: E.Ç.
Final approval of the version to be published: E.Ç, İ.S., C.H.

REFERENCES

[1] Edwards L, Guilliams TG. Chronic stress and the HPA axis: Clinical assessment and therapeutic considerations. The Standard. 2010;9(2):1-12.

[2] Mawdsley JE, Rampton DS. Psychological Stress in IBD: New insights into pathogenic and therapeutic implications. Gut. 2005;54(10):1481-1491. DOI: 10.1136/gut.2005.064261.

[3] Bradesi S, Schwetz I, Ennes HS, Lamy CM, Ohning G, Fanselow M, Pothoulakis C, McRoberts JA, Mayer EA. Repeated exposure to water avoidance stress in rats: A new model for sustained visceral hyperalgesia. Am J Physiol Gastrointest Liver Physiol. 2005;289(1):42-53. DOI: 10.1152/ajpgi.00500.2004.

[4] Ersoy Y, Çikler E, Çetinel Ş, Şener G, Ercan F. Leukotriene D4 receptor antagonist montelukast alleviates water avoidance stress-induced degeneration of the gastrointestinal mucosa. PLEFA. 2008;78(3):189-197. DOI: 10.1016/j.plefa.2008.01.007.

[5] Nandam LS, Brazel M, Zhou M, Jhaveri DJ. Cortisol and major depressive disorder-translating findings from humans to animal models and back. Front Psychiatry. 2020;10:974. DOI: 10.3389/fpsyt.2019.00974.

[6] Theoharides TC, Cochrane DE. Critical role of mast cells in inflammatory diseases and the effect of acute stress. J Neuroimmunol. 2004;146(1-2):1-12. DOI: 10.1016/j.jneuroim.2003.10.041.

[7] Aschbacher K, O'Donovan A, Wolkowitz OM, Dhabhar FS, Su Y, Epel E. Good stress, bad stress and oxidative stress: Insights from anticipatory cortisol reactivity. Psychoneuroendocrinology. 2013;38(9):1698-1708. DOI: 10.1016/j.psyneuen.2013.02.004.

[8] Sogut I, Oglakcı A, Kartkaya K, Ol KK, Sogut MS, Kanbak G, Inal ME. Effect of boric acid on oxidative stress in rats with fetal alcohool syndrome. Exp Ther Med. 2015;9(3):1023-1027. DOI: 10.3892/etm.2014.2164.

[9] Cetinel S, Ercan F, Cikler E, Contuk G, Sener G. Protective effect of melatonin on water avoidance stress induced degeneration of the bladder. J Urol. 2005;173(1):267-270. DOI: 10.1097/01.ju.000.014.5891.35810.56.

[10] Overman EL, Rivier JE, Moeser AJ. CRF induced intestinal epithelial barrier injury via the releasevof mast cell proteases and TNF-α. PlosOne. 2012;7(6):e39935. DOI: 10.1371/journal.pone.0039935.

[11] Schepetkin I, Khlebnikov A, Kwon BS. Medical drugs from humus matter: Focus on mumie. Drug Develop Res. 2002;57:140-159. DOI: 10.1002/ddr.10058.

[12] Sparks DL. Environmental soil chemistry. San Diego: Academic Press; 2003. p.1-267.

[13] Gandy JJ, Meeding JP, Snyman JR, Van Rensburg CE. Phase I clinical study of the acute and subacute safety and proof of concept efficacy of carbohydrate-derived fulvic acid. Clin Pharmacol. 2012;4:7-11. DOI: 10.2147/CPAA.S25784.

[14] Afify AE-MMR, Konswa OK. Natural antioxidants of humic and fulvic acids against oxidative stress induced by hydrogen peroxide: in-vivo studies. Fresen Env Bull. 2017;26(10):5841-5848.

[15] Bahçıvan C, Söğüt İ, Hürdağ C, Çikler-Dülger E. Investigation on the therapeutic effect of the fulvic acid upon the testicular damage occurred by the water avoidance stress (WAS). Bozok Medical Journal. 2018;8(4):60-68. DOI: 10.16919/bozoktip.421473.

[16] Çikler-Dülger E, Söğüt İ. Investigation of the protective effects of boric acid on ethanol induced kidney injury. Biotech Histochem. 2020;95(3):186-193. DOI: 10.1080/10520.295.2019.1662086.

[17] Chrosus JP. Stress and disorders of the stress system. Nat Rev Endocrinol. 2009;5(7):374-381. DOI: 10.1038/nrendo.2009.106.

[18] Lassnig R. Hofmann P. Life crisis as a consequence of depression and anxiety. Wien Med Wochenschr. 2007;157(17-18):435-444. DOI: 10.1007/s10354.007.0455-1.

[19] Patki G, Solanki N, Atrooz F, Allam F, Salim S. Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. Brain Res. 2013;1539:73-76. DOI: 10.1016/j.brainres.2013.09.033.

[20] Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, Mayer EA, Niesler B, Quigley EM, Rajilić-Stojanović M, Schemann M, Schwille-Kiuntke J, Simren M, Zipfel S, Spiller RC. Irritable bowel syndrome. Nat Rev Dis Prim. 2013;2:16014. DOI: 10.1038/nrdp.2016.14.

[21] Jacob C, Yang PC, Darmoul D, Amadesi S, Saito T, Cottrell GS, Coelho AM, Singh P, Grady EF, Perdue M, Bunnett NW. Mast cell tryptase controls paracellular permeability of the intestine. J Biol Chem. 2005;280(36):31936–31948. DOI: 10.1074/jbc.M506338200.

[22] Söderholm JD, Yang PC, Ceponis P, Vohra A, Riddell R, Sherman PM, Perdue MH. Chronic stress induced mast cell-dependent bacterial adherence and initiates mucosal inflammation in rat intestine. Gastroenterology 2002;123(4):1099-1108. DOI: 10.1053/gast.2002.36019.

[23] Nozu T, Kumei S, Takakusaki K, Okumura T. Water avoidance stress enhances gastric contractions in freely moving conscious rats: Role of peripheral CRF receptors. J Gastroenterol. 2014;49(5):799-805. DOI: 10.1007/s00535.013.0828-8.

[24] Vivinus-Nébot M, Dainese R, Anty R, Saint-Paul MC, Nano JL, Gonthier N, Marjoux S, Frin-Mathy G, Bernard G, Hébuterne X, Tran A, Theodorou V, Piche T. Combination of allergic factors can worsen diarrheic irritable bowel syndrome: Role of barrier defects and mast cells. Am J Gastroenterol Suppl. 2012;107(1):75-81. DOI: 10.1038/ajg.2011.315.

[25] Sun Y, Zhang M, Chen CC, Gillilland M 3rd, Sun X, El-Zaatari M, Huffnagle GB, Young VB, Zhang J, Hong SC, Chang YM, Gumucio DL, Owyang C, Kao JY. Stress-induced corticotropin-releasing hormone-mediated NLRP6 inflammasome nhibition and transmissible enteritis in mice. Gastroenterology. 2013;144(7):1478-1487. DOI: 10.1053/j.gastro.2013.02.038.

[26] Zeybek A, Ercan F, Cetinle S, Cikler E, Sağlam B, Sener G. Taurine ameliorates water avoidance stress-induced degenerations of

gastrointestinal tract and liver. Dig Dis Sci. 2006;51(10):1853-1861. DOI: 10.1007/s10620.006.9425-5.

[27] Duthie GG, Arthur JR, Nicol F, Walker M. Increased indices of lipid peroxidation in stress-susceptible pigs and effects of vitamin E. Res Vet Sci. 1989;46(2):226-230.

[28] Sogut I, Paltun OS, Tunçdemir M, Ersoz M, Hurdag C. The antioxidant and antiapoptotic effect of boric acid on hepatoxicity in chronic alcohol-fed rats. Can J Physiol Pharmacol. 2018;96(4):404-411. DOI: 10.1139/cjpp-2017-0487.

[29] Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol. 2002;35(5):523-534. DOI: 10.1590/s0100-879x200.200.0500003.

[30] Goel RK, Banerjee RS, Acharya SB. Antiulcerogenic and antiinflammatory studies with shilajit. J Ethnopharmacol. 1990;29(1):95-103. DOI: 10.1016/0378-8741(90)90102-y.

How to cite this article: Çikler E, Söğüt I, Aydoğan Gizem S, Kırmızıkan S, Hürdağ C. The Effects of Fulvic Acid Against Water Avoidance Stress-Induced Damage of Rat Colon Mucosa. Clin Exp Health Sci 2023; 13: 137-*142*. DOI: 10.33808/clinexphealthsci.1036048

Clinical and Experimental Health Sciences

Relationship Between Nutritional Status, Anthropometric Measurements and Dietary Inflammatory Index in Professional Football Players

Emre Batuhan Kenger[1], Fatih Eren[2], Fatma Esra Güneş[3]

[1] Bahçeşehir University, Faculty of Health Sciences, İstanbul, Türkiye.
[2] Marmara University, Faculty of Medicine, Department of Medical Biology, İstanbul, Türkiye.
[3] İstanbul Medeniyet University, Faculty of Health Sciences, İstanbul, Türkiye.

Correspondence Author: Emre Batuhan Kenger
E-mail: emrebatuhan.kenger@hes.bau.edu.tr
Received: 01.02.2022 **Accepted:** 15.04.2022

ABSTRACT

Objective: This study was carried out to evaluate the relationship between the nutritional status, anthropometric measurements and dietary inflammatory index (DII) of professional football players exposed to long-term intense exercise.

Method: Twenty-one professional male football players with a mean age of 26.00±5.69 years playing in the same club participated in the study. The nutritional status of the football players was evaluated with 3-day food consumption record (2 days of training and 1 match day). DII scores were calculated using data on 34 nutrient/nutritional ingredients obtained from the food consumption records. Body fat percentage in the anthropometric evaluations were determined by caliper and skinfold thicknesses.

Results: The median DII scores of the football players were found as – 3.42 (-9.95 – 0.95), and their nutritional intake were found to be anti-inflammatory. When the relationship between the DII scores of the football players and their anthropometric measurements was examined, a positive and significant correlation (R: .476; p: .029) was found between their DII score and their abdominal adiposity. However, there was no significant correlation (p> .05) between the DII scores and the other anthropometric measurements. In addition, there was a significant negative correlation (R: – .468; p: .032) between fiber consumption and abdominal adiposity, and a significant positive correlation between carbohydrate and fat consumption and body weight (respectively R= .730 p= .000; R= .526 p= .014).

Conclusion: It has been revealed that the football players participating in our study generally have an anti-inflammatory diet. It was also found that abdominal adiposity was higher in the football players with high DII scores.
Keywords: Football player, dietary inflammatory index, anthropometry

1. INTRODUCTION

Football is a challenging game that includes irregular changes in speed and anaerobic activities, as well as long-term moderate-intensity exercise in which various physiological systems are combined, and it includes non-cyclical and intermittent, high-intensity activities (1). In football, which is the most popular sport in the world, it is of crucial importance to maintain and improve performance and prevent injuries. Nutrition is considered to be a vital part of performance and recovery in both young and elite athletes (2). It also has a very important effect on the general health of athletes. It is emphasized that optimal nutrition for athletes includes adequate intake of energy, macro and micronutrients, and fluid during training and competition periods (2,3). However, it is stated that the immune system is at risk in football, which is an endurance sport. (4). Long-term and intense exercises are associated with psychological, metabolic and physiological stress, immune dysfunction, inflammation, oxidative stress and muscle damage (5). In addition, it is stated that there is an increase in inflammation biomarkers, especially after high-intensity exercises (6).

Nutrition affects inflammation positively or negatively (7). Cavicchia et al. created a special scoring system called the dietary inflammatory index (DII) by measuring the nutrients or nutritional ingredients thought to affect inflammation (8). In the study by Shivappa et al. (2014), the validity of DII was achieved (9). DII has been used in many studies on nutrition and inflammation (10,11). The aim of the study is to evaluate the relationship between the nutritional status, anthropometric measurements and DII scores of professional football players exposed to long-term intense exercise.

2. METHODS

The sample of this cross-sectional study consisted of male football players of a professional club based in Istanbul that plays in the Turkish Football Federation Second League.

DOI: 10.33808/clinexphealthsci.1066512

After necessary permissions were obtained from the club, the players meeting the inclusion criteria were made to sign voluntary consent forms. Those who did not have any chronic diseases and were not receiving any medication or nutritional support were included in the study. Having been prepared in accordance with the ethical standards of the Declaration of Helsinki, the present study received ethical approval from the clinical research ethics committee of Marmara University (16.09.2020 no: 09.2020.952).

Anthropometric variables include body height (BH), body weight (BW), body mass index (BMI), and body fat percentage (BF%). The measurements were performed according to the anthropometric measurement standards recommended by the International Society for the Advancement of Kinanthropometry (ISAK) (12). BH was measured with an accuracy of 0.1 cm using a Harpenden anthropometer (Holtain Ltd, Croswell, UK). BW was evaluated using an electronic scale (Sinbo^{R}) with an accuracy of 0.1 kg. Skinfold thickness was measured with a caliper (Holtain Ltd, Croswell, UK). Skinfold thicknesses were taken from four areas (anterior thigh, abdominal, triceps and medial calf sites) suggested for football players, and were calculated using the following formula to estimate BF% (13). BMI was calculated by dividing the body weight by the square of the height in meters. However, it is stated that the use of BMI parameter in athletes causes problematic results (14). For this reason, BMI was not included when examining the relationship between nutrient intake and anthropometric measurements of football players.

BF%= 5.174 + (0.124 × thigh) + (0.147 × abdominal) + (0.196 × triceps) + (0.130 × calf)

The food consumption records of the football players were obtained by interviewing them face to face and showing them the food atlas (15). The food consumption of the football players was determined by taking the average of their 3-day diet records (2 days of training and 1 match day). The food consumption data were analyzed using Nutrition Information Systems (Beslenme Bilgi Sistemleri – BeBiS) version 8.1 (Pasific Ltd. Şti., Istanbul, Turkey). The results obtained based on the food consumption records were evaluated by using the DII scoring that Shivappa et al. developed and revised (8,9).

The obtained data were evaluated with SPSS software package program version 21.0 (IBM Inc., Chicago). Statistical significance was accepted as $p<0.05$ in all analyzes. Numbers, percentages, medians and minimum-maximum values were included in descriptive statistics. The relationship between the DII scores and anthropometric measurements was determined using Spearman's correlation.

3. RESULTS

A total of 21 volunteer professional football players with a mean age of 26.00±5.69 years were included in the study. While 76.2% of the football players were high school graduates, 23.8% were university graduates. When their dietary intakes were examined, all of the participants (100%) were found to consume 3 main meals, while 57.1% had only 1 snack. On the training days, all of them (100%) had their meals 3-4 hours before the training. They had meals within the first 2 hours following the training (Table 1).

Table 1. *Demographic characteristics and dietary habits of football players*

	n	%
Characteristics		
Education		
High school	16	76.2
Bachelor's degree	5	23.8
Marital status		
Married	8	38.1
Single	13	61.9
Nutrition habits		
Number of main meals		
2	0	0.0
3	21	100.0
Number of snacks		
1	12	57.1
2	9	42.9
Pre-workout meal timing (hours)		
≤2 hours	0	0.0
3-4 hours	21	100.0
>4 hours	0	0.0
Post-workout meal timing (hours)		
≤2 hours	21	100.0
>2 hours	0	0.0

When the anthropometric measurements of the football players were evaluated, the median BW was found to be 72.00 kg (61.20-88.50), while the median BMI to be 23.07 kg/m^2 (19.32-26.05). The median body fat percentage of football players whose skinfold thickness was measured with caliper was 9.66% (7.71-11.25) (Table 2).

Table 2*. Anthropometric measurements of football players*

Anthropometric measurements	Median	Minimum	Maximum
Height (cm)	178.0	164.0	189.0
Body weight (kg)	72.0	61.2	88.5
Body mass index (kg/ m^2)	23.0	19.3	26.0
Thigh (mm)	8.3	4.8	15.0
Abdominal (mm)	9.4	4.9	16.2
Triceps (mm)	6.8	3.4	11.0
Calf (mm)	4.0	2.4	5.3
Total fat (%)	9.6	7.7	11.2

**cm:centimeters, kg: kilograms, m: meters, mm: milimeters, %: percentage*
** statistical significance level p<0.05*

When the nutritional intakes of the football players was analyzed, the median energy was found 3053.79 kcal (2820.23-3197.05), and the median rates of carbohydrate, protein and fat consumption were 50% (46.0-52.0), 20%

(18.0-21.0) and 30% (28.0-33.0), respectively. The median DII scores of the football players were found to be – 3.42 (-9.95-0.95) (Table 3).

Table 3. *Nutritional intakes and dietary inflammatory index scores of football players*

	Median	Minimum	Maximum
Energy (kcal)	3053.7	2820.2	3197.0
Protein (g)	143.8	132.7	157.7
Protein (%)	20.0	18.0	21.0
Fat (g)	103.9	87.2	116.3
Fat (%)	30.0	28.0	33.0
Saturated fatty acids (g)	43.3	36.7	48.2
Monounsaturated fatty acids (g)	36.7	31.6	42.2
Polyunsaturated fatty acids (g)	15.2	12.3	22.3
Omega 3 (g)	2.1	1.8	4.0
Omega 6 (g)	12.3	9.4	17.5
Cholesterol (mg)	882.3	606.4	925.2
Carbohydrate (g)	374.7	331.3	398.0
Carbohydrate(%)	50.0	46.0	52.0
Fiber (g)	31.9	24.0	39.9
Caffeine (mg)	32.0	29.0	36.0
Vitamin A (mcg)	2126.9	2011.0	2465.3
Carotene (mcg)	5930.0	5260.0	8430.0
Vitamin D (mcg)	5.2	3.2	5.4
Vitamin E (mg)	15.2	12.7	21.9
Vitamin B1 (mg)	1.3	1.0	1.7
Vitamin B2 (mg)	2.4	2.0	2.8
Vitamin B6 (mg)	2.6	2.0	3.4
Niacin (mg)	28.5	25.9	34.0
Folate (mcg)	482.9	386.2	545.8
Vitamin B12 (mcg)	12.3	11.0	13.8
Vitamin C (mg)	219.7	141.8	345.6
Iron (mg)	17.8	14.8	26.0
Magnesium (mg)	426.7	348.0	495.1
Zinc (mg)	50.0	46.0	52.0
Selenium (mcg)	22.0	20.0	26.0
Alcohol (g)	0.0	0.0	0.0
Total DII score	-3.4	-9.9	0.9

**kcal: kilocalories, g: grams, mg:miligrams, mcg: micrograms, %: percentage DII: Dietary inflammatory index*
** statistical significance level p<0.05*

The relationship between the nutritional intakes of the football players and their anthropometric measurements is given in Table 4. A significant positive correlation was found between the energy, carbohydrate and fat consumption of the football players and their body weights (p< .05). In addition, there was a significant negative correlation (R: – .468; p: .032) between fiber consumption and abdominal adiposity, and a significant positive correlation between carbohydrate and fat consumption and body weight (respectively R= .730 p= .000; R= .526 p= .014). However, no significant correlation was observed between the consumption of protein, saturated fatty acids and other micronutrients and the anthropometric measurements.

Table 4. *The relationship between the nutritional intakes of football players and their anthropometric measurements*

	Body weight (kg)	Thigh (mm)	Abdominal (mm)	Triceps (mm)	Calf (mm)	Total fat (%)
Energy (kcal)	R=0.729 **p=0,000**	R=-0.125 p=0.589	R= – 0.087 p= 0.709	R=-0.195 p= 0.397	R= 0.152 p=0.511	R= – 0.135 p=0.559
Protein (g)	R=0.379 p=0,090	R= – 0.122 p= 0.598	R= – 0.241 p= 0.292	R= – 0.171 p= 0.460	R= 0.082 p= 0.725	R= – 0.188 p= 0.415
Protein (%)	R=-0.242 p=0,291	R= – 0.011 p= 0.963	R= – 0.057 p= 0.806	R= 0.087 p= 0.708	R= 0.088 p= 0.703	R= 0.019 p= 0.934
Fat (g)	R=.526 **p=0.014**	R= – 0.272 p= 0.233	R= – 0.105 p= 0.651	R= – 0.260 p= 0.255	R= 0.165 p= 0.475	R= – 0.246 p= 0.282
Fat (%)	R=-0.059 p= 0.799	R= – 0.249 p= 0.277	R= – 0.032 p= 0.889	R= – 0.139 p= 0.548	R= 0.052 p= 0.823	R= – 0.155 p= 0.502
Monounsaturated fatty acids (g)	R= 0.184 p= 0.424	R= – 0.068 p= 0.771	R= 0.334 p= 0.139	R= 0.015 p= 0.949	R= – 0.014 p= 0.951	R= 0.099 p= 0.670
Carbohydrate (g)	R=.730 **p= 0,000**	R= 0.165 p= 0.474	R= 0.115 p= 0.621	R= – 0.006 p= 0.980	R= 0.095 p= 0.683	R= 0.105 p= 0.650
Carbohydrate (%)	R= 0.307 p= 0.176	R= 0.397 p= 0.075	R= 0.238 p= 0,299	R= 0.193 p= 0.402	R= 0.064 p= 0.782	R= 0.304 p= 0.180
Fiber (g)	R= 0.314 p= 0.165	R= – 0.279 p= 0.220	R= – .468 p= **0.032**	R= – 0.347 p= 0.123	R= 0.107 p= 0.644	R= – 0.412 p= 0.064

**kcal: kilocalories; g: grams; %: percentage; * statistical significance level p<0.05*

When the relationship between the DII scores of the football players and their anthropometric measurements was examined, a moderate positive correlation (R: .476; p: .029) was found between the DII score and abdominal adiposity. No significant correlation (p> .05) was found between the DII scores and the other anthropometric measurements (Table 5).

Table 5. *The relationship between the football players' dietary inflammatory index scores and anthropometric measurements*

	Dietary inflammatory index score	
	R	p
Anthropometric measurements		
Body weight (kg)	-0.276	0.226
Body mass index (kg/m^2)	-0.339	0.133
Thigh (mm)	0.233	0.309
Abdominal (mm)	0.476	**0.029**
Triceps (mm)	0.194	0.400
Calf (mm)	-0.083	0.721
Total fat (%)	0.337	0.135

** kg: kilograms, m: meters, mm: milimeters, %: percentage; * statistical significance level p<0.05*

4. DISCUSSION

The relationship between the nutritional status of professional football players, their anthropometric measurements and their DII scores was investigated in this study. The nutritional status of football players is of great importance as it positively affects performance parameters and reduces the risk of injury (16).

When the participants' nutritional intakes before training were examined, it was found that all of them consumed a meal 2-4 hours before training. Considering the intensity of training in football players, they need to receive nutrition before training in order to prevent fatigue, increase performance and muscle strength. However, it is stated that they should consume meals 3-4 hours before training due to gastrointestinal problems that may occur during training (17). The football players participating in our study ate in accordance with this statement. When the football players' nutritional intakes after training were examined, all of them were found to consume meals during the first 2 hours following training. Nutrition is essential in recovery after training in football. The main goal in post-training nutrition is to replenish glycogen stores and repair muscle damage for the next match or training. It is stated that the timing of eating is very important at this point so as to ensure rapid recovery (18). It is also stated that a meal containing sufficient carbohydrates (1-1.5 g/kg) and protein (0.4 g/kg) should be consumed within the first 2 hours after training/exercise for optimal muscle glycogen and protein synthesis (19).

Body composition is of significance for optimal performance and protection from injuries in football (20). The fat percentage of the football players participating in our study was found as 9.66% (7.71-11.25). In a study conducted on elite Australian football players, their average fat percentage was found as 12.8±1.9% (21). When the body compositions of professional football players playing in different leagues in Turkey were examined, it was found that the average body fat percentage of those playing in the 2nd league was 16.5±3.26%, which is in accordance with our study (22). It has been revealed that an increase in body fat percentage decreases football players' performance (23) and causes an increase in the risk of injury (24).

The nutritional intakes of football players are stated to be of great importance for their performance and health. In the study Anderson et al. conducted with football players, the average energy intake was 2956±374 kcal (25), and this result is similar to ours [median 3053.79 kcal (2820.23 – 3197.05)]. The distribution of energy to macronutrients is as important as the total energy intake in the diet. Carbohydrate consumption is crucial for the optimal performance of team sports athletes (26). The carbohydrate consumption of the football players participating in the study was found as 50% of the energy (46.0-52.0). It has been shown in studies conducted with football players that carbohydrate consumption is 38±12% (19) and 43.9±4.8% of energy (22). However, a positive correlation was found between carbohydrate consumption and body weight, which is in accordance with the literature (27). It is stated that an increase in carbohydrate consumption above the recommended levels has negative effects on body composition (28). In addition, a negative significant correlation was found between fiber consumption and abdominal skinfold thickness in this study. In a cohort study, a negative correlation was determined between increased fiber consumption and abdominal adiposity (29). It is stated that fiber consumption should be encouraged in athletes due to its positive effects on health (30).

Proteins increase muscle protein synthesis and endothelial regeneration, especially after exercise, and reduce muscle damage (31). It was found that the protein consumption of the football players participating in the study corresponded to 20% of the energy (18.00-21.00). Although there are studies in the literature indicating that the daily protein consumption of football players is in accordance with this study (32,33), there are also those reporting lower protein consumption (34,35). It is stated that the total amount of daily protein intake should be between 1.4-2.0 g/kg/day in football players, although it varies according to training/match frequency (36). The protein consumption of the football players participating in the study met the recommendations.

Although the benefits of fat for exercise performance are not clear, fat consumption is essential in maintaining health (37). The fat consumption of the football players participating in the study was found to correspond to 30% (28.0-33.0) of the energy. In the study Brinkmans et al. conducted with a group of professional football players, it was revealed that their fat consumption was 30.8±4.9% on average, which is similar to our study (38). Furthermore, a positive correlation was observed between fat consumption and body weight in the present study. In another study examining its effects on athletes' nutrition intakes and body composition, a positive correlation was found between fat consumption and body weight (27). Although carbohydrate and fat consumption are positively correlated with body weight, body fat mass/percentage is not correlated. It is believed that carbohydrate and fat consumption do not have a negative effect on body composition. For football players, fat consumption is recommended to be between 20-35% of the daily energy intake. That fat consumption exceeds the recommended level is stated to have negative effects on performance and body composition (39).

It has been stated that inflammation and oxidative damage in football players can negatively affect their performance and increase the risk of diseases such as upper respiratory tract infections (40). DII score has been found to be associated with inflammation parameters. In this study, the median DII scores of the football players were found to be low (-3.42). A study indicated that soldiers with low DII scores had higher maximum VO_2 levels, which is very important in sportive performance (41). Another study reported that the maximum VO_2 levels of individuals with pro-inflammatory (high DII score) eating habits were 7% less (42).

In our study, a moderate positive correlation was found between DII score and abdominal adiposity. A study with a large sample size revealed that individuals with low DII scores (anti-inflammatory) had lower waist circumferences (43). Another study conducted with soldiers reported that individuals with high DII scores had higher fat percentages. It is stated that an increase especially in abdominal adiposity increases inflammation and health risks (44). In addition, it has been shown that abdominal adiposity increases the risk of

injury in athletes (45) and decreases endurance performance (46). Considering these effects, it can be recommended that football players gain anti-inflammatory (low DII score) eating habits.

When the literature is reviewed, this study is the first to evaluate the nutritional status of professional football players through DII, and it is of significance to obtain detailed food consumption records through face-to-face interviews and using the food atlas (15). On the other hand, since only one professional football team was studied, the number of samples was limited and the DII scores could not be compared with the biochemical findings because the football players did not want to undergo an invasive procedure during the season.

5. CONCLUSION

In conclusion, it was found that the football players participating in the study had an anti-inflammatory diet. Besides, individuals with high DII scores were found to have more abdominal adiposity. In addition, a negative correlation was determined between fiber consumption and abdominal adiposity, and a positive correlation between carbohydrate and fat consumption and body weight. Considering the risks posed by the training loads of professional athletes, it is thought that more studies should be carried out on the relationship between inflammation and nutritional intakes. Further studies are needed to determine the relationship between biochemical measurements and inflammatory status, in addition to sex, which is one of the main contributors to inflammatory status.

Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Marmara University, Clinical Research Ethics Committee (Date and number: 16.09.2020, 09.2020.952).*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: EBK
Design of the study: EBK, FEG
Acquisition of data for the study: EBK
Analysis of data for the study: EBK, FE
Interpretation of data for the study: EBK, FE
Drafting the manuscript: EBK
Revising it critically for important intellectual content: FEG, FE
Final approval of the version to be published: EBK, FE, FEG

REFERENCES

[1] Camerino OF, Chaverri J, Anguera MT, Jonsson GK. Dynamics of the game in soccer: Detection of T-patterns. Eur J Sport Sci. 2012;12(3):216-224. DOI:10.1080/17461.391.2011.566362.

[2] Purcell LK, Canadian Paediatric Society, & Paediatric Sports and Exercise Medicine Section. Sport nutrition for young athletes. J Paediatr Child Health 2013;18(4):200-202. DOI: 10.1093/pch/18.4.200.

[3] Briggs MA, Cockburn E, Rumbold PL, Rae G, Stevenson EJ, Russell M. Assessment of energy intake and energy expenditure of male adolescent academy-level soccer players during a competitive week. Nutrients 2015;7(10):8392-8401. DOI: 10.3390/nu7105400.

[4] Baralic I, Andjelkovic M, Djordjevic B, Dikic N, Radivojevic N, Suzin-Zivkovic V, Radojevic-Skodric S, Pejic S. Effect of astaxanthin supplementation on salivary IgA, oxidative stress, and inflammation in young soccer players. Evid Based Complement Alternat Med. 2015; 2015: 1-9 DOI: 10.1155/2015/783761.

[5] Cicchella A, Stefanelli C, Massaro M. Upper respiratory tract ınfections in sport and the ımmune system response. A Review. Biology 2021;10(5):362-375. DOI: 10.3390/biology10050362.

[6] Cerqueira É, Marinho DA, Neiva HP, Lourenço O. Inflammatory effects of high and moderate intensity exercise-A systematic review. Front Physiol. 2020;10(1550):1-14. DOI: 10.3389/fphys.2019.01550.

[7] Suzuki K. Recent progress in applicability of exercise ımmunology and ınflammation research to sports nutrition. Nutrients 2021;13(12):1-14-. DOI: 10.3390/nu13124299.

[8] Cavicchia PP, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, Hébert JR. A new dietary inflammatory index predicts interval changes in serum high-sensitivity C-reactive protein. J Nutr. 2009;139(12):2365-2372. DOI: 10.3945/jn.109.114025.

[9] Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr. 2014;17(8):1689-1696. DOI: 10.1017/S136.898.0013002115.

[10] Shivappa N, Hebert JR, Marcos A, Diaz LE, Gomez S, Nova E, Michels N, Arouca A, González-Gil E, Frederic G, González-Gross M, Castillo MJ, Manios Y, Kersting M, Gunter MJ, De Henauw S, Antonios K, Widhalm K, Molnar D, Moreno L, Huybrechts I. Association between dietary inflammatory index and inflammatory markers in the HELENA study. Mol Nutr Food Res. 2017;61(6):1-18. DOI: 10.1002/mnfr.201600707.

[11] Moludi J, Shivappa N, Alisgharzadeh S, Hébert JR, Alizadeh M. Dietary inflammatory index is related to heart failure risk and cardiac function: A Case–Control Study in Heart Failure Patients. Front Nutr. 2021;8(605396):1-9. DOI: 10.3389/fnut.2021.605396.

[12] Portao J, Bescos R, Irurtia A, Cacciatori E, Vallejo L. Assessment of body fat in physically active young people: Anthropometry vs bioimpedance. Nutr Hosp. 2009;24(5):529-534. DOI: 10.3305/nh.2009.24.5.4463.

[13] Reilly T, George K, Marfell-Jones M, Scott M, Sutton L, Wallace JA. How well do skinfold equations predict percent body fat in elite soccer players?. Int J Sports Med. 2009;30(8):607-613. DOI: 10.1055/s-0029.120.2353.

[14] Nevill AM, Winter EM, Ingham S, Watts A, Metsios GS, & Stewart AD. Adjusting athletes' body mass index to better reflect adiposity in epidemiological research. J Sports Sci. 2010;28(9):1009-1016.DOI:10.1080/02640.414.2010.487071.

[15] İmeryüz N, Güneş FE. Besin Atlası. 1th ed. Istanbul:AdaYayıncılık;2008.

[16] Ersoy N, Kalkan I, Ersoy G. Assessment of nutrition status of Turkish elite young male soccer players in the pre-competition period. Prog Nutr. 2019;21(1):12-18. DOI: 10.23751/pn.v21i1.7127.

[17] Ormsbee MJ, Bach CW, Baur DA. Pre-exercise nutrition: The role of macronutrients, modified starches and supplements on metabolism and endurance performance. Nutrients 2014;6(5):1782-1808. DOI: 10.3390/nu6051782.

[18] Poulios A, Fatouros IG, Mohr M, Draganidis D, Deli CK, Papanikolaou K, Sovatzidis A, Nakopoulou T, Ermidis G, Tzatzakis T, Laschou VC, Georgakouli K, Koulouris A, Tsimeas P, Chatzinikolaou A, Karagounis LG, Batsilas D, Krustrup P, Jamurtas AZ. Post-game high protein intake may improve recovery of football-specific performance during a congested game fixture: Results from the PRO-FOOTBALL study. Nutrients 2018;10(494):1-26. DOI: 10.3390/nu10040494.

[19] Arent SM, Cintineo HP, McFadden BA, Chandler AJ, Arent MA. Nutrient timing: A garage door of opportunity?. Nutrients 2020;12(7):1-19. DOI: 10.3390/nu12071948.

[20] Devlin BL, Kingsley M, Leveritt MD, Belski R. Seasonal changes in soccer players' body composition and dietary intake practices. J Strength Cond Res. 2017;31(12):3319-3326. DOI: 10.1519/JSC.000.000.000000175.

[21] Devlin BL, Leveritt MD, Kingsley M, Belski R. Dietary intake, body composition, and nutrition knowledge of Australian football and soccer players: Implications for sports nutrition professionals in practice. Int J Sport Nutr Exerc Metab. 2017;27(2):130-138. DOI: 10.1123/ijsnem.2016-0191

[22] Köse B, Kızıltan G, Turnagöl H. Farklı liglerdeki futbolcuların vücut kompozisyonu, beslenme ve hidrasyon durumlarının sezon içi dönemde değerlendirilmesi. Spor Bilimleri Dergisi 2021;32(4):183-196. DOI:10.17644/sbd.817101. (Turkish)

[23] Nikolaidis PT, Ruano MAG. De Oliveira NC, Portes LA, Freiwald J, Leprêtre PM, Knechtle B. Who runs the fastest ? Anthropometric and physiological correlates of 20 m sprint performance in male soccer players. Res Sports Med. 2016;24(4):341-351. DOI: 10.1080/15438.627.2016.1222281.

[24] Bongiovanni T, Mascherini G, Genovesi F, Pasta G, Iaia FM, Trecroci A, Ventimiglia M, Alberti G, Campa, F. Bioimpedance vector references need to be period-specific for assessing body composition and cellular health in elite soccer players: A brief report. J Funct Morphol Kinesiol. 2020;5(4):73-80. DOI: 10.3390/jfmk5040073.

[25] Anderson L, Orme P, Naughton RJ, Close GL, Milsom J, Rydings D, O'Boyle A, Di Michele R, Louis J, Hambly C, Speakman JR, Morgans R, Drust B, Morton JP. Energy intake and expenditure of professional soccer players of the English premier league: Evidence of carbohydrate periodization. Int J Sport Nutr Exerc Metab. 2017;27(3):228-238. DOI: 10.1123/ijsnem.2016-0259.

[26] Heaton LE, Davis JK, Rawson, ES, Nuccio RP, Witard OC, Stein KW, Baar K, Caret JM, Baker LB. Selected in-season nutritional strategies to enhance recovery for team sport athletes: A practical overview. Sports Med. 2017;47(11):2201-2218. DOI: 10.1007/s40279.017.0759-2.

[27] Shriver LH, Betts NM, Wollenberg G. Dietary intakes and eating habits of college athletes: Are female college athletes following the current sports nutrition standards?. J Am Coll Health 2013;61(1):10-16. DOI: 10.1080/07448.481.2012.747526.

[28] Santos FL, Esteves SS, da Costa Pereira A, Yancy Jr WS, Nunes JP. Systematic review and meta-analysis of clinical trials of the effects of low carbohydrate diets on cardiovascular risk factors. Obes Rev. 2012;13(11):1048-1066. DOI: 10.1111/j.1467-789X.2012.01021.x.

[29] Jaimie N Davis, Katharine E Alexander, Emily E Ventura, Claudia M Toledo-Corral, Michael I Goran. Inverse relation between dietary fiber intake and visceral adiposity in overweight Latino youth. Am. J. Clin. Nutr. 2009; 90(5): 1160–1166. DOI: 10.3945/ajcn.2009.28133.

[30] Son J, Jang LG, Kim BY, Lee S, Park H. The effect of athletes' probiotic intake may depend on protein and dietary fiber intake. Nutrients 2020; 12(10): 2947-2960. DOI: 10.3390/nu12102947.

[31] Yang C, Jiao Y, Wei B, Yang Z, Wu JF, Jensen J, Jean WH, Huang CY, Kuo CH. Aged cells in human skeletal muscle after resistance exercise. Aging 2018;10(6):1356-1365. DOI: 10.18632/aging.101472.

[32] Bettonviel AE, Brinkmans NY, Russcher K, Wardenaar FC, Witard OC. Nutritional status and daytime pattern of protein intake on match, post-match, rest and training days in senior professional and youth elite soccer players. Int J Sport Nutr Exerc Metab. 2016;26(3):285-293. DOI: 10.1123/ijsnem.2015-0218.

[33] Raizel R, da Mata Godois A, Coqueiro AY, Voltarelli FA, Fett CA, Tirapegui J, de Paula Ravagnani FC, de Faria Coelho-Ravagnani C. Pre-season dietary intake of professional soccer players. Nutr Health. 2017;23(4):215-222. DOI: 10.1177/026.010.6017737014.

[34] Iglesias-Gutiérrez E, García Á, García-Zapico P, Pérez-Landaluce J, Patterson ÁM, García-Rovés PM. Is there a relationship between the playing position of soccer players and their food and macronutrient intake?. Appl Physiol Nutr Metab. 2012;37(2):225-232. DOI: 10.1139/h11-152.

[35] Caruana Bonnici D, Akubat I, Greig M, Sparks A, Mc Naughton LR. Dietary habits and energy balance in an under 21 male international soccer team. Res Sports Med 2018;26(2):168-177. DOI: 10.1080/15438.627.2018.1431537.

[36] Caruana Bonnici DC, Greig M, Akubat I, Sparks SA, Bentley D, Mc Naughton LR. Nutrition in soccer: A brief review of the issues and solutions. J Sci Med Sport 2019;1(1):3-12. DOI: 10.1007/s42978.019.0014-7.

[37] Burke LM. Ketogenic low-CHO, high-fat diet: The future of elite endurance sport?. J Physiol. 2021;599(3):819-843. DOI: 10.1113/JP278928.

[38] Brinkmans NY, Iedema N, Plasqui G, Wouters L, Saris WH, van Loon LJ, van Dijk JW. Energy expenditure and dietary intake in professional football players in the Dutch Premier League: Implications for nutritional counselling. J Sports Sci. 2019;37(24):2759-2767. DOI: 10.1080/02640.414.2019.1576256.

[39] Oliveira CC, Ferreira D, Caetano C, Granja D, Pinto R, Mendes B, Sousa M. Nutrition and supplementation in soccer. Sports 2017;5(2):28-63. DOI: 10.3390/sports5020028.

[40] Farjallah MA, Hammouda O, Ben Mahmoud L, Graja A, Ghattassi K, Boudaya M, Jammoussi K, Sahnoun Z, Souissi N. Melatonin supplementation ameliorates oxidative stress, antioxidant status and physical performances recovery during a soccer training camp. J Biol Rhythms 2020;51(3):441-452. DOI: 10.1080/09291.016.2018.1533749.

[41] Ramezani A, Parastouei K, Delkhosh M, Rostami H. The relationship between dietary inflammatory index, physical performance and anthropometric indices in marines. Res Sq 2021; DOI: 10.21203/rs.3.rs-596924/v1.

[42] Shahinfar H, Payandeh N, Shahavandi M, Mohammadpour S, Babaei N, Ebaditabar M, Djafarian K, Shab-Bidar, S. The Combined Association of Dietary Inflammatory Index and Resting Metabolic Rate on Cardiorespiratory Fitness in Iranian Adults. Res Sq 2021; DOI: 10.21203/rs.3.rs-970709/v1.

[43] Ruiz-Canela M, Zazpe I, Shivappa N, Hébert JR, Sánchez-Tainta A, Corella D, Salas-Salvadó J, Fitó M, Lamuela-Raventós

RM, Rekondo J, Fernández-Crehuet J, Fiol M, Santos-Lozano JM, Serra-Majem L, Pinto X, Martínez JA, Ros E, Estruch R, Martínez-González MA. Dietary inflammatory index and anthropometric measures of obesity in a population sample at high cardiovascular risk from the PREDIMED (PREvencion con DIeta MEDiterranea) trial. Br J Nutr. 2015;113(6):984-995. DOI: 10.1017/S000.711.4514004401

[44] Bawadi H, Katkhouda R, Tayyem R, Kerkadi A, Raad SB, Subih H. Abdominal fat is directly associated with inflammation in persons with type-2 diabetes regardless of glycemic control–a Jordanian study. Diabetes Metab Syndr. 2019;12(?):2411-2417. DOI: 10.2147/DMSO.S214426.

[45] Toomey CM, Whittaker JL, Nettel-Aguirre A, Reimer RA, Woodhouse LJ, Ghali B, Doyle-Baker PK, Emery CA. Higher fat mass is associated with a history of knee injury in youth sport. J Orthop Sports Phys Ther. 2017;47(2):80-87. DOI: 10.2519/jospt.2017.7101.

[46] Islam MS. Relationship of abdominal muscle endurance with selected anthropometric measurements in soccer players. Int J Physiol Nutr Phys Educ. 2018;3(2):1088-1090.

How to cite this article: Kenger EB, Eren F, Güneş FE. Relationship between Nutritional Status, Anthropometric Measurements and Dietary Inflammatory Index in Professional Football Players. Clin Exp Health Sci 2023; 13: 143-149. DOI: 10.33808/clinexphealthsci.1066512

Clinical and Experimental Health Sciences

Time-Dependent Change of Color and Translucency of Recent Restorative Materials in Various Beverages

Seda Nur Karakaş[1], Cihan Küden[2]

[1] Çukurova University, Faculty of Dentistry, Department of Restorative Dentistry, Adana, Türkiye.
[2] Çukurova University, Faculty of Dentistry, Department of Endodontics, Adana, Türkiye.

Correspondence Author: Seda Nur Karakaş
E-mail: skarakas@cu.edu.tr
Received: 04.02.2022 **Accepted:** 16.05.2022

ABSTRACT

Objective: The aim of this study was to evaluate the time-dependent changes in optical properties of recent tooth-colored restorative materials in commonly used coloring beverages.

Method: A total of 200 specimens of four different materials (Alkasite, ACTIVA, Equia Forte, Zirconomer) and a composite material as the control group were prepared in Teflon molds and the materials were immersed in four different solutions (coffee, cola, ice tea and saliva) (n=10). Color measurements were performed with a spectrophotometer at baseline, 7, 14 and 28 days. Color changes, translucency parameters (TP) and contrast ratios of the materials were calculated. The data were analyzed by repeated measurements and three-way ANOVA and post hoc Tukey tests.

Results: Alkasite showed the greatest color change for all solutions in all immersion periods compared to other materials ($p<0.001$). The experimented solutions caused a perceptible color change in all materials after 28 days ($p<0.05$). Coffee and ice tea caused more staining of resin-based materials for all immersion periods ($p<0.05$). Initial TP and ΔTP of glass ionomer-based materials were found to be lower than the resin-based materials.

Conclusion: Exposure of the recent materials to different solutions for a certain period of time caused significant changes in optical properties. Resin-based alkasite, ACTIVA and composite showed the highest change the optical properties in coffee and ice tea.
Keywords: Alkasite, color change, composite resin, contrast ratio, translucency.

1. INTRODUCTION

The resin-based materials are popular materials in modern dentistry due to their esthetic properties, mechanical strength and low cost (1). However, composite resins have disadvantages such as incremental application, insufficient degree of conversion, weak cavity adaptation, lack of remineralization ability and absence of antibacterial properties (2,3). Glass ionomer cements (GIC), which are routinely used as tooth-colored restorative materials. GICs can be considered as the first-choice materials, especially in the restorations of patients with high cariogenic activity, due to their fluoride release feature that gives them a cariostatic property (4,5). In addition, current restorative materials are being developed by combining the strength and esthetics of composites with the benefits of glass ionomers. One of these materials, alkasite is a bulk-fill tooth-colored resin-based restorative material which uses alkaline filler, capable of releasing acid-neutralizing ions and fluorides (6). Another recent resin-based material, ACTIVA contains bioactive ionic resin, patented rubberized resin, and bioactive ionomer glass (7). Bioactive ionic resin content of the material provides the properties of moisture tolerant and high release and recharge of calcium, phosphate, and fluoride ions (8).

One of the main goals in the current improvement of the dental restorative materials is to ensure the optical properties of the tooth to reflect its original nature (9). In order to obtain esthetically successful restorative filling materials, they are able to imitate the natural tooth structure and are able to maintain their color and translucency of the initial application (10). Light reaching a tooth surface can be reflected, diffused, absorbed or transmitted (11). Also, restorative materials should exhibit similar behavior in order to provide acceptable esthetics. Translucency is defined as the ability of a material to transmit light and it was reported to be the most essential parameter after primary color properties (12). Also, it is one of the key factors affecting the esthetic performance of the dental restorations (13). Translucency parameter (TP) and contrast ratio (CR) are two commonly used indicators to determine translucency values

DOI: 10.33808/clinexphealthsci.1068359

and changes (14). CR is a wavelength dependent parameter based on the luminescence and reflection calculations (15).

A change in color or shade can also affect translucency and change the initial optical properties (16) The longevity of the restorative materials in the oral cavity was extended by the improvements in their structures. One of the important factors in restoration renewal is the loss of color harmony between the restoration and the tooth (17). Therefore, long-term color stability of dental materials is a necessity (18). With the increasing esthetic demands of the patients, the color match and the stability of the restorative materials became important in determining the longevity of the restoration even in the posterior region. Color changes in composites occur due to the internal and external factors. Internal coloration occurs in the inner layers as a result of the structure of the composite resins and incomplete polymerization, while external coloration occurs as a color change on the outer surfaces of the composite resins (19).

It was determined that the coloration caused by diet containing coloring pigment and chemical coloring agents is more prominent than the coloration caused by intrinsic factors due to water absorption in polymerized materials (20). Moreover, the pH value of the aging solution can also affect the surface degradation, causing changes in water absorption (21). Also, it was reported that the beverages containing coloring pigments such as tea, coffee, cola, red wine, fruit juices and energy drinks, which were widely consumed, caused discoloration in resin-based materials (1, 20). It was also reported that when the resin-containing glass ionomers were subjected to immersion in various solutions, more discoloration occurred comparing the composites (22,23). A recent study evaluating the translucency of the different GICs found that they had low translucency (13).

However, there is not enough information in the literature about the color stability and translucency of recent restorative materials, whose demand for use was increased with the progress in their structure. The aim of this study is to evaluate the time-dependent changes in optical properties of recent fluoride-released tooth-colored restorative materials in commonly used colorant beverages by comparing them with composite. In this study, two null hypotheses were tested: (i) that the type of restorative materials and (ii) the type of the beverage and the duration of the immersion would not affect the color, translucency and contrast of the materials.

2. METHODS

2.1. Preparation of Specimens

This study was found medically appropriate with the ethics committee report numbered 2021/108.43 and date 21.02.2021 of Çukurova University, Faculty of Medicine, Clinical Research Ethics Committee. The specification, composition and filler ratio of the materials (Alkasite, ACTIVA, Equia Forte, Zirconomer and composite) used in this study were given in Table 1. The microhybrid composite was included as the control group. A total of 200 disc-shaped specimens were fabricated for 40 of each material. In accordance with the manufacturer's instructions, all materials were prepared in disk-shaped Teflon molds with a diameter of 8 mm and a height of 2 mm, by pressing gently from the top with mylar strip and glass to promote removal of the excess material and to obtain a standard surface. The thickness of each disc was measured with a digital caliper (Liaoning MEC, Dalian, China) placed in the center of the material. Only samples with a thickness of 2 mm were used in the study.

Table 1. *The Brand and the contents of the restorative materials*

Materials	Specification	Composition	Filler ratio (weighted)
Alkasite (Cention N; Ivoclar Vivadent, Schaan Liechtenstein) Lot: Z0054T	Alkasite, self-adesive restorative material	Powder: Ca fluorosilicate glass (25-35%, Ba-Al silicate glass (20-30%), Ca-Ba-Al fluorosilicate glass (10-20%), ytterbium trifluoride (5-10%), iso-fillers, initiators and pigments. Liquid: UDMA (95-97%), DCP, Aromatic aliphatic – UDMA, PEG-400 DMA, initiators (hydroperoxide – self cure), stabilizers, additives and mint flavour	78.4%
ACTIVA (Pulpdent, Watertown, MA, USA) Lot: 191212	Bioactive glass ionomer	Blend of diurethane and other methacrylates with modified polyacrylic acid (44.6%), Bioactive glass filler (21.8%), patented rubberized resin (Embrace), amorphous silica (6.7%), and sodium fluoride (0.75%)	56%
Equia Forte Fil (GC, Corp., Tokyo, Japan) Lot: 1803121	High viscosity glass ionomer cement (HVGIC)	Fluoroalumino silicate glass (95%), hybrid glass particles, polyacrylic acid powder, 5% polyacrylic acid, polybasic carboxylic acid, distilled water	75%
Zirconomer (Shofu, Kyoto, Japan) Lot: 10200185	High viscosity glass ionomer cement (HVGIC)	Powder: Fluoro-Aluminosilicate glass, Zirconium oxide, pigments and others Liquid: polyacrylic acid solution and Tartaric acid	15 – 20%
Filtek Z250 (3M ESPE, St.Paul, MN, USA) Lot: NA53476	Microhibrit composite resin	TEGDMA (1 – 5%), Bis-GMA (1 – 5%), Bis-EMA (5 – 10%), UDMA (5 – 10%), Zirconia/silica inorganic fillers 60%	60%

Ca: Calcium, Ba: Barium, Al: Aliminum, UDMA: Urethane dimethacrylate, DCP: Tricyclodecan-dimethanol dimethacrylate, Aromatic aliphatic – UDMA: Tetramethyl-xylylendiurethane dimethacrylate, PEG-400 DMA: Polyethylene glycol 400 dimethacrylate, TEGDMA: Trietyhlenglycol dimetacrylate, Bis-GMA: Bisphenol-A-glycidylmethacrylate, Bis-EMA: Bisphenol-A polyethylenglycol dietherdimethacrylate

Group 1 (Alkasite): One measuring spoon of powder and one drop of liquid, corresponding to a powder/liquid weight ratio of 4.6g to 1g, were manually mixed until a smooth consistency. The mixing time was completed in 60s and the prepared material placed in the mold. The setting time for the self-curing mode was achieved in 5min.

Group 2 (ACTIVA): The material applied into the mold with a gun and polymerized with a 1200 mW/cm^2 LED curing unit (Freelight Elipar II, 3M, St. Paul, MN, USA) for 20s.

Group 3 (Equia Forte): Capsule of the material was activated just before mixing and was placed in the amalgamator immediately. The restorative material mixing time was 10s and the setting time of the material was 2min 30s after placing into the mold. Equia Forte Coat (GC, Tokyo, Japan) was applied to the surfaces using a disposable microtip applicator. The coated surface was light-cured with the same curing unit.

Group 4 (Zirconomer): Two measures of powder and one drop of liquid with a powder liquid ratio of 3.6 g/1.0 g were placed on the mixing paper. The first half of 2 equal parts of powder was added to the spread liquid by using a plastic spatula and mixing in 5-10s. Then the remaining half was added and mixed until a paste-like consistency was achieved. Mixing was completed in a total of 30s. The prepared material was placed in the mold and the 3min curing time was completed.

Group 5 (Composite): The material was applied into mold and was polymerized with a 1200 mW/cm^2 LED curing unit for 20s.

The upper surfaces of all samples were polished with medium, thin and superfine aluminum oxide impregnated discs (Sof-Lex, 3M, St. Paul, MN, USA), respectively, using a low-speed handpiece rotating at 12,000rpm, according to manufacturer's instructions. The samples were kept in distilled water at 37°C for 24h in an incubator (Memmert, Schwabach, Germany) to complete their polymerization. Then, the baseline color measurements of the materials were determined.

2.2. Storage in Colored Beverages and Artificial Saliva

The groups of the restorative materials were divided into four subgroups (n=10) according to the type of the solution (coffee, cola, ice tea and artificial saliva). The composition and pH values of the coloring agents used in this study and the salivary fluid used as the control group were given in Table 2. The specimens were immersed for 7, 14, 28 days in four different solutions in the incubator at 37°C. The specimens were positioned inside 48-well plates containing 3 mL of solution during the immersion process. All solutions were renewed every 24h. Before putting the samples into the newly prepared solution, they were washed with 5ml of distilled water and dried with blotting paper.

2.3. Measurement of the Color Change, Translucency Parameter and Contrast Ratio

The optical analyzes were carried out by using a spectrophotometer (VITA Easyshade® V; VITA Zahnfabrik, Bad Sackingen, Germany). A saturated sucrose solution (refractive index n=1.5 approximately) was used as contact between samples and background. The values of CIELAB coordinates (L*, a*, b*) were determined by using the CIE D65 illuminator and the CIE 2° standard colorimetric observation at initial, 7, 14 and 28 days on a white background [24]. It was completed by taking three consecutive measurements from each sample. The color change (ΔE_{00}) was calculated for each sample according to the CIEDE2000 formula [25,26]:

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)\right]^{1/2}$$

where ΔL′, ΔC′ and ΔH′ in the CIEDE2000 system are the differences in lightness, chroma and hue, respectively. The weighting functions (SL, SC, and SH) determine the total color difference for variation in the location. The parametric factors (KL, KC and KH) are correction terms for experimental conditions. For this study, each KL, KC and KH were taken as 1.0. A rotation function (RT) defines the interaction between chroma and hue differences in the blue region.

Table 2. *The brand, content and pH value of the solutions*

Solutions	Composition	pH
Cola (The Coca Cola, İstanbul Turkey)	Carbonated water, sugar, caramel color, phosphoric acid, natural flavors, caffeine	2.53
Ice-Tea (Unilever, Lipton, İstanbul, Turkey)	Water, sugar, fructose, acids (citric acid, malic acid), black tea extract (0.14%), peach juice concentrate (0.1%), acidity regulator (trisodium citrate), flavorings (peach flavor), antioxidant (ascorbic acid), sweetener (steviol glycosides).	3.8
Coffee (Nescafe Classic, Nestle, Vevey, Switzerland)	10g coffee / 200 ml of boiling water and cooled to 60°C	4.50
Artificial saliva	1.160 g/l sodium chloride, 0.600 g/l calcium chloride, 0.600 g/l potassium phosphate, 1.491 g/l potassium chloride, 0.050 g/l sodium fluoride, trace of sodium hydroxide	6.93

Translucency changes of the samples were compared by using the relative translucency parameter (27). Translucency measurements were completed on white and black backgrounds at the beginning and at the end of the 28th day. Translucency parameters determined by calculating the color difference between measurements on black (L: 8.0, a: 0.3, b: 1.6) and white (L: 96.2, a: 0.9, b: 6.2) backgrounds for the same sample:

$$TP_{00} = \left[\left(\frac{L'_B - L'_W}{K_L S_L}\right)^2 + \left(\frac{C'_B - C'_W}{K_c S_c}\right)^2 + \left(\frac{H'_B - H'_W}{K_H S_H}\right)^2 + R_T\left(\frac{C'_B - C'_W}{K_C S_C}\right)\left(\frac{H'_B - H'_W}{K_H S_H}\right)\right]^{1/2}$$

where subscripts w and b refer to measurements on white and black backgrounds, respectively. The change in translucency parameter (ΔTP_{00}) at the end of the 28th day was calculated according to the formula following:

$$\Delta TP_{00} = TP_{28} - TP_0$$

The calculation of CR was carried out by using the L* values measured on white and black backgrounds. The spectral reflectance, Y values were calculated for black and white background as following formula and YN value was taken as 100 (15):

$$Y = (L + 16/116)^3 x\, Y_N$$

The values of CR were obtained as follows:

$$CR = Y_B / Y_W$$

The change in contrast ratio (ΔCR) at the end of the 28th day was calculated according to the formula following:

$$\Delta CR = CR_{28} - CR_0$$

2.4. Statistical Analysis

Statistical analyzes were performed by using the SPSS program for Mac version 26 (IBM SPSS, Chicago, IL, USA). Homogeneity of the data was evaluated by using the Shapiro Wilks test. Intergroup and intragroup comparisons of the color change, TP and CR were analyzed by one-way analysis of variance (ANOVA) and Tukey post hoc test. The correlation between CR and TP of the materials was assessed with the Pearson correlation test. Time-dependent color changes of the materials were compared by using repeated measure ANOVA and paired sample t-test, respectively. A three-way ANOVA (restorative material at five levels, immersion solution at four levels and time at three levels) evaluated the effects of material type, storage solution and time as well as their interaction, on ΔE00 values. All statistical testing was performed at a preset alpha of 0.05.

3. RESULTS

The mean color change (ΔE_{00}), standard deviations, comparison of the materials according to the type of immersed liquid and comparison of the immersion times were shown in Table 3. Comparison of the effect of type of immersed liquid on color change of the material was shown in Figure 1. ΔE_{00} increased with time in all solutions and the difference was statistically significant in all groups (p≤0.002). There was a significant difference between 7 and 28 days for all groups in pairwise comparisons. All solutions caused a perceptible color change in all materials after 28 days.

Alkasite, ACTIVA and composite materials, which are resin-based materials, showed the highest ΔE_{00} in coffee and ice tea solutions. Cola caused significantly less color change compared to coffee and ice tea for alkasite at the measurement times of the 14 and 28 days, for ACTIVA at all measurement times and for composite at the measurement of the 7th days. (p≤0.007) Coffee and cola caused more coloration of Equia Forte than ice tea and saliva. The difference among groups was statistically significant for all the measurement times. (p<0.001). Cola caused more coloration of Zirconomer than coffee and ice tea for all the measurement times (p≤0.004). Saliva caused the lowest ΔE_{00} at all measurement times and for all materials (p<0.001). Three-way ANOVA test (Table 4) revealed difference in the mean value of ΔE_{00} that was significantly affected by the material type, solution type and time (p<0.001).

Table 3. *Mean color differences (ΔE_{00}), standard deviations and statistical analysis for specimens immersed in different liquids at different times*

Liquids	Days	Alkasite	ACTIVA	Equia Forte	Zirconomer	Composite	p
Coffee	**7**	$12.31 \pm 2.99^{A, a}$	$6.32 \pm 1.48^{A, b}$	$7.30 \pm 2.65^{A, b}$	$3.60 \pm 0.73^{A, c}$	$6.67 \pm 1.94^{A, b}$	<0.001
	14	$16.63 \pm 2.90^{B, a}$	$7.89 \pm 1.70^{A, c}$	$11.27 \pm 2.61^{B, b}$	$4.80 \pm 0.95^{B, d}$	$8.18 \pm 1.69^{B, c}$	<0.001
	28	$21.18 \pm 3.34^{C, a}$	$10.78 \pm 2.15^{B, c}$	$14.88 \pm 4.77^{C, b}$	$6.09 \pm 1.08^{C, d}$	$10.09 \pm 2.33^{C, c}$	<0.001
p		<0.001	<0.001	<0.001	<0.001	<0.003	
Cola	**7**	$8.90 \pm 1.63^{A, a}$	$2.24 \pm 0.94^{A, d}$	$6.70 \pm 1.66^{A, b}$	$5.73 \pm 1.36^{A, bc}$	$4.60 \pm 1.43^{A, c}$	<0.001
	14	$11.91 \pm 4.01^{AB,a}$	$3.14 \pm 1.16^{B, c}$	$9.46 \pm 2.64^{B, ab}$	$6.76 \pm 1.61^{A, b}$	$6.64 \pm 1.34^{B, b}$	<0.001
	28	$13.12 \pm 2.33^{B, ab}$	$5.12 \pm 1.55^{C, d}$	$15.54 \pm 2.14^{C, b}$	$10.65 \pm 2.22^{B, ac}$	$8.41 \pm 1.90^{B, c}$	<0.001
p		<0.007	<0.001	<0.001	<0.001	<0.001	
Ice tea	**7**	$10.09 \pm 2.69^{A, a}$	$6.48 \pm 1.44^{A, b}$	$2.56 \pm 0.92^{A, c}$	$2.78 \pm 1.15^{A, c}$	$6.39 \pm 1.09^{A, b}$	<0.001
	14	$17.85 \pm 3.05^{B, a}$	$7.98 \pm 1.3^{B, b}$	$4.36 \pm 1.32^{B, c}$	$4.88 \pm 1.29^{B, c}$	$7.63 \pm 1.78^{A, b}$	<0.001
	28	$23.33 \pm 3.36^{C, a}$	$9.83 \pm 2.09^{C, bc}$	$7.69 \pm 1.06^{C, bc}$	$7.45 \pm 1.83^{C, c}$	$10.25 \pm 1.8^{B, b}$	<0.001
p		<0.001	<0.003	<0.001	<0.001	<0.001	
Saliva	**7**	$4.68 \pm 0.83^{A, a}$	$1.58 \pm 0.42^{A, b}$	$1.73 \pm 0.39^{A, b}$	$1.55 \pm 0.38^{A, b}$	$1.48 \pm 0.63^{A, b}$	<0.001
	14	$5.62 \pm 1.78^{AB, a}$	$2.62 \pm 0.74^{B, b}$	$3.05 \pm 0.71^{B, b}$	$2.25 \pm 0.28^{B, b}$	$2.01 \pm 0.58^{B, b}$	<0.001
	28	$7.40 \pm 1.18^{B, a}$	$3.45 \pm 0.92^{B, bc}$	$4.27 \pm 0.62^{C, c}$	$2.73 \pm 0.34^{C, b}$	$2.60 \pm 0.64^{C, b}$	<0.001
p		<0.001	<0.001	<0.001	<0.001	<0.001	

Different capital letters in each column indicate statistical differences depicted by paired samples t test and repeated measures ANOVA (p=0.05). Different small letters in each row indicate statistical differences depicted by one-way ANOVA and Tukey's post-hoc test (p=0.05).

Table 4. *Three-way ANOVA test for the influence of material, liquid and time on the color difference (ΔE_{00})*

Source	Type III Sum of Squares	df	Mean Square	F	p
Corrected Model	13743.080[a]	59	232.934	65.869	<0.001
Intercept	32779.824	1	32779.824	9269.485	<0.001
material	4705.701	4	1176.425	332.670	<0.001
Liquid	3913.547	3	1304.516	368.891	<0.001
Time	2084.243	2	1042.121	294.691	<0.001
material * Liquid	2131.820	12	177.652	50.236	<0.001
material * Time	318.747	8	39.843	11.267	<0.001
Liquid * Time	257.850	6	42.975	12.152	<0.001
material * Liquid * Time	331.172	24	13.799	3.902	<0.001

a. R Squared=0.878 (Adjusted R Squared=0.865)

Table 5. *Mean ΔTP_{00} values and standard deviations of restorative materials after 28 days of immersion in liquids*

ΔTP_{00}	Alkasite	ACTIVA	Equia Forte	Zirconomer	Composite	p
Coffee	$4.6 \pm 1.9^{A, a}$	$3.9 \pm 1.0^{A, a}$	$1.5 \pm 0.6^{A, b}$	$1.3 \pm 0.6^{A, b}$	$3.5 \pm 1.8^{A, a}$	<0.001
Cola	$3.1 \pm 1.3^{AB, a}$	$2.0 \pm 0.9^{B, ab}$	$1.6 \pm 0.9^{A, b}$	$1.5 \pm 0.8^{A, b}$	$3.2 \pm 1.3^{A, a}$	<0.001
Ice tea	$4.6 \pm 1.5^{A, a}$	$3.4 \pm 1.0^{A, ab}$	$1.0 \pm 0.4^{A, c}$	$1.1 \pm 0.4^{AB, c}$	$3.3 \pm 1.0^{A, b}$	<0.001
Saliva	$2 \pm 0.4^{B, a}$	$1.4 \pm 0.6^{B, b}$	$0.9 \pm 0.2^{A, c}$	$0.5 \pm 0.2^{B, c}$	$0.8 \pm 0.2^{B, c}$	<0.001
p	<0.001	<0.001	<0.053	<0.005	<0.001	

Different capital letters in each column indicate statistical differences depicted by one-way ANOVA and Tukey's post-hoc test (p=0.05). Different small letters in each row indicate statistical differences depicted by one-way ANOVA and Tukey's post-hoc test (p=0.05).

Table 6. *Mean ΔCR values and standard deviations of restorative materials after 28 days of immersion in liquids*

ΔCR	Alkasite	ACTIVA	Equia Forte	Zirconomer	Composite	p
Coffee	$0.19 \pm 0.08^{A, a}$	$0.12 \pm 0.05^{A, b}$	$0.11 \pm 0.05^{A, b}$	$0.04 \pm 0.02^{A, c}$	$0.10 \pm 0.5^{A, b}$	<0.001
Cola	$0.10 \pm 0.04^{B, a}$	$0.08 \pm 0.05^{A, a}$	$0.10 \pm 0.4^{A, a}$	$0.05 \pm 0.03^{A, b}$	$0.07 \pm 0.04^{AB, a}$	<0.037
Ice tea	$0.13 \pm 0.07^{AB, a}$	$0.11 \pm 0.06^{A, a}$	$0.08 \pm 0.02^{A, ab}$	$0.03 \pm 0.01^{AB, b}$	$0.11 \pm 0.05^{A, a}$	<0.001
Saliva	$0.08 \pm 0.04^{B, a}$	$0.6 \pm 0.2^{B, ab}$	$0.02 \pm 0.01^{B, cd}$	$0.01 \pm 0.00^{B, d}$	$0.04 \pm 0.01^{B, bc}$	<0.001
p	<0.001	<0.031	<0.001	<0.004	<0.007	

Different capital letters in each column indicate statistical differences depicted by one-way ANOVA and Tukey's post-hoc test (p=0.05). Different small letters in each row indicate statistical differences depicted by one-way ANOVA and Tukey's post-hoc test (p=0.05).

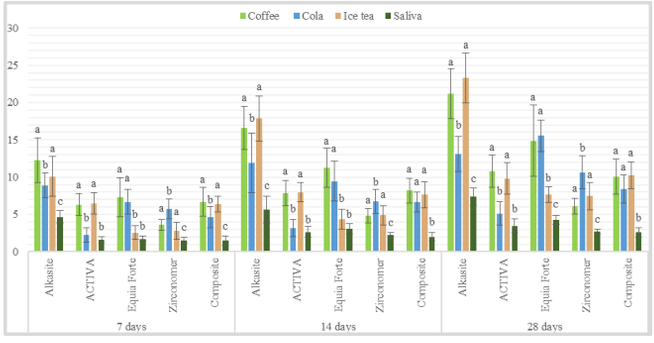


Figure 1. *Comparison of the effect of solution type on the color change of the material. Different letters indicate statistically significant differences among the solutions in the same measurement time and material.*

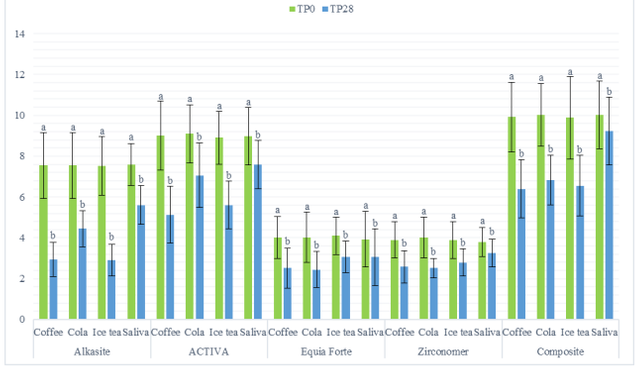


Figure 2. *Pairwise comparison of translucency parameters at the initial and 28 days. Different letters denote statistically significant difference between the initial and 28 days in the same solution and material.*

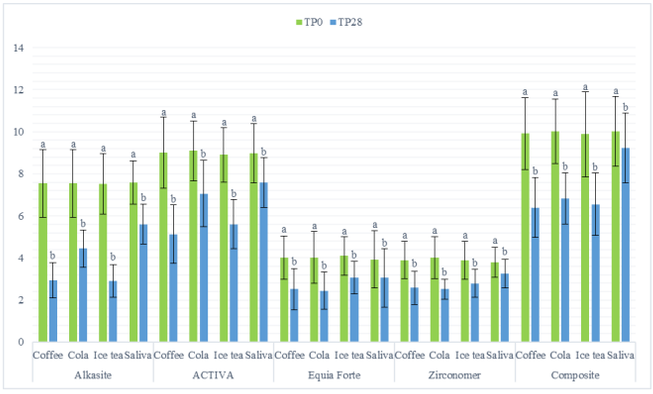


Figure 3. *Pairwise comparisons of contrast ratio at the initial and 28 days. Different letters denote statistically significant difference between the initial and 28 days in the same solution and material.*

Translucency parameters and pairwise comparisons at the beginning and at the end of the 28th day were shown in Figure 2. There were significant differences for all groups ($p<0.05$). TP values of all materials decreased after immersion in solutions. The statistical analysis of mean and standard deviations of ΔTP_{00} according to the type of the immersion liquid and material were represented in Table 5. Equia Forte and Zirconomer had lower initial translucency than the other materials. ΔTP_{00} of these materials congruently was lower than the other materials immersed in coffee and ice tea solutions. Alkasite, ACTIVA and composite showed similar ΔTP_{00} in all liquids except saliva.

The values of CR and pairwise comparisons of CR at the initial and 28 days were shown in Figure 3 and significant differences were found for all groups except the Equia Forte group, which was immersed in saliva ($p<0.05$). HVGIC-based materials had significantly higher initial CR than the resin-based materials, consistent with TP results ($p<0.001$). The mean CR change (ΔCR), standard deviations, statistical analysis according to the immersed solution type and the material were shown in Table 6. Zirconomer showed statistically significant lower ΔCR than the other materials in all investigated solutions ($p<0.05$). Alkasite showed the highest ΔCR value. However, the change in coffee and cola was not statistically significant compared to the other resin-based materials ($p>0.05$). The Pearson correlation test revealed a strong ($r=-0.71$) and statistically significant negative correlation between TP and CR.

4. DISCUSSIONS

There is a lack of studies in the literature evaluating the color stability and optical properties of recent bulk-fill restorative materials. In this in-vitro study, conventional resin composite and recent bulk-fill restorative materials were tested for ΔE_{00}, TP_{00}, ΔTP_{00}, CR and ΔCR after immersion in different aging solutions. As a result of this study, ΔE_{00}, ΔTP_{00} and ΔCR of the materials were found to be different from each other. Alkasite showed the most ΔE_{00} and ΔTP_{00} while Zirconomer was the least. Thus, the first hypothesis was rejected. The color changes of the materials increased during the immersion periods of 7, 14 and 28 days. At the end of 28 days, TP of the materials decreased and CR increased. While coloration occurred mostly in beverages such as coffee and ice tea with resin-based materials, cola was the beverage that most influenced the HVGIC-based materials. Thus, the second hypothesis of the study, which is the time of immersion in the type of beverage, was rejected.

Tooth-colored restorative materials should have excellent color matching and high color stability during the clinical treatment (28). The immersion in cola and coffee beverages with high staining potential are considered a proper test for predicting the tendency of the materials to change color (29). In this study, the effect of immersion for 7, 14 and 28 days on the color stability of the materials in coffee, cola, ice tea and saliva, which are consumed frequently, was investigated. The CIELab color formula, which is widely used in dentistry, was used to evaluate the colors and color differences between various natural and restorative esthetic materials (24). The $CIEDE_{2000}$ formula was shown to define color differences recognized by the human eye superior to the CIELab formula in recent studies (30). The $CIEDE_{2000}$ was used to determine the color change in this study. The $CIEDE_{2000}$ and color differences were evaluated for detectability and clinical acceptability thresholds. Paravina et al. (31) reported that the color perceptibility threshold was between 0.8 and 1.8. In the study, all materials showed a color change over time in beverages above clinical acceptability.

In this study, the time-dependent color changes were determined mostly in coffee and ice tea. ΔE_{00} was occurred especially on Alkasite, ACTIVA and composite. Alkasite is one of the recently introduced tooth-colored materials and is classified as a subgroup of composite materials (32). ACTIVA, which combines the positive properties of GIC and composite resins, can be an alternative to composite resins in anterior and posterior teeth due to its positive properties such as fluoride release and low polymerization shrinkage (33).

The hydrophilic nature and sensitivity to absorb water of the resin matrix can increase the probability of staining materials. The sensitivity to absorb water is a property of the resin ingredient of the material and the resistance of the resin-filler matrix. Excessive water absorption causes expansion and plasticization of the resin matrix, which results in hydrolysis of the silane and, accordingly, the formation of microcracks. Thus, the life of the composite resin is decreased. Microcracks or interfacial gaps between the filler and the matrix cause color penetration and discoloration (34). Coffee and tea contain yellow colorants of different polarities which could explain the discoloration of composite samples (35, 36). Previous studies were shown that composite resins are sensitive to discoloration when exposed to various coloring beverages (23, 34). Although alkasite is a resin-based material, which is a subgroup of composite resin, it was very sensitive to coloration.

The type and particle size of the fillers can also affect the discoloration of composite resin (19). Composite resins with smaller particles have been reported to have less tendency to stain (37). The fact that the resin matrix type of the alkasite is different from the composite resin and the particle size of the alkasite is larger may explain the greater discoloration than the composite resin. Furthermore, it was stated that all resin composition properties such as chemical differences and concentration of resin monomers, type of initiators and inhibitors, oxidation of unreacted monomers affect the discoloration potential of the composite resins (35). The discoloration in self-cured and dual-cure resins is more pronounced than in light-cured resins because tertiary aromatic amines are more likely to oxidize than aliphatic amines used in light-cured resins (38). In addition, since self-cure initiators can induce colored oxidation products that lead to the staining of resins by the time, self-curing of alkasite may cause more intrinsic coloration than the composite resin and ACTIVA (39).

The resin matrix mostly absorbs water directly, but glass fillers do not show the water absorption into the bulk of the material. It can only absorb water to the surface of the material (35). This situation could explain that the color changes of Equia Forte and Zirconomer with glass filler content in coffee and ice tea were less than that of alkasite, ACTIVA and composite with the resin matrix. When immersion in cola was examined, resin-containing materials did not show discoloration as much as coffee and tea. Although cola has the lowest pH value and damages the surface integrity of the resin materials, it has been reported that it does not cause discoloration as much as coffee and tea, probably because it does not contain yellow colorants (40). However, the most

color changes occurred in cola with glass filler Equia Forte and Zirconomer. It can be caused by discoloration, possibly as a result of the low pH damaging the integrity of the materials.

Translucency and contrast are important properties for dental tissues and materials. TP and CR are two indicators commonly used to determine the transparency and opacity values and changes of the material (14). The values of TP range from 0 to 100, indicating that the material is completely transparent and completely opaque, respectively (41). The values for CR range from 0 to 1, indicating that the material is completely transparent and completely opaque, respectively (42). The strong correlation was found between the two values in this study.

The translucency of human teeth should be a reference in the evaluation of the translucency of dental restorative materials (43). The translucency of natural teeth tends to decrease from the cutting edge (TP=15) towards the cervical (TP=5) (44). Mean TP values of 1 mm thick bovine enamel, bovine dentin, human enamel and human dentin were 14.7, 15.2, 18.7, and 16.4, respectively (45). In this study, the values closest to enamel translucency were seen in microhybrid composite resin, while the translucency of HVGIC materials was quite low. The translucency of the flowable and universal resin composites of the same brand prepared with a thickness of 2 mm were compared, and the TP values were found to be between 10-15 for the flowable composite and between 9-12 for the universal composite, respectively (46). Uchimura et al. (13) found the TP values of 18 different conventional glass ionomers after 7 days without immersion in any liquid, varying between 3.9-20. The translucency values of resin-modified glass ionomers varied depending on whether the material was light-cured or cured by acid-base reaction alone (47). Light-cured samples were found to be marginally more translucent than the samples allowed to cure without irradiation.

Quek et al. (48) found that red wine and coffee decreased the translucency of composite restorative materials. Stawarczyk et al. (49) evaluated ΔE and TP of five different composite resin materials for different colorant solutions for 14 days and reported that the internal structure and composition of the material affected the translucency of the materials. Barutçugil et al. (14) showed that immersion of three different materials in red wine and coffee caused significant changes in color and translucency compared to immersion in water. In the present study, the solutions affected ΔE, ΔTP and ΔCR of the materials and the changes were higher in coffee, cola and ice tea compared to saliva.

The detectability and acceptability thresholds of ΔTP were reported as 0.62 and 2.62, respectively (16). Considering the ΔTPs of the materials, the changes caused by the beverages at the end of 28 days showed unacceptable changes in the translucency of the resin-containing ones, while the changes in HVGIC-based materials are not clinically significant. This study stands out with its evaluation of ΔE, ΔTP and ΔCR values as a result of time-dependent aging of current restorative materials in different solutions.

In a study evaluating the CR of different GICs, the contrast values were found to be between 0.7-1 (13). A study evaluating time-dependent of ΔCR found that all materials tested statistically increased their opacity during the 1-month immersion period (14). Similar to the above studies, CR values of the materials were found to be between 0.73-0.90 in this study, while their values approached 1 at the end of 28 days. These results may present a poor clinical picture due to the opacities of the materials. These materials may be preferred for clinical uses where masking with low thickness is required.

Limitations of this study involve allowing both sides of the material to be exposed to coloring agents, unlike clinical staining conditions. In the oral cavity, restorative materials are constantly exposed to colorants from foods and beverages and are rinsed with saliva and are cleaned with oral hygiene procedures. The present study experimented to simulate the clinical setting but did not comprise the effect of cleaning and thermal change on restorative materials and specimens were in beverages nonstop during the immersion period.

5. CONCLUSIONS

Within the limitations of this in vitro study, exposing recent restorative materials to different beverages produced significant changes in color, translucency and contrast. Resin-based alkasite, ACTIVA and composite showed the highest change the optical properties in coffee and ice tea. HVGIC-based materials, Equia Forte and Zirconomer were mostly affected by cola and coffee. HVGICs presented very low TP and high CR values, which remains thought-provoking in supplying esthetic demands.

Acknowledgments: *The authors desire to thank all the participants in the study.*
Funding: *This research was supported by the Scientific Research Projects of Çukurova University with the project number TSA-2020-13339.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Çukurova University, Faculty of Medicine, Non-interventional Clinical Research Ethics Committee (Decision date and number: 2021/108.43)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: SNK, CK
Design of the study: SNK, CK
Acquisition of data for the study: SNK, CK
Analysis of data for the study: CK
Interpretation of data for the study: CK, SNK
Drafting the manuscript: SNK
Revising it critically for important intellectual content: SNK, CK
Final approval of the version to be published: SNK, CK

REFERENCES

[1] Ren Y-F, Feng L, Serban D, Malmstrom HS. Effects of common beverage colorants on color stability of dental composite

resins: the utility of a thermocycling stain challenge model in vitro. J Dent. 2012;40:48-56.

[2] Ilie N, Bucuta S, Draenert M. Bulk-fill resin-based composites: an in vitro assessment of their mechanical performance. Oper Dent. 2013;38:618-625.

[3] Tarle Z, Attin, T, Marovic D, Andermatt L, Ristic M, Tauböck TT. Influence of irradiation time on subsurface degree of conversion and microhardness of high-viscosity bulk-fill resin composites. Clin Oral Investig. 2015;19:831-840.

[4] Chau NPT, Pandit S, Cai JN, Lee M-H, Jeon J-G. Relationship between fluoride release rate and anti-cariogenic biofilm activity of glass ionomer cements. Dent Mater. 2015;31:100-108.

[5] Hegde NN, Attavar SH, Hegde MN, Priya G. Antibacterial activity of dental restorative material: An in vitro study. J Conserv Dent. 2018;21:42-46.

[6] Amalavathy RK, Sahoo HS, Shivanna S, Lingaraj J, Aravinthan S. Staining effect of various beverages on and surface nano-hardness of a resin coated and a non-coated fluoride releasing tooth-coloured restorative material: An in-vitro study. Heliyon 2020;6:1-5.

[7] Karakaş SN, Küden C. Comparative evaluation of cavities disinfected with ozone gas and photodynamic therapy and restored with two different reinforced glass ionomer cements on microleakage and color change. Ozone Sci Eng. 2021:1-10.

[8] Bhadra D, Shah NC, Rao AS, Dedania MS, Bajpai N. A 1-year comparative evaluation of clinical performance of nanohybrid composite with Activa™ bioactive composite in Class II carious lesion: A randomized control study. J Conserv Dent. 2019;22:92-96.

[9] Atri F, Safi M, Salehzadeh S, Izadi J, Fallah PE, McLaren EA. Analysis of color and fluorescence of different dentin ceramics and natural human dentin's to enhance shade matching. J Esthet Dent. 2021;33:1029-1034.

[10] Miletic V, Stasic JN, Komlenic V, Petrovic R. Multifactorial analysis of optical properties, sorption, and solubility of sculptable universal composites for enamel layering upon staining in colored beverages. J Esthet Dent. 2021;33:943-952.

[11] Çakmak G, Donmez MB, Kashkari A, Johnston WM, Yılmaz B. Effect of thickness, cement shade, and coffee thermocycling on the optical properties of zirconia reinforced lithium silicate ceramic. J Esthet Dent. 2021;1-7.

[12] Winter R. Visualizing the natural dentition. J Esthet Dent. 1993;5:103-118.

[13] Uchimura JYT, Sato F, Santana RG, Menezes-Silva R, Bueono LS Borges AFS, Pascotto RC. Translucency parameter of conventional restorative glass-ionomer cements. J Esthet Dent. 2021;33:935-942.

[14] Barutçugil Ç, Bilgili D, Barutcigil K, Dündar A, Büyükkaplan UŞ, Yılmaz B. Discoloration and translucency changes of CAD-CAM materials after exposure to beverages. J Prosthet Dent. 2019;122:325-331.

[15] Nogueira AD, Della Bona A. The effect of a coupling medium on color and translucency of CAD–CAM ceramics. J Dent. 2013;41:18-23.

[16] Salas M, Lucena C, Herrera LJ, Yebra A, Della-Bona A, Perez MN. Translucency thresholds for dental materials. Dent Mater. 2018;34:1168-1174.

[17] Blum IR, Özcan M. Reparative Dentistry: Possibilities and Limitations. Curr Oral Health Rep. 2018;5:264-269.

[18] Villalta P, Lu H, Okte Z, Garcia-Godoy F, Powers JM. Effects of staining and bleaching on color change of dental composite resins. J Prosthet Dent. 2006;95:137-142.

[19] Barutcigil Ç, Barutcigil K, Özarslan MM, Dündar A, Yılmaz B. Color of bulk-fill composite resin restorative materials. J Esthet Dent. 2018;30:3-8.

[20] Erdemir U, Yıldız E, Eren MM. Effects of sports drinks on color stability of nanofilled and microhybrid composites after long-term immersion. J Dent. 2012;40:55-63.

[21] Örtengren U, Andersson F, Elgh U, Terselius B, Karlsson S. Influence of pH and storage time on the sorption and solubility behaviour of three composite resin materials. J Dent. 2001;29:35-41.

[22] Duke E, Trevino D. A resin-modified glass ionomer restorative: three-year clinical results. J Indiana Dent Assoc 1998;77:13-16.

[23] Abu-Bakr NE, Han L, Okamoto A, Iwaku M. Color stability of compomer after immersion in various media. J Esthet Dent. 2000;12:258-263.

[24] Johnston WM. Color measurement in dentistry. J Dent. 2009;37:2-6.

[25] Colorimetry C, Commission Internationale de l'Èclairage: Vienna. 2004, Austria.

[26] Luo MR, Cui G, Rigg B. The development of the CIE 2000 colour-difference formula: CIEDE2000. Color Res Appl. 2001;26:340-350.

[27] Kürklü D, Azer SS, Yilmaz B, Johnston WM. Porcelain thickness and cement shade effects on the colour and translucency of porcelain veneering materials. J Dent 2013;41:1043-1050.

[28] Watts A, Addy M. Tooth discolouration and staining: a review of the literature. Br Dent J. 2001;190:309-316.

[29] Kim JH, Lee YK, Powers JM. Influence of a series of organic and chemical substances on the translucency of resin composites. J Biomed Mater Res B Appl Biomaters. 2006;77:21-27.

[30] Gómez-Polo C, Muñoz MP, Luengo MC, Vicente P, Galindo P, Casado AMM. Comparison of the CIELab and CIEDE2000 color difference formulas. J Prosthet Dent. 2016;115:65-70.

[31] Paravina RD, Ghin R, Herrera LJ, Bona AD, Igiel C, Lininger M, Mar-Perez MD. Color difference thresholds in dentistry. J Esthet Dent. 2015;27:1-9.

[32] Samanta S, Das UK, Mitra A. Comparison of microleakage in class V cavity restored with flowable composite resin, glass ionomer cement and cention N. Imp J Interdiscip Res 2017;3:180-183.

[33] Croll TP, Berg JH, Donly KJ. Dental repair material: a resin-modified glass-ionomer bioactive ionic resin-based composite. Compend Contin Educ Dent. 2015;36:60-65.

[34] Ertas E, Güler AU, Yücel AC, Köprülü H, Güler E. Color stability of resin composites after immersion in different drinks. Dent Mater J. 2006;25:371-376.

[35] Barutcigil Ç, Yıldız M. Intrinsic and extrinsic discoloration of dimethacrylate and silorane based composites. J Dent. 2012;40:57-63.

[36] Ardu S, Braut V, Gutemberg D, Krejci I, Dietschi D, Feilzer AJ. A long-term laboratory test on staining susceptibility of esthetic composite resin materials. Quintessence Int. 2010;41:695-702.

[37] Fontes ST, Fernández MR, Moura CM, Meireles SS. Color stability of a nanofill composite: effect of different immersion media. J Appl Oral Sci. 2009;17:388-391.

[38] Janda R, Roulet JF, Kaminsky M, Steffin G, Latta M. Color stability of resin matrix restorative materials as a function of the method of light activation. Eur J Oral Sci. 2004;112:280-285.

[39] Krishnan VK, Yamuna V. Effect of initiator concentration, exposure time and particle size of the filler upon the mechanical properties of a light-curing radiopaque dental composite. J Oral Rehabil. 1998;25:747-751.

[40] Tekçe N, Tuncer S, Demirci M, Serim ME, Baydemir C. The effect of different drinks on the color stability of different restorative materials after one month. Restor Dent Endod. 2015;40:255-261.

[41] Paravina RD, Ontiveros JC, Powers JM. Curing-dependent changes in color and translucency parameter of composite bleach shades. J Esthet Dent. 2002;14:158-166.

[42] Vichi A, Sedda M, Fabian-Fonzar R, Carrabba M, Ferrari M. Comparison of Contrast Ratio, Translucency Parameter, and Flexural Strength of Traditional and "Augmented Translucency" Zirconia for CEREC CAD/CAM System. J Esthet Dent. 2016;28:32-39.

[43] Lee Y-K. Translucency of human teeth and dental restorative materials and its clinical relevance. J Biomed Opt. 2015;20:045002.

[44] Hasegawa A, Ikeda I, Kawaguchi S. Color and translucency of in vivo natural central incisors. J Prosthet Dent. 2000;83:418-423.

[45] Yu B, Ahn J-S, Lee Y-K. Measurement of translucency of tooth enamel and dentin. Acta Odontol Scand. 2009;67:57-64.

[46] Yu B, Lee Y-K. Differences in color, translucency and fluorescence between flowable and universal resin composites. J Dent. 2008;36:840-846.

[47] Mount G, Patel C, Makinson O. Resin modified glass-ionomers: Strength, cure depth and translucency. Aust Dent J. 2002;47:339-343.

[48] Quek SH, Yap AU, Rosa V, Tan KBC, Teoh KH. Effect of staining beverages on color and translucency of CAD/CAM composites. J Esthet Dent. 2018;30:9-17.

[49] Stawarczyk B, Liebermann A, Eichberger M, Güth JF. Evaluation of mechanical and optical behavior of current esthetic dental restorative CAD/CAM composites. J Mech Behav Biomed Mater. 2016;55:1-11.

How to cite this article: Karakaş SN, Küden C. Time-Dependent Change of Color and Translucency of Recent Restorative Materials in Various Beverages. Clin Exp Health Sci 2023; 13: 150-158. DOI: 10.33808/clinexphealthsci.1068359

Clinical and Experimental Health Sciences

Determination of the Effect of the Fowler and Prone Position on Oxygen Saturation in Patients Diagnosed with COVID-19

Yeşim İşler Işıldak, Fatma Eti Aslan, Göknur Parlak

Bahçeşehir University, Faculty of Health Sciances, Department of Nursing, İstanbul, Türkiye.

Correspondence Author: Yeşim İşler Işıldak
E-mail: yesimisler60@gmail.com
Received: 14.02.2022 **Accepted:** 26.09.2022

ABSTRACT

Objective: This study was conducted to investigate the effect of the Fowler position and prone position on oxygen saturation in patients receiving treatment in clinics with the diagnosis of COVID-19 disease.

Method: A total of 40 patients, admitted to the pandemic ward who met the inclusion criteria, were included in the quasi-experimental type study without any sampling. The patients were first given the Fowler position and then the prone position. There was a time interval of 15 minutes wait between the two positions. For each position, peripheral oxygen saturation, heart rate, respiratory and blood pressure values were obtained at initial position placement, after the 30th minute and every hour for the first four hours.

Results: The mean age of the participants was 57.57±12.64 years. Respiratory distress, cough, fever, weakness, sweating and headache were the main symptoms. A total of 22.5% of them had a diagnosis of hypertension and Diabetes Mellitus. The requirement for the positioning was found to be 95% in the first five days after admittance. After treatment, 85% of them were discharged home. The mean oxygen saturation values of the patients for every hour in the Prone position were 93.15±1.718 (p=0.035), 93.60±1.809 (p=0.019), 93.93±1.774 (p=0.006) and 94.15±1.718 (p=0.002), respectively in the first four hours. These findings were statistically significant compared to the Fowler position. Respiratory values in the prone position were 17.30±1.159 (p=0.005), 17.20±1.344 (p=0.010), 17.20±1.181 (p=0.005), and 17.05±1.280 (p=0.001), respectively in the first four hours, which were statistically lower than in the Fowler position. There was no significant difference in the mean heart rate and blood pressure in both positions (p>0.05).

Conclusion: The prone position was found to have a positive effect on oxygen saturation levels when Fowler and Prone positions were applied in patients receiving treatment with the diagnosis of COVID-19 in hospital wards. Therefore, it is recommended that patients admitted with the diagnosis of COVID-19 be placed in the prone position at regular intervals.
Keywords: COVID-19, Prone position, Fowler position, Oxygen Saturation

1. INTRODUCTION

Coronavirus-2019(COVID-19) disease is a clinical picture caused by SARS-CoV-2 (Acute Respiratory Syndrome Coronavirus-2) (1). COVID-19 affects many systems, but primarily the respiratory system. It often has the symptoms of fever, dry cough, weakness, myalgia and dyspnea. It travels from the upper respiratory tract to the lower respiratory tract. With the increase in the severity of the disease, the symptoms also become more severe, and hypoxia and severe shortness of breath are observed (2-4). While 14% of the admitted patients had a severe case (dyspnea, hypoxemia and presence of more than 50% lung involvement in imaging) and 5% had a critical case (respiratory failure, shock, multiorgan failure) (5,6).

For clinical patients to continue to breathe effectively, ensuring that the oxygen saturation is at 95-100% should be among the first goals of healthcare professionals. Oxygen therapy should be started with a 5 L/ min nasal or standard face mask for the patient and oxygen saturation should be adjusted to >95% (7,8).

With the development of pulmonary inflammation in COVID-19, impaired lung ventilation/perfusion leads to hypoxemia. Oxygen therapy is included in the first step of follow-up and treatment in the clinical setting before intensive care in the hypoxemia picture (9). Along with the COVID-19 treatment protocol, the importance and effectiveness of patient positioning have also been stated in the literature (2,10). Lung circulation and ventilation may be performed more effectively with the patient positioning, which is the independent role of nursing. The purpose of the prone position in patients with respiratory distress is to make breathing more effective and relieve the pulmonary circulation by reducing abdominal pressure (11). The prone position has been used to improve hypoxemia since 1974 (12). By eliminating the compressive weight of the abdominal region with this position, the alveoli in the dorsal region merge with the pulmonary blood flow, relieving lung perfusion (13,14). This position is known to be important for

DOI: 10.33808/clinexphealthsci.1186086

more homogeneous ventilation of the lungs and it contributes significantly to the improvement of oxygenation. The prone position increases the functional residual capacity, opens the atelectatic lung areas, leads to an increase in chest wall elastance, corrects the ventilation/perfusion ratio, and ensures the mobility of secretions (1,10,15). This positioning facilitates the redistribution of pulmonary blood flow rather than opening the collapsed alveoli, thereby reducing the formation of shunts. In this way, the pulmonary circulation is relieved (16). Many studies have been conducted related to the prone position, however, there was not enough clinical evidence available in the literature showing the effectiveness of the prone position in the clinical treatment process of patients with COVID-19 (1).

By evaluating the effectiveness of position practice, which is the independent role of nursing, this study is thought to make contributions to the quality of nursing care (12). The study was planned to determine the effect of the Fowler and prone position on oxygen saturation values in patients diagnosed with COVID-19.

The hypothesis of the study: There is a difference between the peripheral oxygen saturation values of the patients who were given the Fowler and Prone positions.

2. METHODS

2.1. Ethic Aspects

Ethics Committee approval of Bahçeşehir University (Decision date and number:14.01.2021-KAEK 2021/1), institutional permission and informed consent of patients were obtained to conduct the study which has been carried out in accordance with the 1964 Helsinki Declaration and its later amendments.

2.2. Design and Setting of the Study

The study was carried out using a quasi-experimental model in the pandemic ward of a public hospital.

2.3. Population and Sample of the Study

The population of the study consisted of patients admitted to the pandemic ward between 14.01.2021 and 30.03.2021. The sample group consisted of individuals who were admitted to the ward within the specified period, who met the inclusion criteria and who agreed to participate in the study. The effect size standardized by Cohen was used due to the unavailability of any study that could be used as a reference in the study. Therefore, the minimum number of samples was determined as 34 by taking the effect size of the study as, the alpha value of 0.05 and the theoretical power of 0.80. Given that there may be losses during the study, it was started with a number of participants (n=40) more than the required (17,18).

2.4. Inclusion and Exclusion Criteria for the Study

From the individuals who were admitted to the pandemic ward and were between the age of 18-79 (young, middle age and early-old age group) with an oxygen saturation level below 95%;

- Those without a history of malnutrition, Parkinson's, dementia, or stroke,
- Those with ground-glass opacity in their lungs and positive PCR test result,
- Those who were literate and could be communicated and were willing to participate were included.
  - Excluded individuals during the study:
- Patients who passed away,
- Patients who needed intubation and transferred to the intensive care unit,
- Patients who could not do the positioning during the study were excluded.
- Pregnant individuals were not included in the study.

This study has been prepared per the TREND guidelines (19).

2.5. Data Collection Tools and Data Collection

The data collection and demographic characteristics forms, prepared by the researchers after the literature review, were used for the data collection (20). Before the data collection process, institutional permission, and ethics committee approval were obtained. The effectiveness of the Fowler and prone positions which were given to the individuals, who were included in the study according to the study criteria were compared. The positioning was started after providing the necessary training to the individuals and obtaining their consent. In the Fowler position, individuals were positioned sitting in the bed. In the prone position, the individuals were positioned lying on their face down. A total of eight-hour positioning, first four-hour Fowler position and then four-hour prone position, was applied during the day (24 hours) determined by using the literature information and vital signs (oxygen saturation, respiratory rate, blood pressure and heart rate) were measured and recorded in this period. Oxygen saturation and heart rate measurements were performed peripherally. Blood pressure was measured with a manual sphygmomanometer. The respiratory rate was counted from the movement of the thoracic cage after the position was given. Participants rested for 15 minutes between two positions. It is stated in the literature that the prone position can be applied to a patient for a maximum of 12 hours with 4-hour rotations (3,7, 15,21). Participants were informed about the benefit of using this positioning during the COVID-19 treatment and they were asked to continue the practice in this process. Data collection was performed on the first day that the positioning was given by the researchers. The process was not repeated until the patients left the clinic. The oxygen saturation values were measured with a pulse oximeter brand device during the positioning period. It was a brand-new device and calibrated by the hospital biomedical unit. Parameters were recorded in the data form. Measurements were planned as such: initial measurement

when the positioning was performed, measurement after 30 minutes, measurement after one hour and every one hour for the next three hours (21).

2.6. Data Analysis

SPSS (Statistical Package for Social Sciences) for Windows 25.0 (Z125-5543-05) was used for the analysis of the data in this study. Descriptive statistical methods (number, percentage, mean, standard deviation) were used in the evaluation of the data. In addition to normality tests, distribution measures such as histogram, Q-Q plot and box-plot graphics, Skewness and Kurtosis and coefficient of variation may be used to evaluate whether the data is normally distributed or not (22). In order to ensure normality, the values of the data should be observed close to 45 degrees in the scattering diagram and positioned by centring the median line of the box on the box line chart (23). The normal distribution was checked by conformity normality tests and Skewness and Kurtosis values. In the analysis of the data, the dependent sample t – test was used for comparison of quantitative data when the assumption of the normal distribution is ensured, and Wilcoxon signed-rank test was used in cases where normal distribution was not achieved.

3. RESULTS

Personal information of the patients (n=40) included in the study and admitted to the pandemic ward is given in Table 1. According to the results, 60% of the participants were male patients. While 50% (n=20) of them were in the 45-64 (middle age) age range, 47.5% (n=19) had a bodyweight between 70-84 kg. Looking at the symptoms, 60% (n=26) of them were observed to have respiratory distress, cough, fever, weakness, sweating and headache. There was no chronic disease in 37.5% (n=15) of the patients. While steroid was used in the treatment of 67.5% (n=27) of them, 95% (n=38) of them needed positioning in the first five days after admittance. A total of 85% (n=34) of them were discharged home.

In all of the hourly as well as the initial and 30th-minute measurements for the oxygen saturation values of the patients given Fowler and Prone positions, no statistical significance was found according to the descriptive characteristics of the patients such as whether the patients had a chronic disease, weight or age (p>0.05).

There was no statistically significant difference between the initial and 30th-minute oxygen saturation measurements of the patients given Fowler and Prone positions (p>0.05). The first – hour mean oxygen saturation value of the patients was 92.33±2.043 in the Fowler position and 93.15±1.718 in the prone position and a statistically significant difference was found between the positions (p=0,035). The second-hour mean oxygen saturation value of the patients was 92.58±2.024 in the Fowler position and 93.60±1.809 in the prone position and a statistically significant difference was found between the positions (p=0.019). The third-hour mean oxygen saturation value of the patients was 92.70±1.937 in the Fowler position and 93,93±1,774 in the prone position and a statistically significant difference was found between the positions(p=0.006). The fourth-hour mean oxygen saturation value of the patients was 92.78±1.968 in the Fowler position and 94.15±1.718 in the prone position and a statistically significant difference was found between the positions (p=0.002). It was statistically significant that the oxygen saturation values of the patients in the prone position were higher than the oxygen saturation values in the Fowler position in all of the hourly measurements. It was concluded that the longer the prone position was applied, the more effective it was (Table 2).

Table 1. *Descriptive characteristics and health information of patients*

Descriptive characteristics and health information		n	%
Gender	Female	16	40
	Male	24	60
Age (years)	<= 44	6	15
	45-64	20	50
	>= 65	14	35
Weight	55 – 69 kg	8	20
	70-84 kg	19	47.5
	>=85 kg	13	32.5
Presence of symptoms	Respiratory distress, cough, fever, weakness, sweating, headache	26	65
	Cough, weakness, back pain, nausea, vomiting	9	22.5
	Respiratory distress, cough, fever, weakness, sweating, headache, cough, fatigue, back pain, nausea, vomiting, fainting, loss of appetite, loss of smell and taste	4	10
	No symptoms	1	2.5
	Chest pain and headache	1	2.5
Chronic diseases	No	15	37.5
	Hypertension Hypertension and diabetes Hypertension, diabetes, heart diseases Cancer and infections Diabetes mellitus DM and chronic heart failure Hypertension and hypothyroidism Chronic renal failure Hypertension and asthma	5 9 2 2 3 1 1 1 1	12.5 22.5 5 5 7.5 2.5 2.5 2.5 2.5
Use of Steroids	Yes	27	67.5
	No	13	32.5
The need for positioning	The need for positioning in the first five days of admittance	38	95
Discharge outcome	The need for positioning five days after the admittance	2	5
	Discharged to home	34	85
	Transferred to intensive care	6	15

Table 2. *Oxygen saturation measurement values in Fowler and prone positions*

	Mean	SD	Test value	p-value
Fowler position initial SPO2	91.33	1.992	-0.919 ^{t}	0.364
Prone position initial SPO2	91.63	1.807		
Fowler position 30th minute SPO2	92.05	2.075	-1.308 ^{t}	0.199
Prone position 30th minute SPO2	92.55	1.724		
Fowler position 1st hour SPO2	92.33	2.043	-2.718 ^{t}	**0.035***
Prone position 1st hour SPO2	93.15	1.718		
Fowler position 2nd hour SPO2	92.58	2.024	-2.441 ^{t}	**0.019***
Prone position 2nd hour SPO2	93.60	1.809		
Fowler position 3rd hour SPO2	92.70	1.937	-2.939^{t}	**0.006***
Prone position 3rd hour SPO2	93.93	1.774		
Fowler position 4th hour SPO2	92.78	1.968	-3.049^{z}	**0.002***
Prone position 4th hour SPO2	94.15	1.718		

t: Dependent sample t-test; z: Wilcoxon signed-rank test, SD: Standard Deviation, SPO2: Oxygen saturation

There was no statistically significant difference in the heart rates at all measurement times of the patients between the positions (p>0.05).

There was no statistically significant difference between the initial and 30th-minute respiratory rates of the patients given Fowler and Prone positions (p>0.05). The first-hour mean respiratory rates of the patients were 17.93±1.328 per minute in the Fowler position and 17.30±1.159 in the prone position and a statistically significant difference was found between the positions (p=0,005). The second-hour mean respiratory measurement value of the patients was 17.75±1.214 in the Fowler position and 17.20±1.344 in the prone position and a statistically significant difference was found between the positions (p=0,010). The third-hour mean respiratory measurement value of the patients was 19.25±9.604 in the Fowler position and 17.20±1.181 in the prone position and a statistically significant difference was found between the positions (p=0,005). The fourth-hour mean respiratory measurement value of the patients was 19.25±9.604 in the Fowler position and 17.05±1.280 in the prone position and a statistically significant difference was found between the positions (p=0,001). According to the findings, the respiratory values of the patients in the prone position were found to be lower (Table 3). The respiratory rate of the patients given Fowler and Prone position was found to be statistically significantly lower (p=0.001). The mean respiratory rate of 25% of the patients in the prone position was 20 (per minute) while it was 18 in 50% of the patients and 16 in 25% of them. The respiratory rate is lower in the prone position. As the time of the prone position increases, the respiratory rates of the patients get lower.

There was no statistically significant difference in the systolic blood pressure values at all measurement times of the patients between the positions (p>0.05).

There was no statistically significant difference in the diastolic blood pressure values at all measurement times of the patients between the positions (p>0.05).

Table 3. *Respiratory rates in Fowler and prone positions*

	Mean	SD	Test value	p-value
Fowler position initial RR	18.05	0.959	-0.577 ^{z}	0.564
Prone position initial RR	17.95	1.154		
Fowler position 30th minute RR	17.85	1.231	0.572 t	0.570
Prone position 30th minute RR	17.45	1.197		
Fowler position 1st hour RR	17.93	1.328	2.964 ^{t}	**0.005***
Prone position 1st hour RR	17.30	1.159		
Fowler position 2nd hour RR	17.75	1.214	2.718 ^{t}	**0.010***
Prone position 2nd hour RR	17.20	1.344		
Fowler position 3rd hour RR	19.25	9.604	-2.837 ^{z}	**0.005***
Prone position 3rd hour RR	17.20	1.181		
Fowler position 4th hour RR	19.25	9.604	-3.257 ^{z}	**0.001***
Prone position 4th hour RR	17.05	1.280		

RR: Respiratory rate, t: Dependent sample t-test; z: Wilcoxon signed-rank test

When oxygen saturation measurements were compared during the Fowler and prone positioning of the individuals with COVID 19, we see that oxygen saturation levels were higher in the prone position (Fig. 1).

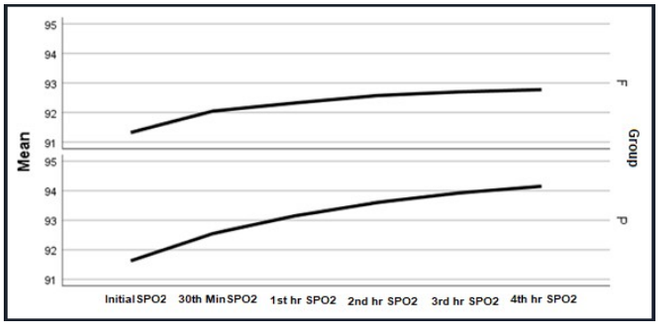


Figure 1. *Oxygen saturation levels of the individuals with COVID 19 during the positioning (Initial, 1st, 2nd, 3rd, 4th hour).*

4. DISCUSSION

In the literature review, the prone position was seen to be mostly given to intubated patients and those diagnosed with Acute Respiratory Distress Syndrome (ARDS) in the intensive care settings. There have been few studies in which positioning was used for awake patients with COVID-19 in the clinical setting outside the intensive care unit (15,20,24,25,26).

In our experimental study, ward patients with a diagnosis of COVID-19 were able to perform prone positioning continuously for four hours. No major complications were observed to develop during the positioning. It was concluded that the prone position was more effective on oxygen saturation levels. In the study of Elharrar et al (26), 63% of hypoxemic patients with a diagnosis of COVID-19 who received treatment outside the intensive care unit were able to tolerate the prone position for more than three hours. However, they reported that oxygen saturation levels increased by 25% during the prone position.

In our study, while 60% of the patients were male, the mean age was 57.57±12.64 (min: 27 max: 78). Hypertension and

Diabetes were the most common chronic diseases with 22.5%. In a similar study by Coppo et al (27), the mean age was 57.4 years and 78% of the patients were male. The same study reported that most of the patients had hypertension and diabetes. Our study supports the literature in this aspect. In our study, 95% of the patients admitted to the clinic were started early positioning (in the first five days), and each patient was given positioning for a total of eight hours. In all the hourly measurements, the oxygen saturation values of the patients in the prone position were found to be higher than the values in the Fowler position. Golestani-Eraghi et al (28) evaluated the effectiveness of the positions on oxygen saturation levels by giving supine and prone positions to 10 awake patients in the intensive care unit. The patients were monitored in the prone position for an average of nine hours. Based on the results of their study, the oxygen saturation efficiency of the prone position in the first hour was found to be 60%, and the oxygenation efficiency has been moderate in the measurements of the next hourly oxygen saturation. In the cohort study of Solverson et al (29), positioning was applied to 17 awake patients (12 intensive care, 5 clinical patients). Prone and supine positions were applied for 75 minutes every day for a week. While the mean oxygen saturation measurement in the supine position was 91% (84 – 95), it was 98% (92-100) in the prone position. Our study supports the studies that show that prone positioning is more effective in patients with COVID-19 with hypoxemic, non – intubated high-risk and with a critical illness (22,24,25,30). The respiratory rate of the patients given Fowler and Prone position was found to be statistically significantly lower (p=0.001). The mean respiratory rate of 25% of the patients in the prone position was 20 (per minute) while it was 18 in 50% of the patients and 16 in 25% of them. The respiratory rate is lower in the prone position. As the time of the prone position increases, the respiratory rates of the patients get lower. In the cohort study of Solverson et al (29), positioning was applied to 17 awake patients. Prone and supine positions were applied for 75 minutes every day for a week. The respiratory rate measured during the positioning was 28 minutes in the supine position and 22 minutes in the prone position. In this respect, our results are consistent with the literature. In the study of Golestani-Eraghi et al (28), the rate of patients who could tolerate the prone position and who were discharged was reported as 80%, while the rate of patients given this position and discharged was 85% in our study. Studies have shown that prone positioning can be used in COVID-19 patients, especially in awake clinical patients who are not intubated (20,31,32), and that the use of prone positioning improves oxygenation and lung capacity at high tolerance (20,31,32). The use of the prone position in non – intubated patients is increasing. The prone positioning has been stated to be beneficial in preventing and delaying the need for intubation by improving oxygenation in awake and spontaneously breathing patients (15,26,33).

In our study, the prone position was found to be more effective on oxygen saturation in non – intubated COVID-19 patients. However, further studies investigating the effect of prone position on delaying and preventing the intubation, need for intensive care unit, length of hospital stay and respiration are needed to support our study. Furthermore, the effectiveness of the prone positioning in non-intubated COVID-19 patients has not yet been determined due to the lack of adequate studies (26,27,34) and there is a need for further studies on this subject.

During the positioning, there were difficulties with the patient follow-up due to isolation conditions. There were patients who experienced low back pain. To solve this, the pelvis area was supported with a pillow. There were difficulties in communication and nutritional needs in the prone position. Measurements were performed over a four-hour period and the patient's needs were checked in between the measurements. The positioning time of the patients who had difficulties with the positioning was rescheduled and their measurements were taken from the beginning. This led to an extended time of stay for the researchers inside the isolated rooms which may possibly have increased the transmission risk to the researchers. The study was carried out in a public hospital in one of the cities therefore the results cannot be generalized to all the COVID-19 patients.

5. CONCLUSION

When Fowler and prone positions were given to the clinical patients with COVID-19, the prone position was observed to have a positive effect on oxygen saturation. Considering the results of this study, giving prone position at certain intervals until discharge to the admitted patients with COVID-19 is recommended.

Since the beginning of the pandemic, prone positioning has become important as a supportive treatment method that can reduce the burden on the health sector both by improving short – term outcomes and also by reducing or delaying the need for intubation. There are studies on prone positioning in intensive care patients with COVID-19. However, there is a need for studies that will reveal more clinical evidence with a larger number of patients in order to apply prone positioning routinely to clinical patients outside the intensive care unit.

Acknowledgments: *This article has been submitted as an oral presentation to the 4th International & 12th National Congress of Turkish Surgical and Operating Room Nurses.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics Committee of Bahçeşehir University (Decision date and number:14.01.2021-KAEK 2021/1)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: YİI, FEA, GP
Design of the study:YİI, FEA
Acquisition of data for the study: YİI, GP
Analysis of data for the study: YİI, FEA, GP
Interpretation of data for the study: YİI, FEA, GP
Drafting the manuscript: YİI, GP

Revising it critically for important intellectual content: Yİl, FEA, GP
Final approval of the version to be published: Yİl, FEA, GP

REFERENCES

[1] Özbilen F, Altunkan AA. COVID-19 and awake prone position. Turk J Intensive Care 2020;18:50-56 (Turkish).

[2] Rollas K, Şenoğlu N. Management of Covid-19 patients in intensive care unit. Tepecik Eğit. ve Araşt. Hast. Dergisi 2020;30:142-155 (Turkish).

[3] Akyar I. COVID-19 hastalarında Akut Solunum Sıkıntısı Sendromu (ARDS) yönetimi ve hemşirelik bakımı. Hacettepe Üniversitesi Hemşirelik Fakültesi Dergisi. 2020;7:8-14 (Turkish).

[4] Goh KJ, Choong MCM, Cheong EHT, Kalimuddin S, Wen DS, Phua GC. Rapid progression to acute respiratory distress syndrome: Review of current understanding of critical illness from coronavirus disease 2019 (COVID-19) infection. Ann Acad Med Singap. 2020;49:108-118.

[5] Yalçın A. COVID-19 sürecinde yoğun bakım yönetimi. Med Res Rep. 2020;3:66-85 (Turkish).

[6] Wu ZY, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020;323:1239 – 1242.

[7] Uzelli Yılmaz D, Ceylan B, Yıldırım D, Karatekin Erkut D, Akın Palandöken E. Care priorities and critical nursing interventions in patients with COVID-19: Four different case reports. Izmir Kâtip Çelebi Üniversitesi Sağlık Bilimleri Fakültesi Dergisi 2020;5:235-242. (Turkish).

[8] Türkiye enfeksiyon hastaliklari ve klinik mikrobiyoloji uzmanlik derneği. COVID-19 erişkin hasta yönetimi ve tedavisi. Available from https://www.ekmud.org.tr/haber/328-eriskin-hasta-yonetimi-vetedavisi-COVID-19 – virus-algoritmalari. Sept 21, 2021.

[9] Kansu A. Covid 19 pandemisinde akciğer ve göğüs hastalıkları. Med Res Rep 2020;3:11-16 (Turkish).

[10] Fan E, Del Sorbo L, Goligher EC, Hodgson CL, Munshi L, Walkey AJ. An official American Thoracic Society/European Society of Intensive Care Medicine/Society of Critical Care Medicine clinical practice guideline: mechanical ventilation in adult patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 2017;195:1253-1263.

[11] Gürün Kaya A, Öz M, Erol S, Çiftçi F, Çiledağ A. Kaya A. Prone positioning in non – intubated patients with COVID-19. Tuberk Toraks 2020;68:331-336.

[12] Anchala AM. A study to assess the effect of therapeutic positions on hemodynamic parameters among critically ill patients in the intensive care unit at Sri Ramachandra medical centre. J Nurs Care 2016;5:1000348.

[13] Scholten EL, Beitler JR, Prisk GK, Malhotra A. Treatment of ARDS with prone positioning. Chest 2017;151:215-224.

[14] Gattinoni L, Taccone P, Carlesso E, Marini JJ. Prone position in acute respiratory distress syndrome. Rationale, indications, and limits. Am J Respir Crit Care Med 2013;188:1286-1293.

[15] Jiang LG, LeBaron J, Bodnar D, Caputo ND, Chang BP, Chiricolo G. Conscious proning: An introduction of a proning protocol for nonintubated, awake, hypoxic emergency department COVID-19 patients. Acad Emerg Med 2020;27:566-569.

[16] Kocayigit H, Ülgen AM, Tuna Tas A. Respiratory failure management in the patient with COVID-19 in intensive care. Journal of Human Rhythm 2020;6:101-107.

[17] Çapık C. İstatistiksel güç analizi ve hemşirelik araştırmalarında kullanımı: Temel bilgiler. Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi 2014;17:4 (Turkish).

[18] Cohen J. Statistical power analysis. Curr Dir Psychol Sci 1992;1:98-101.

[19] Des Jarlais DC, Lyles C, Crepaz N, the Trend Group. Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. Am J Public Health 2004;94:361-366.

[20] Qadri SK, Ng P, Toh TSW, Loh SW, Tan HL, Lin CB. Critically ill patients with COVID-19: A narrative review on prone position. Pulm Ther 2020;6:233-246.

[21] Tuncer M, Khorshtd L. The effect of positions on oxygen saturation in obese individuals. Ege Üniversitesi Hemşirelik Fakültesi Dergisi 2018;34:54 – 65 (Turkish).

[22] Hayran M, Hayran M. Sağlık Araştırmaları Için Temel Istatistik. 1. Basım. Ankara: Omega Araştırma; 2011 (Turkish).

[23] Büyüköztürk Ş. Veri Analizi El Kitabı. Ankara: Pegem Akademi; 2011 (Turkish).

[24] Xu Q, Wang T, Qin X, Jie Y, Zha L, L We. Early awake prone position combined with high-flow nasal oxygen therapy in severe COVID-19: A case series. Crit Care 2020;24:250.

[25] Ding L, Wang L, Ma W, He H. Efficacy and safety of early prone positioning combined with HFNC or NIV in moderate to severe ARDS: A multi-center prospective cohort study. Crit Care 2020;24:28.

[26] Elharrar X, Trigui Y, Dols AM, Touchon F, Martinez S, Prud'homme E. Use of prone positioning in nonintubated patients with COVID-19 and hypoxemic acute respiratory failure. JAMA 2020;323:2336-2338.

[27] Coppo A, Bellani G, Winterton D, Di Pierro M, Soria A, Faverio P. Feasibility and physiological effects of prone positioning in non-intubated patients with acute respiratory failure due to COVID-19 (PRON-COVID): A prospective cohort study. Lancet Respir Med 2020;8:765-774.

[28] Golestani-Eraghi M, Mahmoodpoor A. Early application of prone position for management of Covid-19 patients. J Clin Anesth 2020;66:109917.

[29] Solverson K, Weatherald J, Parhar KKS. Tolerability and safety of awake prone positioning COVID-19 patients with severe hypoxemic respiratory failure. J Can Anesth 2021;68:64-70.

[30] Nakos G, Tsangaris I, Kostanti E, Nathanail C, Lachana A, Koulouras V. Effect of the prone position on patients with hydrostatic pulmonary edema compared with patients with acute respiratory distress syndrome and pulmonary fibrosis. Am J Respir Crit Care Med 2000;161:360-368.

[31] Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E. Surviving sepsis campaign: Guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). Crit Care Med 2020;48:e440-e469.

[32] World Health Organization. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: Interim guidance. Available from https://www.who.int/docs/default-source/ coronaviruse/clinical-management-of-novel – cov. pdf. Apr 6, 2020.

[33] Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan,

China: A single – centered, retrospective, observational study. Lancet Respir Med. 2020; 8:475-481.

[34] Thompson AE, Ranard BL, Wei Y, Jelic S. Prone positioning in awake, nonintubated patients with COVID-19 hypoxemic respiratory failure. JAMA Intern Med 2020;180:1537-1539.

How to cite this article: İşler Işıldak Y, Eti Aslan F, Parlak G. Determination of the Effect of the Fowler and Prone Position on Oxygen Saturation in Patients Diagnosed with COVID-19. Clin Exp Health Sci 2023; 13: 159-165. DOI: 10.33808/clinexphealthsci.1186086

Clinical and Experimental Health Sciences

Influence of Finishing Procedures on Surface Roughness and Biaxial Flexural Strength of High-translucent 4Y-PSZ, 5Y-PSZ, and 6Y-PSZ Monolithic Zirconia

Gülsüm Doğru[1], Elif Demiralp[1], Emre Koçak[2], H. Hasan Orkçu[2], Cemal Aydın[1], Handan Yılmaz[1]

[1] Gazi University, Faculty of Dentistry, Department of Prosthodontics, Ankara, Türkiye.
[2] Gazi University, Faculty of Science, Department of Statistics, Ankara, Türkiye.

Correspondence Author: Handan Yılmaz
E-mail: hozkula@gmail.com
Received: 15.02.2022 **Accepted:** 30.11.2022

ABSTRACT

Objective: This study aims to investigate the effects of glazing, polishing and polishing with a polishing paste of newly developed highly translucent materials on the flexural strength of these materials and aims to illuminate where there is not much study yet on the finishing procedures to be done.

Methods: Three different high translucency Y-PSZ (Yittria partially stabilized zirconia): KST (Katana STML), KUT (Katana UTML), NCQ (Nacera Pearl Q3 Multi-Shade) and one translucent 3Y-TZP (3-yttria stabilized tetragonal zirconia polycrystalline): NCMS (Nacera Pearl Multi-Shade) zirconia system were used. A total of 120 specimens were prepared in the form of discs with a diameter of 14 mm and dimensions of 1.2 ± 0.2 mm. Three experimental groups (n = 30) were formed from each type of material, using three finishing protocols: Diamond Polishing system (DP); Diamond Polishing system followed by Polishing Paste (PP); Glaze Application (GP). Surface Roughness (Ra) was measured by using a contact profilometer, and a biaxial flexural strength test was applied to determine their flexural strength. The obtained data were analyzed using the Weibull distribution. All results were evaluated statistically.

Results: For Ra values, there was a statistical difference between all the procedures applied in the KST material as in the NCQ material. However, there was no statistical difference between GP and PP procedures in Ra values in the NCMS material and between DP and PP procedures in the KUT material. The characteristic strengths of DP applied to NCMS and NCQ material, PP applied to KST, and KUT material had the highest value. The highest m values for DP were determined in KST, NCMS, NCQ materials, while in KUT material, PP was determined in the finishing procedure.

Conclusion: Finishing procedures have significant effects on surface roughness and flexural strength values for translucent zirconia materials. The lowest Ra value and the highest flexural strength were found in the DP group of NCMS. In KST and KUT materials, the highest flexural strength results were found in the PP procedure while NCQ was not affected by finishing procedures.

Keywords: Translucent zirconia, monolithic, surface roughness, biaxial flexural strength

1. INTRODUCTION

Yttria stabilized tetragonal zirconia polycrystalline (Y-TZP) have become superior in use in dental restorations due to their excellent mechanical properties (1,2). Zirconia is a polymorphic material that can be found in three different phases: monoclinic (m), tetragonal (t), and cubic (c) (2,3). External stresses such as grinding, sandblasting, polishing, and low-temperature degradation can stimulate the t-m phase transformation of Y-TZP. This conversion causes 3 – 4% volume expansion which seals the crack tip and prevents further crack propagation. However, this protective effect is not fixed and predictable (4). This transformation can also be triggered by occlusal adjustments such as airborne particles, polishing, and grinding. In addition, Y-TZP can be degraded by the t→m transformation, which can occur spontaneously in humid environments. This phenomenon is called low-temperature degradation and begins at minor surface defects (1). Also, the biggest disadvantage of Y-TZP is its high opacity (5). Translucent monolithic zirconias have been developed to solve these problems (6).

New methods were used to improve Y-TZP; increasing the amount of yttria by adding cubic phase zirconium; decreasing the amount of Al_2O_3 from 0.25% to 0.1% by weight; adding 0.2 mol% La2O3 to Y-TZP is to change the sintering time and temperature, and reduce the grain size, which can increase the translucency of zirconia (7-9). However, increased yttria content inhibited the transformation hardening mechanism and affected flexural strength (9-12).

The microstructure difference between conventional zirconia and partially stabilized zirconia (PSZ) was due to the amount

DOI: 10.33808/clinexphealthsci.1073010

of cubic phase in their content. 3Y-TZP consisted of ~20% by weight cubic zirconia, while PSZ stabilized with 4-6% mol yttria contained 40−70% by weight cubic zirconia (8). Cubic zirconia was non-birefringent (6) and did not undergo phase transformation under stresses and water conditions (13).

Occlusal adjustment may be required to obtain an occlusal relationship. Due to the hardness of zirconium, adjustments were usually made with diamond burs, which affected the uppermost glaze layer and the original smoothness of the surface. When monolithic zirconia was exposed to the oral environment in this way, saliva and other factors could disrupt the structure of the zirconia restoration (1). Occlusal adjustment caused more stress on the surface indirectly increasing the deterioration of the material and reducing its aesthetics and longevity (14). Therefore, if zirconia is to be used monolithic, it is important that it is well polished (15).

Various techniques have been advanced to restore the smoothness and gloss of monolithic zirconia surfaces, such as grinding, polishing, polishing with diamond rubber polishers, and the use of fine diamond burs. Considering the benefits of a polished surface, some polishing procedures can lead to the improvement of the cracks that can weaken the structure (14). Khayat et al. reported that the polishing procedure by reducing the irregularities on the surface contributed to increasing the flexural strength of the restorations (7).

Various manufacturers offer dentists polishing sets used in the rough (grinding) to fine (high gloss finish) stages, which are made especially for zirconia, usually consisting of a set of two or three stages. These sets are also suggested with the use of different polishing pastes, and it is possible to obtain a smooth surface depending on the successive application of all polishing steps (16). However, information on the effects of different surface treatments on newly developed high translucent zirconia ceramics is limited in the literature.

The null hypothesis of this study is that different finishing procedures applied will not affect the surface roughness and flexural strength of the tested materials.

This study aims to investigate the effects of glazing, polishing, polishing with a paste of different highly translucent materials on the flexural strength of these materials, and illuminate the places where there has not been much work on the finishing procedures to be made on these newly developed materials.

2. METHODS

2.1. Specimen Preparation

Four different yttria content monolithic zirconia material; 3Y-TZP (Nacera Pearl Multi Shade, Doceram Medical Ceramics Gmbh, Dortmund, Germany) (NCMS), 4Y-PSZ (Katana STML, Kuraray Noritake Dental Inc., Tokyo, Japan) (KST), 5Y-PSZ (Katana UTML, Kuraray Noritake Dental Inc., Tokyo, Japan) (KUT), and 6Y-PSZ (Nacera Pearl Q^3, Doceram Medical Ceramics Gmbh, Dortmund, Germany) (NCQ) are used in this study and shown in Table 1. A total of 120-disc shaped specimens (n=30/ for each material) were prepared according to ISO 6872-2015 guidelines with absolute dimensions of 1.2 ± 0.2 mm in thickness and 14 mm in diameter.

Table 1. *Composition of the materials tested in this study.*

Monolithic Zirconia Materials	Content	Manufacturer	Sintering cycle	Indications	Lot No
Katana STML (Ultra Translucent Multi-Layered) Zirconia	ZrO_2+HfO_2 %88-93 (Y_2O_3) %7-10 Other oxides %0-2	Kuraray Noritake Dental Inc., Tokyo, Japan	2 h at 1550 Cº	Frameworks, monolithic crowns, monolithic bridges, inlays, onlays and veneers	EAUWN
Katana UTML (Ultra Translucent Multi-Layered) Zirconia	ZrO_2 + HfO 2 %87-92 (Y_2O_3) %8-11 Other oxides %0-2	Kuraray Noritake Dental Inc., Tokyo, Japan	2 h at 1550 Cº	Frameworks, monolithic crowns, monolithic bridges, inlays, onlays and veneers	DOZBT
Nacera Pearl Multi-Shade	ZrO_2+HfO_2+ Y_2O_3 > %99, Y_2O_3 %4,5 – %6	Doceram Medical Ceramics Gmbh, Dortmund, Germany	2 h at 1450 Cº	Single crowns, bridges consisting of up to 16 units. In the posterior region, the span between the abutments must not exceed two units	5146158
Nacera Pearl Q^3 Multi-Shade	Yitriya-stabilize %40 Tetragonal, %60 cubic zirkonya polikristal (%6 mol Y_2O_3)	Doceram Medical Ceramics Gmbh, Dortmund, Germany	2 h at 1450 Cº	-Single crowns -Bridges in the front and side tooth areas consisting of up to 3 units.	5057862

The specimens were designed using Solidworks CAD software (Solidworks, Dassault Systems SolidWorksCorp., Waltham MA). The design was transferred with 3Shape CAM Software (3Shape, Copenhagen, Denmark) and the position of the samples in the zirconia blocks was arranged. . Then presinterized zirconia discs were milled using CAM system (imes-icore GmbH Im Leibolzgraben 16 D-38132 Eiterfeld/ Germany). Before the sintering procedure, all specimens were ground using 800 grit and 1200 grit silicon carbide paper to remove any irregularities and obtain standardized and smooth surfaces. Specimens were sintered in the furnace (Tabeo – 1/S/Zirkon-100, Germany) according to the instructions of the whole material manufacturer (Table 1). All specimens were ground with 1200 grit silicon carbide paper. The specimens' diameters and thicknesses were measured with a digital caliper (Alpha Tools Digital Caliper, Alpha Professional Tools, Oakland, USA) and were checked for standardization.

According to the ISO 6872-2015 standard, the specimens prepared with the final dimensions of

1.2 ± 0.2 mm in thickness and 14 mm in diameter were divided into 3 subgroups (n = 10) of each material according to the finishing procedures to be applied.

2.2. Finishing Procedures

The materials, manufacturers, and lot number used in the finishing procedure are shown in Table 2.

Table 2. *The materials, manufacturers, and lot number used in the finishing procedure*

Finishing Procedure	Materials	Manufacturers	Lot No
Diamond Polishing System	Diacera Medium, Diacera Fine G&Z Instrumente	G&Z Instrumente GmbH, Lustenau, Austria	434914
Glaze	IPS e.max Ceram Glaze powder and IPS e.max Ceram Glaze and stain liquid	Ivoclar Vivadent AG	Z00B5Z
Polishing Paste	Nacera Shine Zr	Doceram Medical Ceramics Gmbh, Dortmund, Germany	7777YO118

Finishing procedure were performed by the same operator according to the manufacturers recommendations. Diamond polishing system (DP): Samples were sampled using a micromotor handpiece (NSK Micromotor ULTIMATE XL-DT, Japan) using a medium followed by fine diamond bur (Fig. 1) (Diacera Medium, Diacera Fine G&Z Instrumente GmbH, Lustenau, Austria) polished. Each diamond bur was applied at 10,000 rpm for 60 seconds. The diamond polishing system followed by polishing paste (PP): The specimens were polished with the same diamond polishing system and techniques as a diamond polishing system. The polishing paste (Nacera Shine Zr, Doceram Medical Ceramics Gmbh, Dortmund, Germany) was applied with a goat hair brush for 60 seconds at 10,000 rpm. Glaze application (GP): The zirconia discs were glazed with glaze powder and liquid (IPS e.max Ceram Glaze powder and IPS e.max Ceram Glaze and stain liquid, Ivoclar, Vivadent) following to the manufacturer's instructions. A thin glaze layer was applied to one surface with a brush and fired in the ceramic furnace at 925 Cº (Programat P300, Ivoclar Vivadent, Schaan, Liechtenstein).

After all finishing procedures, all specimens were cleaned in an ultrasonic bath (Euronda; Eurosonic Energy, Italy) containing distilled water for 10 minutes and allowed to dry at room temperature before the analysis.

2.3. Surface Roughness (Ra) Analysis

Ra (roughness average) and Rz (average maximum height of profile) values of surface roughness of all specimens before the finishing procedure and after the finishing procedure were measured using a contact profilometer device (SJ-301, Mitutoyo Corporation, Takatsu-Ku, Kawasaki, Kanagawa, Japan) (λ=5x0.25 mm). Before the measurement of each group, the device was calibrated. Measurements were made from the surface of each specimen 3 times in 3 different directions.

2.4. Biaxial Flexural Strength Test

A biaxial flexural strength test was applied to determine the flexural strength of the specimens whose surface roughness were determined. Biaxial flexural strength was conducted following by ISO 6872-2015 in a universal testing machine (Lloyd Instruments, Ametek Inc, Florida, ABD) with a piston on a three-ball system. The finishing surface of disc-shaped specimens was placed on the support circle to balance the tension load during the test. Specimens were loaded at a crosshead speed of 1 mm/min until failure in the universal test machine. The load at the point of the fracture for each material was recorded. The biaxial flexural strength values of the specimens were calculated with the following equation:

$$\sigma = -0.2387\ P(X-Y)/b^2$$

$$X = (1 + v)\ \ln(r2/r3)^2 + ((1-v)/2)\ (r2/r3)^2$$

$$Y = (1 + v)\ (1 + \ln(r1/r3)^2) + (1-v)\ (r1/r3)^2$$

Where; σ represents the maximum tensile stress (MPa), P is the total load at fracture (N), and b is the thickness at the fracture origin (mm), respectively. In which; v= Poisson's ratio (0.3 for zirconia), r1 (5 mm) is the radius of the support circle, r2 (0.7 mm) is the radius of the loaded area, and r3 (7 mm) is the radius of the specimen.

2.5. Statistical Analysis

Initially, it was examined whether the data discussed in the study came from a normal distribution before starting the analysis. Kruskal Wallis was used at the 5% significance level to measure the differences between the experimental groups according to the normality test result because both the surface roughness data and the flexural strength data assumed a non-parametric distribution.

The reliability of the materials used in the study was obtained by Weibull analysis of flexural strength data according to the following equation:

$$P = 1 - e^{-\left(\frac{\sigma}{\sigma_0}\right)^m}$$

where *P* is the failure probability; *m*, Weibull modulus; σ is the material fracture tension and σo is the characteristic strength. Statistical analysis was performed using MATLAB (R2018b) software, and the significance level was 5%.

3. RESULTS

The Average Surface Roughness (Ra) values of materials are listed in Table 3. In Table 3, the results show that there is a significant difference between the finishing procedures applied with each material in terms of Ra values. Different capital letters indicate statistical differences within the same material group in columns. For Ra values, there is a statistical difference between all the procedures applied in the KST material and in the NCQ material. However, there is no statistical difference between GP and PP procedures in Ra values in the NCMS material (*p= .1583*). There is also no statistical difference in the polishing and paste procedures applied to the KUT material in terms of Ra (*p= .2206*) values.

According to the Ra values given in Table 3, different small letters indicate statistical differences per surface treatment across different materials in rows. Ra values of the Glaze procedure applied to Group KST, KUT, and NCQ showed the lowest mean values except for Group NCMS. The lowest value of NCMS is the polishing procedure applied to the NCMS Group.

Table 3. *Mean (SD) R_a values for all experimental groups.*

	KST	KUT	NCMS	NCQ
GP	0.05 (0.02) ^{A,a*}	0.07 (0.02) ^{A,b*}	0.16 (0.06) ^{A,c*}	0.07 (0.03) ^{A,b*}
DP	0.14 (0.06) ^{B,a*}	0.09 (0.01) ^{B,b*}	0.09 (0.02) ^{B,b*}	0.09 (0.02) ^{B,c*}
PP	*0.11 (0.04)* ^{C,a*}	*0.12 (0.07)* ^{B,a*}	*0.15 (0.06)* ^{A,b*}	*0.15 (0.08)* ^{C,b*}

GP: Glaze application, DP: Diamond Polishing system , PP: The diamond polishing system followed by polishing paste, KST: Katana STML, KUT: Katana UTML NCMS: Nacera Pearl Multi Shade, NCQ: Nacera Pearl Q³ SD: Standard Deviation

-Similar letters indicate lack of statistically difference (p> .05)

-Capital letters indicate statistical differences within the same material group.

-Small letters indicate statistical differences per surface treatment across different materials.

**Significant difference between pre-procedure and post-procedure roughness values (p< .05).*

Table 4. *Mean (SD) flexural strength values for all experimental groups.*

	KST	KUT	NCMS	NCQ
GP	732.70 (158.76) ^{A,a}	715.08 (175.46) ^{A,a}	753.56 (123.50) ^{A,a}	644.71 (119.13) ^{A,a}
DP	728.94 (48.73) ^{A,a}	554.14 (146.72) ^{B,b}	1344.22 (154.16) ^{B,c}	665.86 (92.13) ^{A,a}
PP	1474.72 (246.12) ^{B,a}	1402.23 (180.71) ^{C,a,b}	1028.76 (421.09) ^{A,B,b}	542.93 (138.15) ^{A,c}

GP: Glaze application, DP: Diamond Polishing system , PP: The diamond polishing system followed by polishing paste, KST: Katana STML, KUT: Katana UTML NCMS: Nacera Pearl Multi Shade, NCQ: Nacera Pearl Q³ SD: Standard Deviation

Similar letters indicate lack of statistically difference (p>0.05)

Capital letters indicate statistical differences within the same material group in columns.

Small letters indicate statistical differences per surface treatment across different materials in rows.

The descriptive analysis of the flexural strength data is made to obtain mean and standard deviation (SD) listed in Table 4. It was examined whether there was a significant difference between procedure group flexural strength values within the same materials in columns. According to the results, it was observed that there was no significant difference between the finishing procedures applied to NCQ material (*p= .0612*).

The flexural strength measurements in terms of finishing procedures applied to materials determined by the Weibull analysis are summarized in Table 5 and Figure 2. The highest m values for DP and KST was determined in NCMS and NCQ materials, while in KUT material, PP was determined in the finishing procedure. The characteristic strengths of PP applied to KST material had the highest value, whereas the paste applied to NCQ material had the lowest value. In addition, the lower and upper limit values corresponding to these characteristic strengths at 95% reliability are given in Table 4. Breaking probabilities for applied power values in finishing procedures applied to materials are given in Figure 3.

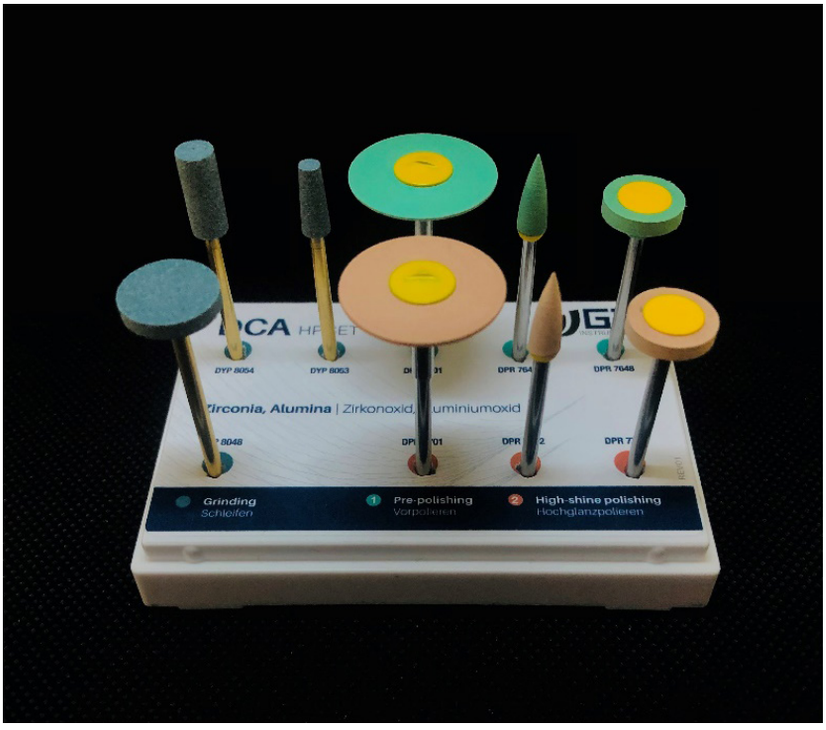


Figure 1: *Diamond Polishing system*

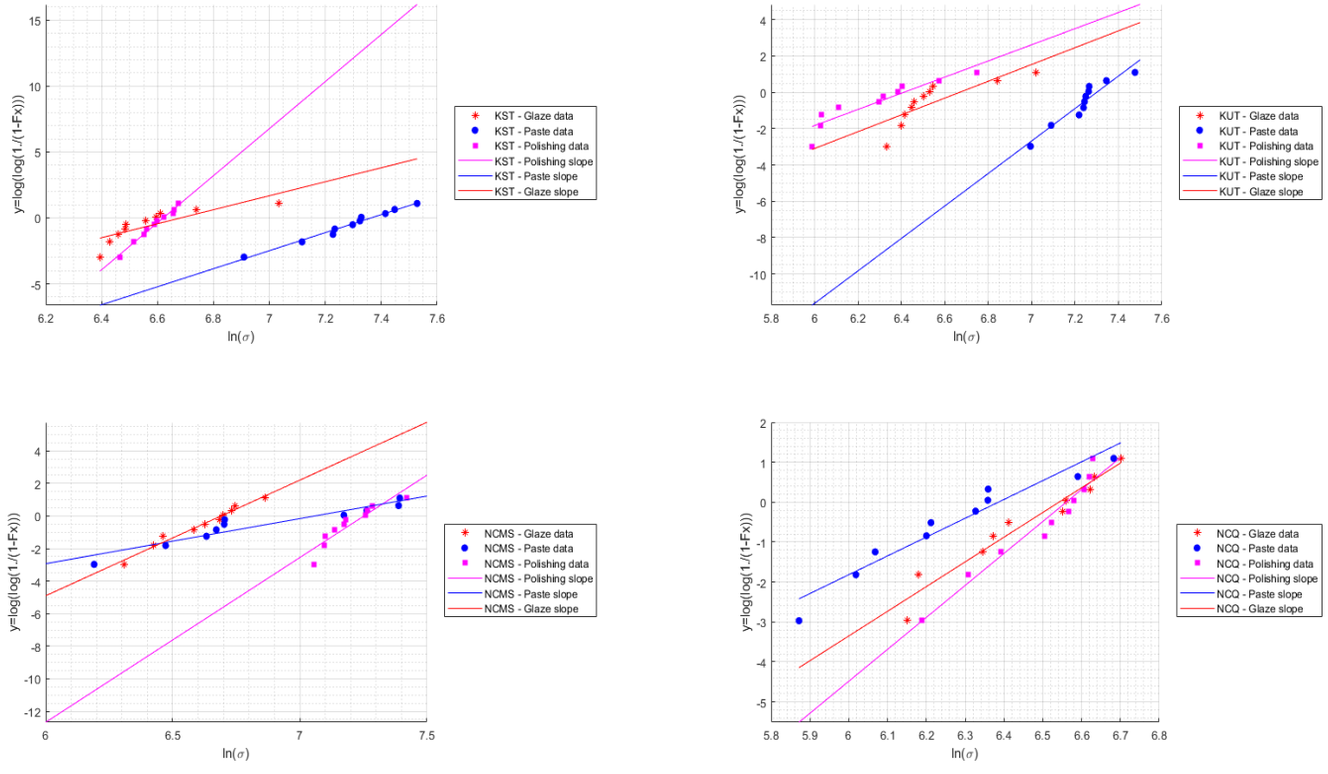


Figure 2. *Weibull analysis*

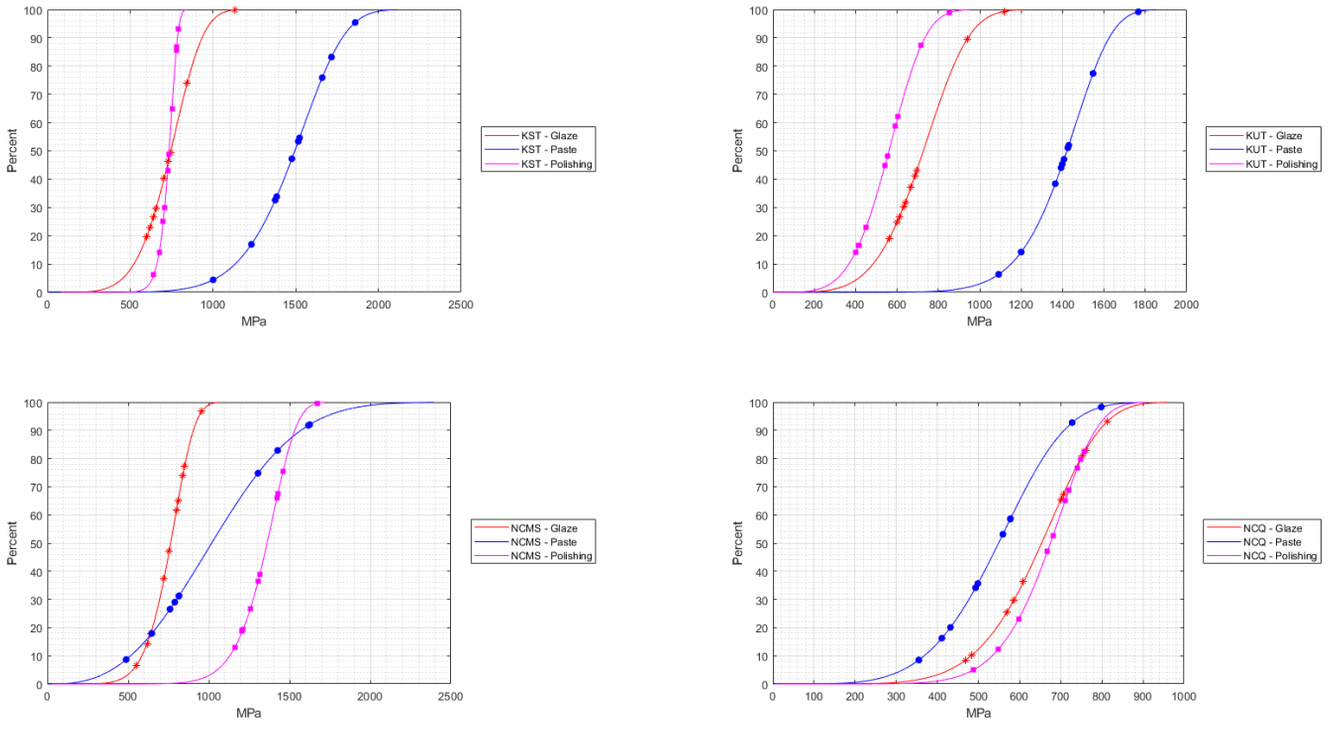


Figure 3. *Breaking probabilities*

Table 5. *The mean strenght (MPa), Standard deviation, Characteristic strength, Weibull moduli and Confidence interval (CI) (95%) results in terms of finishing procedures applied to materials.*

Material	Procedure	Mean strenght (MPa) (S.D)	Characteristic strength			Weibull modulus			R^2
			MPa	95% CI		m	95% CI		
				Lower limit	Upper limit		Lower limit	Upper limit	
KST	GP	732.6981 (158.7640)	798.5423	399.4504	1021.2093	5.3071	2.3286	8.2855	0.6785
	DP	728.9360 (48.7267)	750.2224	610.5613	807.1507	17.8464	15.8334	19.8594	0,9812
	PP	1474.7190 (246.1157)	1576.8351	921.1885	1908.4135	6.8393	6.1017	7.5769	0.9828
KUT	GP	715.0781 (175.4614)	787.2420	355.6381	1043.8597	4.6263	1.9669	7.2858	0.6679
	DP	554.1400 (146.7236)	608.8148	266.4571	816.4020	4.4490	2.9242	5.9738	0.8498
	PP	1402,2290 (180.7140)	1480.0624	981.1647	1712.6679	8.9424	6.5356	11.3491	0.9017
NCMS	GP	753.5593 (123.5000)	804.0369	479.1653	966.2552	7.1024	6.1655	8.0393	0.9745
	DP	1344.2160 (154.1631)	1411.3278	981.4208	1605.6388	10.1193	6.5865	13.6521	0.8451
	PP	1028.7646 (421.0887)	1162.0274	307.9341	1862.1072	2.7681	1.9837	3.5526	0.8922
NCQ	GP	644.7087 (119.1350)	693.3943	382.7744	856.2508	6.1873	4.9706	7.4040	0.9450
	DP	665.8634 (92.1274)	706.5187	446.3447	831.6547	8.0046	6.5128	9.4965	0.9503
	PP	542.9318 (138.1498)	593.1163	271.8564	782.4145	4.7124	3.6814	5.7434	0.9328

GP: Glaze application, DP: Diamond Polishing system , PP: The diamond polishing system followed by polishing paste, KST: Katana STML, KUT: Katana UTML NCMS: Nacera Pearl Multi Shade, NCQ: Nacera Pearl Q3

4. DISCUSSION

In this study, the effects of 3 different finishing treatments applied to 3 high translucent and one translucent 3Y-TZP zirconia materials with different yttria content on the surface roughness and flexural strength were investigated. It was concluded that 3 different finishing procedures applied in the study had different effects on the surface roughness of the materials. While glaze showed the lowest surface roughness value in highly translucent materials (KST, KUT, NCQ), 3 different surface roughness values were determined for all materials in glaze, polishing, and polishing paste procedures. The different microstructures of the materials in the study led to different results in terms of Ra values between the finishing procedures. For this reason, the hypothesis that the different finishing treatments applied would not affect the surface roughness of the tested materials was rejected.

The highly translucent materials used in the study were used as an alternative material to 3Y-TZP for monolithic restorations due to their increased translucency and adequate mechanical properties (17). Y_2O_3 constituted 8-11% of the content of ultratranslucent Katana UTML, while supertranslucent Katana STML contained 7-10% Y_2O_3 (18). Another material Nacera Pearl Q^3 MS contains 6 mol% Y_2O_3 (19). Previous studies showed that the higher the stabilizer content in the materials, the higher the conversion hardening. They reported that it was responsible for the elimination of the mechanism and also the emergence of a large amount of cubic crystal in its microstructure (6,20,21). Hatanaka et al. stated that this difference in microstructure affected the polishability of the materials (1). On the other hand, Khayat et al. reported that the surface roughness of restorations could be reduced with appropriate polishing methods in high translucent zirconia (7).

When the results of this study were examined, a significant difference was found between the surface treatments in KST and NCQ material in terms of mean Ra values, while no significant difference was found between the DP and PP groups in KUT and between the GP and PP groups in NCMS, and a significant difference was found between all other groups. In this study, while the polishing procedure was applied to the samples, first a medium-grained polishing bur was used. The Ra values of the DP group in this study were the Ra obtained by Happe et al. (22) and Alkimavičius et al. (23). While the Ra values of the PP group were lower than those of the polishing-pat group in the study of Happe et al. (22), Alkimavičius et al. (23) determined that it was higher. The reason for these differences is thought to be related to the application method of the polishing protocols and the different microstructures of the materials.

Since monolithic zirconia was directly exposed to the oral environment, finishing and polishing were performed after occlusal adjustments to prevent the wear of the antagonist enamel (4). Various manufacturers offer polishing sets made specifically for zirconia restorations, usually consisting of a two or three-stage set, used in coarse (grinding) and fine (high-gloss) stages (16). Khayat et al. (7) determined the Ra value of high translucent Y-TZP samples to be 1.00 µm in the group to which they finished with a 2-stage Brasseler polishing set, and 0.81 µm in the group in which they finished with a 2-stage Komet polishing set. In this study, the Ra values of the samples polished with the 2-stage Diacera polishing set were found to be lower. Kurt et al. (24) in their study with monolithic zirconiareported that the Ra values of the samples in the group in which they applied polishing paste with a brush after the polishing kit were lower than the samples in which the paste was applied in this study. Park et al. (25), on the other hand, applied 2-stage polish to Y-TZP zirconia samples and found the Ra values higher than the values in the polishing group of this study.

In this study, it was determined that the GP procedure had significantly lower Ra values in general (except for Nacera MS material). These results are similar to the results of the study by Al Hamad et al. (26) and Manzuic et al. (27). However, it wasreported in other studies that the glaze layer wore out quickly and caused the rough milled surface to be exposed (21,26). Therefore, polishing was recommended in areas with high chewing pressure (28).

Studies reported that a smoother surface was required to prevent biofilm formation and reduce aging (1,7,29). Bollen et al. (30) suggested a threshold surface roughness value (Ra=0.2 µm) for bacterial retention on dental materials as a result of their in vivo studies. In this study, it was determined that all finishing procedurees had roughness below this value and it was determined that it could provide information about suitable surface finishing procedures for translucent zirconias.

The fact that the surface of the restoration is polished im addition to reducing roughness, it was reported that it could have 2 different effects on flexural strength; polishing could reduce surface defects and increase flexural strength, or it could reduce flexural strength by removing the pressure layer (1,31).

When the flexural strength results of different finishing procedurees for the same material were examined, no significant difference was found between the 3 surface finishing procedures in only NCQ material, while a significant difference was determined between the finishing procedures in KUT material. There was no significant difference between GP and DP groups in KST material, and between GP and PP groups and DP and PP groups in NCMS material. For this reason, the second hypothesis that different finishing procedures would not affect the flexural strength of the tested materials was also rejected.

De Souza et al. (32) in their study with translucent zirconia, Vila-Nova et al. (14) and Carvalho et al. (33) in their study with ultratranslucent zirconia, they stated that the flexural strength of the zirconia increased after the 3-stage polishing procedure compared to the control group.

Furthermore Mohammadi-Bassir et al. (31) reported that the flexural strength of the groups to which they applied the 2-stage polishing procedure was higher than the groups in

which the standard single-stage polishing procedure was applied.

Pfefferle et al. (16) reported that the application of polishing paste significantly increased the flexural strength values of the material. Similarly, in this study, the flexural strength values obtained in the polishing paste finishing group in KST and KUT materials were found to be significantly higher than the flexural strength values obtained in the polishing procedure. While Lee et al. (34) reported that the flexural strength decerased as the surface roughness increased, Khayat et al. (7) in their study in which they applied a 2-stage polishing finishing procedure stated that there was no correlation between the roughness and flexural strength of zirconia following the previous studies.

According to the results of Weibull analysis; In KST and KUT material, the highest characteristic strength results were found in the PP group (KST=1474.7 MPa, KUT=1402.2 MPa), while the highest values for NCMS and NCQ were found in the DP group (NCMS=1344.2 MPa, NCQ=665.8 MPa) Pittayachawan et al. (35) noted that the lower the value of the Weibull modulus, the more defects and microcracks in the material, thus reducing reliability. Conversely, they reported that higher values of the Weibull modulus indicated a smaller error range and therefore greater structural reliability. While they reported that most ceramics had weibull modulus values in the range of 5-15, they reported in their study that the Weibull modulus values of the samples were in the range of 9.3-12.9, which is acceptable for dental ceramics. In this study, Weibull modulus values were determined to be in the range of 2.7 to 17.8. Weibull modulus was found to be higher in DP groups of materials except for KUT. In line with these results, it is thought that the polishing procedure applied may have increased the reliability.

The flexural strength of the high translucent zirconia tested in the study was found to be above 500 MPa, which is the minimum value accepted for class 5 restorations in fixed prostheses according to ISO 6872, while Nacera MS had higher flexural strength values than high translucent zirconias. The result was found to be compatible with class 6 materials recommended in ISO 6872.

Differences in grain size and microstructure of zirconia materials with different yttria content affect the surface structure and polishability. In this study, it was determined that different surface finishing procedures had different effects on the surface roughness and flexural strength of translucent zirconias. In the study, 3 high translucent zirconia materials with different yttria content were compared with translucent 3Y-TZP zirconia in terms of surface roughness values and flexural strength. In subsequent studies, the roughness and flexural strength of materials could be compared by using different high-translucent zirconias and different surface treatments.

5. CONCLUSION

Considering the limitations of the study, the following conclusions can be drawn;

1. In terms of finishing procedures, the Ra values were determined in the GP group in KST, KUT, and NCQ materials. In the NCMS material, the highest Ra value was determined in the GP group.

2. The Ra values obtained for all materials were found below the reference threshold value of

0.2 µm. While a significant difference was found between the groups in terms of Ra values in the KST and NCQ material, there was no significant difference between the GP and PP groups in the NCMS material and between the DP and PP groups in the KUT.

3. In terms of flexural strength results, there was no significant difference between all groups in the NCQ material with different finishing procedures, andthere was no significant difference between the GP and PP groups, DP and PP groups in the NCMS material and between the GP and DP groups in the KST material. A significant difference was found between all groups in the KUT material.

4. According to Weibull modulus results, the highest Weibull modulus values were determined in the paste group in KUT, while it was determined in the polishing group in all other materials. The characteristic strengths of the high translucent zirconias tested in the study were determined to be above 500 MPa, which is the minimum accepted value for class 5 restorations in fixed prostheses according to ISO 6872.

Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: GD, ED, HY
Design of the study: GD, ED, CA, HY
Acquisition of data for the study: GD, ED, EK, HY
Analysis of data for the study: EK, HHÖ
Interpretation of data for the study: GD, ED, EK, HHÖ, HY
Drafting the manuscript: GD, ED, EK, HHÖ, CA, HY
Revising it critically for important intellectual content: GD, ED, EK, HHÖ, CA, HY
Final approval of the version to be published: ED, CA, HY

REFERENCES

[1] Hatanaka GR, Polli GS, Adabo GL. The mechanical behavior of high-translucent monolithic zirconia after adjustment and finishing procedures and artificial aging. J Prosthet Dent. 2020;123(2):330-337. DOI: 10.1016/j.prosdent.2018.12.013

[2] Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. Biomaterials. 1999;20(1):1-25. DOI: 10.1016/s0142-9612(98)00010-6

[3] Karakoca S, Yılmaz H. Influence of surface treatments on surface roughness, phase transformation, and biaxial flexural strength of Y-TZP ceramics. Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal

of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2009;91(2):930-937. DOI: 0.1002/jbm.b.31477

[4] Zucuni CP, Guilardi LF, Rippe MP, Pereira GKR, Valandro LF. Fatigue strength of yttria-stabilized zirconia polycrystals: Effects of grinding, polishing, glazing, and heat treatment. J Mech Behav Biomed Mater. 2017;75:512-520. DOI: 10.1016/j.jmbbm.2017.06.016

[5] Zucuni CP, Guilardi LF, Rippe MP, Pereira GKR, Valandro LF. Fatigue strength of yttria-stabilized zirconia polycrystals: Effects of grinding, polishing, glazing, and heat treatment. J Mech Behav Biomed Mater. 2017;75:512-520. DOI: 10.1016/j.jmbbm.2017.06.016

[6] Zhang Y. Making yttria-stabilized tetragonal zirconia translucent. Dent Mater. 2014;30(10):1195-1203. DOI: 10.1016/j.dental.2014.08.375

[7] Khayat W, Chebib N, Finkelman M, Khayat S, Ali A. Effect of grinding and polishing on roughness and strength of zirconia. J Prosthet Dent. 2018;119(4):626-631. DOI: 10.1016/j.prosdent.2017.04.003

[8] Zhang F, Van Meerbeek B, Vleugels J. Importance of tetragonal phase in high-translucent partially stabilized zirconia for dental restorations. Dent Mater. 2020;36(4):491-500. DOI: 10.1016/j.dental.2020.01.017

[9] Zhang F, Inokoshi M, Batuk M, Hadermann J, Naert I, Van Meerbeek B, Vleugels J. Strength, toughness and aging stability of highly-translucent Y – TZP ceramics for dental restorations. Dent Mater. 2016;32(12):327-337. DOI: 10.1016/j.dental.2016.09.025

[10] Zhang F, Reveron H, Spies BC, Van Meerbeek B, Chevalier J. Trade-off between fracture resistance and translucency of zirconia and lithium – disilicate glass ceramics for monolithic restorations. Acta biomaterialia. 2019;91:24-34. DOI: 10.1016/j.actbio.2019.04.043

[11] Pereira GK, Guilardi LF, Dapieve KS, Kleverlaan CJ, Rippe MP, Valandro LF. Mechanical reliability, fatigue strength and survival analysis of new polycrystalline translucent zirconia ceramics for monolithic restorations. J Mech Behav Biomed Mater. 2018;85:57-65. DOI: 10.1016/j.jmbbm.2018.05.029

[12] Zucuni CP, Venturini AB, Prochnow C, Pereira GKR, Valandro LF. Load-bearing capacity under fatigue and survival rates of adhesively cemented yttrium-stabilized zirconia polycrystal monolithic simplified restorations. J Mech Behav Biomed Mater. 2019;90:673-680. DOI: 10.1016/j.jmbbm.2018.11.009

[13] Zhang F, Inokoshi M, Batuk M, Hadermann J, Naert I, Van Meerbeek B, Vleugels J. Strength, toughness and aging stability of highly-translucent Y – TZP ceramics for dental restorations. Dent Mater. 2016;32(12):327-337. DOI: 0.1016/j.dental.2016.09.025

[14] Vila-Nova TEL, de Carvalho IHG, Moura DMD, Batista AUD, Zhang Y, Paskocimas CA, Bottino MA, e Souza ROdA. Effect of finishing/polishing techniques and low temperature degradation on the surface topography, phase transformation and flexural strength of ultra-translucent ZrO2 ceramic. Dent Mater. 2020;36(4):126-130. DOI: 10.1016/j.dental.2020.01.004

[15] Preis V, Schmalzbauer M, Bougeard D, Schneider-Feyrer S, Rosentritt M. Surface properties of monolithic zirconia after dental adjustment treatments and in vitro wear simulation. J Dent. 2015;43(1):133-139. DOI: 10.1016/j.dental.2020.01.004

[16] Pfefferle R, Lümkemann N, Wiedenmann F, Stawarczyk B. Different polishing methods for zirconia: impact on surface, optical, and mechanical properties. Clin Oral Investig. 2020;24(1):395-403. DOI: 10.1007/s00784.019.02953-6

[17] Carrabba M, Keeling AJ, Aziz A, Vichi A, Fonzar RF, Wood D, Ferrari M. Translucent zirconia in the ceramic scenario for monolithic restorations: A flexural strength and translucency comparison test. J Dent. 2017;60:70-76. DOI: 10.1016/j.jdent.2017.03.002

[18] Websites: BEGO. 3D Printing, CAD/CAM Solutions, Conventional Solutions, Implantology Solutions. Published [10 February 2020]. Updated [30 October 2021]. Accessed [31 October 2022]. https://www.bego.com/cad-cam-solutions/materials/ceramic/katana-zirconia-stml/

[19] Websites: Dentaurum. Nacera Pearl Q3 Multi-Shade Ultra-highly translucent–multi-layered Published [10 February 2020]. Updated [30 October 2021]. Accessed [31 October 2022]. https://www.dentaurum.de/eng/nacera-pearl-q3-multi-shade-32309.aspx

[20] Zhang F, Spies BC, Vleugels J, Reveron H, Wesemann C, Müller W-D, van Meerbeek B, Chevalier J. High-translucent yttria-stabilized zirconia ceramics are wear-resistant and antagonist-friendly. Dent Mater. 2019;35(12):1776 – 1790. DOI: 10.1016/j.dental.2019.10.009

[21] Miyazaki T, Nakamura T, Matsumura H, Ban S, Kobayashi T. Current status of zirconia restoration. Int J Prosthodont. 2013;57(4):236-261. DOI: 10.1016/j.jpor.2013.09.001

[22] Happe A, Röling N, Schäfer A, Rothamel D. Effects of different polishing protocols on the surface roughness of Y-TZP surfaces used for custom-made implant abutments: a controlled morphologic SEM and profilometric pilot study. J Prosthet Dent. 2015;113(5):440-447. DOI: 10.1016/j.prosdent.2014.12.005

[23] Alkimavičius J, Ginevičiūtė E, Valantiejienė V, Andrijauskas R, Linkevičius T. The effect of polishing protocols on the surface roughness of zirconium oxide. Clin Oral Implants Res. 2018;29. DOI: 10.1111/clr.10_13358

[24] Kurt M, Bal BT. Farklı yüzey işlemleri ve hızlandırılmış yapay yaşlandırmanın monolitik seramik sistemlerin yüzey pürüzlülüğü ve topografisi üzerine etkileri. Selcuk Dent J. 2017;4(2):68-76. DOI: 10.15311/selcukdentj.328716 (Turkish)

[25] Park C, Vang M-S, Park S-W, Lim H-P. Effect of various polishing systems on the surface roughness and phase transformation of zirconia and the durability of the polishing systems. J Prosthet Dent. 2017;117(3):430-437. DOI: 10.1016/j.prosdent.2016.10.005

[26] Al Hamad KQ, Abu Al-Addous AM, Al-Wahadni AM, Baba NZ, Goodacre BJ. Surface roughness of monolithic and layered zirconia restorations at different stages of finishing and polishing: an in vitro study. J Prosthodont. 2019;28(7): 818-825. DOI: 10.1111/jopr.13071

[27] Manziuc MM, Gasparik C, Burde AV, Colosi HA, Negucioiu M, Dudea D. Effect of glazing on translucency, color, and surface roughness of monolithic zirconia materials. J Esthet Restor Dent. 2019;31(5):478-485. DOI: 10.1111/jerd.12493

[28] Janyavula S, Lawson N, Cakir D, Beck P, Ramp LC, Burgess JO. The wear of polished and glazed zirconia against enamel. J Prosthet Dent. 2013;109(1): 22-29. DOI: 10.1016/S0022-3913(13)60005-0

[29] Nakamura K, Kanno T, Milleding P, Örtengren U. Zirconia as a dental implant abutment material: a systematic review. Int J Prosthodont. 2010;23(4).

[30] Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: A review of the literature. Dent Mater. 1997;13(4):258-269. DOI: 10.1016/s0109-5641(97)80038-3

[31] Mohammadi-Bassir M, Babasafari M, Rezvani MB, Jamshidian M. Effect of coarse grinding, overglazing, and 2 polishing systems on the flexural strength, surface roughness, and phase transformation of yttrium-stabilized tetragonal zirconia. J Prosthet Dent. 2017;118(5):658-665. DOI: 10.1016/j.prosdent.2016.12.019

[32] De Souza RH, Kaizer MR, Borges CEP, Fernandes ABF, Correr GM, Diógenes AN, Zhang Y, Gonzaga CC. Flexural strength and crystalline stability of a monolithic translucent zirconia subjected to grinding, polishing and thermal challenges. Ceram Inter. 2020;46(16):26168-26175. DOI: 10.1016/j.ceramint.2020.07.114

[33] de Carvalho IHG, da Silva NR, Vila-Nova TEL, de Fatima Dantas de Almeida L, Veríssimo AH, de Melo RM, Zhang Y, de Assunção e Souza RO. Effect of finishing/polishing techniques and aging on topography, C. albicans adherence, and flexural strength of ultra-translucent zirconia: An in situ study. Clin Oral Investig. 2021;1-12. DOI: 10.1007/s00784.021.04068-3

[34] Lee J-Y, Jang G-W, Park I-I, Heo Y-R, Son M-K. The effects of surface grinding and polishing on the phase transformation and flexural strength of zirconia. J Adv Prosthodont. 2019;11(1):1 DOI: 10.4047/jap.2019.11.1.1

[35] Pittayachawan P, McDonald A, Petrie A, Knowles JC. The biaxial flexural strength and fatigue property of Lava™ Y-TZP dental ceramic. Dent Mater. 2007;23(8):1018-1029. DOI: 0.1016/j.dental.2006.09.003

How to cite this article: Doğru G, Demiralp E, Koçak E, Orkçu HH, Aydın C, Yılmaz H. Influence of Finishing Procedures on Surface Roughness and Biaxial Flexural Strength of High-translucent 4Y-PSZ, 5Y-PSZ, and 6Y-PSZ Monolithic Zirconia. Clin Exp Health Sci 2023; 13: 166-175. DOI: 10.33808/clinexphealthsci.1073010

Clinical and Experimental Health Sciences

Association Between Etiological Factors and Dentin Hypersensitivity: A Cross-Sectional Study in Turkey

Ömer Hatipoğlu[1], Fatma Pertek Hatipoğlu[2]

[1] Niğde Ömer Halisdemir University, Faculty of Dentistry, Department of Restorative Dentistry, Niğde, Türkiye.
[2] Niğde Ömer Halisdemir University, Faculty of Dentistry, Department of Endodontics, Niğde, Türkiye.

Correspondence Author: Ömer Hatipoğlu
E-mail: omerhtp@gmail.com

Received: 03.03.2022 **Accepted:** 30.11.2022

ABSTRACT

Objective: This study purposed to estimate the prevalence of DH and how effective the etiological factors are in the development of DH in Turkey.

Methods: Demographic features, hygiene habits, bruxism, beverage habits, parafunctional habits, smoking, and other medical problems were asked. Air was blasted to the teeth via the air-water syringe for 3 seconds. The Visual Analogue Scale was used to measure DH sensitivity, and data were recorded in the 0-100 mm range, and 5 mm and higher were considered DH. The attrition, abrasion, erosion, abfraction per dental type (Incisal OR Canine OR Premolar OR Molar) were recorded. The same procedure was applied for abnormal tooth position and gingival recession.

Results: A total of 4476 teeth and 236 individuals were evaluated. Significantly higher DH frequency was observed in females (p= .034), the 40-55 age range (p= .009), and non-smokers (p= .016). Those who brushed their teeth three times a day or more (p< .001), preferred horizontal technique (p= .017), used toothbrushes with a hard bristle (p< .001) exhibited higher DH frequency. There was no significant difference in DH regarding bruxism, acidic beverage consumption, vomiting, and reflux (p> .05).

Conclusion: DH is a multi-etiological symptom affected by demographic attributes, hygiene, and other habits. Clinicians should painstakingly distinguish the source of DH to gain the long-term success of DH treatment, which depends on many etiological factors.

Keywords: Dentin hypersensitivity, dentin sensitivity, cross-sectional studies

1. INTRODUCTION

Dentin hypersensitivity (DH) is a clinical finding frequently encountered in the population, characterized by short-term sharp pain due to thermal, chemical, or mechanical stimuli, not associated with dental defects or caries (1). Commonly, patients state that pain occurs when they drink cold or hot beverages, brush their teeth, or eat sweet foods (2). The most accepted theory explaining the mechanism of DH is based on the stimulation of baroreceptors in the pulp and dentin due to the movement of the fluids in the exposed dentinal tubules after chemical and physical changes. Accordingly, pain sensitivity occurs (3).

Dentin tubules are generally covered with enamel and cementum to be isolated from the external environment. Dentin tubules can be exposed in cases where the enamel or cementum is corrupted due to erosion, abrasion, attrition, and abfraction (4). Erosion is a type of wear on the teeth caused by excess acid in the mouth due to heartburn, gastritis reflux, constant vomiting, excessive acidic drinks, and food consumption. As a result of bruxism, attrition may occur in the tubercules and incisal of the teeth, and abfraction in the cervical regions. Another critical etiological factor for DH is the gingival recession. It has been stated that the root surface exposed due to gingival recession is the highest risk factor for DH (5).

Studies report that DH prevalence ranges from 2.8% to 74% in adult populations (2,6-8). It has been hypothesized that this wide variation in prevalence may link to the different methodologies and populations in the studies. The only study measuring the prevalence of DH among patients in Turkey was conducted in Kırıkkale in 2012 (9) and a novel study was required. This study aimed to measure the prevalence of DH and how effective the etiological risk factors are in the development of DH in Turkey.

DOI: 10.33808/clinexphealthsci.1071585

2. METHODS

The Clinical Research Ethics Committee of Sutcu Imam University approved this study (2019/68) on 06.03.2019. A written consent form was obtained from the patients who were involved.

2.1. Study Population

This study was carried out by conducting a questionnaire and clinical examination on patients who applied to Sutcu Imam University between April 2019 and April 2020. Turkish patients older than 18 years, in good health, and who approved to participate in the study were included. Patients who had previously undergone bleaching or currently undergoing orthodontic treatment or receiving professional dentin sensitivity treatment were excluded from the study. All teeth which did not have caries, cracks, fractures, and restorations were included in the study, except the third molar tooth.

2.2. Power Analysis

Based on a previous study conducted in Turkey, the prevalence of DH was found to be 7.6% (9). It was calculated with 95% confidence that at least 108 patients should be attended with a 5% alpha margin of error. It was decided to include at least 108 patients in the study.

2.3. Calibration and Intra-reliability of the Examiner

Before the study protocol, the examiner (O.H.) was initially trained and calibrated to detect DH. Afterward, an oral examination of 10 subjects (not part of the study sample) was carried out by the examiner (O.H.) at Sutcu Imam University to test the intra-reliability through Intra-class Correlation Coefficient (ICC). The examiner was trained and calibrated until ICC was higher than 0.70 (between 0.70 and 0.90 is considered sufficient).

2.4. Questionnaire

A researcher (F.P.H.) formulated the structured questionnaire. The questionnaire had adequate reliability with a Cronbach alpha coefficient of 0.833. Demographic features [age, gender (male OR female), hygiene habits [Tooth-brushing frequency (Once daily OR twice daily OR more) and tooth-brushing technique (horizontal+vertical OR horizontal OR vertical), bristle hardness (soft OR medium OR hard)], bruxism (none OR sometimes OR often), acidic beverage (none OR sometimes OR often), vomiting (none OR sometimes OR often), reflux (none OR sometimes OR often), parafunctional habits (none OR sometimes OR often), smoking (none OR sometimes OR often) were asked (Table 1).

Table 1. *The questionnaire that was used in the study*

1. Age?
2. Gender?
3. How often do you brush your teeth?
1
2
3
4. With which method do you brush your teeth?
Horizontal+Vertical
Vertical
Horizontal
5. What is the hardness of the toothbrush you use?
Soft
Medium
Hard
6. Do you have bruxism?
None
Sometimes
Often
7. How much do you consume acidic beverages?
None
Sometimes
Often
8. How much do you vomit?
None
Sometimes
Often
9. How much do you experience reflux?
None
Sometimes
Often
10.Do you have a habit of smoking?
None
Sometimes
Often

2.5. Evaluation of Dentin Hypersensitivity and Non-Carious Lesions

The Visual Analogue Scale (VAS) was used to measure DH in participants. VAS was formed as a horizontal line of 0-100 mm, and “0 mm” was accepted as “no pain” and “100 mm” as severe pain. Air was blasted for 3 seconds, 2 mm away and perpendicular to the tooth, through the air-water syringe. The patient was asked to mark which value corresponded to the severity of the pain between 0-100 mm. Teeth with values of 5 mm and higher were considered as having DH. A Google form page was created on the internet to save the data. In order to determine the non-carious lesions that may affect the DH, the number of attrition, abrasion, erosion, and abfraction per dental type (Incisal OR Canine OR Premolar OR Molar) were recorded. The same procedure was applied for abnormal tooth position and gingival recession.

2.6. Statistical Analysis

Jamovi 1.6.23 statistical program was used for the statistical analysis. The frequencies of non-caries lesions and DH were determined. Chi-squared test was used to detect the relationship between etiologic factors and DH. Significance was set at p<.05.

3. RESULTS

A total of 236 individuals, 156 (66%) female, and 80 (34%) male were recruited and 4476 teeth were examined. The mean age of the individuals was 35.13±14.06. The age pyramid according to gender is presented in Figure 1. Of them 7.69 % (n=344) of teeth and 12.3% (n=29) of individuals were diagnosed with DH (Figure 2). The frequencies of NCLs, gingival recession, and abnormal tooth position were 14.63% (n=655), 9.07% (n=406), 4.83% (n=216), respectively. Also, in the same sequence, the frequencies of DH were 15.42%, 3.9%, and 8.81%, respectively. While attrition was the most frequent NCL (9.34%, n=418), abfraction was the least (0.16%, n=7). While abfraction caused the highest DH (100%), attrition caused the least (13.10%) (Table 2).

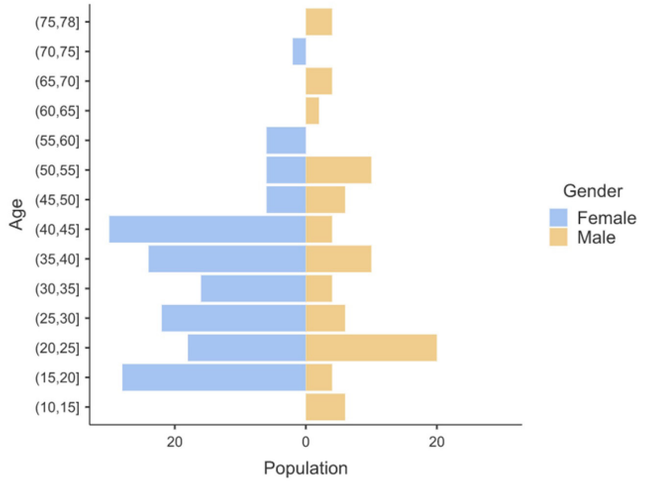


Figure 1. *The age pyramid according to gender.*

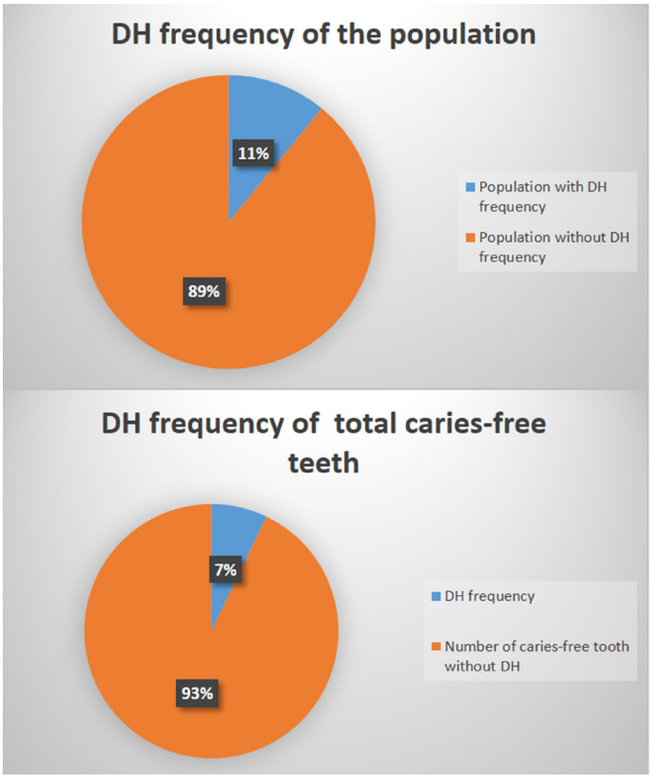


Figure 2. *The frequency of DH according to population and number of caries-free tooth*

Table 2. *Frequency and rate of DH in terms of non-carious lesions, other etiological factors, and tooth type.*

	Frequency %	Rate of DH %
Non-carious lesions		
Abfraction (n=7)	0.16%	100%
Attrition (n=418)	9.34%	13.10%
Erosion (n=20)	0.45%	53.33%
Abrasion (n=210)	4.69%	33.43%
Total	14.63%	15.42%
Other Etiological Factors		
Gingival recession (n=406)	9.07%	3.9%
Abnormal tooth position (n=216)	4.83%	8.81%
Tooth Type		
Incisal (n=808)	18.05%	5.69%
Canine (n=1604)	35.84%	6.98%
Premolar (n=1218)	27.21%	11.33%
Molar (n=846)	18.90%	5.67%
Total DH frequency		
DH frequency of total caries-free teeth (n=344)	-	7.69%
DH frequency of population (n=29)	-	12.3%

In terms of gender, a significantly higher DH frequency was observed in females compared to males in premolar (p= .04), molar (p= .04), and total (p= .03). In terms of age range, a significantly higher DH frequency was found in the 40-55 years compared to the other age ranges in canine (p< .01),

premolar (p< .01), molar (p< .01), and total (p= .04). In terms of tooth brushing frequency, individuals who brush their teeth three times a day or more exhibited significantly higher DH than others (p< .05)

In terms of tooth brushing technique, significantly higher DH frequency was observed in individuals brushing teeth with the horizontal technique in incisal (p< .01), molar (p< .01), and total (p= .07). In terms of bristle hardness, a significantly higher DH was observed in individuals who use a toothbrush with a hard bristle in all tooth types and total (p< .01) (Table 3).

There was no significant difference in DH frequency in all dental groups and total regarding bruxism, acidic beverage consumption, vomiting, and reflux (p> .05). In terms of smoking, although there was no significant difference in all dental groups, a significant difference was observed in total (p< .05). Higher DH frequency was observed in non-smokers (Table 4).

Table 3. *The presentation of the association between etiologic factors and DH frequency using the Chi-squared test (Part 1)*

		Incisal		Canine		Premolar		Molar		Total	
Age Range		DH	None	DH	None	DH	None	DH	None	DH	None
18-40	(N=152)	22 (55%)	130 (66%)	10 (38%)	142 (68%)	28 (58%)	124 (66%)	12 (46%)	140 (67%)	54 (66%)	98 (64%)
40-55	(N=66)	16 (40%)	50 (26%)	16 (62%)	50 (24%)	20 (42%)	46 (24%)	14 (54%)	52 (25%)	26 (32%)	40 (26%)
55-78	(N=20)	2 (5.0%)	16 (8.2%)	0 (0%)	18 (8.6%)	0 (0%)	18 (9.6%)	0 (0%)	18 (8.6%)	2 (2.4%)	16 (10%)
Test Statistic		X^2=3.86, p=.14		X^2=17.43, p<.01*		X^2=9.55, p=.01*		X^2=11.06, p<.01*		X^2=6.09, p=.04*	
Gender											
Female	(N=158)	28 (70%)	128 (65%)	20 (77%)	136 (65%)	38 (79%)	118 (63%)	22 (85%)	134 (64%)	62 (76%)	94 (61%)
Male	(N=80)	12 (30%)	68 (35%)	6 (23%)	74 (35%)	10 (21%)	70 (37%)	4 (15%)	76 (36%)	20 (24%)	60 (39%)
Test Statistic		X^2=.28, p=.60		X^2=1.45, p=.23		X^2=4.40, p=.04*		X^2=4.35, p=.04*		X^2=4.77, p=.03*	
Tooth-brushing Frequency											
1 per day	(N=122)	22 (55%)	98 (50%)	8 (31%)	112 (53%)	20 (42%)	100 (53%)	10 (38%)	110 (52%)	36 (44%)	84 (55%)
2 per day	(N=104)	12 (30%)	92 (47%)	14 (54%)	90 (43%)	22 (46%)	82 (44%)	12 (46%)	92 (44%)	36 (44%)	68 (44%)
3 per day	(N=12)	6 (15%)	6 (3.1%)	4 (15%)	8 (3.8%)	6 (12%)	6 (3.2%)	4 (15%)	8 (3.8%)	10 (12%)	2 (1.3%)
Test Statistic		X^2=11.65, p<.01*		X^2=9.27, p=.01*		X^2=7.78, p=.02*		X^2=7.17, p=.03*		X^2=14.02, p<.01*	
Tooth-brushing Technique											
Horizantal+vertical	(N=166)	16 (40%)	148 (76%)	16 (62%)	148 (70%)	28 (58%)	136 (72%)	16 (62%)	148 (70%)	50 (61%)	114 (74%)
Vertical	(N=44)	14 (35%)	30 (15%)	4 (15%)	40 (19%)	10 (21%)	34 (18%)	2 (7.7%)	42 (20%)	18 (22%)	26 (17%)
Horizantal	(N=28)	10 (25%)	18 (9.2%)	6 (23%)	22 (10%)	10 (21%)	18 (9.6%)	8 (31%)	20 (9.5%)	14 (17%)	14 (9.1%)
Test Statistic		X^2=2.35, p<.01*		X^2=3.61, p=.162		X^2=5.51, p=.06		X^2=11.08, p<.01*		X^2=5.19, p=.07	
Bristle-Hardness											
Soft	(N=46)	4 (10%)	42 (21%)	0 (0%)	46 (22%)	12 (25%)	34 (18%)	6 (23%)	40 (19%)	16 (20%)	30 (19%)
Medium	(N=160)	20 (50%)	140 (71%)	12 (46%)	148 (70%)	22 (46%)	138 (73%)	6 (23%)	154 (73%)	44 (54%)	116 (75%)
Hard	(N=30)	16 (40%)	14 (7.1%)	14 (54%)	16 (7.6%)	14 (29%)	16 (8.5%)	14 (54%)	16 (7.6%)	22 (27%)	8 (5.2%)
Test Statistic		X^2=32.69, p<.01*		X^2=46.61, p<.01*		X^2=18.06, p<.01*		X^2=47.70, p<.01*		X^2=23.41, p<.01*	

**p<0.05, statistically significant*

Table 4. *The presentation of the association between etiologic factors and DH frequency using the Chi-squared test (Part 2)*

		Incisal		Canine		Premolar		Molar		Total	
Bruxism		DH	None	DH	None	DH	None	DH	None	DH	None
None	(N=130)	16 (40%)	112 (57%)	8 (31%)	120 (57%)	26 (54%)	102 (54%)	12 (46%)	116 (55%)	38 (46%)	90 (58%)
Sometimes	(N=62)	14 (35%)	48 (24%)	14 (54%)	48 (23%)	14 (29%)	48 (26%)	8 (31%)	54 (26%)	28 (34%)	34 (22%)
Often	(N=46)	10 (25%)	36 (18%)	4 (15%)	42 (20%)	8 (17%)	38 (20%)	6 (23%)	40 (19%)	16 (20%)	30 (19%)
Test Statistic		X^2=4.16, p=.12		X^2=11.93, p=.07		X^2=.45, p=.80		X^2=.84, p=.66		X^2=4.72, p=.09	
Acidic Beverage											
None	(N=150)	22 (55%)	126 (64%)	20 (77%)	128 (61%)	34 (71%)	114 (61%)	20 (77%)	128 (61%)	48 (59%)	100 (65%)
Sometimes	(N=68)	14 (35%)	54 (28%)	6 (23%)	62 (30%)	14 (29%)	54 (29%)	6 (23%)	62 (30%)	30 (37%)	38 (25%)
Often	(N=20)	4 (10%)	16 (8.2%)	0 (0%)	20 (9.5%)	0 (0%)	20 (11%)	0 (0%)	20 (9.5%)	4 (4.9%)	16 (10%)
Test Statistic		X^2=1.33, p=.51		X^2=3.66, p=.16		X^2=5.64, p=.06		X^2=3.66, p=.16		X^2=5.06, p=.08	
Vomiting											
None	(N=228)	38 (95%)	188 (96%)	26 (100%)	200 (95%)	46 (96%)	180 (96%)	24 (92%)	202 (96%)	78 (95%)	148 (96%)
Sometimes	(N=10)	2 (5.0%)	8 (4.1%)	0 (0%)	10 (4.8%)	2 (4.2%)	8 (4.3%)	2 (7.7%)	8 (3.8%)	4 (4.9%)	6 (3.9%)
Test Statistic		X^2=.08, p=.78		X^2=1.28, p=.26		X^2=.00, p=.99		X^2=.88, p=.35		X^2=.14, p=.71	
Reflux											
None	(N=172)	26 (65%)	146 (74%)	20 (77%)	152 (72%)	36 (75%)	136 (72%)	18 (69%)	154 (73%)	58 (71%)	114 (74%)
Sometimes	(N=54)	10 (25%)	42 (21%)	4 (15%)	48 (23%)	8 (17%)	44 (23%)	6 (23%)	46 (22%)	16 (20%)	36 (23%)
Often	(N=12)	4 (10%)	8 (4.1%)	2 (7.7%)	10 (4.8%)	4 (8.3%)	8 (4.3%)	2 (7.7%)	10 (4.8%)	8 (9.8%)	4 (2.6%)
Test Statistic		X^2=2.81, p=.25		X^2=1.18, p=.55		X^2=2.32, p=.31		X^2=.45, p=.80		X^2=6.11, p=.05	
Parafunctional habits											
None	(N=220)	40 (100%)	178 (91%)	24 (92%)	194 (92%)	48 (100%)	170 (90%)	26 (100%)	192 (91%)	80 (98%)	138 (90%)
Sometimes	(N=10)	0 (0%)	10 (5.1%)	0 (0%)	10 (4.8%)	0 (0%)	10 (5.3%)	0 (0%)	10 (4.8%)	0 (0%)	10 (6.5%)
Often	(N=8)	0 (0%)	8 (4.1%)	2 (7.7%)	6 (2.9%)	0 (0%)	8 (4.3%)	0 (0%)	8 (3.8%)	2 (2.4%)	6 (3.9%)
Test Statistic		X^2=3.93, p=.14		X^2=2.86, p=.24		X^2=4.92, p=.09		X^2=2.39, p=.30		X^2=5.93, p=.05	
Smoking											
None	(N=198)	36 (90%)	160 (82%)	24 (92%)	172 (82%)	44 (92%)	152 (81%)	26 (100%)	170 (81%)	76 (93%)	120 (78%)
Sometimes	(N=12)	2 (5.0%)	10 (5.1%)	2 (7.7%)	10 (4.8%)	2 (4.2%)	10 (5.3%)	0 (0%)	12 (5.7%)	2 (2.4%)	10 (6.5%)
Often	(N=28)	2 (5.0%)	26 (13%)	0 (0%)	28 (13%)	2 (4.2%)	26 (14%)	0 (0%)	28 (13%)	4 (4.9%)	24 (16%)
Test Statistic		X^2=2.14, p=.34		X^2=4.13, p=.13		X^2=3.56, p=.17		X^2=5.90, p=.05		X^2=8.08, p=.02*	

**p<0.05, statistically significant*

4. DISCUSSION

DH is a symptom, not a disease. In detecting DH, a careful anamnesis should be taken from the patient to exclude all differential diagnoses, and then the patient should be examined in detail radiographically and clinically (10). Since the pain due to DH will disrupt the quality of life, the etiological factors leading to DH should be determined painstakingly.

The prevalence of DH in the population examined in the present study was 12.3%, which seems to increased compared to the previous study (7.6%) conducted in 2012 in Turkey.(9) Probably, dental hygiene habits that enhanced gradually may have caused this increase. Interestingly, our value is almost the same as the study conducted (11) in the United States in 2013. However, many studies found very low prevalence (Nigeria 1.3% (12), UK 2.8% (13)) as well as very high prevalence (China 34.5% (14), Brazil 46% (15)). In a meta-analysis conducted in 2019, the frequency of DH was reported to range between 4.8% and 62.3% (16). It can be supposed that the various diagnostic methods used to detect DH cause high variation outcomes between studies (17). DH can be clinically confused with the acute pains obtained from caries, restoration, cracked teeth, and a reversible or irreversible inflammatory process of the pulp. In order to eliminate the negative impacts of these factors, only healthy, decayed, and unrestored teeth were examined in our study.

Consistent with the results of several studies (13,18,19), the frequency of DH was found higher in females than in males. However, some studies indicate no significant difference between genders (17,20). The difference between genders may be attributed to females paying more attention to oral hygiene than males. Seymour et al. (21) found lower tolerance and higher sensitivity to toothache in females and reported that females more frequently applied to clinics. Walters (22) stated that the frequency of DH in females might be determined at higher rates since females' number of applications to dental practitioners is higher than that of males. In addition, diet differences and higher consumption of acidic foods and beverages in females may affect the consequence.

In the present study, a higher DH frequency was detected in individuals aged 40-55 years. This result is consistent with several studies (2,23). However, some studies found higher DH rate in 20-39 years (24). Our result may be associated with the more significant presence of gingival recession in adults (23,25). The low DH frequency obtained in olders, on the other hand, may explain the changes that occur in the dentin-pulp complex with age, dentin sclerosis, and secondary and tertiary dentin formation (18,26).

The present study observed that individuals who brushed teeth three or more a day experienced four times higher DH frequency than those who brushed less frequently. Consistent with the present study, many studies have reported that the risk of experiencing DH complaints can be minimized by brushing the teeth twice a day (27,28). Excessive frequency of brushing may cause a gingival recession, resulting in the development of cervical defects (29). Dentin abrasion occurring in the cervical region due to excessive brushing is frequently encountered. A study reported that the initial tooth was brushed longer than the last tooth during the toothbrushing cycle. Hence, it is considered that the frequency of sensitivity increases in teeth that are initiated to be brushed, and these teeth are generally premolar and canines (30).

Consistent with numerous studies (13,31,32), individuals who use toothbrush with a hard bristle exhibited significantly higher DH frequency in the present study. However, some studies have reported no relationship between brush bristle hardness and DH (17,33). This difference is more pronounced in the sound enamel tissue but not for affected and demineralized tissues. Therefore, the enamel surface properties investigated in the studies may have induced heterogeneity among outcomes.

Dietary acid is suspected for DH frequency because of its potential to erode cervical dentin (34). The present study found that the consumption of acidic drinks does not affect DH. In line with these outcomes, Rahiotis et al. (33) stated that even consuming acidic foods more than once a day did not induce DH. However, unlike these results, there are also studies reporting that acidic beverages such as orange juice, apple juice, cola, and wine increase tooth wear and are influential in forming DH by causing rapid dissolution of the smear layer (35,36). Differences in age ranges, pain threshold, psychological and physiological state, salivary buffering and flow rate, pellicle thickness and load, movements of soft tissues, distribution and duration of acidic fluid in the oral cavity, tooth structure, and remineralization potential may cause this varied outcomes among studies (37).

Consistent with some studies (31), non-smokers exhibited higher DH than smokers in our study, but some studies found the opposite (2,33). Smoking is a significant risk factor for periodontal disease and attachment loss, and it is assumed that it may increase DH by indirectly increasing gingival recession (17). However, some substances in cigarette may block dentinal tubules and reduce sensitivity; more detailed studies are needed to verify this.

DH is a common clinical condition that presents many associated factors that should be considered in diagnosis and treatment (38). Studies have shown that factors mainly associated with DH include hard tissue loss leading to dentin exposure (39,40). In the present study, the highest DH frequency was observed in erosion cases after abfraction and the lowest in attrition. Several studies have associated non-carious lesions with DH (28,41). In a study that did not evaluate the attrition parameter, erosion exhibited the highest frequency of DH and abfraction lowest (39); consistent with our study, the frequency of abfraction teeth was lower than other NCLs.

Varying the effects of acidic agents on enamel due to different biological, chemical, and behavioral factors (38) may cause some teeth to exhibit less erosion than others, even if they are exposed to the same acid challenge in the diet (37). Abrasion due to inappropriate brushing, wedge-shaped defects in teeth caused by poorly directed occlusal forces, parafunctional habits, stress occurring in the cervical, the erosion caused by the acids may lead to the dentinal tubules to be exposed (42).

The present study found no association between reflux, vomiting, and acidic beverages with DH. It is mistaken to presume that acidic beverages will yield DH in all patients. The surface of the exposed dentin area, the thickness of the remaining dentin layer, the condition of the root and coronal dentin, the molecular size of the agent that will pass through the dentin, the formation of dentin in the periphery, the size of the dentinal tubules, the presence of tertiary dentin near the pulp play an essential role in the formation of DH. The teeth with DH exhibit the number of tubules per unit area is eight times higher than non-sensitive teeth, and the diameter of the tubules is approximately two times larger (43,44).

This research revealed a relationship between DH and gingival recession, in line with the study conducted in Turkey by Yaylı et al. (45). Several studies have pointed to gingival recession as the main etiological factor for DH (33,34,39). In the United Kingdom, DH was observed in 50% of individuals with gingival recession (46). It has been shown in previous studies that areas with gingival recession tend to occur DH (47,48), and this sensitivity decreases with the closure of the recession area (49). Aesthetic, functional problems, and DH that may accompany areas with gingival recession disrupt the quality of life (50).

In the present study, premolars are the teeth where the highest DH occurs, consistent with several studies (2,51) due to their position in the dental arch. DH is most common in premolars since they are the teeth most exposed to brush forces, thus occurring gingival recession and hard tissue loss (11). A meta-analysis revealed that the greater force is applied to the premolars during tooth brushing, and therefore, orthodontic bracket loss is mainly seen in this region (52).

This study had some limitations; It can not be generalized because it was conducted on a specific ethnicity. The tooth groups were classified regardless of whether they were in the

maxilla or mandibula. Furthermore, the severity of DH was ignored; only the focus was given to its presence or absence.

5. CONCLUSION

The frequency of DH in Turkey corresponds to 12.3% of the population, and an increase is remarkable compared to 10 years ago. DH is more common in middle-aged individuals and females. Those who use toothbrushes with a hard bristle or brush their teeth three or more times a day or prefer horizontal technique experienced significantly higher DH. Interestingly, the DH frequency is lower in smokers. Also, NCLs and gingival recession are the main etiological factors for DH. Clinicians should accurately determine the source of DH to achieve the long-term success of DH treatment, which relies on many etiological factors.

Acknowledgments: *We thank all the nurses who participated in this study.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Clinical Research Ethics Committee of Sütçü İmam University approved this study (2019/68) on 06.03.2019*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: ÖH
Design of the study: ÖH
Acquisition of data for the study: FPH
Analysis of data for the study: ÖH
Interpretation of data for the study: ÖH, FPH
Drafting the manuscript: FPH
Revising it critically for important intellectual content: ÖH
Final approval of the version to be published: ÖH

REFERENCES

[1] Markowitz K, Pashley D. Dentine Hypersensitivity. 1st ed. Switzerland: Springer; 2015.
[2] Rees J, Addy M. A cross-sectional study of dentine hypersensitivity. J Clin Periodontol. 2002;29(11):997-1003. DOI:1.1034/j.1600-051X.2002.291104.x
[3] Brännström M. The hydrodynamics of the dentine; its possible relationship to dentinal pain. Int Dent J. 1972;22(2):219-227.
[4] Jacobsen PL, Bruce G. Clinical dentin hypersensitivity: understanding the causes and prescribing a treatment. J Contemp Dent Pract. 2001;2(1):1-12. DOI: 1.5005/jcdp-2-1-27
[5] Chabanski M, Gillam D, Bulman J, Newman H. Prevalence of cervical dentine sensitivity in a population of patients referred to a specialist Periodontology Department. J Clin Periodontol. 1996;23(11):989-992. DOI:1.1111/j.1600-051X.1996.tb00525.x
[6] Murray L. The prevalence of selfreported hypersensitive teeth Arch. Oral Biol. 1994;39:129S. DOI:1.11263/jsotp1982.14.165
[7] Udoye C. Pattern and distribution of cervical dentine hypersensitivity in a Nigerian tertiary hospital. Odontostomatol Trop. 2006;29(116):19-22.
[8] Taani SQ, Awartani F. Clinical evaluation of cervical dentin sensitivity (CDS) in patients attending general dental clinics (GDC) and periodontal specialty clinics (PSC). J Clin Periodontol. 2002;29(2):118-122. DOI:1.1034/j.1600-051x.2002.290205.x
[9] Çolak H, Demirer S, Hamidi M, Uzgur R, Köseoğlu S. Prevalence of dentine hypersensitivity among adult patients attending a dental hospital clinic in Turkey. West Indian Med J. 2012;61(2):174-179.
[10] Porto IC, Andrade AK, Montes MA. Diagnosis and treatment of dentinal hypersensitivity. J Oral Sci. 2009;51(3):323-332. DOI:1.2334/josnusd.51.323
[11] Cunha-Cruz J, Wataha JC, Heaton LJ, Rothen M, Sobieraj M, Scott J, Berg J, Northwest Practice-based Research Collaborative in Evidence-based D. The prevalence of dentin hypersensitivity in general dental practices in the northwest United States. J Am Dent Assoc. 2013;144(3):288-296. DOI:1.14219/jada.archive.201.0340
[12] Bamise CT, Olusile AO, Oginni AO, Dosumu OO. The prevalence of dentine hypersensitivity among adult patients attending a Nigerian teaching hospital. Oral Health Prev Dent. 2007;5(1):49-53. DOI: 1.3290/j.ohpd.a11958
[13] Rees J. The prevalence of dentine hypersensitivity in general dental practice in the UK. J Clin Periodontol. 2000;27(11):860-865. DOI:1.1034/j.1600-051x.200.027.01186.x
[14] Liang X, Wei Z, Hu D, Ruan J. Prevalence of dentin hypersensitivity among the residents of Xi'an city, China. Acta Odontol Scand. 2017;75(6):387-393. DOI:1.1080/00016.357.2017.1323348
[15] Scaramucci T, de Almeida Anfe TE, da Silva Ferreira S, Frias AC, Sobral MA. Investigation of the prevalence, clinical features, and risk factors of dentin hypersensitivity in a selected Brazilian population. Clin Oral Investig. 2014;18(2):651-657. DOI:1.1007/s00784.013.1008-1
[16] Zeola LF, Soares PV, Cunha-Cruz J. Prevalence of dentin hypersensitivity: Systematic review and meta-analysis. J Dent. 2019;81:1-6. DOI:1.1016/j.jdent.2018.12.015
[17] Costa RS, Rios FS, Moura MS, Jardim JJ, Maltz M, Haas AN. Prevalence and risk indicators of dentin hypersensitivity in adult and elderly populations from Porto Alegre, Brazil. J Periodontol. 2014;85(9):1247-1258. DOI:1.1902/jop.2014.130728
[18] Garg N, Garg A. Textbook of operative dentistry. 2nd ed. New Delhi:Boydell & Brewer Ltd; 2013.
[19] Rees J, Jin L, Lam S, Kudanowska I, Vowles R. The prevalence of dentine hypersensitivity in a hospital clinic population in Hong Kong. J Dent. 2003;31(7):453-461. DOI:1.1016/S0300-5712(03)00092-7
[20] Orchardson R, Gillam DG. Managing dentin hypersensitivity. J Am Dent Assoc. 2006;137(7):990-998. DOI:1.14219/jada.archive.2006.0321
[21] Seymour RA, Simpson JM, Charlton JE, Phillips ME. An evaluation of length and end-phrase of visual analogue scales in dental pain. Pain. 1985;21(2):177-185. DOI:1.1016/0304-3959(85)90287-8
[22] Walters PA. Dentinal hypersensitivity: a review. J Contemp Dent Pract. 2005;6(2):107-117. DOI: 1.5005/jcdp-6-2-107
[23] Toy VE, Eltas A. Malatya bölgesinde dişeti çekilmesinin yaygınlığının, şiddetinin ve etkenlerinin araştırılması (Turkish). Ann med health sci res. 2013;2(1):?-?. (Turkish)

[24] Al-Khafaji H. Observations on dentine hypersensitivity in general dental practices in the United Arab Emirates. Eur J Dent. 2013;7(4):389. DOI: 1.4103/1305-7456.120634
[25] Susin C, Haas AN, Oppermann RV, Haugejorden O, Albandar JM. Gingival recession: epidemiology and risk indicators in a representative urban Brazilian population. J Periodontol. 2004;75(10):1377-1386. DOI:1.1902/jop.2004.75.1.1377
[26] Bartold P. Dentinal hypersensitivity: A review. Aust Dent J. 2006;51(3):212-218. DOI:1.1111/j.1834-7819.2006.tb00431.x
[27] West N, Hooper S, O'sullivan D, Hughes N, North M, Macdonald E, Davies M, Claydon N. In situ randomised trial investigating abrasive effects of two desensitising toothpastes on dentine with acidic challenge prior to brushing. J Dent. 2012;40(1):77-85. DOI:1.1016/j.jdent.2011.1.010
[28] Que K, Guo B, Jia Z, Chen Z, Yang J, Gao P. A cross-sectional study: non-carious cervical lesions, cervical dentine hypersensitivity and related risk factors. J Oral Rehabil. 2013;40(1):24-32. DOI:1.1111/j.1365-2842.2012.02342.x
[29] Volpe A, Mooney R, Zumbrunnen C, Stahl D, Goldman H. A long term clinical study evaluating the effect of two dentifrices on oral tissues. J Periodontol. 1975;46(2):113-118. DOI: 1.1902/jop.1975.46.2.113
[30] MacGregor I, Rugg-Gunn A. A survey of toothbrushing sequence in children and young adults. J Periodontal Res. 1979;14(3):225. DOI:1.1111/j.1600-0765.1979.tb00227.x
[31] Vijaya V, Sanjay V, Varghese RK, Ravuri R, Agarwal A. Association of dentine hypersensitivity with different risk factors–A cross sectional study. J Int Oral Health. 2013;5(6):88.
[32] Rossi V, Weidlich P, Oppermann R. Comparative analysis between hard-and soft-filament toothbrushes related to plaque removal and gingival abrasion. J Clin Dent. 2007;18(3):61-64.
[33] Rahiotis C, Polychronopoulou A, Tsiklakis K, Kakaboura A. Cervical dentin hypersensitivity: A cross-sectional investigation in Athens, Greece. J Oral Rehabil. 2013;40(12):948-957. DOI:1.1111/joor.12109
[34] Amarasena N, Spencer J, Ou Y, Brennan D. Dentine hypersensitivity in a private practice patient population in Australia. J Oral Rehabil. 2011;38(1):52-56. DOI:1.1111/j.1365-2842.201.02132.x
[35] Bamise CT, Olusile AO, Oginni AO. An analysis of the etiological and predisposing factors related to dentin hypersensitivity. J Contemp Dent Pract. 2008;9(5):52-59.
[36] Corrêa FOB, Sampaio JEC, Rossa Júnior C, Orrico SRP. Influence of natural fruit juices in removing the smear layer from root surfaces-an in vitro study. J Can Dent Assoc. 2004;:697-702.
[37] Larsen M, Pearce E. Saturation of human saliva with respect to calcium salts. Arch Oral Biol. 2003;48(4):317-322. DOI:1.1016/S0003-9969(03)00007-4
[38] West NX, Lussi A, Seong J, Hellwig E. Dentin hypersensitivity: pain mechanisms and aetiology of exposed cervical dentin. Clin Oral Investig. 2013;17(Suppl 1):9-19. DOI:1.1007/s00784.012.0887-x
[39] Alcantara PM, Barroso NFF, Botelho AM, Douglas-de-Oliveira DW, Goncalves PF, Flecha OD. Associated factors to cervical dentin hypersensitivity in adults: a transversal study. BMC Oral Health. 2018;18(1):155. DOI:1.1186/s12903.018.0616-1
[40] Martens LC. A decision tree for the management of exposed cervical dentin (ECD) and dentin hypersensitivity (DHS). Clin Oral Investig. 2013;17(Suppl 1):77-83. DOI:1.1007/s00784.012.0898-7
[41] West NX, Sanz M, Lussi A, Bartlett D, Bouchard P, Bourgeois D. Prevalence of dentine hypersensitivity and study of associated factors: a European population-based cross-sectional study. J Dent. 2013;41(10):841-851. DOI:1.1016/j.jdent.2013.07.017
[42] Ladalardo TCCGP, Pinheiro A, Campos RAdC, Brugnera Júnior A, Zanin F, Albernaz PLM, Weckx LLM. Laser therapy in the treatment of dentine hypersensitivity. Braz Dent J. 2004;15:144-145. DOI:1.1590/S0103.644.0200400.020.0011
[43] Dababneh R, Khouri A, Addy M. Dentine hypersensitivity—An enigma? A review of terminology, mechanisms, aetiology and management. Br Dent J. 1999;187(11):606-611. DOI:1.1038/sj.bdj.4800345
[44] Christensen GJ. Desensitization of cervical tooth structure. J Am Dent Assoc. 1998;129(6):765-766. DOI: 1.14219/jada.archive.1998.0319
[45] Yaylı NZA, Sarı ET. Dişeti çekilmesi: Yetişkin bir alt popülasyonda prevalans ve ilişkili faktörler (Turkish). Van Sağ Bil Derg.14(2):216-27. DOI:1.52976/vansaglik.942756
[46] Martínez-Ricarte J, Faus-Matoses V, Faus-Llácer VJ, Flichy-Fernández AJ, Mateos-Moreno B. Dentinal sensitivity: Concept and methodology for its objective evaluation. Med Oral Patol Oral Cir Bucal. 2008;13(3):E201-206.
[47] Francetti L, Weinstein R, Taschieri S, Corbella S. Coronally advanced flap with or without subepithelial connective tissue graft for the treatment of single recession: 5-Year Outcomes from a Comparative Study. Int J Periodontics Restorative Dent. 2018;38(6): DOI: 1.11607/prd.3036
[48] Schlee M, Rathe F, Bommer C, Bröseler F, Kind L. Self-assembling peptide matrix for treatment of dentin hypersensitivity: A randomized controlled clinical trial. J Periodontol. 2018;89(6):653-656. DOI:1.1002/JPER.17-0429
[49] Santamaria MP, Silveira CA, Mathias IF, Neves FLdS, Dos Santos LM, Jardini MAN, Tatakis DN, Sallum EA, Bresciani E. Treatment of single maxillary gingival recession associated with non-carious cervical lesion: Randomized clinical trial comparing connective tissue graft alone to graft plus partial restoration. J Clin Periodontol. 2018;45(8):968-976. DOI:1.1111/jcpe.12907
[50] Seong J, Bartlett D, Newcombe R, Claydon N, Hellin N, West NJJod. Prevalence of gingival recession and study of associated related factors in young UK adults. J Dent. 2018;76:58-67. DOI:1.1016/j.jdent.2018.06.005
[51] Que K, Ruan J, Fan X, Liang X, Hu D. A multi-centre and cross-sectional study of dentine hypersensitivity in China. J Clin Periodontol. 2010;37(7):631-637. DOI:1.1111/j.1600-051X.2009.01513.x
[52] Hatipoğlu O, Küçükönder A, Oral E. Positional factors affecting the bond failure rates in orthodontic treatment: A systematic review and meta-analysis. Orthod Waves. 2019;78(3):93-101. DOI:1.1016/j.odw.2019.06.001

How to cite this article: Hatipoğlu Ö, Pertek Hatipoğlu F. Association Between Etiological Factors and Dentin Hypersensitivity: A Cross-Sectional Study in Turkey. Clin Exp Health Sci 2023; 13: 176-*183*. DOI: 10.33808/clinexphealthsci.1071585

Clinical and Experimental Health Sciences

Complementary and Alternative Medicine Uses of Individuals Diagnosed with Chronic Diseases

Didem Oktar[1], Selma Metintaş[1], Muhammed Fatih Onsuz[1], Ece Elif Öcal[2], Seval Çalışkan Pala[3]

[1] Eskişehir Osmangazi University, School of Medicine, Department of Public Health, Eskişehir, Türkiye.
[2] Uşak University, School of Medicine, Department of Public Health, Uşak,Türkiye.
[3] Eskişehir Provincial Health Directorate, Eskişehir, Türkiye.

Correspondence Author: Didem Oktar
E-mail: ddmoktar@hotmail.com

Received: 07.03.2022 **Accepted:** 26.09.2022

ABSTRACT

Objective: The aim of the study was to evaluate the use of CAM practices by individuals who have been admitted to health institutions and have been diagnosed with chronic diseases.

Methods: Patients with a diagnosis of chronic disease were included in this cross-sectional observational study. The use of 15 CAM modalities were evaluated in accordance with the Traditional and Complementary Medicine Regulations. Logistic regression analysis was further performed the association between CAM use and related factors.

Results: The mean age of 692 people in the study group was 50.6±13.6 years. In this study, the frequency of CAM use was found to be 37.1%. Univariate and age adjusted univariate logistic regression analysis performed to determine the correlation of the use of CAM practices with respect to sociodemographic variables and health-related variables revealed that being a woman, admitting to faculty of medicine, not having an income-generating job and living in an extended family were found to be positive predictors of CAM use.

Conclusion: The fact that one out of every three patients diagnosed with a chronic disease and who have admitted to a health care institution had already experienced CAM practices indicate that the orientation towards CAM practices is quite high. Further comprehensive research is necessary with regard to planning the integration of CAM practices into health services.

Keywords: Complementary therapies, chronic disease, prevalence

1. INTRODUCTION

Gradually increasing incidence of the chronic diseases as well as the fact that they trigger 7 out of 10 every death case in the world and account for a significant portion of the Disability-Adjusted Life Years (DALY) make chronic diseases an important public health priority (1, 2). Recent decrease in fertility rates and the increase in the prevalence of chronic diseases exposed by the aging population due to the prolongation of the life expectancy at birth impose a significant burden on the health system and may lead individuals to seek different treatments (2, 3). Accordingly, health care professionals assume significant roles in the evidence-based delivery of health services.

Complementary and Alternative Medicine (CAM) practices, which are based on beliefs and experiences specific to different cultures, are referred to in order to protect health, prevent, diagnose and treat physical and mental illnesses (4). The World Health Organization has developed and launched the 2014-2023 Traditional Medicine Strategy in response to the World Health Assembly resolution on traditional medicine. The strategy aims to support Member States in developing proactive policies and implementing action plans that will strengthen the safe and effective use of traditional medicine by conducting research on integrating traditional medicine products into health systems to exploit the potential contribution of traditional medicine to human-centered health care services (5). This strategy document is the main document explaining the current global situation and priorities for CAM and constitutes a road map for further research on CAM (6). The Regulation Governing CAM practices in Turkey was introduced in 2014. This regulation, stipulating the purpose, scope and the law constituting the legal grounds stipulates the qualifications of practitioners and medical institutions to practice CAM and clarifies the methods to be applied in different diseases (7).

There are a wide range of CAM practices depending on the strength of the cultural influence, the structure of the health

DOI: 10.33808/clinexphealthsci.1185236

care system and the current regulations in the country (8). Besides, an increasing number of health professionals in various parts of the world are doing research on issues related to the safety, effectiveness, quality and accessibility of different CAM practices (5). High-quality evidence have been obtained in the increasing number of randomized controlled research studies published in recent years. Accordingly, the interest towards CAM practices has been scaling up for reasons such as meeting well-being needs of people with chronic diseases which could not yet been met with in the current health system (9,10). Other reasons why these practices are gaining more and more space in the delivery of health care services are considered as cultural identity, a holistic healing approach and closer communication between patient-practitioner (11). On the other hand, it should be noted that the use of CAM practices which is not based on scientific evidence, may cause serious health problems that can lead to death.

The aim of the study was to evaluate the use of CAM practices by individuals who have been admitted to health institutions and have been diagnosed with chronic diseases.

2. METHODS

2.1. Study Design, Setting and Population

This cross-sectional research was conducted between March – June 2019 with individuals who had been diagnosed with chronic diseases, applied to the health institutions for treatment.

Eskişehir province consists of two central and twelve semi-rural/rural districts and has a population of 887,475 in accordance with 2019 TURKSTAT data. Male individuals make up 49.9% of the population, 78.5% are 18 years of age or older and 88.3% live in the city center (12). There is one Level 3 (Tertiary) and two Level 2 (Secondary) hospitals in Eskişehir city center. In addition, 74 Family Health Centers (FHC) (56 FHCs in the provincial center and 18 FHCs in the semi-rural areas) provide primary health care services (13).

2.2. Sample Size

A two-step sampling method was used in the study. At the first stage, the health institutions included in the study were selected via cluster sampling, which is one of the Probabilistic Sampling Methods. Accordingly, a total of five clusters were formed: three clusters were drawn up for Family Health Centers (FHC), which provide primary health care services, one each from two central districts and one from the semi-rural district in addition to one cluster each for Level 2 (secondary) and Level 3 (Tertiary) health institutions. In case there is more than one health care institutions in any level of health care institutions, a simple, random sampling method with a closed envelope was used. At the next stage of the study, a non-probabilistic sampling method was used and individuals aged 18 years and over, who admitted to the selected health institutions for treatment and who had provided their consent to participate in the research were included. As the number of patients who would admit to health institutions for treatment throughout the term of our research cannot be accurately estimated, we used the sample size calculation formula for unknown population. The sample size was calculated, taking into consideration 25% as the percentage of using CAM practices in accordance with the findings of similar studies conducted earlier, accepting 95% of confidence interval, a 5% margin of error and a pattern effect of '2' (14). Minimum sample size was calculated to be at least 580, with 116 individuals from each cluster and the study group consisted of 692 individuals.

2.3. Data Collection

Consecutive patients who admitted to the designated health institutions throughout the term of the study and who agreed to participate in the survey were informed about the subject and purpose of the study; subsequently their verbal consent was obtained. The question of whether the participant had any chronic disease was resolved by asking 'Have you been diagnosed with a chronic disease that requires constant use of any medication?'. Multimorbidity and comorbidity were not questioned. Having used GETAT applications at least one time in a life period was counted as using these applications. The questionnaire was filled out personally by the participants under observation during a face-to-face interview. This procedure took about 10-15 minutes. Individuals who did not agree to participate in the study, who had insufficient cognitive functions or who could not be communicated were excluded from the research.

The questionnaire, which was prepared by the researchers as a data collection tool by referring to the literature, consists of 2 sections (15-16). Section 1 included questions on sociodemographic variables, health-related variables and questions that are considered to be related with the use of CAM practices whereas Section 2 included questions asking the participants whether they have heard of, used or wanted to have further information about 15 CAM practices included in the regulation.

2.4. Statistical Analysis

Data collected were statistically evaluated with the SPSS (v15.0) statistical software. Descriptive statistics of all the data in the study were provided in numbers and percentages. Chi-square test was used to compare the categorical variables between the groups. The relation of the use of CAM practices, which is a dependent variable, with respect to significant independent variables of the Chi-Square test was examined by univariate and age-adjusted univariate Logistic Regression Analysis.

2.5. Ethics Approval

The research was initiated upon receiving the approval of the Non-Interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University dated 15.02.2019 and numbered 25403353-050.99-E.20.484 and the necessary administrative permits of relevant health care institutions.

3. RESULTS

The ages of 692 individuals who made up the study group ranged between 18 to 87 and the mean age was 50.6±13.6 years. Chronic diseases of the individuals included in the study group are exhibited in Table 1.

Table 1. *Chronic diseases of the individuals included in the study group*

Chronic Diseases	n	%
Hypertensive disorders	186	26.9
Impaired glucose regulation and diabetes mellitus	163	23.6
Diseases of the thyroid gland	81	11.7
Chronic lower respiratory tract diseases	52	7.5
Diseases of the musculoskeletal system and connective tissue	39	5.6
Ischemic heart diseases	33	4.8
Diseases of the nervous system	32	4.6
Mental and behavioral disorders	31	4.5
Malignant neoplasms	20	2.9
Benign diseases of the esophagus, stomach and duodenum	11	1.6
Other	44	6.4
Total	692	100.0

Most common chronic disease that adversely affect individuals' daily live was reported as hypertensive disorders (26.9%).

The results of the univariate and age adjusted univariate logistic regression analysis performed to determine the frequency of the use of CAM practices in the study group and related factors are exhibited in Table 2.

Research findings indicated the frequency of CAM use as 37.1% (n:257). Univariate and age adjusted univariate logistic regression analysis performed to determine the correlation of the use of CAM practices with respect to sociodemographic variables and health-related variables revealed that being a woman, admitting to faculty of medicine, not having an income-generating job and living in an extended family were found to be positive predictors of CAM use.

Individuals who want CAM practices to be prescribed by a physician and to be applied within a health institution were found to be more likely to use CAM practices (p<0.001). The individuals in the study group stated that they wanted CAM practices to be applied within the family health centers (40.9%).

Responses of the participants regarding whether they have heard of, used or wanted to have further information about CAM practices included in the regulation are exhibited in Figure 1.

Table 2. *The results of the univariate and age adjusted univariate logistic regression analysis performed to determine the frequency of the use of CAM Practices in the study group and related factors*

Sociodemographic Variables and Health-related Variables		Use of CAM practices n (%)	Univariate OR (95% CI)	Age Adjusted Univariate OR (95% CI)
Age Group	18-39	33 (12.8)	1	
	40-60	155 (60.3)	2.06 (1.32-3.20)[b]	
	61 and above	69 (26.9)	1.95 (1.19-3.21)[b]	
Gender	Male	88 (34.2)	1	1
	Female	169 (65.8)	1.55 (1.12-2.12)[b]	1.69 (1.22-2.34)[b]
Place of Referral	Level 2	30 (11.7)	1	1
	Level 1	151 (58.8)	1.65 (1.04-2.61)[a]	1.51 (0.95-2.41)
	Level 3	76 (29.5)	2.53 (1.51-4.25)[c]	2.43 (1.45-4.10)[b]
Marital Status	Single	17 (6.6)	1	1
	Married	180 (70.1)	1.98 (1.12-3.51)[a]	1.55 (0.81-2.99)
	Widow/ Divorced	60 (23.3)	2.47 (1.31-4.67)[b]	1.84 (0.88-3.87)
Income Generating Job	Yes	144 (56.0)	1	1
	No	113 (44.0)	1.42 (1.04-1.94)[a]	1.45 (1.05-1.98)[a]
Type of Family	Extended family	58 (22.6)	1.80 (1.02-3.18)[a]	1.86 (1.05-3.28)[a]
	Nuclear Family	172 (66.9)	1.35 (0.83-2.20)	1.49 (0.91-2.45)
	Single Parent Family	27 (10.5)	1	1
Alcohol Consumption	Yes	25 (9.7)	1	1
	No	232 (90.3)	1.66 (1.02-2.71)[a]	1.50 (0.91-2.46)
	Total	257 (100.0)		

a: p<0.05, b: p<0.01, c: p<0.001
OR: Odds ratio, CI: Confidence interval, CAM: Complementary and alternative medicine, Level 1: Family Health Centers (FHC), Level 2: State Hospital, Level 3: Faculty of Medicine

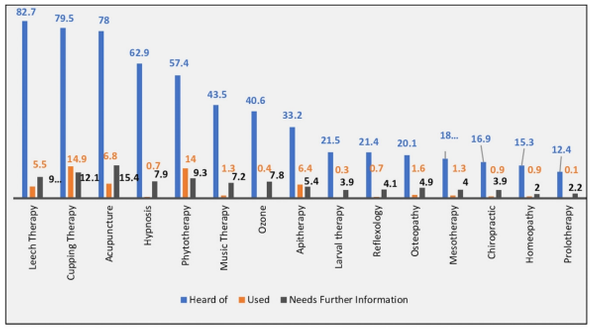


Figure 1. *Responses of the participants regarding whether they have heard of, used or wanted to have further information about complementary and alternative medicine practices included in the regulation. *Percentages are calculated based on the responses.*

The most CAM practice frequently heard of in the study group was Leech Therapy (82.7%), the most commonly used CAM practice was found to be Cupping Therapy (14.9%). Acupuncture (15.4%), Cupping Therapy (12.1%) and Leech Therapy (9.9%) were the top three CAM practices that the participants wanted to learn more about.

The first three most commonly used CAM practices in the presence of chronic diseases were compared with the chi-square test for each chronic disease with respect to the preference of the individuals diagnosed with and without the disease. The frequency of using the top 3 CAM in the presence of chronic diseases on the basis of chronic diseases is exhibited in Table 3.

Table 3. *The frequency of using the top 3 CAM in the presence of chronic diseases on the basis of chronic diseases*

Chronic Diseases	CAM Practice Used n (%)		
	Cupping Therapy n (%)	Phytotherapy n (%)	Acupuncture n (%)
Hypertensive disorders	24 (12.9)	16 (8.6)*	14 (7.5)*
Impaired glucose regulation and diabetes mellitus	21 (12.9)	29 (17.8)	8 (4.9)
Diseases of the thyroid gland	10 (12.3)	15 (18.5)	7 (8.6)
Chronic lower respiratory tract diseases	5 (9.6)	3 (5.8)*	2 (3.8)
Diseases of the musculoskeletal system and connective tissue	9 (23.1)	7 (17.9)	2 (5.1)
Ischemic heart diseases	6 (18.2)	4 (12.1)	3 (9.1)
Diseases of the nervous system	10 (31.3)**	5 (15.6)	5 (15.6)
Mental and behavioral disorders	6 (19.4)	2 (6.5)	2 (6.5)
Malignant neoplasms	1 (5.0)	4 (20.0)	1 (5.0)
Benign diseases of the esophagus, stomach and duodenum	2 (18.2)	3 (27.3)	2 (18.2)

CAM: Complementary and alternative medicine
** CAM Practice is more frequently used by individuals who do not have the related chronic disease (p<0.05).*
*** CAM Practice is more frequently used by individuals who have the related chronic disease (p<0.05).*

Phytotherapy was found out to be used frequently by individuals without any chronic lower respiratory tract disease and hypertensive disorder, whereas Acupuncture was found out to be used by individuals who did not have hypertensive disorders. Cupping Therapy was found out to be used frequently by individuals with diseases of the nervous system.

Most common reasons underlying the individuals' application to CAM practices were reported as rheumatic complaints (37.1%). The reasons of individuals in the study group applying to CAM practices and their frequency of application are exhibited in Figure 2.

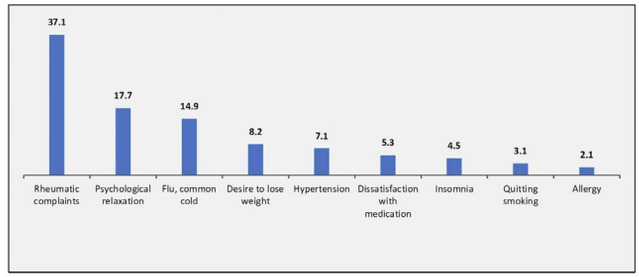


Figure 2. *The reasons of individuals in the study group applying to complementary and alternative medicine practices and their frequency of application. *Percentages are calculated based on the responses.*

The reasons underlying the perceptions of individuals in the study group towards CAM practices are exhibited in Table 4.

Table 4. *The reasons underlying the perceptions of individuals in the study group towards CAM Practices*

Reasons for Using CAM Practices	n=417*	%
There is no medical treatment for my disease/disorder	89	21.4
Interest	71	17.0
Recommendations of the Health care Professional	70	16.8
These are economic interventions	63	15.1
Side effects of the medical treatment	56	13.4
Access to health care services is challenging	39	9.4
High expenses of the medical treatment	29	6.9
Reasons for not considering CAM Practices	**n=585****	**%**
I did not need	254	43.4
Lack of confidence on CAM	59	10.1
Worries about side effects	56	9.6
Doesn't believe in its benefits	49	8.4
Worries about possible harms	48	8.2
Practitioners are not experienced	35	6.0
Insufficient research	28	4.8
Lack of the practitioner's referral	23	3.9
Not covered by medical insurance	15	2.6
Expensive	10	1.7
Negative views of the family	8	1.3

CAM: Complementary and alternative medicine
**The figures were derived on the basis of multiple answers provided by the individuals who have used CAM Practices before.*
***The figures were derived on the basis of multiple answers provided by the individuals who have not used CAM Practices before.*

The most common reasons reported by 257 (37.1%) individuals in the study group for using CAM practices at any time of their lives were "There is no medical treatment for my disease/disorder" (21.4%), "interest" (17.0%) and "Recommendations of the Health care Professional" (16.8%). The most common reasons reported by 435 individuals (62.9%) in the study group for not considering CAM practices at any time of their lives were "I did not need" (43.4%),

"Uncertainty" (10.1%) and "Worries about side effects" (9.6%).

4. DISCUSSION

Reasons such as the aging population, the increasing prevalence of chronic diseases caused by inactivity and irregular nutrition, the inability of physicians to devote sufficient time to their patients due to the heavy burden on the health system, side effects of the drugs due to the use of multiple medications and new health needs brought about by the age has revealed the need to expand the scope of health services. Bearing the qualifications compatible with the cultural background of society, accompany the individuals to accept and frequently use of CAM practices. Having sufficient knowledge about CAM practices is accordingly suggested for the physicians to accurately guide patients' demands.

Most common reported chronic diseases within the scope of the study were found to be hypertensive disorders, impaired glucose regulation and diabetes mellitus and diseases of the thyroid gland, respectively. Most common diseases reported in the study conducted in Hong Kong were cardiovascular, musculoskeletal and diabetes (17). Most common diseases reported in the study conducted in Ireland were hypertension and hyperlipidemia (18). Different results obtained may have been attributed to the age and gender differences of the participants. Research findings indicated the frequency of CAM use as 37.1%. In the studies conducted on patients diagnosed with chronic diseases in Australia, Bangladesh and India, the frequency of CAM use was reported as 32-38% (10,19,20). Another study reported that CAM was used by 44.0% of participants during the COVID-19 pandemic (17). The frequency of CAM use by hypertensive patients in Nigeria was found to be 29% (21). This figure is noted as 33-46% for diabetic patients from different countries (19,22,23). It was reported as 87% for patients diagnosed with cancer in the United States (24). Accordingly, the frequency of CAM use was concluded to be similar with the literature.

Research findings indicated that CAM use was rare in single patients under the age of 40, admitted to a Level 2 health facility and who consumed alcohol. Furthermore female participants who lived in extended families and who did not have an income generating job were found to be less frequently using CAM. After evaluating the effect of the age variable; being female, having admitted to a tertiary health facility, not working in an income-generating job and living in an extended family were found to be positively related variables with using CAM. In a study conducted with adults diagnosed with a chronic disease in Australia, it was reported that regular use of CAM is more frequent in females aged ≥ 60 years and with a low-income (25). Another research conducted in Pakistan on patients diagnosed with Type 2 diabetes revealed that CAM use was frequent among females, divorced individuals, unemployed and illiterate individuals (26). In the study conducted in Saudi Arabia, being over the age of 50, being unemployed and knowing the advantages of CAM practices were mentioned as effective variables with regard to the use of them (27). Another research conducted in Turkey revealed that the use of CAM practices are associated with female gender, being married, not working in an income-generating job, low income and high levels of education (28). In studies conducted in Malaysia and Turkey, the use of CAM practices has been reported to be more frequent within individuals with family members who have experienced CAM before (15,29). Another research conducted in Turkey revealed no correlation between smoking habit and alcohol consumption and CAM use (30). Considering that individuals who have admitted to a faculty of medicine have not received a definitive treatment for their suffering or have multiple health needs, it is expected that they will seek different treatments. The presence of individuals who have previously experienced CAM practices in an extended family is expected to reduce family members' prejudices about the issue and raise awareness.

It is important to prevent the public from applying to people who are not experts about GETAT practices and to provide equal and accessible health services to everyone.

During the research, individuals who prefer CAM practices to be prescribed by a physician and to be applied within a health institution were found to be more likely to use CAM practices. Accordingly, the participants stated that they wanted CAM practices to be applied within the family health centers. Similar result was reported in the previously conducted a study (31).

In this research, it was concluded that the most commonly heard CAM practices are Leech Therapy, Cupping Therapy and Acupuncture however, the most commonly used CAM practices are Cupping Therapy, Phytotherapy and Acupuncture. In studies conducted on the general population in the United States and Malaysia, most frequently used CAM practices were indicated as biological-based applications (plant-based products, vitamins and supplements) (24,32). The research conducted in Saudi Arabia revealed the most commonly used CAM practices as herbs, wet cupping and vitamin-minerals (27). The research conducted in Brazil noted that the use of CAM practices varies depending on the income status and it was reported that individuals with a high income level tend to use acupuncture and homeopathy whereas individuals with a lower income tend to refer to herbal medication and medicinal plants (33). It is observed in studies that the most frequently used CAM practices differ, however herbal products occupy the first place in majority of these studies and individuals tend to use and experience the practices that they know or have heard about. In addition, being cost-effective and easily accessible may also be important reasons underlying preference.

Research on the use of CAM practices in diagnosed diseases are limited. This research indicated that Cupping Therapy has frequently been applied to individuals with diseases of the nervous system and Phytotherapy and Acupuncture have frequently been applied to individuals without hypertensive disorders. In a study conducted in Canada it was reported that chiropractic has frequently been applied to individuals

diagnosed with migraine and asthma whereas acupuncture and reflexology have frequently been applied to individuals diagnosed with diabetes (34). In a study conducted in India it was reported that ayurveda has frequently been applied to individuals diagnosed with epilepsy, rheumatoid arthritis and HIV whereas natural remedies prepared at home has frequently been applied to individuals diagnosed with diabetes (20).

Research indicated that the most common reasons for applying to CAM practices are rheumatic complaints, the need for psychological relief and flu-colds. A research conducted in the Germany indicated that the most common reasons for applying to CAM practices are flu infection, sleeping disturbances and musculoskeletal issues (35). Another research conducted in Iran reported that 23% of the participants referred to CAM practices due to digestive system diseases, 14.4% due to colds and 13.2% due to migraines (36). In a study conducted with patients with cancer in Uganda it was reported that reasons for referring to CAM practices are cancer treatment, strengthening the immune system and relieving pain (37). Rheumatic complaints that persist for a long time, reducing the quality of life, may lead a person to seek different treatments. However, dissatisfaction from medical treatment and believing that some complaints are not important enough to require a doctor's referral may have caused these results.

The cultural viewpoint of the society, the level of knowledge of individuals about CAM practices and the inclusiveness of the health care system are important predictors affecting the orientation towards these practices. Research findings indicated most common reasons for applying CAM practices were believing that there is no medical treatment for the disease/disorder, interest and recommendations of the Health care Professional, while the most common reasons for not considering CAM practices were lack of need, uncertainty and worries about side effects. Another research conducted in Czechia indicated that individuals have more often referred to CAM practices to prevent and treat diseases (38). Similar results were reported in another research conducted in Zimbabwe (39). Another research conducted in Saudi Arabia on patients diagnosed with Type 2 diabetes indicated that the most common reasons for applying to CAM practices are less side-effects, benefits for controlling diabetes, being cost-effective and ease of access (27). Several other research conducted in Turkey indicated that the most common reasons for applying to CAM practices are need for relief, treatment, seeking support for current treatment, complaints about insufficient benefit from medical treatment, inability to access health care providers and medical treatment for reasons such as distance, desperation related to chronic diseases that are inadequately treated with current medical methods and no worry about possible harms/disadvantages/damages (15,40). In another research participants reported the reasons underlying their indifference towards CAM practices as not believing in their benefits and worries about possible harms/disadvantages/damages (15). Most common reasons underlying individuals' orientation towards CAM practices were observed to be disease/disorder related reasons such as considerations with regard to ineffective medical treatment and worries about side effects of the medical treatment. The findings, in particular, suggest the need for a thorough questioning of the medical history of the patient in the presence of chronic diseases that require long-term treatment and follow-up as well as the need for further information about CAM practices.

5. CONCLUSION

For the purpose of health-related decisions, it is important to take into consideration the perceptions and orientations of individuals with regard to CAM practices. Most commonly reported chronic diseases within the scope of the study were hypertension, diabetes and thyroid gland dysfunction. Research findings indicated the frequency of CAM use as 37.1%. Variables such as being female, having admitted to a tertiary health facility, not working in an income-generating job and living in an extended family were found to be more likely to affect CAM use. Most frequently used CAM practices were Cupping Therapy, Phytotherapy and Acupuncture.

The fact that one out of every three patients diagnosed with a chronic disease and who have admitted to a health care institution had already applied CAM practices indicate that the orientation towards CAM practices is quite positive. Allowing CAM practices to be applied within the health care institutions, ensuring that they are prescribed by physicians and are covered by insurance policies are considered to improve their reliability and acceptability and shall ensure health care professionals to have control over preventing possible complications. In addition, we think that health care professionals should able to advise their patients when to/when not to apply CAM practices and that they should have received adequate training to apply them when necessary.

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

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

Ethics Committee Approval: *This study was approved by Non-Interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University dated 15.02.2019 and numbered 25403353-050.99-E.20.484.*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: DO, SM
Design of the study: DO, SM, MFO
Acquisition of data for the study: DO, SM, EEO
Analysis of data for the study: DO, SM, MFO, EEO
Interpretation of data for the study: MFO, EEO, SÇP
Drafting the manuscript: DO, SM, MFO, EEO, SÇP
Revising it critically for important intellectual content: DO, SM,
Final approval of the version to be published: DO, SM, MFO, EEO, SÇP

REFERENCES

[1] World Health Organization, Global Health Estimates, Leading Causes of Death and Disability, A Visual Summary of Global

and Regional Trends 2000-2019. Accessed [19 March 2022]. https://www.who.int/data/stories/leading-causes-of-death-and-disability-2000-2019-a-visual-summary

[2] The Lancet Infographics Global Burden of Disease 2019. Accessed [30 September 2021]. https://www.thelancet.com/infographics/gbd-2019

[3] World Health Organization. Global Status Report on Noncommunicable Diseases 2014. Accessed [2 February 2020]. https://www.who.int/publications/i/item/978.924.1564854

[4] World Health Organization. Traditional, Complementary and Integrative Medicine. Overview. Accessed [2 February 2020]. https://www.who.int/health-topics/traditional-complementary-and-integrative-medicine#tab=tab_1

[5] World Health Organization. WHO Traditional Medicine Strategy: 2014-2023. Accessed [3 February 2020]. https://www.who.int/publications/i/item/978.924.1506096

[6] Burton A, Smith M, Falkenberg T. Building WHO's Global strategy for traditional medicine. Eur J Integr Med. 2015;7(1):13-5. DOI: 10.1016/j.eujim.2014.12.007

[7] Mevzuat Bilgi Sistemi. Geleneksel ve Tamamlayıcı Tıp Uygulamaları Yönetmeliği. Accessed [6 February 2020]. https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=20164&MevzuatTur=7&MevzuatTertip=5

[8] Ung COL, Harnett J, Hu H. Community pharmacist's responsibilities with regards to traditional medicine/complementary medicine products: A systematic literature review. Res Social Adm Pharm. 2017;13(4):686-716. DOI: 10.1016/j.sapharm.2016.08.001

[9] Wode K, Henriksson R, Sharp L, Stoltenberg A, Hök Nordberg J. Cancer patients' use of complementary and alternative medicine in Sweden: A cross-sectional study. BMC Complement Altern Med. 2019;19(1):1-11. DOI: 10.1186/s12906.019.2452-5

[10] Foley H, Steel A, McIntyre E, Harnett J, Sibbritt D, Wardle J, Adams J. Complementary medicine practitioner consultations amongst 1,314 individuals with chronic conditions: Characteristics of users, reasons for and predictors of use. Complement Ther Clin Pract. 2020;40:101194. DOI: 10.1016/j.ctcp.2020.101194

[11] Gyasi R, Buor D, Adu-Gyamfi S, Adjei PO-W, Amoah PA. Sociocultural hegemony, gendered identity, and use of traditional and complementary medicine in Ghana. Women Health 2018;58(5):598-615. DOI: 10.1080/03630.242.2017.1321608

[12] Türkiye İstatistik Kurumu, Adrese Dayalı Nüfus Kayıt Sistemi Sonuçları, Adrese Dayalı Nüfus Kayıt Sistemi İstatistikleri. Accessed [1 May 2020]. http://tuik.gov.tr/PreTablo.do?alt_id=1059

[13] T.C. Sağlık Bakanlığı Eskişehir İl Sağlık Müdürlüğü, Aile Sağlığı Merkezleri, Hastaneler. Accessed [1 May 2020]. https://eskisehirism.saglik.gov.tr/TR,39889/aile-sagligi-merkezleri.html

[14] Daye M, Durmaz K, Durduran Y. Traditional and complementary medicine practices in patients admitted to dermatology outpatient clinic. Konuralp Medical Journal 2020;12(2):247-252. DOI: 10.18521/ktd.639063

[15] Yıldız P, Fatih Ü, Balbay EG. Kronik obstrüktif akciğer hastalarında geleneksel ve tamamlayıcı tıp yöntemi kullanımının değerlendirilmesi. Online Turkish Journal of Health Sciences 2020;5(1):147-54. DOI: 10.26453/otjhs.638683

[16] T.C. Resmi Gazete, Sağlık Bakanlığı Geleneksel Ve Tamamlayıcı Tıp Uygulamaları Yönetmeliği. Accessed [16 August 2020]. https://www.resmigazete.gov.tr/eskiler/2014/10/20141027.pdf

[17] Lam CS, Koon HK, Chung VCH, Cheung YT. A Public survey of traditional, complementary and integrative medicine use during the COVID-19 outbreak in Hong Kong. PloS One 2021;16(7): 1-15. DOI: 10.1371/journal.pone.0253890

[18] Kearns K, Dee A, Fitzgerald AP, Doherty E, Perry IJ. Chronic disease burden associated with overweight and obesity in Ireland: The effects of a small BMI reduction at population level. BMC Public Health 2014;14(1):1-10. DOI: 10.1186/1471-2458-14-143

[19] Shahjalal M, Chakma SK, Ahmed T, Yasmin I, Mahumud RA, Hossain A. Prevalence and determinants of using complementary and alternative medicine for the treatment of chronic illnesses: A multicenter study in Bangladesh. PLoS One 2022;17(1): 1-13 DOI: 10.1371/journal.pone.0262221.

[20] Bhalerao MS, Bolshete PM, Swar BD, Bangera TA, Kolhe VR, Tambe MJ, Wade MP, Bhowate SD, Sonje UB, Gogtay NJ, Thatte UM. Use of and satisfaction with complementary and alternative medicine in four chronic diseases: A Cross-sectional study from India. Natl Med J India 2013;26(2):75-77.

[21] Osamor PE, Owumi BE. Complementary and alternative medicine in the management of hypertension in an urban Nigerian community. BMC Complement Altern Med 2010;10(1):1-9. DOI: 10.1186/1472-6882-10-36

[22] Ogbera AO, Dada O, Adeleye F, Jewo PI. Complementary and alternative medicine use in diabetes mellitus. West Afr J Med. 2010;29(3):158-162 DOI: 10.4314/wajm.v29i3.68213

[23] Radwan H, Hasan H, Hamadeh R, Hashim M, AbdulWahid Z, Hassanzadeh Gerashi M, Al Hilali M, Naja F. Complementary and alternative medicine use among patients with type 2 diabetes living in the United Arab Emirates. BMC Complement Med Ther. 2020;20(1):216. DOI: 10.1186/s12906.020.03011-5.

[24] Judson PL, Abdallah R, Xiong Y, Ebbert J, Lancaster JM. Complementary and alternative medicine use in individuals presenting for care at a comprehensive cancer center. Integr Cancer Ther. 2017;16(1):96-103. DOI: 10.1177/153.473.5416660384

[25] Armstrong AR, Thiébaut SP, Brown LJ, Nepal B. Australian adults use complementary and alternative medicine in the treatment of chronic illness: A national study. Aust N Z J Public Health 2011;35(4):384-390. DOI: 10.1111/j.1753-6405.2011.00745.x

[26] Raja R, Kumar V, Khan MA, Sayeed KA, Hussain SZM, Rizwan A. Knowledge, attitude, and practices of complementary and alternative medication usage in patients of type II diabetes mellitus. Cureus 2019;11(8):e5357 DOI: 10.7759/cureus.5357

[27] Al-Eidi S, Tayel S, Al-Slail F, Qureshi NA, Sohaibani I, Khalil M, Al-Bedah AM. Knowledge, attitude, and practices of patients with type II diabetes mellitus towards complementary and alternative medicine. J Integr Med. 2016;14(3):187-196. DOI: 10.1016/S2095-4964(16)60244-3

[28] Mollaoğlu M, Aciyurt A. Use of complementary and alternative medicine among Patients with Chronic Diseases. Acta Clin Croat. 2013;52(2.):181-188.

[29] Hasan SS, Ahmed SI, Bukhari NI, Loon WCW. Use of complementary and alternative medicine among patients with chronic diseases at outpatient clinics. Complement Ther Clin Pract. 2009;15(3):152-157. DOI: 10.1016/j.ctcp.2009.02.003

[30] Demirci GT, Altunay IK. Use of complementary and alternative medicine and assessment of dermatology quality of life index among dermatology outpatients: A cross-sectional comparative study. Br J Med Med Res. 2014;4(9):1812-1820 DOI: 10.9734/BJMMR/2014/6924

[31] Ee C, Templeman K, Grant S, Avard N, de Manincor M, Reath J, Hunter J. Informing the model of care for an academic integrative health care center: A qualitative study of primary care stakeholder views. J Altern Complement Med. 2020;26(4):300-315. DOI: 10.1089/acm.2019.0321

[32] Siti ZM, Tahir A, Farah AI, Fazlin SA, Sondi S, Azman AH, Maimunah AH, Haniza MA, Siti Haslinda MD, Zulkarnain AK, Zakiah I, Zaleha WW. Use of traditional and complementary medicine in Malaysia: A baseline study. Complement Ther Med. 2009;17(5-6):292-299. DOI: 10.1016/j.ctim.2009.04.002

[33] de Moraes Mello Boccolini P, Siqueira Boccolini C. prevalence of complementary and alternative medicine (CAM) use in Brazil. BMC Complement Med Ther. 2020;20(1):1-10. DOI: 10.1186/s12906.020.2842-8

[34] Metcalfe A, Williams J, McChesney J, Patten SB, Jetté N. use of complementary and alternative medicine by those with a chronic disease and the general population-Results of a national population based survey. BMC Complement Altern Med. 2010;10(1):1-6. DOI: 10.1186/1472-6882-10-58

[35] Welz AN, Emberger Klein A, Menrad K. Why people use herbal medicine: Insights from a focus-group study in Germany. BMC Complement Altern Med. 2018;18(1):1-9. DOI: 10.1186/s12906.018.2160-6

[36] Adib-Hajbaghery M, Hoseinian M. Knowledge, attitude and practice toward complementary and traditional medicine among Kashan Health Care Staff, 2012. Complement Ther Med. 2014;22(1):126-132. DOI: 10.1016/j.ctim.2013.11.009

[37] Mwaka AD, Mangi SP, Okuku FM. Use of traditional and complementary medicines by cancer patients at a national cancer referral facility in a low-income country. Eur J Cancer Care (Engl). 2019;28(6):e13158. DOI: 10.1111/ecc.13158

[38] Pokladnikova J, Selke-Krulichova I. Prevalence of complementary and alternative medicine use in the general population in the Czech Republic. Forsch Komplementmed. 2016;23(1):22-28. DOI: 10.1159/000443712

[39] Chingwaru W, Vidmar J. Prevalence, perceptions and factors influencing the use of traditional and complementary medicine (T&CM) in Zimbabwe's adult population: The case of Bindura District. Eur J Integr Med. 2016;8(4):484-493. DOI: 10.1016/j.eujim.2016.03.007

[40] Şimşek B, Aksoy DY, Basaran NC, Taş D, Albasan D, Kalaycı MZ. Mapping traditional and complementary medicine in Turkey. Eur J Integr Med. 2017;15:68-72. DOI: 10.1016/j.eujim.2017.09.006

How to cite this article: Oktar D, Metintaş S, Onsuz MF, Öcal EE, Çalışkan Pala S. Complementary and Alternative Medicine Uses of Individuals Diagnosed with Chronic Diseases. Clin Exp Health Sci 2023; 13: 184-191. DOI: 10.33808/clinexphealthsci.1185236

Clinical and Experimental Health Sciences

Bergen Insomnia Scale for Adults: The Psychometric Features of the Turkish Version

Tuba Bay Kula[1], İlknur Yalçın[2], Ayşe Ergün[3]

[1] Haydarpaşa Numune Training and Research Hospital İstanbul, Türkiye / Marmara University, Institute of Health Sciences, Public Health Nursing Department, İstanbul, Türkiye.
[2] UNILEVER, İstanbul, Türkiye / Marmara University, Institute of Health Sciences, Public Health Nursing Department, İstanbul, Türkiye.
[3] Marmara University, Faculty of Health Science, Public Health Nursing Department, İstanbul, Türkiye.

Correspondence Author: Tuba Bay Kula
E-mail: tuba_bay@hotmail.com
Received: 12.03.2022 **Accepted:** 20.01.2023

ABSTRACT

Objective: Insomnia is an important health problem affecting physical, spiritual and social well-being of individuals negatively and it should be early diagnosed as well. The purpose is to appraise the psychometric features of the Turkish version of the Bergen Insomnia Scale (BIS) for adults.

Methods: A total of 495 adults were included in the methodological study in Turkey. Turkish form of BIS was designed within the scope of study conducted in adolescent sample. Construct validity was appraised with exploratory, confirmatory factor analysis, convergent and discriminant validity. Internal-consistency and test-retest analysis were used for reliability.

Results: According to results of explanatory, confirmatory factor analysis; it was identified that BIS showed a two-factor structure as daytime symptoms (α=.85) and nocturnal symptoms (α=.80). Item-total correlations were found as ≥.59 and test-retest correlation as .83.

Conclusions: The Turkish version of the Bergen Insomnia Scale for adults was assessed as valid and reliable.

Keywords: Insomnia, reliability and validity, adult

1. INTRODUCTION

Insomnia is an important health problem effecting physical, spiritual and social well-being of individuals negatively. Insomnia is being described a sleep-wake disorder in International Classification of Sleep Disorder (ICSD-3) and The Diagnostic and Statistical Manual of Mental Disorders (DSM-5). According to ICSD-3 and DSM-5 diagnostic measures, sleep-wake disorder is characterized by starting sleep difficulty, continue sleep difficulty and early waking up in the morning symptoms being presented at least three months and three times a week as well as dissatisfaction of the person with the quality of sleep. Such dissatisfaction is accompanied by disturbance and interference at day functions (1,2).

In literature, it was stated that majority of individuals having sleep disorder were not diagnosed medically. According to a study carried out in France, it was demonstrated that 53% of the people suffering from heavy insomnia and only 27% of people with rarely experiences sleep difficulties go to the doctor for such a problem (3). Such a result means that insomnia should be defined properly for the whole society. Variety of self-report questionnaire exist in literature in order to assess insomnia. Pallesen et al. (4) developed Bergen Insomnia Scale (BIS) within the basis of DSM-4 insomnia diagnosis criteria. Additionally, this scale meet diagnosis criteria of DSM-5.

According to DSM-4, the first four question of the scale met criteria A and the last two ones met criteria B. Latest studies expressed that 5 items of BIS might be used to identify insomnia regarding DSM-5 criteria. The first 3 questions of the scale met criteria A and the last two ones met criteria B. Scoring 3+ from at least one of the "A" criteria, additionally scoring 3+ from criteria "B" is defined as insomnia. According to DSM-5, if insomnia symptoms are lasted more than 3 months, it is defined as chronic insomnia, between 1-3 months as episodic insomnia, less than 1 month as acute insomnia (5). BIS is considered to define acute insomnia if assessed in terms of DSM-5 since it questioned only the last month (6,7).

The scale consists of six items and it is suitable for epidemiological studies and surveys since it is brief and easy-to-implement. Unlike other scales, BIS use 30 min cut-off value which has been suggested as clinical marker to measure time period to fall asleep and stay awake at night. In addition, the

DOI: 10.33808/clinexphealthsci.1084321

scale utilizes number of days experienced a sleep problem instead of expressions like “never” or “sometimes” used by many other scales (4). The scale was used in many studies having various age and sample groups to define insomnia based on self-report strategy (8-12).

The purpose of present study is to appraise the psychometric features of the Turkish version of BIS among the adults.

2. METHOD

2.1. Sample and Setting

This was a methodological study was carried out in March 2018 with adults working in a Fast-Moving Consumer Goods Company in 4 different provinces of Turkey. Instead of sample selection, the study was carried out on a volunteer basis. It was recommended in scale adaptation studies that 10-20 participants should be involved for each item of the scale (13). The study was completed with 495 (41.6%) volunteer adults by taking it into consideration. Two weeks following initial data collection, 108 of the participants were retested.

2.2. Data Collection

The required data were collected with descriptive questionnaire and BIS via e-mail basing on self-report. Descriptive questionnaire consists of 5 questions containing overall information about age, gender, education, marital status and employment status. Since the purpose of present study was to test the validity and reliability of the scale in Turkish, only basic socio-demographic questions were used. The questionnaires were web-based using an online platform belonging to the company and were sent to the employees via e-mail. In a preliminary section, the purpose of the study was introduced and the participants were inquired to give their consent for participation.

2.2.1. Bergen Insomnia Scale

BIS include 6 items in order to measure nocturnal and daytime symptoms of insomnia. Individuals indicated the number of having sleep disorder days in a week between 0 and 7; 8-point measurement in total. Psychometric analysis of original study of BIS were realized with three separate sample group as student, community and patient. In student and patient samples, two factor construct was detected whereas in community sample, single factor construct was identified. In original study, Cronbach’s alpha coefficient of community sample was .87 and test-retest reliability was .77 (4).

Turkish version of the BIS was developed by Bay and Ergun (14) and its language equivalency-cultural adaptation, content validity and pilot test were conducted with an adolescent sample group. In the study, content validity index was determined as .99; internal consistency as α= .72 and test-retest coefficient as r= .74. The BIS items are available in English and Turkish (See supplementary material 1).

2.3. Ethical Considerations

A written permission was obtained from the author who developed the scale, namely Pallesen via e-mail. Additionally, an approval was obtained from the authors who conducted the Turkish version of BIS in the adolescent sample. Ethical approval was obtained from University Ethical Committee (05.03.2018-86). In addition, written permission was obtained both from the institution and from authorities in order to practice the scale in the workplaces.

2.4. Data Analysis

By using SPSS 21 (Statistical Package for Social Sciences Inc, IL, USA) and LISREL 9.20 (Scientific Software International [SSI]) programs, the data were evaluated. Initially, data set was examined to investigate univariate and multivariate outliers, and normality. Univariate outliers were examined with standardized scores (Z). Excess 3.29 of scores Z is outliers and there are no outliers in our data set. The criterion for multivariate outliers was Mahalanobis distance at p< .001. Mahalanobis distance was evaluated as $\chi 2$ with degrees of freedom equal to the number of variables (15); in our data set, it was 6. According to $\chi 2(6)= 22.458$, 4 outliers was found and removed from data set. The univariate frequency distributions of all items in scale were examined, and both skewness and kurtosis values were found to be within normality. Furthermore, in adult sample; convergent validity, discriminant validity, confirmatory and explanatory factor analysis were utilized for construct validity of the scale and internal consistency and test-retest analysis for reliability of the scale.

2.4.1. Construct Validity

In order to ensure construct validity, the data set including 495 participants were divided into 2 groups randomly by statistic programmed as n_1=244 and n_2=251. The first group (n=244) was implemented explanatory factor analysis (EFA) in order to examine structural relations of the scale in Turkish culture. The second group (n=251) was used confirmatory factor analysis (CFA) to confirm the factor analysis results obtained in the first group to ensure cross-validation. In CFA, by using maximum likelihood (ML) method; chi square ($\chi 2$), degrees of freedom (df), The Root Mean Square Error of Approximation (RMSEA), Standardized Root Mean Square Residual (SRMR), fit/unfit coefficient values of comparative fit index were assessed together with relation of theoretical model’s data set.

Convergent validity and discriminant validity were evaluated by calculating composite reliability (CR), average variance extracted (AVE) and maximum shared variance (MSV) using the results obtained from CFA.

2.4.2. Reliability

As for reliability analysis; Cronbach's Alpha reliability coefficient and McDonald's omega (ω) coefficient were calculated to ensure internal consistency. Omega coefficient was calculated by OMEGA macro for SPSS (16). Test-retest was utilized to assess invariance in time and Pearson correlation coefficient was benefited to assess the relation between test-retest scores. Descriptive results were evaluated with standard deviation, average and percentage.

3. RESULTS

3.1. Descriptive Results

The mean ages of participants were 32.71±6.97 and 44.8% of them were females. 55.6% of them were bachelor graduate; 56.0% of them married and 65.1% of them were working shiftlessly (Table 1).

Table 1. *Socio-demographic Characteristic of Adults (N:495)*

Variables		Min. – Max.	M±Sd
Age (N=491)		19-58	32.71±6.97
		n	**%**
Gender	Female	222	44.8
	Male	273	55.2
Educational status	Secondary School	2	.4
	High School	136	27.5
	Bachelor graduate	275	55.6
	Postgraduate	82	16.6
Marital Status	Single	201	40.6
	Married	277	56.0
	Divorced	17	3.4
Employment Status	In Shift	173	34.9
	Shiftlessly	322	65.1

Min*.:Minimum,* ***Max****.:Maximum,* ***M****:Mean,* ***Sd****:Standard deviation*

3.2. Construct Validity-related Results

In order to ensure construct validity, Kaiser-Meyer-Olkin (KMO) coefficient value, assessing the size of the sample according to explanatory factor analysis implemented to primary sample group (N=244), was found .82 and Bartlett's Sphere Test chi square value implemented to test the suitability of sample group for factor analysis was found 752.247, df=15 (p< .001). Explanatory Factor Analysis (EFA) results demonstrated that the scale was two factor structure explaining 77.38% of the total variant of the scale. The first factor included three questions (4th, 5th and 6th questions) about daytime symptoms of insomnia with a factor load ranging between .84 and .88 and the second factor involved three questions (1st, 2nd and 3rd questions) about nocturnal symptoms of insomnia with a factor load between .80 and .87. (Table 2).

Table 2. *Factor loads of the BIS Turkish form for adults (N=244)*

Factors	Items	Two Factors	
		1	2
Nocturnal Symptoms	BIS 1		.81
	BIS 2		.87
	BIS 3		.80
Daytime Symptoms	BIS 4	.86	
	BIS 5	.84	
	BIS 6	.88	
Variance (%)		59.7	17.6

BIS*: Bergen Insomnia Scale*

The fit/unfit coefficient values of two factor structure of the scale determined by the result of confirmatory factor analysis implemented to secondary sample group (n=251) was reported as better than single factor structure. When the overall fit/unfit coefficient values related with theoretical model were examined, it was indicated that fit/unfit coefficient values were perfect (CFI= .99, SRMR= .03 and χ2/SD=1.96) and acceptable (RMSEA= .06) except for chi square test results considered to be effected by the number of samples (Table 3).

Table 3. *The fit/unfit coefficient obtained by the BIS confirmatory factor analysis (N=251)*

Model	χ^2	p	df	χ^2/df	CFI	RMSEA	SRMR
Theoretical (two factors)	**15.68**	**.04**	**8**	**1.96**	**.99**	**.06(.01-.11)**	**.03**
Single factors	90.05	.00	9	10.0	.86	.19(.16-.22)	.08
***Perfect fit**	-	>.05	-	χ2/sd<3	.97≤CFI≤1	.00< RMSEA<.05	.00≤SRMR≤ .05
***Acceptable fit**	-	>.05	-	χ2/sd<5	.95≤CFI≤.97	.05< RMSEA<.1	.05≤ SRMR≤ .1

BIS*: Bergen Insomnia Scale; χ2; Chi-square* ***df****: Degrees of freedom;* ***RMSEA****: The Root Mean Square Error of Approximation;* ***SRMR****: Standardized Root Mean Square Residual;* ***CFI****: Comparative Fit Index (17).*

The factor loads of items in daytime symptoms subscale was between .73 and .85; in the nocturnal symptoms subscale ranged between .64 and .77 (Figure 1).

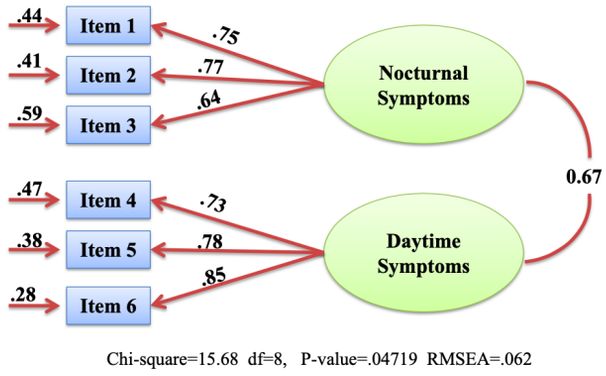


Figure. 1 *BIS confirmatory factor analysis graphic*

Composite reliability (CR) was .83 for daytime symptoms subscale and .76 for nocturnal symptoms subscale. Average variance extracted (AVE) for the daytime symptoms and nocturnal symptoms were .62 and .52, respectively. Maximum Shared Variance (MSV) was .43.

3.3. Results Related with Item Analysis and Reliability

Cronbach's alpha coefficient of daytime symptoms subscale was .85, for nocturnal symptoms subscale was .80 and for total BIS .85. Cronbach's Alpha coefficient was found age interval 19-30 (n=218) .85, age interval 31-40 (n=204) .84 and age interval 41-58 (n=69) .89; shiftlessly operator .81, in shift operator .89. McDonald's omega coefficients for the daytime symptoms, nocturnal symptoms and the whole scale were .85, .80 and .85, respectively (Table 4).

Scale's test-retest correlation was identified as r= .78 for daytime symptoms subscale and r= .77 for nocturnal symptoms subscale (p< .001). In addition, item sub-dimension correlations of the BIS varied between .70-.76 for daytime symptoms and .60-.69 for nocturnal symptoms (p< .001) (Table 4). 48.7% of the adults experienced insomnia according to DSM-5. As for mean scores of BIS sub-dimensions; the mean score of daytime symptoms sub dimension was 3.93±2.13 and it was 2.07±1.94 for nocturnal symptoms sub-dimension. The lowest score was for the item 2 (staying awake more than 30 minutes at night after waking up) and the highest score was item 4 (feeling not rested enough after waking up). It was stated that 73.3% of the participants felt unrested after waking for three days or more in a week; 72.1% of them were not satisfied with their sleep and 57.6% of them felt tired and sleepy effecting their daily functions. Not any statistically significant differences occurred for insomnia in terms of gender (Table 5).

Table 4. *Psychometric Properties of Bergen Insomnia Scale for Adults (N=495)*

Factors	Scale Items	Item-factors r	Item-Total r	Test-retest r	Cronbach's alpha (α)	McDonald's omega (ω)
Nocturnal symptoms	Item 1	.64	.61	.78	.80	.80
	Item 2	.69	.60			
	Item 3	.60	.59			
Daytime symptoms	Item 4	.70	.64	.77	.85	.85
	Item 5	.70	.64			
	Item 6	.76	.71			
Total	-	-	-	.83	.85	.85

r: *Correlation*

Table 5. *Introductory Features of Adults According to BIS and Factors*

Items	≥3 day n (%)	M±Sd
Nocturnal Symptoms	-	2.07[a]±1.94
I1: You are not able to fall asleep within 30 minutes, after you leave your phone/tablet and switched off the light although you wanted to sleep	196 (39.6)	2.34±2.31
I2: You stay awake for more than 30 minutes when you woke up at night	145 (29.3)	1.81±2.24
I3: You wake up at least 30 minutes earlier than you are supposed to wake up and then could not fall asleep again	165 (33.3)	2.07±2.36
Daytime Symptoms		3.93[a]±2.13
I4: You feel not rested appropriately after waking	363 (73.3)	4.29±2.46
I5: You feel sleepy/tired in a manner that shall affect your school/job or private life	285 (57.6)	3.39±2.43
I6: You dissatisfied with your sleep	357 (72.1)	4.12±2.39
BIS Total		3.00[a]±1.78
Person who has insomnia according to DSM-5[b]	241 (48.7)	
Female	104 (46.8)	p=.471[c]
Male	137 (50.2)	

[a]The average score divided by the number of questions. [b] questioned only the past month. [c] Chi-square test
BIS*: Bergen Insomnia Scale,* ***M****:Mean,* ***Sd****:Standard Deviation*

4. DISCUSSION

The results of the present study suggested that BIS is a valid and reliable instrument to assess insomnia for Turkish adults. BIS consisted of two sub-dimensions as daytime symptoms and nocturnal symptoms.

The term "validity" is related with test or a scale actually measures what it sets out to, or how well it reflects the reality (13,18). In order to assess construct validity, EFA and CFA are being used. EFA leads to some new structures and factors utilizing the relations between variables (13). On the other hand, confirmatory factor analysis assesses whether items' relation with factors are sufficient or not. Furthermore, it is validity analysis method that can be used in cultural adaptation studies (18). At present study, it was identified that Turkish version of BIS showed a two-factor structure and it was confirmed by CFA.

In CFA, chi square value which is one of the fit indices is expected to be insignificant however chi square value is rather sensitive to size of the sample. Thus, it is recommended in the assessment of the model that the value obtained from chi square value's dividing independence degree ($\chi 2/df$) should be taken into consideration (19). At present study CFI, SRMR and χ^2/df fit indices related with theoretical model were ascertained as perfect and RMSEA fit index were identified as acceptable (19,20). In original study, Bergen Insomnia Scale had two-factor structure in adolescent sample and one-factor structure in adult sample. It was determined in our study that Turkish version of BIS was compatible with two factor structure in adults like adolescents (4,14). The first subscale included daytime symptoms of insomnia and the second subscale included nocturnal symptoms similar to adolescent sample of Turkish version.

Factor loads are commended to be ≥ .40 to ensure construct validity (13,21). In adult sample of the original study factor loads were determined between .69 and .88. Similar to original study, rather high factor loads in our study (.80-.88 in the first sample; .64-.85 in the second one) indicated a supportive result regarding to construct validity.

Hair et al (22) proposed using AVE to evaluate the convergent validity for each construct. AVE is calculated as the mean variance extracted for the item loading on a construct (derived from CFA). It is expected that AVE is supposed to be ≥.50 and <CR. Discriminant validity was established where MSV was lower than AVE. In the present study, convergent validity was well for both nocturnal and daytime factors and MSV was low enough to propose independence between the factors in the model and so, it shows that discriminant validity is well.

In order to realize reliability analysis, each item of the tool should be identified to what degree they are related with the tool in total and its correlation coefficient should be calculated through item analysis. Higher correlation coefficient for each item mean that the item is efficient and sufficient to measure the intended theoretical model. Literature suggested that item-total correlation should be >.30 (23). At present study, item-subscale correlations for daytime symptoms were found between .70 and .76; it is between .60 and .69 in nocturnal symptoms subscale and it is between .59 and .71 for item-total correlation that is similar to original study (r= .57 – .80). These results demonstrated that the correlation of items with total score and subscales' item scores with total score of their subscales correlation were sufficient and reliability of the scale together with its subscales was rather high.

Cronbach's alpha and omega coefficient is being used to evaluate internal consistency, which is, how closely related a set of items are as a group. It is considered to be a measure of scale reliability. Furthermore, in literature, alpha and omega reliability coefficient is suggested to be over .70 (24-26).

At present study, Cronbach's Alpha value of BIS was .85 that is similar to community sample of original study (α= .87) and it showed a high level of reliability (4). Moreover, reliability coefficients obtained from subscales were found .85 for daytime symptoms and .80 for nocturnal symptoms that meant a high level of reliability. Additionally, according to employment status and age interval, Alpha reliability coefficient was found out > .81 which is very high level in terms of reliability. For the BIS Turkish version, the omega and alpha coefficients of the scale and sub-dimensions were same and higher than .70. These findings showed that the Turkish version of BIS had good reliability. Since the McDonald's Omega coefficient was not calculated in the original scale, the findings of present study were not able to be compared (4).

Test-retest reliability is a measure of reliability acquired by applying the same test twice over a period of time to a group of people. Literature suggested that at least two weeks and four weeks at most (27) should be between two measurements and it should be held at least with 100 people (23). The correlation coefficient of test retest is recommended to be ≥ .40 (24). In our study, test-retest total correlation of BIS was .83 as rather high and .77-.88 in two subscales respectively that is fairly good. According to these results, it was identified that daytime and nocturnal symptoms of insomnia for adults were consistent in two weeks period.

When the descriptive results were examined, daytime symptoms subscale mean score was higher than that of nocturnal symptoms for adults. Thus, adults experience daytime symptoms more often than nocturnal symptoms. In this study, as similar to original one, the question "How many days a week do you feel not rested enough after waking?" had the highest score.

In Literature, 33%-50% of the adults suffer from insomnia and 10%-15% of them experience sleep disorder (5). Sleep disorder rate was 14.9% in Denmark (28), objective insomnia prevalence was 32% in Brazil and subjective insomnia prevalence was 45% and it was 15% in terms of DSM-4 criteria (29), according to a study conducted in Sweden, insomnia symptoms were 24.6% (30) and insomnia prevalence according to DSM-4 criteria was 15.3% in our country (31). Insomnia prevalence was determined between 49.8% and

66.8% in studies conducted with the help of Bergen Insomnia Scale. Bjorvatn et al (8) found out the rate of insomnia as 53.6% in 1346 patients awaiting their doctor appointments; Katsifaraki et al (12) found the rate as 49.8% in 1032 nurses; Blagestad et al (32) found it as 54.0% in 291 patients admitted to orthopedics clinic; Brevik et al (33) found it as 66.8% in 268 adults. In present study, subjective insomnia prevalence was determined 48.7%. We used BIS for assessment of insomnia, and insomnia was defined according to DSM-5 diagnostic criteria. These findings are in line with other studies.

There are various limitations for the present study. The first limitation was that due to collection of data by self-report, it might affect the quality of data with the recall bias and social desirability bias limitations of participants. Another one is that the number of individuals working in the company operator age between 41-58 is low. In further studies, its reliability can be tested for adults over 41 years old and elderly people. In addition, no evaluation was made between BIS and study variables in our study and this can be considered as a limitation.

5. CONCLUSION

To conclude; Turkish version of BIS, developed by Pallesen et al (4) in English, was confirmed as a valid and reliable tool. Turkish version of BIS might be utilized in studies focusing on sleep disorders and mental health studies in order to assess adults' insomnia conditions.

Acknowledgments: *Written informed consent was obtained from of the adults who participated in this study and we would like to share our thankfulness with all participants.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics committee of Marmara University, Institute of Health Sciences (05.03.2018-86)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: TBK, İY, AE
Design of the study: TBK, İY, AE
Acquisition of data for the study: TBK, İY
Analysis of data for the study: TBK, İY, AE
Interpretation of data for the study: TBK, İY, AE
Drafting the manuscript: TBK, İY, AE
Revising it critically for important intellectual content: TBK, İY, AE
Final approval of the version to be published: TBK, İY, AE

REFERENCES

[1] American Academy of Sleep Medicine. International Classification of Sleep Disorders. Diagnostic and Coding Manual. 3rd ed. Westchester: IL; 2014.

[2] American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington: American Psychiatric Publishing; 2013.

[3] Bayard S, Lebrun C, Maudarbocus KH, Schellaert V, Joffre A. Validation of a French version of the sleep condition indicator: A clinical screening tool for insomnia disorder according to DSM-5 criteria. J Sleep Res. 2017;26(6):702-708.

[4] Pallesen S, Bjorvatn B, Nordhus IH, Sivertsen B, Hjørnevik M. A new scale for measuring insomnia: The Bergen insomnia scale. Percept and Mot Skills 2008;107:691-706.

[5] Sateia MJ, Buysse DJ, Krystal AD, Neubauer DN, Heald JL. Clinical practice guideline for the pharmacologic treatment of chronic insomnia in adults: An American Academy of sleep medicine clinical practice guideline. J Clin Sleep Med. 2017;13(2):307-349.

[6] Bjorvatn B, Berge T, Lehmann S, Pallesen S, Saxvig IW. No effect of a self-help book for insomnia in patients with obstructive sleep apnea and comorbid chronic insomnia–a randomized controlled trial. Front Psychol. 2018;9:2413.

[7] Bjorvatn B, Waage S, Pallesen S. The association between insomnia and bedroom habits and bedroom characteristics: An exploratory cross-sectional study of a representative sample of adults. Sleep Health. 2018;4(2):188-193.

[8] Bjorvatn B, Meland E, Flo E, Mildestvedt T. High prevalence of insomnia and hypnotic use in patients visiting their general practitioner. Fam Pract. 2017;34(1):20-24.

[9] Fossum IN, Nordnes LT, Storemark SS, Bjorvatn, B, Pallesen, S. The association between use of electronic media in bed before going to sleep and insomnia symptoms, daytime sleepiness, morningness, and chronotype. Behav Sleep Med. 2014;12(5):343-357.

[10] Brunborg GS, Mentzoni RA, Molde H, Myrseth H, Skouverøe KJM. The relationship between media use in the bedroom, sleep habits and symptoms of insomnia. J Sleep Res. 2011;20:569–575.

[11] Sivertsen B, Petrie KJ, Skogen JC, Hysing M, Eberhard-Gran M. Insomnia before and after childbirth: The risk of developing postpartum pain—A longitudinal population-based study. Eur J Obstet Gynecol Reprod Biol. 2017;210:348-354.

[12] Katsifaraki M, Nilsen KB, Wærsted M, Knardahl S, Lie JAS. The association of sleepiness, insomnia, sleep disturbance and pain: A study amongst shift working nurses. Sleep Biol Rhythm. 2018;16(1):133-140.

[13] Aksayan S, Gözüm, S. Kültürlerarası ölçek uyarlaması için rehber I: Ölçek uyarlama aşamaları ve dil uyarlaması. Hemşirelikte Araştırma Geliştirme Dergisi 2002;4(1):9-14. (Turkish)

[14] Bay T, Ergün A. Validity and reliability of Bergen Insomnia Scale (BIS) among adolescents. Clin Exp Health Sci. 2018;8(4):268-275.

[15] Tabachnick BG, Fidell LS. Using multivariate statistics. 6th ed. Boston, MA: Pearson Education; 2013.

[16] Hayes AF, Coutts JJ. Use omega rather than Cronbach's alpha for estimating reliability. Communication Methods and Measures 2020;14(1):1-24.

[17] Dursun Y, Kocagöz E. Yapısal eşitlik modellemesi ve regresyon: Karşılaştırmalı bir analiz. Erciyes Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi 2010;35:1-17. (Turkish)

[18] Büyüköztürk S, Kılıç EK, Akgün ÖE, Karadeniz S, Demirel F. Bilimsel Araştırma Yöntemleri. 15th ed. Ankara, Turkey: Pegem Akademi; 2013.

[19] İlhan M, Çetin B. LISREL ve AMOS programları kullanılarak gerçekleştirilen yapısal eşitlik modeli (YEM) analizlerine ilişkin sonuçların karşılaştırılması. Egit Psikol Olcme Deger Derg. 2014;5(2):26-42. (Turkish)

[20] Browne MW, Cudeck R. Alternative ways of assessing model fit. Bollen KA & Long JS, editors. Testing structural equation models: Beverly Hills, CA: Sage; 1993. p. 136-162.

[21] Şencan H. Sosyal ve davranışsal ölçümlerde güvenilirlik ve geçerlilik. İstanbul, Turkey: Seçkin Yayıncılık; 2005.(Turkish)

[22] Hair JF, Sarstedt M, Ringle CM, Gudergan SP. Advanced Issues in Partial Least Squares Structural Equation Modeling (PLS-SEM). Thousand Oaks, CA: Sage Publications; 2018.

[23] Kline P. Handbook of Psychological Testing. 2th ed. London and New York: Routledge; 2013.

[24] Streiner DL, Norman GR, Cairney J. Health measurement scales: A practical guide to their development and use. USA: Oxford University Press; 2015.

[25] Dunn TJ, Baguley T & Brunsden V. From alpha to omega: A practical solution to the pervasive problem of internal consistency estimation. Br J Psychol. 2014;105(3):399–412.

[26] Green SB & Yang Y. Evaluation of dimensionality in the assessment of internal consistency reliability: Coefficient alpha and omega coefficients. Educational Measurement: Issues and Practice 2015;34:14–20.

[27] Aksayan S, Gözüm S. Kültürler arası ölçek uyarlaması için rehber II: Psikometrik özellikler ve kültürlerarası karşılaştırma. Hemşirelikte Araştırma Geliştirme Dergisi 2003;5:3-14.

[28] Andersen LL, Garde AH. Sleep problems and computer use during work and leisure: Cross-sectional study among 7800 adults. Chronobiol Int. 2015;32(10):1367-1372.

[29] Castro LS, Poyares D, Leger D, Bittencourt L, Tufik S. Objective prevalence of insomnia in the São Paulo, Brazil epidemiologic sleep study. Ann Neurol. 2013;74(4):537-546.

[30] Mallon L, Broman JE, Åkerstedt T, Hetta J. Insomnia in Sweden: a population-based survey. Sleep Disord 2014.

[31] Demir AU, Ardıç S, Fırat H, Karadeniz D, Aksu M. Prevalence of sleep disorders in the Turkish adult population epidemiology of sleep study. Sleep Biol Rhythm. 2015;13(4):298–308.

[32] Blågestad T, Pallesen S, Grønli J, Tang NK, Nordhus IH. How perceived pain influence sleep and mood more than the reverse: a novel, exploratory study with patients awaiting total hip arthroplasty. Front Psychol. 2016;7:1689.

[33] Brevik EJ, Lundervold AJ, Halmøy A, Posserud MB, Instanes JT. Prevalence and clinical correlates of insomnia in adults with attention-deficit hyperactivity disorder. Acta Psychiatr Scand. 2017;136(2):220-227.

How to cite this article: Bay Kula T, Yalçın İ, Ergün A. Bergen Insomnia Scale for Adults: The Psychometric Features of the Turkish Version. Clin Exp Health Sci 2023; 13: 192-198. DOI: 10.33808/clinexphealthsci.1084321

Clinical and Experimental Health Sciences

Motor Functional Level and Quality of Life According to Feeding Types in Children With Cerebral Palsy

Emre Cengiz[1], Selen Serel Arslan[1], Numan Demir[1], Aynur Ayşe Karaduman[2]

[1] Hacettepe University, Faculty of Physical Therapy and Rehabilitation, Ankara, Türkiye.
[2] Lokman Hekim University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Ankara, Türkiye.

Correspondence Author: Emre Cengiz
E-mail: pt.emrecengiz@gmail.com
Received: 24.03.2022 **Accepted:** 22.09.2022

ABSTRACT

Objective: The study aimed to compare motor functions and quality of life of children with cerebral palsy (CP), as well as anxiety and depression levels of caregivers according to the feeding types of the children.

Methods: The study included children with CP aged between 5-17 years. There were two groups: children with oral feeding (n=19) and children with non-oral feeding (n=16). Gross Motor Function Classification System (GMFCS) to classify children according to gross motor function, Gross Motor Function Measurement (GMFM) to evaluate gross motor functions, and parent-reported Pediatric Quality of Life Inventory to evaluate the quality of life of children were used. Beck Depression Inventory and Beck Anxiety Inventory were used to measuring caregivers' anxiety and depression levels.

Results: Significant differences were found between groups regarding the GMFCS, GMFM, and parent-reported Pediatric Quality of Life scores of children in favor of children with oral feeding ($p<0.05$). Depression levels of caregivers of children with non-oral feeding were higher than children with oral feeding ($p=0.006$).

Conclusion: It was concluded that children with CP who fed non-orally had lower motor functional levels and lower quality of life compared to children who fed orally, as well as their caregivers reported higher depression scores.

Keywords: Cerebral Palsy, Deglutition Disorders, Dysphagia, Enteral Nutrition, Motor Skills.

1. INTRODUCTION

Cerebral Palsy (CP) is defined as a condition that causes movement and postural impairment due to nonprogressive damage in the developing brain. Neurological damage in CP may affect muscle control, movement, posture, and balance (1). Children with CP are prone to experiencing various problems such as development and growth, mobility, cognitive speech, and respiratory issues (1, 2).

The oral motor and swallowing problems in children with CP include poor lip closure, inadequate tongue function, increased bite reflex, drooling, chewing disorders, delayed swallow initiation, decreased pharyngeal motility and inadequate airway closure (3). These symptoms may cause difficulties in providing adequate nutrition, growth and developmental problems in children with CP (4). In particular, children with CP who have problems with airway protection are at the greatest risk of experiencing significant nutritional problems and respiratory complications (5). It is also important to identify the children with CP who has swallowing dysfunction and determine the appropriate feeding method (6).

Swallowing evaluation of children with CP involves both clinical and instrumental approaches. In addition, several factors, such as their medical history, communication, and caregiver concerns should also be considered. This process can help determine the appropriate feeding method that will provide adequate nutrition and hydration (7). In addition to determine the appropriate feeding method, instrumental techniques such as the videofluoroscopic swallowing study and/or fiberoptic endoscopic evaluation of swallowing should be used (8, 9). If children with CP cannot swallow safely and efficiently and are having difficulty meeting nutritional requirements by mouth, enteral nutrition may be initiated (10). Enteral feeding strategies include intragastric (orogastric, nasogastric, gastrostomy tubes) or transpyloric (nasoduodenal, nasojejunal) types. The enteral feeding type is selected whether the enteral feeding requirement

DOI: 10.33808/clinexphealthsci.1093054

is short or long-term (11). Orogastric or nasogastric feeding types are used for children who are predicted to have a short-term need for enteral feeding. Gastrostomy tube feeding is chosen if long-term enteral feeding is required. In addition, texture modification might be selected for children with dysphagia risk or for those who experience only liquid aspiration (12).

Gross motor functions of children with CP who had feeding problems are discussed in the literature (13-16). A percentage of 85 of children with CP have been diagnosed with oropharyngeal dysphagia which is related to lower levels of the Gross Motor Function Classification System (GMFCS) (14). It was also reported that feeding problems are observed in children with various levels of the GMFCS, especially at the level of IV and V. According to these studies, the frequency, and severity of swallowing and feeding problems increase as GMFCS level increases (13-16). The literature, however, provides little information about the quality of life of children with CP who fed enterally. There is one study evaluating quality of life related to usage of gastrostomy (17). This study reported improvement in the quality of life scores after gastrostomy was performed in adults with severe CP, but the statistical significance was not tested due to the limited population. In another study conducted on children with neurological dysfunction, parent reports indicated no change in their children's quality of life at both 6 and 12 months post-G-tube insertion (18). There are also studies investigating the depression and anxiety levels of the caregivers of children with CP. However, there are no studies on the same issue regarding the caregivers of children with non-oral feeding.

Likewise, despite some studies on the relationship between gross motor function and feeding ability, there is no study investigating the motor functional level and quality of life children with CP, as well as anxiety and depression levels of caregivers according to their children's feeding types.

This current study aimed to compare (a) motor function levels of the children, (b) parent-reported quality of life of the children, and (c) anxiety and depression levels of caregivers according to the feeding types of children with CP. We hypothesized that children with CP who fed orally would be better in terms of motor skills and quality of life, and there would be low levels of anxiety and depression among their caregivers.

2. METHODS

2.1. Design

This prospective cross-sectional study included 35 children (19 males and 16 females) with CP aged between 5 to17 years. The study was carried out at Hacettepe University Faculty of Physical Therapy and Rehabilitation and Hacettepe University Swallowing Disorders Application and Research Center. Informed consent was obtained from the patients and their parents who agreed to participate in the study. The study did not include any intervention. Ethics committee approval was obtained from Hacettepe University Non-Invasive Ethics Committee (Approval number = GO18/265-06).

2.2. Participants

Children who were diagnosed with CP, aged between 5-17 years, fed by orally or non-orally and had sufficient cooperation to carry out the commands were included in the study. Children with having any other accompanying neurodegenerative diseases, and situations including having an acute respiratory tract infection and/or complications of enteral feeding such as malposition of the feeding tube, perforation of the intestinal tract, infection in the tube placement area, peritonitis that may affect the quality of life were excluded.

Children were divided into two groups as children with oral feeding (Group 1, n=19) and children with non-oral feeding (Group 2, n=16). Grouping of children was made according to the feeding type at the time of admission to the clinic.

2.3. Measures

Descriptive information including age, height, weight, gender, and CP subtype was noted. Observational evaluation of oral motor structures was performed. Open mouth refers to the chronic opening of the lip seal at rest. Open bite is a type of malocclusion, which means upper and lower incisor teeth do not meet properly when the jaws are closed. A high arched palate is high and narrow palate. Micrognathia is a condition in which the jaw is undersized (19).The presence of problems in oral structures, including the open mouth, open bite, high palate, and micrognathia was noted as 'present' or 'absent'.

The Gross Motor Function Classification System (GMFCS) was used to classify the level of gross motor functions in children with CP (20), and levels were based on child-initiated movement abilities, with emphasis on sitting, displacement, and mobility. The GMFCS uses a rating system of Level I to Level V. Level I shows the most independent functional motor level and Level V shows the most dependent functional motor level.

The Gross Motor Function Measurement (GMFM) is an observational clinical tool to evaluate the motor function of children with CP (21). It has five basic sections that evaluate motor function, including lying and rolling; sitting; crawling and kneeling; standing; and walking, running, and jumping, with a total of 88 items. Each evaluation is scored according to the level of achieving gross motor function without considering the quality of movement. While performing each task in the GMFM, a physical therapist scored each evaluation on a Likert scale between 0 to 3. 0 means 'Does not initiate' and 3 means 'Completes'.

Pediatric Quality of Life Inventory (PedsQL) was used to measure the parent-reported quality of life of children (22). The items of the PedsQL were scored between 0 to 100, of which 100 means "never", 75 means "almost never", 50

means “sometimes”, 25 means “often”, and 0 means “almost always”. Points are collected and divided by the number of items filled to obtain the total score. Higher scores indicate better health-related quality of life. Parent proxy-report versions of PedsQL were applied and recorded as physical health summary score (PHSS), psychosocial health summary score (PSHSS), and total scale score (TSS).

The Turkish version of the Beck Depression Inventory (BDI), and Beck Anxiety Inventory (BAI) were used to measure anxiety and depression levels of caregivers of children with CP (23, 24). The BDI scale is a tool that measures the symptoms of depression. It consists of 21 questions that are related to the emotional, cognitive, and motivation aspects of the disorder. The questions are scored between 0 and 3, and the highest score is 63. Areas evaluated in BDI are unhappiness, crying, self-blame, feeling of failure, irritability, social withdrawal, changes in body image, indecision, weight loss, fatigue, anorexia, insomnia, somatic efforts, and libido reduction. The scores of 17 and above could distinguish between depression with more than 90% accuracy. The BAI is a self-report measure of anxiety. It has 21 items and each one is scored on a Likert scale between 0 to 3. The total score ranges between 0 to 63. Higher scores indicate more severe anxiety in an individual.

Each evaluation was performed by a physical therapist with five years of experience in the field of dysphagia rehabilitation. All assessments were carried out in a silent and comfortable environment. Each evaluation session took 45 minutes for each child.

2.4. Data Analysis

Power of the study was calculated via G*Power version 3.1 as a total of 34 cases with 19 for oral feeding group and 16 for non-oral feeding group as a result of two-way post-hoc hypothesis testing with 5% type I error margin, and evaluated using PedsQL TSS mean scores and standart deviations of each group. Effect size was found 1.05 and the power of the study was found 85%.

The IBM-SPSS Statistics 20 for Windows was used for calculations. Mean (X), standard deviation (SD), minimum and maximum values are used for continuous variables, while number (n) and percentage (%) are used for categorical variables. The Shapiro-Wilk test (n <50) was used to determine whether the continuous variable averages were distributed normally, and the non-parametric tests were applied because the variables were not normally distributed. Mann-Whitney U test was used to compare the continuous variables according to the nutritional status of oral and enteral feeding groups. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

A total of 35 children (19 males and 16 females) were included in the study. Group 1 consisted of children with full oral feeding (n = 11, 31.4%) and children with liquid-restricted oral feeding (n = 8, 42.1%). Group 2 included children with nasogastric tube feeding (n = 6, 17.1%) and children with g-tube feeding (n = 10, 28.6%). The mean duration of feeding with nasogastric tube in group 2 was 7.66 ± 3.26 months and the mean duration of feeding with g-tubes was 36.9 ± 30.8 months. Descriptive information is shown in Table 1. There was no difference in age, height, and weight between groups (p > .05).

Table 1. *Descriptive information of children*

	Group 1 Children with Oral Feeding (n = 19)		Group 2 Children with Non-oral Feeding (n = 16)		p
	X ± SD	min-max	X ± SD	min-max	
Age (year)	8.47 (3.63)	5-17	8.19 (3.19)	5-16	.947
Height (cm)	114.21 (22.60)	77-165	104.44 (20.20)	76-140	.389
Weight (kg)	20.29 (9.23)	8-48	20.75 (10.25)	6.5-43	.921

There was a significant difference between group 1 and group 2 in terms of sub-type of CP, GMFCS levels, and observational oral motor evaluation results (p < .05). The information regarding the sub-type of the CP, GMFCS levels and observational oral motor evaluation results are presented in Table 2.

Table 2. *Comparison of CP sub-type, GMFCS levels and oral structural evaluation.*

	Group 1 Children with Oral Feeding (n = 19)		Group 2 Children with Non-oral Feeding (n = 16)		p
CP sub-type	**n**	**%**	**n**	**%**	
Quadriparetic	4	21.1	13	81.3	.01*
Diparetic	3	15.8	1	6.3	
Hemiparetic	9	47.4	1	6.3	
Choreo-athetotic	2	10.5	1	6.3	
Dystonic	1	5.3	0	0.0	
GMFCS	**n**	**%**	**n**	**%**	.004*
Level I	7	36.8	1	6.3	
Level II	5	26.3	1	6.3	
Level III	2	10.5	1	6.3	
Level IV	2	10.5	0	0.0	
Level V	3	15.8	13	81.3	
Oral structural evaluation	**n**	**%**	**n**	**%**	
Open mouth	11	57.9	16	100	.003*
Open bite	9	47.4	16	100	.001*
High palate	2	10.5	7	43.8	.025*
Micrognathia	1	5.3	1	6.3	.900

** p<0.05; Abbreviations:CP: Cerebral Palsy, GMFCS: Gross Motor Functional Classification System.*

A significant difference was found between groups regarding the GMFM scores of children (p < .05). Children in group 2 had lower scores in all sub-scores and total scores of the GMFM compared to group 1 (Table 3). There was also a statistically significant difference in parent-reported quality

of life of children between groups (p < .05). The parent-reported PSHSS, PHSS, and TSS scores of group 1 were higher than group 2 (p < .05). No difference was found between groups in terms of the anxiety levels of the caregivers (p > .05). Depression levels of caregivers in group 2 were higher than in group 1 (p < .05).

Table 3. *Comparison of GMFM, PedsQL, BAI and BDI means.*

	Group 1 Children with Oral Feeding (n = 19)		Group 2 Children with Non-oral Feeding (n = 16)		p
	X ± SD	Min-Max	X ± SD	Min-Max	
GMFM					
Lying & rolling	87.72±25	0-100	46.32±28.47	13.73-100	.00**
Sitting	80.09±31.56	0-100	21.87±35.20	0-100	.00**
Crawling & kneeling	72.06±38.36	0-100	14.88±34.12	0-100	.00**
Standing	65.85±38.32	0-100	14.58±33.74	0-100	.00**
Walking, running & jumping	55.77±36.65	0-95.83	11.89±29.43	0-100	.001*
Total	72.30±32.64	0-99.17	21.91±31.05	2.75-100	.001*
PedsQL					
PHSS	62.99±28.74	12.5-100	27.54±23.34	0-84.38	.001*
PSHSS	72.81±18.47	21.67-93.33	60.52±19.77	0-83.33	.037*
TSS	69.39±19.81	18.48-93.48	49.05±18.85	0-80.43	.003*
BAI	9.63±7.78	0-28	14.25±10.86	0-41	.135
BDI	9.42±9.22	1-39	17.56±9.96	3-41	.006*

**p<0.05; **p<0.001; Abbreviations: GMFM: Gross Motor Function Measurement, PedsQL: Pediatric Quality of Life Inventory, PHSS: Physical Health Summary Score, PSHSS: Psychosocial Health Summary Score, TSS: Total Scale Score, BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory.*

4. DISCUSSION

The current study shows that children with CP who had non-oral feeding had lower motor functional levels and lower quality of life compared to children who had oral feeding, as well as their caregivers reported higher depression scores.

The demographics including age, height, and weight were similar in groups, which strengthens our study to be able to compare the groups. It was reported that malocclusion, open bite, and biting reflex were more commonly seen in children with CP (25), and the possible reasons were reported as neurological conditions of the children, head hyperextension at rest, atypical swallowing, using pacifiers, and thumb sucking habits (26, 27). Additionally, our study results showed that the prevalence of oral motor structural problems regarding the presence of open mouth, high palate and open mouth were higher in children with CP who had non-oral feeding. It may be explained by the increased neurological impairments of these children. Children with non-oral feeding in our study were mostly quadriparetic CP and children with oral feeding were mostly hemiparetic CP. When neurological impairment increased, optimal control of head, neck, and trunk muscles could not be maintained. Therefore, poor or inadequate control and accompanying kinetic problems of muscles may affect the development of the oral region and may cause inefficient feeding. Also oral structural problems seen more common on children with limited mobility and motor functional skills (28, 29).

Gross motor functional levels were lower in children with CP who had non-oral feeding compared to children with CP who had oral feeding. Children who fed non-orally were mostly in GMFCS level V although children fed orally were generally in GMFCS I and II. Oropharyngeal phase problems were seen in all children with CP at GMFCS levels II to V. In addition, it was found that the frequency and severity of nutritional and feeding disorders increase as the level of GMFCS increases as our study results (13-16, 30). Because children with lower motor functional ability need more support in functional activities including oropharyngeal swallowing and feeding. Similarly, the inability to provide an adequate head position for feeding and inappropriate head positions such as neck hyperextension may be increasing factors related to swallowing problems.

In terms of motor function of children in our study, the GMFM scores were better in children who fed orally compared to children fed non-orally as complementary to our results regarding the GMFCS levels. In a study (14), decreased sub-scores and total scores of GMFM are related to the presence of oropharyngeal dysphagia. It has been shown that approximately 70% of children with hemiparetic or diparetic CP had oropharyngeal dysphagia and their motor function scores were lower than children with hemiparetic/diparetic CP who did not have any feeding problems.

In our study, majority of the children fed non-orally were quadriparetic CP. In the current study, the highest achievement in both groups were seen in the lying and rolling section scores. However, children fed non-orally had lower scores in all sub-scores and total scores of the GMFM. In another study significant correlation was found between trunk control and oral motor functions in children with CP (31). Similarly, in our study, it was observed that the sitting subgroup scores of children fed orally were higher. Namely, the frequency of enteral feeding in other words severity of swallowing problems increased through motor function scores decreased. Therefore, children with poorer motor functional levels should be evaluated in terms of possible swallowing and feeding problems.

This study showed that children who fed non-orally had worse parent-reported quality of life scores compared to children fed orally. In a study on children with non-ambulant CP, factors affecting the health-related quality of life were reported as decreased motor functional levels and inefficient swallowing result in enteral feeding (32). Considering motor functional levels of children who fed non-orally, they have worse motor control and need more support on daily activities. They may be more dependent on their caregivers in terms of daily activities including walking, feeding, self-care, dressing, etc. In addition, they have worse swallowing and feeding difficulties. These factors may decrease the

quality of life in children with non-oral feeding. In addition, the need for hospitalization due to surgical intervention, economical costs, need for routine medical follow-up, risk of infection, the necessity to change at regular intervals, and usage of enteral feeding supplements instead of foods could also contribute to poorer quality of life in children fed non-orally (32). Therefore, more support should be given to children who are fed non-orally. This result does not mean that tube feeding in itself adversely affects quality of life for these children and their caregivers. Even though, there may be an improvement in quality of life after tube placement in children by eliminating the negative consequences of swallowing impairments (18, 33, 34). In the present study, there are different factors contributing to the quality of life including their neurological involvements, independency levels, etc. in our study groups. Therefore, the study design does not allow for interpret the findings in this way.

Anxiety and depression levels of caregivers were also measured. It is crucial to define the anxiety and depression levels of caregivers because caregiver compliance is important for the success of feeding and swallowing management. Despite no difference between anxiety levels of caregivers, depression levels of caregivers of children with non-oral feeding were higher than caregivers of children with oral feeding. Arslan et al. found a relationship between the feeding type of the children with CP and the anxiety levels of the parent's (35). Caregivers of children with feeding disorders spent more than half an hour and more than three hours a day for a single meal (16). Therefore, feeding sessions become dominant in the daily life of caregivers. In addition, children with CP need more care than healthy children due to complex health needs requiring multidirectional problems, hospital visits and follow-ups (36). Therefore, caregivers of children with CP had limited social life (37). In a study, it was reported that the dysphagia problem of children with neurological involvement negatively affects the main activities of daily life and health-related quality of life of caregivers (38). In addition, having children who are fed non-orally may increase the need of care for children with CP, thereby depression symptoms of caregivers of children with non-oral feeding may be higher.

There are also some limitations in the current study. The analysis could also be performed between subgroups if the number of participants will be increases. Thus, different groups including children fed by nasogastric tubes, gastrostomy tubes, etc. could be arranged and intergroup differences between parameters could be examined. Also in our study, the age range of children with CP was wide which may affect the of quality of life, perspective, and responses of parents depending on the age of the child. In future studies, different age groups can be formed, and the difference between feeding type and motor skills also quality of life of children and caregivers can be observed. Also, enteral feeding can be observed in certain time periods. Thus, differences and changes in motor functions and quality of life can be shown. Also, other conditions that could affect the anxiety and depression status of parents including parents' history of anxiety/depression, and medication use were not investigated in the present study.

5. CONCLUSION

The study concluded that children with CP who had non-oral feeding had lower motor functional levels and lower quality of life compared to children who had oral feeding, as well as their caregivers reported higher depression scores. Therefore, children with lower motor functional levels should be supported and followed closely by both theirselves and their caregivers.

Acknowledgments: *The researchers like to thank the children and parents who consented and participated the study.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Hacettepe University Non-Invasive Ethics Committee (Approval number = GO18/265-06)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: EC
Design of the study: EC, SSA
Acquisition of data for the study: EC
Analysis of data for the study: EC, SSA
Interpretation of data for the study: EC, SSA
Drafting the manuscript: EC, SSA
Revising it critically for important intellectual content: SSA, AAK, ND
Final approval of the version to be published: EC, SSA, ND, AAK

REFERENCES

[1] Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M, Damiano D. A report: the definition and classification of cerebral palsy April 2006. Dev Med Child Neurol Suppl. 2007;109(suppl 109):8-14.

[2] Arvedson J. Feeding children with cerebral palsy and swallowing difficulties. Eur J Clin Nutr. 2013;67(2):S9-S12.

[3] Erkin G, Culha C, Ozel S, Kirbiyik EG. Feeding and gastrointestinal problems in children with cerebral palsy. Int J Rehabil Res. 2010;33(3):218-24.

[4] Stevenson RD, Conaway M, Chumlea WC, Rosenbaum P, Fung EB, Henderson RC. Growth and health in children with moderate-to-severe cerebral palsy. Pediatrics. 2006;118(3):1010-8.

[5] Rempel G. The importance of good nutrition in children with cerebral palsy. Phys Med Rehabil Clin N Am. 2015;26(1):39-56.

[6] Marchand V, Motil KJ. Nutrition support for neurologically impaired children:A clinical report of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr. 2006;43(1):123-35.

[7] Arvedson JC. Assessment of pediatric dysphagia and feeding disorders: clinical and instrumental approaches. Dev Disabil Res Rev. 2008;14(2):118-27.

[8] Marrara J, Duca A, Dantas R, Trawitzki L, Lima R, Pereira J. Swallowing in children with neurologic disorders: clinical and videofluoroscopic evaluations. Pro Fono. 2008;20(4):231-6.

[9] Sitton M, Arvedson J, Visotcky A, Braun N, Kerschner J, Tarima S. Fiberoptic endoscopic evaluation of swallowing in children: feeding outcomes related to diagnostic groups and endoscopic findings. Int J Pediatr Otorhinolaryngol. 2011;75(8):1024-31.

[10] Gantasala S, Sullivan PB, Thomas AG. Gastrostomy feeding versus oral feeding alone for children with cerebral palsy. Cochrane Database Syst Rev. 2013(7).

[11] Park R, Allison M, Lang Je, Spence E, Morris A, Danesh B. Randomised comparison of percutaneous endoscopic gastrostomy and nasogastric tube feeding in patients with persisting neurological dysphagia. BMJ. 1992;304(6839):1406-9.

[12] Benfer KA, Weir KA, Bell KL, Ware RS, Davies PS, Boyd RN. Food and fluid texture consumption in a population-based cohort of preschool children with cerebral palsy: relationship to dietary intake. Dev Med Child Neurol. 2015;57(11):1056-63.

[13] Benfer KA, Weir KA, Bell KL, Ware RS, Davies PS, Boyd RN. Oropharyngeal dysphagia in preschool children with cerebral palsy: oral phase impairments. Res Dev Disabil. 2014;35(12):3469-81.

[14] Benfer KA, Weir KA, Bell KL, Ware RS, Davies PS, Boyd RN. Oropharyngeal dysphagia and gross motor skills in children with cerebral palsy. Pediatrics. 2013;131(5):e1553-e62.

[15] Kim J-S, Han Z-A, Song DH, Oh H-M, Chung ME. Characteristics of dysphagia in children with cerebral palsy, related to gross motor function. Am J Phys Med Rehabil. 2013;92(10):912-9.

[16] Sullivan P, Lambert B, Rose M, Ford-Adams M, Johnson A, Griffiths P. Prevalence and severity of feeding and nutritional problems in children with neurological impairment: Oxford Feeding Study. Dev Med Child Neurol. 2000;42(10):674-80.

[17] Davout A, Rech C, Hanachi M, Barthod F, Melchior JC, Crenn P. Feasibility and results of pull-percutaneous endoscopic gastrostomy for enteral nutrition in adults with severe cerebral palsy. Clin Nutr. 2016;35(4):918-23.

[18] Mahant S, Friedman JN, Connolly B, Goia C, Macarthur C. Tube feeding and quality of life in children with severe neurological impairment. Arch Dis Child. 2009;94(9):668-73.

[19] Logemann J. Anatomy and physiology of normal deglutition. Evaluation and treatment of swallowing disorders. 1983:9-36.

[20] El Ö, Baydar M, Berk H, Peker Ö, Koşay C, Demiral Y. Interobserver reliability of the Turkish version of the expanded and revised gross motor function classification system. Disabil Rehabil. 2012;34(12):1030-3.

[21] Russell DJ, Rosenbaum PL, Cadman DT, Gowland C, Hardy S, Jarvis S. The gross motor function measure: a means to evaluate the effects of physical therapy. Dev Med Child Neurol. 1989;31(3):341-52.

[22] Varni JW, Seid M, Rode CA. The PedsQL™: measurement model for the pediatric quality of life inventory. Med Care 1999:126-39.

[23] Hisli N. Beck depresyon envanterinin universite ogrencileri icin gecerliligi, guvenilirligi. J Psychol. 1989;7:3-13 (Turkish)

[24] Ulusoy M, Sahin NH, Erkmen H. The Beck anxiety inventory: psychometric properties. J Cogn Psychother. 1998;12(2):163-72.

[25] Franklin D, Luther F, Curzon M. The prevalence of malocclusion in children with cerebral palsy. Eur J Orthod. 1996;18(6):637-43.

[26] de Castilho LS, Abreu MHNG, Pires e Souza LGdA, Romualdo LTdA, Souza e Silva ME, Resende VLS. Factors associated with anterior open bite in children with developmental disabilities. Spec Care Dentist 2018;38(1):46-50.

[27] Martinez-Mihi V, Silvestre FJ, Orellana LM, Silvestre-Rangil J. Resting position of the head and malocclusion in a group of patients with cerebral palsy. J Clin Exp Dent. 2014;6(1):e1.

[28] Bensi C, Costacurta M, Docimo R. Oral health in children with cerebral palsy: A systematic review and meta-analysis. Spec Care Dentist. 2020;40(5):401-11.

[29] Yogi H, Alves LAC, Guedes R, Ciamponi AL. Determinant factors of malocclusion in children and adolescents with cerebral palsy. Am J Orthod Dentofacial Orthop. 2018;154(3):405-411.

[30] Léonard M, Dain E, Pelc K, Dan B, De Laet C. Nutritional status of neurologically impaired children: Impact on comorbidity. Arch Pediatr. 2020;27(2):95-103.

[31] Acar G, Ejraei N, Turkdoğan D, Enver N, Öztürk G, Aktaş G. The effects of neurodevelopmental therapy on feeding and swallowing activities in children with cerebral palsy. Dysphagia 2021:1-12.

[32] Elema A, Zalmstra TA, Boonstra AM, Narayanan UG, Reinders-Messelink HA, vd Putten AA. Pain and hospital admissions are important factors associated with quality of life in nonambulatory children. Acta Pediatr. 2016;105(9):e419-e25.

[33] Sullivan PB, Juszczak E, Bachlet AM, Thomas AG, Lambert B, Vernon-Roberts A. Impact of gastrostomy tube feeding on the quality of life of carers of children with cerebral palsy. Dev Med Child Neurol. 2004;46(12):796-800.

[34] Sullivan PB, Juszczak E, Bachlet AM, Lambert B, Vernon-Roberts A, Grant HW. Gastrostomy tube feeding in children with cerebral palsy: A prospective, longitudinal study. Dev Med Child Neurol. 2005;47(2):77-85.

[35] Arslan SS, Ilgaz F, Demir N, Karaduman AA. Yutma bozukluğu olan serebral palsili çocuklarda büyüme yetersizliği ve beslenme şeklinin ebeveynlerin kaygı durumu üzerindeki etkisi. Beslenme ve Diyet Dergisi 2017;45(1):28-34.

[36] Craig G, Carr L, Cass H, Hastings R, Lawson M, Reilly S. Medical, surgical, and health outcomes of gastrostomy feeding. Dev Med Child Neurol. 2006;48(5):353-60.

[37] Sloper P, Turner S. Service needs of families of children with severe physical disability. Child: Care, Health and Development 1992;18(5):259-82.

[38] Pérez PO, Arredondo IV, Rubio ET, López AR, Taillifer PG-H, Navas-López VM. Clinicopathological characterization of children with dysphagia, family impact and health-related quality of life of their caregivers. An Pediatr (Engl Ed). 2022;96(5):431-40.

How to cite this article: Cengiz E, Serel Arslan S, Demir N, Karaduman AA. Motor Functional Level and Quality of Life According to Feeding Types in Children With Cerebral Palsy. Clin Exp Health Sci 2023; 13: 199-204. DOI: 10.33808/clinexphealthsci.1093054

Clinical and Experimental Health Sciences

Inflammation in Major Depressive Disorder Patients with and without Attempted Suicide

Gülay Taşçı[1], Filiz Özsoy[2]

[1] Fethi Sekin State Hospital, Department of Psychiatry, Elaziğ, Türkiye.
[2] Gaziosmanpaşa University, Faculty of Medicine, Department of Psychiatry, Tokat, Türkiye.

Correspondence Author: Filiz Özsoy
E-mail: Flzkoseoglu82@gmail.com
Received: 24.03.2022 **Accepted:** 07.10.2022

ABSTRACT

Objective: This study aimed to examine Major Depressive Disorder (MDD) patients with or without attempted suicide history for inflammation parameters.

Methods: The study included 287 participants, 187 had MDD diagnosis (87 patients with a suicide attempt, 100 patients without suicide history) and 100 healthy. All participants were subjected to Beck Depression Inventory (BDI).

Results: Mean BDI score of MDD patients with suicide attempts was higher than the other two groups ($p<0.01$). Compared to the healthy controls, MDD patients with suicide attempts had significantly higher white blood cell and neutrophil levels ($p= .007, .001$; retrospectively). Hematocrit, erythrocyte and PCT value, which represents the percentage of platelet in blood, was significantly lower in MDD patient group with suicide history than in the control ($p= .036, .016, .002$; respectively). Neutrophil/lymphocyte ratios and monocyte/lymphocyte ratio values were significantly higher in MDD patients with suicide attempts compared to the ones without suicide history ($p= .003, .022$; respectively). On the other hand, no difference was found among the study groups for the platelet/lymphocyte ratio ($p= .590$).

Conclusion: The present study indicated that certain inflammatory parameters could be used to predict suicide risk in MDD. More detailed studies are needed to better elucidate the associations of inflammatory processes with MDD and different stages of suicide behavior.

Keywords: Major depressive disorder, suicide, inflammation, neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio.

1. INTRODUCTION

Suicide; intentional termination of one's own life. Suicidal behavior consists of thinking, planning, attempting and committing stages (1). Committed suicides are among the leading causes of death all of the world. The World Health Organization (WHO) reports that about 800.000 people die of suicide each year (2). It was shown that genetic, biological, neurobiological factors, familial factors, personality traits, age, gender, culture, psychiatric diseases, psychosocial stress factors play role in suicide etiology (3). Of psychiatric diseases, major depressive disorder (MDD) has been reported to have an increased risk for suicide (4).

It has been shown that inflammation begins with an altered balance of neurotransmitters in the brain and the suppression of the brain in psychiatric illnesses (5). It has also been detected that inflammation and immune system abnormalities play roles in both MDD (6) and suicide (7) pathophysiology. Leukocytes which could be evaluated by simple blood tests (neutrophil, lymphocyte, eosinophil, monocyte, basophil), red blood cell distribution width (RDW) are considered new markers for evaluation of systemic inflammatory response (8,9). Neutrophil/lymphocyte ratio (NLR), monocyte/lymphocyte ratio (MLR), platelet/lymphocyte ratio (PLR) are chronic inflammation markers that can be easily calculated and examined retrospectively. NLR, PLR and MLR are regarded as highly informative in showing chronic low-grade inflammation (10). NLR, PLR and MLR values have been studied in many psychiatric disorders such as psychotic disorder, bipolar disorder, alcohol and substance use disorders as well as systemic diseases (10-14).

A limited number of studies were conducted in the literature to evaluate NLR and PLR in MDD with/without suicide attempt (15-17) Fewer studies examined MLR value in psychiatric diseases (18-20). On the other hand, there is no study investigating NLR, MLR, PLR, leucocyte and RDW together. In our study, MDD patients with and without suicidal attempts; It was established with the hypothesis that parameters such

DOI: 10.33808/clinexphealthsci.1120081

as NLR, PLR, MLR, which are indicators of chronic low-grade inflammation, may be different from healthy controls. It is thought that all this information should be taken into account in the treatment planning, clinical evaluation and evaluation of complications that may occur. Based on this, we aimed to examine the inflammation values of MDD with/without suicide, such as NLR, PLR, MLR, RDW and leukocyte cell distribution levels, which are easy to calculate and evaluate by comparing with healthy controls.

2. METHOD

2.1. Study Design

This cross-sectional, descriptive study was conducted with patients who applied to Tokat State Hospital and Fethi Sekin State Hospital Outpatient Clinic between 01.05.2020 and 31.12.2020, who were being treated with the diagnosis of MDD according to DSM-5 diagnostic criteria, and a healthy control group. This study was approved from the Elazığ Fethi Sekin State Hospital Ethics Committee (Number: 97132852/050.01.04; Date: 13.04.2020).

2.2. Participants

Patients with general condition addiction, chronic disease requiring medical treatment, mental retardation, alcohol/substance use disorder, other psychiatric diagnoses according to DSM-5 diagnostic criteria (such as bipolar, psychotic disorder), illiterate individuals were excluded from the study. Records of about 300 MDD patients were surveyed for the study. Forty individuals were excluded since they had a manic attack. Of the remaining 270 patients, five were excluded because of substance use, 15 were excluded since they had psychotic type depressive disorder, and 25 were excluded since they did not fill the forms in the clinic where they were being treated as inpatients. Thus, the study included 100 MDD patients without an attempted suicide history and 87 MDD patients with suicide attempted. The patients included in the study were patients who applied for treatment for the first time, and their blood tests were taken for routine control purposes before the treatment started. The healthy control group was among those who applied to the psychiatry outpatient clinic for general psychiatric examination, psychiatric evaluation before job application, military examination and counseling; according to the DSM-5 diagnostic criteria, individuals without psychiatric disease and who met the inclusion criteria were selected. 100 healthy controls who did not have any psychiatric disease demanding treatment and who had matching such as gender distribution, marital status and education level with the patient groups were included as the healthy control group. The diagnosis of additional medical disease and accompanying psychiatric disorder was excluded by clinical examination, patient and patient relatives' statements, hospital records, and previous medications and diagnoses from the medulla pharmacy system. The hospital records and e-pulse system were analyzed in terms of whether all MDD patients who said they had attempted suicide or not. Those with signs of self-mutilation and intentional self-harming behaviors were excluded from the study. The participants' characteristics are presented in Table 1.

Table 1. *The participants' sociodemographic characteristics*

	MDD patient group without attempted suicide history (n=100) n(%)	MDD patient group with attempted suicide history (n=87) n(%)	Healthy control group (n=100) n(%)	p
Age (Mean±SD)	41.92±15.53[a]	30.23±12.26[b]	33.78±12.17[b]	**<0.001***
Gender(Female/Male)	51/49 (51/49%)	51/36 (59/41%)	41//59 (41/59%)	0.053
Marital status	45/45/10	40/41/6	42/48/10	
Married/single/separated	(45/45/10%)	(46/47/7%)	(42/48/10%)	0.122
Living place	48/52	46/41	51/49	
Central town/village	(48/52%)	(53/47%)	(51/49%)	0.610
Educational level				
Primary school graduate	25 (25%)	24 (28%)	23 (23%)	
High school graduate	40 (40%)	37 (42%)	42 (42%)	0.240
College graduate	35 (35%)	26 (30%)	35 (35%)	
Economic status	3/96/1	2/83/2	1/99/0	
Low/moderate/high	(3/96/1%)	(2/96/2%)	(1/99/0%)	0.089
Previous suicide attempt	0/100	87/0	0/100	
Yes/no	(0/100%)[a]	(100/0%)[b]	(0/100%)[a]	**<0.001***

*None of the participants had any additional medical conditions needing treatment. None of the participants had alcohol/substance use. Values in the table are given as n (%). One-way analysis of variance was used. (abc): The means with the same letter on the same line are not significantly different. *p< .05.*

2.3. Data Collection Tools

All participants' demographic data form and Beck Depression Inventory (BDI) scores were recorded during the first psychiatric interview. Then, fasting whole blood counts were performed. The blood parameters of the patient group were determined before medical treatment began.

Sociodemographic data form: This form included demographic data such as age, marital status, education level and clinical questions such as the presence of a suicide in the past, treatment history in a psychiatry clinic as an inpatient, a psychiatric disease in the family requiring treatment and alcohol or substance use.

Beck Depression Inventory (BDI): It is used to determine the depression risk, as well as the level and intensity of depressive symptoms. It is a 21-question, self-reporting scale, and each item is graded by points between 0 and 3. These forms are applied to all patients hospitalized with the diagnosis of MDD during the first psychiatric interview (21).

2.4. Statistical Analyses

SPSS for Windows 19 software (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY) was used to analyze the data obtained from the participants. Data are expressed as mean ± standard deviation, median, quartile 1, quartile 3 or frequency and percent. In order to gain information about the general characteristics, descriptive analyses, frequency and percent distributions, and mean ± standard deviation were calculated. Data of continuous variables were given as mean ± standard deviation, while those of categorical ones were expressed as n (%).

Power analysis was performed to determine the sample size of the study. For this purpose, the G*Power (Foul, Erdfelder, Lang and Buchner. 2007) program was used. The minimum sample size was calculated as 5% tolerance and 95% range of confidence for an effect size of 0.54. In total, at least 80 participants were calculated for each group.

Qualitative variables were demographic data. In addition, the presence of diagnosed psychiatric disease in the family, smoking or alcohol use, and additional medical conditions were other qualitative variables in the study. Cross-table and Chi-square tests were used to evaluate the relationships between the qualitative variables. Quantitative variables were BDI and whole blood counts. Independent sample t test or one way analysis of variance were used to compare the continuous normal data between/among groups. Mann Whitney U test or Kruskal Wallis test was used to compare the continuous non-normal data between/among groups. Normal – data are: hemoglobin, hematocrit, platelet, erythrocyte, monocyte, RDW and PCT. Non – Normal data are: white blood cell, neutrophil, lymphocyte, RDW-SD, RDW-CV, Eosinophil, basophil, NLR and PLR. Pearson correlation coefficient was used for correlation between variables. p values less than 0.05 were considered statistically significant.

3. RESULTS

3.1. Participants' Characteristics

A total of 287 individuals (187 MDD patients and 100 healthy). MDD group were divided into two groups with and without suicide attempt history. The average age was 41.92±15.53 years in the MDD group without an attempted suicide history, 30.23±12.26 years in MDD with suicide attempt history, and 33.78±12.17 years in the healthy control ($p< .001$). There were no differences among the study groups for gender distribution, marital status, living place, educational level and economic status. The patients' characteristics are presented in Table 1. None of the participants used alcohol, and smoking levels were similar ($p> .05$). Methods of suicide used by MDD patients with suicide attempts were taking drugs by 79 individuals (90.8%), using sharp objects by five individuals (5.7%), hanging by two individuals (2.3%), and jumping from height by one individual (1.1%).

3.2. The Results of Quantitative Variables

BDI scores of the MDD with a suicide attempt were significantly higher than the patient group without a suicide attempt and healthy control groups ($p< .001$). In terms of laboratory parameters, hemoglobin, platelet and lymphocyte levels were not different among the study groups ($p= .483, .917, .695$; respectively). Hematocrit, erythrocyte and PCT value which represents the percentage of platelet in blood were lower in MDD patient group with suicide history than the control group ($p= .036, .016, .002$; respectively). Compared to the controls, MDD with suicide attempts had significantly higher white blood cell and neutrophil levels ($p= .007$ and $.001$). Monocyte level, on the other hand, was significantly higher in MDD with suicide attempts compared to the ones without suicide attempts ($p= .014$). Basophil and standard deviation of RDW-SD were significantly higher in both patient groups ($p<0.001$ and $.002$). In addition, compared to the patient group without suicide attempt, MDD with suicide attempt had significantly higher NLR and MLR, while PLR was not different ($p= .003, .022$ and $.590$). No correlation was found between the depression scale used for the patients and any of the laboratory parameters studied (Table 2).

Table 2. *The results of quantitative variables*

	MDD patient group without attempted suicide history (n=100)	MDD patient group with attempted suicide history (n=87)	Healthy control group (n=100)	p
BDI	20.16±9.40[a]	27.42±10.56[b]	8.14±6.12[c]	**<0.001***
Hemoglobin	14.12±1.61	13.81±1.92	14.01±1.82	0.483
Hematocrit	41.46±4.34[ab]	40.3±5.15[b]	42.08±4.77[a]	**0.036***
Platelet	252.88±71.94	256.97±79.5	256.18±65.54	0.917
Erythrocyte	4.87±0.5[ab]	4.78±0.54[b]	4.99±0.51[a]	**0.016***
Monocyte	0.55±0.22[a]	0.68±0.34[b]	0.58±0.37[ab]	**0.014***
RDW	15.05±2.55[ab]	16.82±0.55[b]	13.35±2.43[c]	**<0.001***
PCT	0.23±0.06[ab]	0.21±0.06[b]	0.24±0.06[a]	**0.002***
	Median value	**Median value**	**Median value**	**P**
White blood cell	5.96-9.43[ab]	7.0-10.9[b]	6.8-9.8[a]	**0.007[A]***
Neutrophil	3.28-5.56[ab]	4.11-7.54[b]	3.7-7.0[a]	**0.001[A]***
Lymphocyte	1.76-2.76	1.54-2.79	1.6-2.56	0.695[A]
RDW-SD	39.8-44.5[a]	40.3-44.2[a]	39.0-43.0[b]	**0.002[A]***
RDW-CV	12.8-13.9[a]	13.1-15.0[b]	12.5-13.7[a]	**<0.001[A]***
Eosinophil	0.08-0.27[a]	0.05-0.21[b]	0.07-0.2[b]	**0.001[A]***
Basophil	0.03-0.07[a]	0.04-0.08[a]	0.01-0.03[b]	**<0.001[A]***
NLR	1.83 (1.39-2.6)[a]	2.46 (1.69-3.96)[b]	2.26 (1.54-3.46)[ab]	**0.003[A]***
PLR	111.21 (86.98-147)	125.79 (81.65-157.5)	117 (95.78-155.32)	0.590[A]

*Abbreviations used in the table: BDI: Beck's depression inventory, RDW: Red blood cell distribution width, PCT: Platelet Crit, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio. Values in the table are given as Mean±Standard Deviation. A Kruskal Wallis test was used. For others, one-way analysis of variance was employed. (abc): The means with the same letter on the same line are not significantly different. *p< .05.*

4. DISCUSSION

In this study, NLR, PLR, MLR, RDW and leucocyte levels of MDD with attempted suicide history were evaluated by comparing with MDD without suicide history and with controls.

NLR, which is an indication of chronic low-grade inflammation, was higher in MDD with suicide history compared to the ones without suicide history. Similar to our findings, a study in the literature found higher NLR levels in patients with suicide attempts compared to the controls. In that study, there was no difference between the MDD patients with/without suicide attempts for NLR levels (15). There are conflicting results in the studies dealing with NLR levels in major depressive patients (16,22,23). In a meta-analysis, NLR levels of major depressive patients were reported to be higher than the healthy controls, but whether the patients were receiving treatment was not mentioned (24). In another study, 27 MDD with attempted suicide were compared with 26 patients without attempted suicide history, and no difference was found between the two groups for NLR and PLR levels (19). In our study; however, blood parameters of MDD with or without suicide attempts were evaluated before the treatment started. Our findings revealed that irrespective of receiving treatment, the NLR level of MDD with suicide attempts was higher than the healthy controls. On the other hand, the NLR level of the patients without suicide attempts was not different from the healthy controls. Although the exact cause-effect relationship was not fully elucidated, it was reported that peripheral inflammation could play role in suicide etiology (4,15,24). Our results seem to support this finding.

There are limited studies in the literature dealing with monocyte values and MLR in psychiatric diseases (5,19,25,26). Higher MLR were reported in schizophrenia and bipolar disorder-manic episode (26). Another study in which monocyte/lymphocyte ratios were examined in different periods of schizophrenia patients revealed that MLR were higher in both remission and relapse periods (19). In a study with bipolar disorder patients, higher monocyte/lymphocyte ratios were reported in the manic episode (25). In the only study, which evaluated MLR in patients with suicide attempts, the medical records of adolescents who attempted suicide were examined retrospectively. As a result, patients with suicide attempts were found to have higher MLR levels compared to healthy controls. In the same study, NLR was found to be high in patients with suicide attempts. In our results, the monocyte value was higher in MDD patient group with a suicide attempt compared to the group without a suicide attempt. Also, MLR levels were elevated in MDD with suicide attempts, which is similar to the study in the literature (5). In the present study, increased monocyte and MLR levels observed only in MDD with attempted suicide history could be due to the suicide attempt/thinking rather than due to depressive disorder. Hence, these parameters, which are an indication of peripheral inflammation, could be used as a predictor for suicide.

It is a parameter, which is inexpensive, easily-evaluated, and is an indicator of chronic inflammation, and fewer studies were conducted on PLR (5,26-28). No difference was found in our study for PLR between the MDD groups with / without suicide history compared to the healthy control group. In a study, in which patients with suicide attempts were evaluated retrospectively, the PLR levels of the patients were found to be higher than those of healthy controls (5). Findings similar to ours were observed in a study with adolescents in which the PLR level of depressive disorder patients was not different from that of healthy controls (26). In a study PLR was higher in MDD than the control (27). Higher PLR levels were also found in depressive disorder patients with psychotic character in a study evaluating the NLR and PLR parameters in MDD patients (28). PLR was examined in a limited number of studies in patients with suicide attempts (5), and the results obtained in MDD patients were contradictory, as mentioned above (26-28). In our results, NLR and MLR values were found to be more important in predicting the risk of suicide than PLR value. Although these results contribute to the literature, it is important to support the results with further studies.

Of the leucocyte levels evaluated in the present study, the lymphocyte level was not different among the study groups. White sphere values were high when compared to healthy controls in the suicide group, and basophil value was high in both patient groups. It was found in the present study that RDW-SD, not much encountered during the literature review, were higher in both patient groups. RDW-CV, red blood cell distribution correlation coefficient value was high only in patients with a suicide attempt. RDW-SD value is the most examined parameter in the studies conducted in the literature. In a study, the RDW-SD value was been reported as an indicator of high mortality in trauma-accidents, neurological, psychiatric diseases, and in general population (29). In another study, RDW value was shown to be associated with mortality without distinction as RDW-SD or RDW-CV in the general population (30). Higher RDW values were observed in depressive disorder patients. In the same study, lower hemoglobin and hematocrit values were observed for depressive disorder patients. Based on this finding, it was suggested that MDD patients carry risks for all three types of anemia (31). In another study, RDW values were found to be high and hemoglobin and hematocrit values were low in unipolar depressive disorder patients. With this result, depressive disorder patients were shown to be risky for anemia. In our results, depressive disorder patients were shown to be risky for anemia (32). Although RDW values were high in our results, hemoglobin values did not differ between groups. Hematocrit value, on the other hand, was found to be lower only in patients with suicide attempts than healthy controls. Similar to the value of hematocrit, the value indicating the percentage of erythrocyte and PLT (PCT) was also calculated to be low only in the group with suicide attempts when compared to healthy controls. Together, these findings all together would imply that anemia risk is higher in depressive disorder patients with attempted suicide history or that suicide risk could be higher in anemic patients.

In many studies in the literature, the BDI scores of patients with depressive disorder who attempted suicide were found to be higher than the group without suicide attempt (15,33,34).

Similarly, in our results, the BDI scores of the patients who had attempted suicide were much higher. It was an expected result that patients with suicide attempts were more depressive symptoms.

Our findings should be evaluated with some limitations. These are the retrospective nature of the study and relatively small patient populations. The diagnosis of MDD was made according to the DSM-5, and a clinical examination was performed by senior psychiatrists. But structured psychiatric assessments, such as the SCID-5, were not performed. In addiction, don't knowing the factors that may affect hematological parameters such as smoking., eating habits, body mass index and exercise status is also a limitation. Also, the limitations of our study include not examining the cases who attempted suicide more than once as a separate group, not examining the relationship between the suicide method, BDI scores and inflammation factors, and not specifying the duration of the suicide attempt. Besides, the lack of parameters such as C-reactive protein and inflammatory cytokines could be considered as other limitations. The fact that the study was conducted during the pandemic period can also be counted among the limitations as it may affect psychiatric applications. These limitations prevent a generalized interpretation of our results.

CONCLUSION

Our study is the first to examine peripheral inflammatory parameters in MDD patients with and without suicide attempt. It was found in the present study that irrespective of attempted suicide history, peripheral inflammation parameters were elevated in major depressive patients. NLR and MLR were higher in MDD patients with attempted suicide history compared to the ones without suicide attempts. Similarly, monocyte value was higher in MDD patients with attempted suicide history compared to the ones without suicide attempts. Although basophile and RDW-SD levels were elevated in MDD patients regardless of with or without suicide attempt, RDW-CV, white sphere and neutrophil values were high only in patients with suicide attempts. Also, PDW was found to be much higher in patients with suicide attempts. When all these results were evaluated together, it was considered that some peripheral inflammation markers might be used as a predictor of suicide in patients with major depressive disorders. In order to expand our results, further studies are required in larger sample groups, in which inflammation indicators such as CRP are also evaluated, and the relationship between the suicide method and inflammation factors is examined.

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

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

Ethics Committee Approval: *This study was approved by Elazığ Fethi Sekin State Hospital Local Ethics Committee (Number: 97132852/050.01.04; Date: 13.04.2020).*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: FO
Design of the study: FO, GT
Acquisition of data for the study: GT
Analysis of data for the study: FO
Interpretation of data for the study: FO, GT
Drafting the manuscript: FO
Revising it critically for important intellectual content: GT
Final approval of the version to be published: FO, GT

REFERENCES

[1] Dávila-Cervantes CA, Luna-Contreras M. Suicide attempt in teenagers: Associated factors. Rev Chil Pediatr. 2019;90(6):606-616.

[2] World Health Organization. Preventing suicide: A global imperative. Geneva: World Health Organization (WHO), 2014; First edition: 7-8.

[3] Turecki G, Ernst C, Jollant F, Labonte B, Mechawar N. The neurodevelopmental origins of suicidal behavior. Trends Neurosci. 2012;35(1):14-23.

[4] Serafini G, Parisi VM, Aguglia A, Amerio A, Sampogna G, Fiorillo A, Pompilli M, Amore M. A specific inflammatory profile underlying suicide risk? Systematic review of the main literature findings. Int J Environ Res Public Health. 2020;17(7):2393.

[5] Ucuz İ, Tetik BK. Can suicide behavior and seasonality of suicide be predicted from inflammatory parameters in adolescents? Medical Hypotheses. 2020;143(2020):110.061.110065.

[6] Pandey GN, Rizavi HS, Zhang H, Bhaumik R, Ren X. Abnormal protein and mRNA expression of inflammatory cytokines in the prefrontal cortex of depressed individuals who died by suicide. J. Psychiatry Neurosci. 2018;43(6):376-385.

[7] Courtet P, Giner L, Seneque M, Guillaume S, Olie E, Ducasse D. Neuroinflammation in suicide: Toward a comprehensive model. World J Biol Psychiatry. 2016;17(8):564-586.

[8] Sünbül M, Gerin F, Durmuş E, Kıvrak T, Sarı I, Tigen K, Cincin A. Neutrophil to lymphocyte and platelet to lymphocyte ratio in patients with dipper versus non-dipper hypertension. Clin Exp Hypertens. 2014;36(4):217-221.

[9] Azab B, Zaher M, Weiserbs KF, Torbey E, Lacossiere K, Gaddam S, Gobunsuy R, Jadonath S, Baldari D, McCord D, Lafferty J. Usefulness of neutrophil to lymphocyte ratio in predicting short – and long-term mortality after non-ST elevation myocardial infarction. Am J Cardiol. 2010;106(4):470-476.

[10] Giynas Ayhan M, Çiçek IE, İnanli I, Çalışkan AM, Kirci Ercan S, Eren I. Neutrophil/lymphocyte and platelet/lymphocyte ratios in all mood states of bipolar disorder. Psychiatry Clin Psychopharmacol. 2017;27(3):284-288.

[11] Sünbül EA, Sünbül M, Yanartaş O, Cengiz F, Bozbay M, Increased neutrophil/lymphocyte ratio in patients with depression is correlated with the severity of depression and cardiovascular risc factors. Psychiatry Investig. 2016;13(1):121-126.

[12] Moody G, Miller BJ. Total and differential white blood cell counts and hemodynamic parameters in first-episode psychosis. Psychiatry Res. 2018;260(2018): 307-312.

[13] Demir B, Şahin SK, Özsoy F, Altındağ A, Elboğa G. Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in methamphetamine use disorder. Psychiatr Clin Psychopharmacol. 2021;31(1):34-39.

[14] Kulu M, Özsoy F, Demir B. Alkol ve madde kullanım bozukluğu tanılı hastalarda inflamasyon düzeyleri. J Cukurova Med. 2021;46(4):1558-1565. (Turkish)

[15] Ayhan MG, Dağıstan AA, Tanrıkulu CŞ, Bozdoğan ŞY, Eren I. Increased neutrophil/lymphocyte ratio in suicide attempters. Anatolian J Psychiatr. 2019;20(3):305-312.

[16] Ivković M, Pantović Stefanović M, Dunjić Kostić B, Jurišić V, Lačković M, Totić-Poznanović S, Jovanovic A, Damjanovic D. Neutrophil to lymphocyte ratio predicting sui-cide risk in euthymic patients with bipolar disorder: Moderatory effect of family history. Compr Psychiatry. 2016;66(4):87-95.

[17] Meydaneri GG, Meydaneri S. Can neutrophil lymphocyte ratio predict the likelihood of suicide in patients with major depression? Cureus. 2018;10(4):e2510.

[18] Mazza MG, Lucchi S, Rossetti A, Clerici M. Neutrophil-lymphocyte ratio, monocyte-lymphocyte ratio and platelet-lymphocyte ratio in non-affective psychosis: A meta-analysis and systematic review. World J Biol Psychiatry. 2019;21(5):1-13.

[19] Özdin S, Böke Ö. Neutrophil/lymphocyte, platelet/lymphocyte and monocyte/lymphocyte ratios in different stages of schizophrenia. Psychiatry Res. 2019;271(7):131-135.

[20] Mazza MG, Capellazzi M, Lucchi S, Tagliabue I, Rossetti A, Clerici M. Monocyte count in schizophrenia and related disorders: a systematic review and meta-analysis. Acta Neuropsychiatr. 2020;32(5):229-236.

[21] Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4(6):561-571.

[22] Mazza MG, Lucchi S, Tringali AGM, Rossetti A, Botti ER, Clerici M. Neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in mood disorders: A meta-analysis. Prog Neuro-Psychopharmacol Biol Psychiatry. 2018;84(8):229-236.

[23] Adhikari A, Dikshit R, Karia S, Sonavane S, Shah N, De Sousa A. Neutrophil-lymphocyte ratio and C-reactive protein level in patients with major depressive disorder before and after pharmacotherapy. East Asian Arch Psychiatry. 2018;28(2):53-58.

[24] Miná VAL, Lacerda-Pinheiro SF, Maia LC, Pinheiro RFF Jr, Meireles CB, de Souza SIR, Reis AOA, Bianco B, Rolim MLN. The influence of inflammatory cytokines in physio-pathology of suicidal behavior. J Affect Disord. 2015;172(2):219-230.

[25] İnanli I, Aydın M, Çalışkan AM, Eren I. Neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, and mean platelet volume as systemic inflammatory markers in different states of bipolar disorder. Nord J Psychiatry. 2017;73(6):372-379.

[26] Özdin S, Sarısoy G, Böke Ö. A comparison of the neutrophil-lymphocyte, platelet-lymphocyte and monocyte-lymphocyte ratios in schizophrenia and bipolar disorder patients–a retrospective file review. Nord J Psychiatry. 2017;71(7):509-512.

[27] Cai L, Xu L, Wei L, Chen W. Relationship of mean platelet volume to MDD: a retrospective study. Shanghai Arch Psychiatry. 2017;29(1):21-29.

[28] Kayhan F, Gündüz Ş, Ersoy SA, Kandeğer A, Annagür BB. Relationships of neutrophil–lymphocyte and platelet–lymphocyte ratios with the severity of major depression. Psychiatry Res. 2017;247(7):332-335.

[29] Pan J, Borné Y, Engström G. The relationship between red cell distribution width and all-cause and cause-specific mortality in a general population. Sci Rep. 2019;9(1):16208.

[30] Zalawadiya SK, Veeranna V, Panaich SS, Afonso L, Ghali JK. Gender and ethnic differences in red cell distribution width and its association with mortality among low risk healthy United State adults. American J Cardiol. 2012;109(11):1664-1670.

[31] Demircan F, Gözel N, Kılınç F, Ulu R, Atmaca M. The impact of red blood cell distribution width and neutrophil/lymphocyte ratio on the diagnosis of major depressive disorder. Neurol Ther. 2016;5(1):27-33.

[32] Wysokinski A, Szczepocka E. Red blood cells parameters in patients with acute schizophrenia, unipolar depression and bipolar disorder. Psychiatr Danub. 2018;30(3):323-330.

[33] Sublette ME, Galfalvy HC, Fuchs D, Lapidus M, Grunebaum MF, Oquendo MA, Mann JJ, Postolache TT. Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder. Brain Behav Immun. 2011;25(6):1272-1278.

[34] Grassi-Oliveira R, Brieztke E, Teixeira A, Pezzi JC, Zanini M, Lopes RP, Bauer ME. Peripheral chemokine levels in women with recurrent major depression with suicidal ideation. Braz J Psychiatry. 2012;34(1):71-75.

How to cite this article: Taşçı G, Özsoy F. Inflammation in Major Depressive Disorder Patients with and without Attempted Suicide. Clin Exp Health Sci 2023; 13: 205-211. DOI: 10.33808/clinexphealthsci.1120081

Clinical and Experimental Health Sciences

The Prevalence of Drug-Drug Interactions and Reported Therapy Related Side-Effects in Oncology Out-Patients

Rashida Muhammad Umar[1], Zeynep Yeşim Can[2], Ece Güven Eke[1], Emine Karataş Koçberber[3], Ömer Fatih Ölmez[4]

[1] İstanbul Medipol University Faculty of Pharmacy, Department of Clinical Pharmacy, İstanbul, Türkiye.
[2] University of Health Sciences Faculty of Pharmacy, Department of Clinical Pharmacy, İstanbul, Türkiye.
[3] Marmara University Health Science Institute, Department of Clinical Pharmacy, İstanbul, Türkiye.
[4] İstanbul Medipol University Mega Hospital, Department of Medical Oncology, İstanbul, Türkiye.

Correspondence Author: Rashida Muhammad Umar
E-mail: rmumar@medipol.edu.tr

Received: 07.04.2022 **Accepted:** 27.09.2022

ABSTRACT

Objective: The use of multiple medications in cancer patients is unavoidable; thus, adverse drug-drug interactions are frequent. This study aims to assess the prevalence of potential drug interactions in oncology patients visiting the outpatient chemotherapy unit.

Method: Demographic and health-related information of patients visiting an outpatient chemotherapy unit was recorded using a pre-prepared form. A comprehensive list of all concurrently used medications was compiled and checked for interactions with the Micromedex online drug interaction tool.

Results: A total of 179 adult patients were included. We recorded an average of 9.3 drugs per patient with 79 patients using more than 10 drugs. A total of 1671 drugs including 303 chemotherapeutic agents were assessed for drug-drug interactions. A total of 374 interactions, of which 203 were significant, were recorded in 118 (65.9%) patients with an average of 3.2 interactions per patient. Only 46 major interactions were recorded for anticancer agents. Cyclophosphamide (n=13) and cisplatin (n=12) were involved in most interactions. The number of interactions correlated with the number of drugs used (p=.001) and the presence of comorbidities (p=.002). The presence of comorbidities increased the risk of interaction by 1.21 (p=.04). Recorded side effects were not correlated to drug interactions.

Conclusion: Medication review in cancer patients is essential in establishing all medications used by patients. Routine assessment in terms of potential drug interactions and evaluation of these interactions by a qualified pharmacist may help in optimizing patient outcomes.
Keywords: Cancer patients, Antineoplastic agents, Drug-drug interaction, Side effects, Polypharmacy.

1. INTRODUCTION

Cancer is a major life-threatening condition with a high rate of mortality and morbidity and increasing prevalence around the world. The treatment of cancer involves the use of highly toxic medications with low therapeutic index and serious adverse effects. The rate of drug-related problems is high in cancer patients due to the concurrent use of many drugs. These drugs are used for cancer treatment, side effect management, palliative and supportive care, and comorbidity treatment. Drug-drug interactions make up an important part of the drug-related problems seen in these patients (1,2).

There are different forms of drug interactions. These interactions can be with food, disease, laboratory analysis and other drugs. Drug-drug interactions can lead to changes in the therapeutic effects or adverse effects of drugs. The outcome of interactions is variable. Outcomes are often clinically insignificant, occasionally beneficial or harmful (3,4). Clinically significant interactions are those that have negative impact on patient outcomes (3) which are estimated to be between 3% and 20% (4). Drug interactions can lead to an increase in side effects already present with cancer medications (5). Assessment of drug interactions is fundamental in cancer patients for optimal management of pharmacotherapy. A systematic review of patients' medications at the beginning and with any change in regimen is necessary to prevent interactions (5,6).

The number of drugs used by a patient is an independent factor that increases the risk of drug interactions (7,8). Approximately, at least one drug-drug interaction may be present in the majority of the patients undergoing treatment for cancer. Some of these interactions may require medical intervention. Most potential drug-drug interactions are not detected or prevented due to the inefficient professional relationship between pharmacists and other health care providers. The most common consequences of interactions are gastrointestinal toxicity, QT prolongation, and central

DOI: 10.33808/clinexphealthsci.1099582

nervous system depression. Most interactions are reported to involve unavoidable supportive care medications including antiemetics, analgesics and steroids. The combination of these medication increases the risk of interactions (6,9–11).

In oncology, the main function of a pharmacist is to conduct a comprehensive medication review to prevent drug-related problems. The integration of clinical pharmacy services in the care of oncology patients optimizes therapeutic outcomes by improving medication appropriateness, reducing adverse drug events, increasing patient satisfaction, and reducing health expenditure (1,10,12)

This study aimed to determine the rate and significance of potential drug-drug interactions between concurrently used medications by cancer patients visiting the outpatient chemotherapy unit. And also to assess the rate of chemotherapy-related side effects and their relation to potential drug interaction.

2. METHODS

This prospective study was carried out in the outpatient chemotherapy delivery unit of Medipol Mega university hospital between January and April 2017. Ethics approval was obtained from Istanbul Medipol University Non-Invasive Clinical Trial Ethics Committee with Reference No: 18/2017 before the commencement of the study.

Adult cancer patients visiting the unit for treatment, who gave consent, were included in the study. The patients' demographics and health-related data including primary cancer site, the presence of comorbidities, and home medications were recorded using a pre-prepared form by 5th-year pharmacy students. Treatment protocols administered in the unit during the patients' visits were recorded from the unit's patient records. Under the supervision of a clinical pharmacist, a comprehensive list of all medications used by each patient was compiled and checked for clinically significant potential drug-drug interactions using the Micromedex online drug interaction checker (Access date: January – April 2017). Interactions were classified as contraindicated, major, moderate, minor, and unknown. Contraindicated and major interactions were considered significant interactions. Treatment-related side effects experienced by patients were also recorded and classified based on Common Terminology Criteria for Adverse Events (CTCAE) v5.0. These side effects were compared with major interactions involving chemotherapy agents and the possible significance of interactions were assessed.

Statistical analysis was done with SPSS 22.0 (Statistical Package for the Social Sciences) program. Spearman test and Pearson test were used to analyse correlations between numerical data and categorical data respectively. A logistic regression test was done to predict clinical risk factors. The results with p<.05 in the 95% confidence range were considered significant.

3. RESULTS

A total of 179 patients were included in the study. The majority of the patients were female (n=97, 54.2%). We classified our patients into three groups based on their ages (13). Most patients were in the 31-60 age group. The mean age was 55.8 ± 14.6. Some patients (n=81) reported having at least one comorbidity. Only four of these patients reported not using any medication for their comorbidities. The most common comorbid diseases reported were hypertension (n=35), diabetes (n=29) and hyperlipidemia (n=12). Demographic and health-related data of patients and distribution of interactions are given in Table 1.

Table 1. *Demographic data of patients and distribution of interactions*

Demographic details		Patients with interactions	Patients without interactions	Total n (%)
Gender	Male	54	28	82 (45.8)
	Female	64	33	97 (54.2)
Age	18-30	6	1	7 (3.9)
	30-60	58	38	96 (53.6)
	>60	54	22	76 (42.5)
Comorbidities	None	58	40	98 (54.7)
	At least 1	60	21	81 (45.3)
Number of medications used	< 5	8	23	31 (17.3)
	6-10	42	27	69 (38.5)
	>10	68	11	79 (44.2)
Presence of side effects	None	79	51	130 (72.6)
	At least one	39	11	49 (27.4)

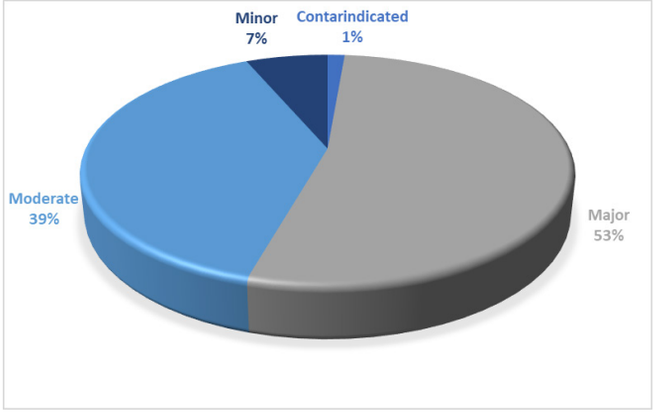


Figure 1*. Types of interactions*

The most common cancer sites were breast (C50) (n=49), lung (C35) (n= 37), and colon (C18) (n=15). At least one-cite metastasis was present in 24 patients. During their visits, most patients (n=163) received chemotherapy drugs while 16 patients received only palliative medications. The most received chemotherapy agents were paclitaxel (n=35), trastuzumab (n=27) and carboplatin (n=22). Cancer-related data and distribution of interactions are given in Table 2.

Table 2: *Patient and interaction distribution based on primary cancer type.*

Cancer type	Code	Sex		Total number of patients		Metastasis		Comorbidity		Average number of total drugs used	Average number of chemotherapy drugs used	Total number of interactions	Significant interactions (n)	Chemotherapy-related major interaction (n)
		Male (n)	Female (n)	n	%	None	At least one site	None	At least one					
Gastric	C16	2	2	4	2.2	4	0	3	1	7.3	2.0	1	1	0
Colon	C18	9	6	15	8.4	12	3	7	8	10.5	2.2	25	11	0
Rectal	C20	4	1	5	2.8	4	1	2	3	10.8	2.2	27	14	0
Liver	C22	3	2	5	2.8	4	1	3	2	11.2	1.4	19	17	1
Pancreas	C325	2	2	4	2.2	3	1	1	3	11.3	2.0	3	2	0
Lung	C35	26	11	37	20.7	33	4	20	17	10.7	1.8	99	56	6
Breast	C50	3	46	49	27.4	37	12	29	20	7.4	1.5	83	40	15
Ovary	C56	0	8	8	4.4	7	1	5	3	8.5	1.9	9	7	2
Prostate	C61	6	0	6	3.3	6	0	2	4	8.3	1.5	13	7	2
Testis	C62	3	0	3	1.7	3	0	3	0	7.7	2.0	1	1	0
Kidney	C64	1	2	3	1.7	3	0	0	3	8.0	1.0	4	1	0
Brain	C71	3	3	6	3.3	6	0	4	2	6.8	1.7	1	1	0
NHL	C85	5	0	5	2.8	5	0	2	3	12.8	2.6	15	8	7
MM	C90	1	2	3	1.7	3	0	2	1	10.0	1.3	15	8	2
AML	C92	2	2	4	2.2	4	0	1	3	9.5	1.0	18	8	0
Others		12	10	22	12.4	21	1	14	8	82.8	9.3	41	21	9
Total		82	97	179	100	155	24	98	81	-	-	374	203	44

AML – acute myelomonocytic leukemia; MM – Multiple myeloma; NHL – Non-Hodgkin lymphoma

Table 3. *Major drug-drug interactions involving agents used for cancer treatment*

Chemotherapy drug	Interacting drug (n)	Frequency (44)	Outcome of interaction
Cyclophosphamide	Allopurinol	6	Increase in cyclophosphamide toxicity
	Doxorubicin	6	Increase in cardiomyopathy risk
	Hydrochlorothiazide	1	Increase in cyclophosphamide effect and myelosuppression
Cisplatin	Furosemide	10	Increase in nephrotoxicity and ototoxicity risk
	Vinorelbine	2	Increase in granulocytopenia risk
Doxorubicin	Aprepitant	5	Increase in doxorubicin effect
	Dexamethasone	3	Decrease in doxorubicin effect
Methotrexate	Trimethoprim	1	Increase in methotrexate toxicity
Paclitaxel	Levonorgestrel	1	Increase in paclitaxel toxicity
Pazopanib	Lansoprazole	1	Reduction in pazopanib bioavailability
	Calcium Carbonate	1	Reduction in pazopanib bioavailability
	Magnesium Hydroxide	1	Reduction in pazopanib bioavailability
Pemetrexed	Diclofenac	1	Increase in pemetrexed toxicity
Tamoxifen	Domperidone	1	Increase in QT elevation risk
	Goserelin	1	Increase in QT elevation risk
Trastuzumab	Epirubicin	1	Increase in cardiac dysfunction risk
Vincristine	Filgrastim	1	Increase in severe peripheral neuropathy risk
	Dexamethasone	1	Decrease in serum vincristine levels

A comprehensive list of all medications used by each patient was compiled. The average number of total drugs used was 9.3 drugs per patient with 79 patients using more than 10 drugs. The use of at least one home medication was recorded in 134 patients with an average of 4.1. Fifty-eight of these patients were using only cancer-related medications while 76 patients were using cancer-related medications and/or medications for their comorbidities. A total of 303 chemotherapy medications were administered to 163 patients during their visit.

A total of 1671 drugs were assessed for drug-drug interactions. These included 303 chemotherapeutic agents, 555 reportedly used home medications and 813 palliative care and pre-medication agents. A total of 374 interactions were recorded in 118 (65.9%) patients with an average of 3.2 interactions per patient. Recorded interactions included five contraindicated, 198 major, 146 moderate, and 24 minor interactions as shown in Figure 1. Contraindicated interactions were recorded between palliative care medications which include ondansetron-posaconazole (n=3), fluconazole-granisetron (n=1) and fluconazole – ondansetron (n=1). Only 44 major interactions involved anticancer agents. A list of these interactions is given in Table 3. The most used anticancer agents included paclitaxel (n=35), trastuzumab (n=27), carboplatin (n=22), fluorouracil (n=19), bevacizumab (n=18), cisplatin (n=17) and cyclophosphamide (n=15). Cyclophosphamide (n=13) and cisplatin (n=12) were involved in most interactions.

The presence of comorbidities significantly correlated to the total number of drugs used (r=0.3; p=.001) and the number of total (r=0.23; p=.002), major (r=0.16; p=.03) and moderate interactions (r=0.2; p=.005). The presence of comorbidity increased the risk of interaction by 1.21 (p=.04). The number of interactions also correlates with the total number of drugs used (r=0.5; p=.001).

***Table 4**. Side effects reported by patients*

MedDRA SOC	CTCAE Term	Frequency
Blood and lymphatic system disorders	Anemia	1
	Neutropenia	2
	Thrombocytopenia	1
Ear and labyrinth disorders	Vertigo	1
Gastrointestinal disorders	Abdominal distension	1
	Abdominal pain	2
	Constipation	6
	Diarrhoea	3
	Dyspepsia	1
	Mucositis oral	4
	Nausea	23
	Rectal ulcer	1
	Salivary duct inflammation	3
	Stomach pain	1
	Toothache	2
	Vomiting	3
General disorders and administration site conditions	Fatigue	16
	Hot flashes	5
	Pain	4
Immune system disorders	Allergic reaction	3
	Anaphylaxis	1
Infections and infestations	Nail infection	4
Metabolism and nutrition disorders	Anorexia	1
	Arthralgia	1
	Hypercalcemia	1
	Hypokalemia	1
Nervous system disorders	Headache	2
	Hypersomnia	1
	Syncope	1
Psychiatric disorders	Anxiety	3
	Hallucinations	1
	Insomnia	3
Reproductive system and breast disorders	Vaginal dryness	1
Respiratory, thoracic, and mediastinal disorders	Hoarseness	1
Skin and subcutaneous tissue disorders	Alopecia	4
	Skin rash	1
	Palmar-plantar erythrodysesthesia syndrome	1
Vascular disorders	Flushing	2
	Hypertension	1
	Hypotension	1

MedDRA SOC – Medical Dictionary for Regulatory Activities System Organ Class: CTCAE-Common Terminology Criteria for Adverse Events

The therapy-related side effects experienced by patients were recorded. A total of 115 side effects were reported by 49 patients, with an average of 2.3 events per patient. Most reported side effects were gastrointestinal-related (n=50). Details are given in Table 4. We evaluated the possible influence of interactions on reported side effects. A possible interaction-related increase in side effects was noticed in only three patients. Two patients using cyclophosphamide and allopurinol reported having nausea and one patient using paclitaxel and Levonorgestrel reported having five different side effects which included nausea, vomiting, neutropenia, fatigue, and hair loss.

4. DISCUSSION

Drug-drug interactions can occur in most patients treated with more than one medication. The clinical significance of these interactions varies based on the severity and time to unset, in addition to patient and/or therapy-related factors. The outcomes of most interactions are unnoticed and unrecorded in the clinical setting. The use of multiple medications in cancer patients increases their susceptibility to drug interactions and outcomes in these patients may be devastating. In this study, we evaluated the incidence and significance of potential drug interaction that may be present in all concurrently used medications. The incidence of drug interactions was 65.95% in our study population with most interactions occurring in our female patients. This may be due to the study's higher number of female patients. Most of them had other diseases and thus polypharmacy was present in many. Similar studies have a comparable number of drug-drug interactions (1,8,14–17).

The presence of comorbidities was recorded as an independent factor that increased drug-drug interaction. Also, the frequency of interactions increased as the concurrent number of drugs used increased. The presence of comorbidities necessitates the use of other medications. Comorbidities and polypharmacy are usually associated with a higher incidence of drug interactions (18,19). A study revealed that interactions were more in patients receiving 7 or more medications (8). This is similar to our result as interactions were more common in patients receiving more than five drugs. The selection of chemotherapy regimens with drugs that have more interacting potential also predisposes patients to interactions (20). Comorbidities and polypharmacy are more common in elderly patients. We recorded at least one interaction in 22 patients above 60 years. Studies carried out mainly in geriatric cancer patients have revealed high incidences of drug-drug interactions and risk increases with the presence of comorbidities and polypharmacy (11,21,22).

We prepared a comprehensive medication list of all patients which included all medications the patients were taking at home. Most of these medications were involved in drug interactions. A study revealed a high prevalence of drug interactions among medications independently dispensed to the same set of cancer patients by a hospital pharmacist and

community pharmacist (23). There is a need for optimum medication reconciliation and surveillance in cancer patients due to the use of various sets of medications at the different levels of health care.

We also evaluated the frequency of reported chemotherapy side effects. Side effects were reported by 27.4% of the patients. This rate is lower when compared to other studies. In their retrospective study, Bayraktar et al reported a total of 9080 chemotherapy-related side effects in 347 patients (14). A similar study also recorded high levels of side effects in geriatric patients (24). Both studies recorded all symptoms that may be attributed to chemotherapy from doctor-filled patient information charts. This may have given them the opportunity to gain more verified data. The other study was mainly on geriatric patients who are more liable to side effects. Also, improvements in clinical practice aimed at improving better use of drugs, adequate prophylaxis and improving quality of life over the years may have led to decrease in the incidence of side effects.

Assessment of drug interactions in cancer patients is essential for the management of pharmacotherapy. To prevent interactions, routine evaluation of all patients' medications is required (5,25). The use of a computer-aided interaction check system in the prescription system has been shown to reduce incidences of interactions. These systems provide accurate proactive information enhancing quick decision-making (26,27). This can help in the early identification of possible threatening interactions. But the significance of identified interactions to individual patients' therapeutic outcomes needs to be assessed and verified by a qualified pharmacist. The positive impact of clinical pharmacy services on patient outcomes under different conditions has been shown in various studies (12, 28).

This study had some limitations. It was a single center study and as such there were limited number of patients. Only patient reported-therapy side effects were evaluated in terms of possible association to drug interactions. Clinical proof of this association was not established. Also patients were not monitored for clinical outcomes that may be associated to drug interactions. No interventions were made for all interactions.

5. CONCLUSION

Drug-drug interactions are quite common in cancer patients. Pharmacists can improve therapy by identifying potential drug interactions and drug-related problems in cancer patients. A clinical pharmacist as a member of a multidisciplinary healthcare team can ensure the provision of the safest chemotherapy regimens, effective treatment of comorbidities, and effective supportive and palliative care through comprehensive medication management.

Acknowledgments: *We want to thank the chemotherapy unit pharmacist and other staff for their support.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by Ethics committee of Istanbul Medipol University Non-Invasive Clinical Trial Ethics Committee (Decision date and number:18.01.2017; 18)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: RMU
Design of the study: RMU, EKK, ÖFÖ
Acquisition of data for the study: ZYC, EGE
Analysis of data for the study: RMU
Interpretation of data for the study: ZYC
Drafting the manuscript: RMU
Revising it critically for important intellectual content: RMU, ZYC, EKK
Final approval of the version to be published: RMU

REFERENCES

[1] Umar RM, Apikoglu-Rabus S, Yumuk PF. Significance of a clinical pharmacist-led comprehensive medication management program for hospitalized oncology patients. Int J Clin Pharm. 2020;42(2):652–661.
[2] Delpeuch A, Leveque D, Gourieux B, Herbrecht R. Impact of clinical pharmacy services in a hematology/oncology inpatient setting. Anticancer Res. 2015;35(1):457–460.
[3] Thanacoody R. Drug interactions. Whittlesea C, Hodson K, editors. Clinical Pharmacy and Therapeutics. China: Elsevier; 2019.p53-65.
[4] Wiffen P, Mitchell M, Snelling M, Stoner N. Oxford Handbook of Clinical Pharmacy. 3rd ed. United Kingdom: Oxford Press; 2017. p17-26.
[5] van Leeuwen RW, Swart EL, Boom FA, Schuitenmaker MS, Hugtenburg JG. Potential drug interactions and duplicate prescriptions among ambulatory cancer patients: a prevalence study using an advanced screening method. BMC Cancer 2010;10:679. DOI: 10.1186/1471-2407-10-679.
[6] Lopez-Martin C, Garrido Siles M, Alcaide-Garcia J, Faus Felipe V. Role of clinical pharmacists to prevent drug interactions in cancer outpatients: A single-centre experience. Int J Clin Pharm. 2014 Dec;36(6):1251-1259.
[7] Conde-Estévez D. Targeted cancer therapy: interactions with other medicines. Clin Transl Oncol. 2017;19(1):21-30.
[8] Ismail M, Khan S, Khan F, Noor S, Sajid H, Yar S. Prevalence and significance of potential drug-drug interactions among cancer patients receiving chemotherapy. BMC Cancer 2020;20(1):1–9.
[9] Umar RM. Drug-drug interactions between antiemetics used in cancer patients. J Oncol Sci. 2018;4:142-146.
[10] Leveque D, Delpeuch A, Gourieux B. New anticancer agents: Role of clinical pharmacy services. Anticancer Res. 2014;34(4):1573–1578.
[11] Popa MA, Wallace KJ, Brunello A, Extermann M, Balducci L. Potential drug interactions and chemotoxicity in older patients with cancer receiving chemotherapy. J Geriatr Oncol. 2014;5(3):307–314.
[12] Han JM, Ah YM, Suh SY, Jung SH, Hahn HJ, Im SA, Lee JY. Clinical and economic impact of pharmacists' intervention in a large volume chemotherapy preparation unit. Int J Clin Pharm. 2016;38(5):1124–1132.
[13] Chaudhari SJ. Methodology for gender identification, classification and recognition of human age. Int J Comput

Appl. 2015;(NCAC):5–10. Available from: https://research.ijcaonline.org/ncac2015/number2/ncac175023.pdf LK – https://academic.microsoft.com/paper/237.510.4003

[14] Bayraktar-Ekincioglu A, Demirkan K, Keskin B, Aslantas O, Ozdemir E. Potential drug interactions and side effects in an outpatient oncology clinic: A retrospective descriptive study. Eur J Hosp Pharm. 2014;21(4):216–221.

[15] Holweger K, Bokemeyer C, Lipp HP. Accurate measurement of individual glomerular filtration rate in cancer patients: An ongoing challenge. J Cancer Res Clin Oncol. 2005;131(9):559–567.

[16] Vayalil RK, Shetty KJ, Mateti UV. Assessment of potential drug-drug interactions in an oncology unit of a tertiary care teaching hospital. Indian J Med Paediatr Oncol. 2018;39(4):436–442.

[17] Rashdan S, Yang H, Le T, Selby C, Gerber DE, Alvarez CA. Prevalence and significance of potential pharmacokinetic drug-drug interactions among patients with lung cancer: Implications for clinical trials. Clin Drug Investig. 2021;41(2):161–167.

[18] Akbulut M, Urun Y. Onco-cardiology: Drug-drug interactions of antineoplastic and cardiovascular drugs. Crit Rev Oncol Hematol. 2020;145:102822.

[19] Ramos-Esquivel A, Víquez-Jaikel A, Fernández C. Potential drug-drug and herb-drug interactions in patients with cancer: A prospective study of medication surveillance. J Oncol Pract. 2017;13(7): e613–620.

[20] Bibi R, Azhar S, Iqbal A, Jabeen H, Kalsoom UE, Iqbal MM, Nazeer M. Prevalence of potential drug-drug interactions in breast cancer patients and determination of their risk factors. J Oncol Pharm Pract. 2021;27(7):1616–1622.

[21] Alkan A, Yaşar A, Karcı E, Köksoy EB, Ürün M, Şenler FÇ, Ürün Y, Tuncay G, Ergün H, Akbulut H. Severe drug interactions and potentially inappropriate medication usage in elderly cancer patients. Support Care Cancer 2017;25(1):229–236.

[22] Ramasubbu SK, Mahato SK, Agnihotri A, Pasricha RK, Nath UK, Das B. Prevalence, severity, and nature of risk factors associated with drug-drug interactions in geriatric patients receiving cancer chemotherapy: A prospective study in a tertiary care teaching hospital. Cancer Treat Res Commun. 2021;26:100277.

[23] Voll ML, Yap KD, Terpstra WE, Crul M. Potential drug-drug interactions between anti-cancer agents and community pharmacy dispensed drugs. Pharm World Sci. 2010;32(5):575–580.

[24] Paul T, Palatty P, Adnan M, George T, Rao S, Baliga M. Audit of drug-drug interactions and adverse drug reactions due to polypharmacy in older cancer patients: First report from India. Indian J Cancer 2020;57(4):405–410.

[25] Lopez-Martin C, Garrido Siles M, Alcaide-Garcia J, Faus Felipe V. Role of clinical pharmacists to prevent drug interactions in cancer outpatients: a single-centre experience. Int J Clin Pharm. 2014;36(6):1251–1259.

[26] Vecchia S, Orlandi E, Confalonieri C, Damonti E, Riva A, Sartori A, Cavanna L. Prevalence study on potential drug–drug interaction in cancer patients in Piacenza hospital's Onco-Haematology department. J Oncol Pharm Pract. 2018;24(7):490–493.

[27] Xiong G, Yang Z, Yi J, Wang N, Wang L, Zhu H, Wu C, Lu A, Chen X, Liu S, Hou T, Cao D. An online drug-drug interaction database towards improving clinical decision-making and patient safety. Nucleic Acids Res. 2022;50(D1):D1200-D1207.

[28] Mousavi M, Hayatshahi A, Sarayani A, Hadjibabaie M, Javadi M, Torkamandi H, Gholami K, Ghavamzadeh A. Impact of clinical pharmacist-based parenteral nutrition service for bone marrow transplantation patients: a randomized clinical trial. Support Care Cancer 2013;21(12):3441–3448.

How to cite this article: Umar RM, Can ZY, Güven Eke E, Karataş Koçberber E, Ölmez ÖF. The Prevalence of Drug-Drug Interactions and Reported Therapy Related Side-Effects in Oncology Out-Patients. Clin Exp Health Sci 2023; 13: 212-217. DOI: 10.33808/clinexphealthsci.1099582

Clinical and Experimental Health Sciences

Clinical and Biochemical Effects of Smoking on Non-Surgical Periodontal Treatment in Grade III Stage C Periodontitis Patients

Volkan Arıkan[1], Nimet Gül Görgülü[1], Başak Doğan[2]

[1] Marmara University, Institute of Health Science, Department of Periodontology, İstanbul, Türkiye.
[2] Marmara University, Faculty of Dentistry, Department of Periodontology, İstanbul, Türkiye.

Correspondence Author: Başak Doğan
E-mail: basakdogan@marmara.edu.tr
Received: 08.06.2022 **Accepted:** 07.08.2022

ABSTRACT

Objective: The purpose of this study was to evaluate the effect of smoking on clinical parameters and the serum and saliva levels of RANKL, OPG, and IL-34 in periodontitis stage III grade C (III-C) patients after non-surgical periodontal treatment (NSPT).

Methods: A total of 60 subjects, 40 periodontitis-III-C patients (20 smokers and 20 non-smokers) and 20 non-smoker periodontally healthy individuals, were included. All clinical periodontal parameters were recorded, and unstimulated saliva and serum samples were collected from all patients at baseline, but at 1 and 3 months only from periodontitis patients (N=40). Saliva and serum levels of RANKL, OPG, and IL-34 were analyzed by ELISA.

Results: At baseline only whole mouth probing depth (PD) and percent of sites with PD>5mm were higher in smokers than non-smoker periodontitis patients ($p<.05$). All periodontal measurements significantly improved in both periodontitis groups after NSPT ($p<.001$). After NSPT, the reduction in gingival index (GI), bleeding on probing (BOP), and mean PD in sites initially PD≥5mm were lower in smokers than non-smoker periodontitis patients ($p>.05$). Only saliva IL-34 levels were higher in all-periodontitis patients than healthy individuals ($p=.001$) and decreased in both periodontitis groups after NSPT ($p<.05$). Moreover, elevated serum RANKL level was detected in smokers compared to non-smoker periodontitis or healthy ones at baseline ($p<.05$). Serum RANKL levels exhibited no change after NSPT in either periodontitis groups.

Conclusions: The smokers are less responsive to NSPT, and saliva IL-34 can be a potential inflammatory marker of periodontitis-III-C. Moreover, high serum RANKL levels are associated with smoking.

Keywords: periodontitis, smoking, interleukin-34, receptor activator of nuclear factor-kappa B ligand, osteoprotegerin

1. INTRODUCTION

Periodontitis is caused by an inflammatory response to periopathogens in periodontal tissues that results in periodontal attachment loss and bone resorption around the teeth (1). Several risk factors, such as smoking, gender, obesity, metabolic syndrome, and genetic factors, play a crucial role in periodontal disease progression. Previous studies revealed that smokers are 2 to 8 times more prone to periodontal disease (2) and have more severe tissue destruction, insufficient wound healing due to reduced vascularization, and lesser response to periodontal treatments (3). Although some studies reported higher whole mouth PD reductions in non-smokers than smokers after non-surgical periodontal treatment (NSPT) (4,5), some studies reported similar results (6,7).

Most research supports that smoking affects the periodontal tissues by microcirculatory and host immune systems and bone metabolism (8,9). However, the exact mechanisms of alveolar bone are still unclear. Nicotine is the primary compound in tobacco, which inhibits osteogenesis and angiogenesis. Nicotine can bind to nicotinic receptors in osteoblasts. This binding promotes cell proliferation when nicotine levels are low; however, increased nicotine levels inhibit osteoblast formation, leading to cell death (10). In the osteoclastogenesis and bone turnover cycle, the balance of three molecules, receptor activator of nuclear factor kappa B (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG) is essential (11). The proliferation and differentiation of osteoclasts is stimulated by the attachment of RANKL to RANK on osteoclast or osteoclast precursor cells (12,13). RANKL can be detected in two forms: a cell membrane-bound variant (mRANKL) and a primary soluble form (sRANKL) (12). As for OPG, a naturally soluble decoy receptor generated by osteoclasts has the opposite effect and suppresses osteoclast differentiation. RANKL is bound by OPG, which prevents it from interacting with the RANK receptor (14).

DOI: 10.33808/clinexphealthsci.1128101

Increased RANKL/OPG ratio caused by bone loss and due to increase in RANKL or decrease in OPG levels, or both. Therefore, the balance between OPG and RANKL activity can accelerate bone resorption or bone formation (13). Many studies investigate the relationship between smoking and RANKL–OPG system. However, the results of these studies were controversial. Some studies reported that smokers had a lower serum or gingival crevicular fluid (GCF) level of OPG and a higher RANKL/OPG ratio than non-smokers (15-17). On the other hand, some studies reported no statistical differences in GCF, saliva, and serum levels of OPG between smoker and non-smoker periodontitis patients (18,19). Regarding the effect of NSPT on OPG, studies showed that its level in GCF was decreased after NSPT, but in saliva or serum either increased or showed no difference (20-22).

Recently, functional screening of proteins secreted from an embryonic kidney cell has led to the recognition a new molecule called interleukin 34 (IL-34). It has been observed that this cytokine stimulates macrophages to create colonies from bone marrow cells (23). In RANKL-induced osteoclastogenesis, IL-34 can replace macrophage colony-stimulating factor (M-CSF) and promote osteoclast differentiation as M-CSF does. A recent study showed that serum and GCF IL-34 levels in smokers were higher than in non-smoker patients, which suggested that smoking may be an essential factor in releasing IL-34 (24). To our knowledge, no study that reported the relationship between smoking and the salivary IL-34 before and after NSPT. In the present study, we hypothesize that elevated salivary IL-34 levels are associated with smoking and periodontitis, and NSPT might explore a positive impact on salivary IL-34 levels.

Therefore, the purpose of this study is to analyze the impact of smoking on clinical periodontal parameters and the serum and saliva levels of RANKL, OPG, and IL-34 in periodontitis stage III grade C (III-C) patients after NSPT.

2. METHODS

2.1. Study Population

A total of 60 patients were involved in this study. All patients were selected from the Department of Periodontology, Faculty of Dentistry, Marmara University, Istanbul, Turkey. The study was approved by the Clinical Research Ethics Committee of Faculty of Medicine, Marmara University (No: 09.2018.513/ Date: 13.07.2018) and submitted to clinicaltrials.gov with the number NCT05262153. The study protocol was explained to the participants and each participant signed informed consent form.

Medical and dental histories were obtained. All volunteers were selected based on these criteria; (1) systemically healthy adults (aged>18 years), (2) having at least 24 teeth, (3) no periodontal treatment in the last 6 months, (4) no use of any antibiotics or anti-inflammatory drugs that could affect their periodontal status for 3 months, (5) no lactation, and (6) no pregnancy.

The selection of patients was made according to the clinical and radiological criteria proposed by the 2018 classification of periodontal disease (25). The periodontitis patients (N=40, 20 smokers and 20 non-smokers) were stage III in severity and grade C in the progression rate. These patients had at least 5 non-adjacent interdental sites with interdental PD≥ 6mm, clinical attachment level (CAL)≥5mm (due to periodontal causes), and BOP≥30%. They had not lost more than four teeth due to periodontitis. The grade was assessed according to the the indirect evidence of progression due to lack of direct evidence. To calculate the bone loss%/age, the worst affected tooth in the dentition was selected and radiographic bone loss was expressed as a percentage of root length, and divided by the patient's age. All patients were grade C, regardless of smoking risk factor, since bone loss%/age were higher than 1.0 (25). Inclusion criteria of the healthy group presented PD≤ 3mm with no alveolar bone loss or no interproximal attachment loss, BOP<10% (26), and no smoking. Smokers defined as smoking more than 10 cigarettes per day for at least 5 years, while non-smokers as never smoked.

2.2. Sample Size Calculation

In this study, the sample size required to obtain sufficient power was calculated based on the changes in probing depth. In a prior study, (27) the mean difference in PD reduction between treatment groups was 0.44 mm, with an estimated standard deviation of 0.29 mm. Hence, 12 subjects were needed in each group to detect a difference with a power of 95% and an α error of 0.05 error. Twenty individuals were recruited in each group to compensate for any potential dropouts during the trial.

2.3. Non-Surgical Periodontal Treatment

Before NSPT, oral hygiene instructions, including brushing, flossing, and interdental brushing, were given to all patients. In both treatment groups, scaling and root planning was performed by using ultrasonic instruments (Guilin Woodpecker Medicals Ins. Co., China) and Gracey curettes (Hu-Friedy, Chicago, IL, USA) under local anesthesia twice a week in 4 sessions.

2.4. Clinical Periodontal Measurements

Clinical periodontal examinations were performed by one calibrated clinician (V.A.). Five periodontitis patients, who were not included in the study, were subjected to intra-examiner calibration. PD and CAL parameters were recorded with one-day apart. The intraexaminer kappa score was 0.91 for PD and 0.90 for CAL. All clinical measurements were recorded at baseline, 1 and 3 months after NSPT, including plaque index (PI), (28) GI, (29) BOP, PD, and CAL. During these procedures, the UNC-15 periodontal probe (Hu-Friedy, Chicago, IL, USA) was used, and except the third molars, all teeth were measured from six points.

2.5. Saliva and Serum Sampling

Unstimulated saliva samples were obtained from all the patients in the morning after at least 8 hours of fasting to minimize diurnal variations. Oral hygiene, food, and drinking were not allowed 3 hours before the procedure (30). The patients were asked to accumulate their saliva in the oral cavity for 2 minutes and then transfer at least 3.0 ml of saliva directly to a sterile glass beaker. Collected samples were transferred to sterile propylene tubes (Safe-Lock Tubes 2.0 ml, Sigma, Hamburg) with an automatic pipette. Subsequently, samples were stored at – 80° C until the day of analysis.

Serum was collected from the right or left antecubital regions with a vacutainer (BD Vacutainer Safety-Lok Blood Collection Set, USA) into 8.5 ml vacuum tubes (BD Vacutainer SST-II Advance) and centrifuged at 5000 rpm for 10 minutes. Serum samples were transferred to sterile propylene tubes and preserved at – 80 °C till the analysis day.

2.6. Biochemical Analysis

At the day of analyses, all samples were thawed. The salivary samples were centrifuged at 5500 rpm for 15 min at room temperature. Salivary cotinine (BT Labsystems, Shangai, China), salivary and serum RANKL, OPG, and IL-34 (Elabscience, Beijing, China) levels were analyzed with ELISA technique. All samples were tested in duplicate. RANKL, OPG, and IL-34 were detectable in all saliva and serum samples. The limit of detection for the assay was 0.5 ng/ml for cotinine, 0.10 ng/ml OPG, 9.38 pg/ml for RANKL, and 56.25 pg/ml for IL-34.

2.7. Statistical Analysis

The findings of the study were evaluated using the statistical package program (SPSS v22.0 for Windows, IBM, Chicago, IL). Distribution of clinical and biochemical variables was measured by Shapiro Wilk test. Since the variables were not distributed normally nonparametric tests were performed. The Kruskal-Wallis test was used for inter-group multiple comparisons and Bonferroni-corrected Mann-Whitney U test or Mann-Whitney U test for inter-group pairwise comparisons. For multiple intra-group comparisons Friedman test were performed when significant Bonferroni-corrected Wilcoxon test for pairwise comparisons. The Chi-Square test was performed to compare the gender distributions among groups. The Spearman correlation test was used to determine the correlations between clinical and biochemical parameters. The association of periodontitis or smoking with biochemical parameters was assessed using multinomial logistic regression. Statistical significance was accepted as p <.05.

3. RESULTS

During the whole study period, there were no dropouts, and the individuals did not change their smoking habits.

3.1. Clinical Findings

All study groups were similar in gender distribution (p>.05). The periodontitis groups were similar in age distribution (p>.05), but the healthy group was significantly younger than both periodontitis groups (p<.001). The median cigarette consumption/day was 20 and smoking duration/year was 20 in the smoker periodontitis group (Table 1).

***Table 1**. Demographic findings of the study*

Demographic variables	GROUPS (A)Healthy N=20	(B) Non-smoker Periodontitis-III-C N=20	(C) Smoker Periodontitis-III-C N=20	(A-B-C) p[a]	(A-B) p[b]	(A-C) p[b]	(B-C) p[b]
Age (years) median (Q1–Q3)	30.50 (27–33.5)	38 (35–51)	42.5 (38–48)	**<.001**	**<.001**	**<.001**	1.000
Sex N (%) **Female**	12 (60)	10 (50)	7 (35)				
Male	8 (40)	10 (50)	13 (65)	.281			
Cigarette consumption/day median (Q1–Q3)	-	-	20 (20–20)	-			
Smoking duration (year) median (Q1–Q3)	-	-	20 (15–25)	-			

[a]For gender Chi square test, others Kruskal Wallis test [b]Bonferroni-corrected Mann-Whitney U-test, p<.05 Statistically significant differences are marked in bold.

All clinical periodontal parameters in the healthy individuals were lower than in the periodontitis patients at baseline (p<.001) (Table 2). Except for PD, all clinical periodontal parameters at baseline between the non-smoker and the smoker periodontitis patients were similar (p>.05). Mean PD was higher in the smoker periodontitis group than in the non-smoker periodontitis group (p=.030). At 1 month, except for PD≥5mm+BOP and PD sites (%) with PD>6mm, all periodontal parameters were higher in the smoker periodontitis patients than in non-smoker periodontitis ones (p<.05). At 3 months, PD, PD sites with initially PD≥5mm, PD sites (%) with PD≥5mm, and CAL were higher in smoker than non-smoker periodontitis (p<.05).

***Table 2**. Comparison of clinical periodontal parameters among treatment groups at baseline, 1 and 3 months.*

Clinical parameters	Time points	GROUPS (A) Healthy N=20	(B) Non-smoker Periodontitis-III-C N=20	(C) Smoker Periodontitis-III-C N=20	(A-B-C) p[a]	(A-B) p[b]	(A-C) p[b]	(B-C) p[b]
PI	Baseline	0.07 (0.04–0.10)	2.23 (2.06–2.43)	2.25 (2.13–2.43)	**<.001**	**<.001**	**<.001**	1.000
	1 month	-	0.16 (0.04–0.33)[d]	0.38 (0.23–0.55)[d]				**.002**
	3 months	-	0.18 (0.04–0.28)[d]	0.19 (0.14–0.25)[d,e]				.323
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	2.14 (1.95–2.29)	2.02(1.91–2.25)				.892
GI	Baseline	0.04 (0.02–0.07)	1.45 (0.72–1.99)	1.21(0.62–1.48)	**<.001**	**<.001**	**<.001**	.984
	1 month	-	0.17 (0.06–0.41)[d]	0.44 (0.24–0.60)[d]				**.005**
	3 months	-	0.13 (0.08–0.26)[d]	0.20 (0.14–0.36)[d,e]				.072
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	1.24 (0.61–1.84)	0.88 (0.51–1.12)				**.036**
BOP (%)	Baseline	2.38 (1.34–4.74)	72.24 (36.2–95.30)	58.38 (31.35–72.7)	**<.001**	**<.001**	**<.001**	.696
	1 month	-	10.66 (5.7–20.56)[d]	22.34 (12.05–29.73)[d]				**.022**
	3 months	-	12.08 (5.73–15.71)[d]	9.97 (7.08–17.85)[d,e]				.776
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	58.52 (30.80–82.22)	44.15 (25.75–54.39)				**.026**
PD (mm)	Baseline	1.99(1.85–2.07)	3.86 (3.41–4.15)	4.54 (4.12–4.95)	**<.001**	**<.001**	**<.001**	**.030**
	1 month		2.79 (2.51–2.93)[d]	3.47 (3.22–3.95)[d]				**<.001**
	3 months		2.54 (2.37–2.74)[d]	3.33 (3.07–3.71)[d,e]				**<.001**
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	1.34 (0.83–1.44)	1.18 (1.04–1.52)				.960
Mean PD (mm) sites initially PD≥5 mm	Baseline		5.71 (5.5–5.9)	5.66 (5.4–6)				.659
	1 month		3.54 (3.2–3.9)[d]	4.16 (3.8–4.5)[d]				**.006**
	3 months		3.44 (2.9–3.8)[d]	3.93 (3.5–4.3)[d,e]				**.010**
	p[c]		**<.001**	**<.001**				
	Δ 0-3		2.40 (2.2–2.7)	1.84 (1.6–2.1)				**<.001**
Sites with PD≥5 mm (%)	Baseline		35.37 (21.3–42.2)	49.97 (37.6–61.9)				**<.001**
	1 month		7.44 (4.2–12.8)[d]	20.20 (8.4–34)[d]				**.005**
	3 months		4.17 (3–9.4)[d]	14.20 (6.9–31)[d]				**.002**
	p[c]		**<.001**	**<.001**				
	Δ 0-3		23.36 (15.1–35.8)	30.45 (24.5–37.6)				.068
Sites with PD≥5 mm and BOP (%)	Baseline		25.74 (15.1–40.4)	31 (14.3–44.2)				.383
	1 month		3.12 (1.3–6.1)[d]	5.10 (1.6–8.1)[d]				.242
	3 months		1.65 (0.7–4.8)[d]	3.15 (0.9–5.6)[d]				.355
	p[c]		**<.001**	**<.001**				
	Δ 0-3		23.07 (12–35.7)	27.46 (11.9–37.6)				.369
Sites with PD>6 mm (%)	Baseline		6.45 (4–10.1)	9.92 (4.2–17.1)				.102
	1 month		0.70 (0–1.5)[d]	1.25 (0–3.6)				.242
	3 months		0.00 (0–0.7)[d]	0.00 (0–2.4)[d]				.414
	p[c]		**<.001**	**<.001**				
	Δ 0-3		5.60 (4–8.3)	9.20 (3.7–15.1)				.121
CAL (mm)	Baseline	2.01(1.9–2.1)	4.61 (3.9–5.5)	5.32 (4.7–5.9)	**<.001**	**<.001**	**<.001**	.498
	1 month	-	3.54 (3–4.0)[d]	4.28 (3.7–4.9)[d]				**.007**
	3 months	-	3.22 (2.8–3.7)[d]	4.06 (3.4–4.8)[d,e]				**.008**
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	1.14 (0.6–1.8)	1.15 (0.9–1.5)				.522

Data are presented as median (Q1–Q3) values. [a]Kruskal Wallis test. [b]Bonferroni-corrected Mann-Whitney U-test or Mann-Whitney U-test. [c]Friedman test. [d]Significant difference compared to baseline, [e]Significant difference compared to 1 month (posthoc Bonferroni-corrected Wilcoxon sign-rank test), p<.05. Statistically significant differences are marked in bold. Abbreviations: PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

***Table 3**. Comparison of biochemical parameters among groups at baseline, 1 and 3 months.* Data are presented as median (Q1–Q3) values.

Biochemical parameters	Time points	GROUPS (A) Healthy N=20	(B) Non-smoker Periodontitis-III-C N=20	(C) Smoker Periodontitis-III-C N=20	(A-B-C) p[a]	(A-B) p[b]	(A-C) p[b]	(B-C) p[b]
Saliva Cotinine (ng/ml)	Baseline	0.10 (0.05–0.59)	0.28 (0.08–0.60)	10.80 (9.14–19.81)	**<.001**	1.000	**<.001**	**<.001**
Saliva RANKL (pg/ml)	Baseline	729.05 (474.8–948.7)	736.60 (624–948.5)	787.50 (682.4–887.7)	.591	-	-	-
	1 month		607.66 (394–791.4)	806.25 (611.9–1268.3)				**.014**
	3 months		667.29 (458.7–1040.8)	830.81 (609.7–1042.2)				.211
	p[c]		.142	.861				
	Δ 0-3		-23 (-198.2–374.2)	-75.14 (-244.6–235.1)				.758
Saliva OPG (pg/ml)	Baseline	1200 (362.5–2175)	1835 (1037.5–2277.5)	1960 (947.5–3392.5)	.064	-	-	-
	1 month		870 (552.5–1492.5)[d]	1105 (455–1725)				.583
	3 months		875 (587.5–1262.5)[d]	635 (572.5–1080)[d]				.355
	p[c]		**.002**	**.001**				
	Δ 0-3		535 (52.5–1292.5)	850 (465–2572.5)				.091
Saliva RANKL/OPG (pg/ml)	Baseline	0.61 (0.44–1.41)	0.41 (0.4–0.6)	0.43 (0.3–0.7)	.079	-	-	-
	1 month		0.68 (0.3–1.1)	1.07 (0.4–1.4)[d]				.086
	3 months		0.95 (0.6–1.2)[d]	1.43 (0.6–2.0)[d]				.114
	p[c]		**.041**	**.002**				
	Δ 0-3		-0.39 (-0.8 – – 0.2)	-0.59 (-1.7– – 0.2)				.134
Saliva IL-34 (pg/ml)	Baseline	99.45 (17.8–346.4)	431.38 (132.3–1165)	340.38 (184.1–1543.3)	**.001**	**.001**	**.001**	.718
	1 month		299.36 (157.1–502.7)[d]	304.69 (191.4–495.1)[d]		**.018**	**.007**	.779
	3 months		266.87 (172.7–368)[d]	237.02 (169–416.4)[d]		**.024**	**.017**	.947
	p[c]		**.040**	**.047**				
	Δ 0-3		197.87 (79.1–785.5)	168.8 (-82.1–1106.2)				.820
Serum RANKL (pg/ml)	Baseline	13.85 (6.9–42.7)	11.25 (8.5–27.4)	34.56 (15.5–51.7)	**.012**	.989	**.026**	**.003**
	1 month		11.89 (5.7–29.6)	34.52 (11.4–58.1)				**.028**
	3 months		9.39 (4.9–15.1)	25.9 (14.9–48.5)				**.009**
	p[c]		.449	.861				
	Δ 0-3		1.97 (-4.6–21.6)	4.93 (-8.4–20.4)				.989
Serum OPG (pg/ml)	Baseline	195 (122.5–320)	220 (152.5– 965)	515 (257.5–777.5)	.076	-	-	-
	1 month		330 (137.5–660)	290 (157.5–777.5)				.947
	3 months		240 (172.5–330)	485 (152.5–605)				.108
	p[c]		.711	.091				
	Δ 0-3		20 (-50–362.5)	35 (-77.5–155)				.779
Serum RANKL/ OPG (pg/ml)	Baseline	0.07 (0.03–0.2)	0.05 (0.03–0.08)	0.07 (0.05–0.12)	.073	-	-	-
	1 month		0.04 (0.02–0.06)	0.06 (0.03–0.18)				**.043**
	3 months		0.04 (0.03–0.07)	0.05 (0.04–0.10)				.127
	p[c]		.522	.819				
	Δ 0-3		0.01 (-0.02–0.03)	0.01 (-0.04–0.06)				.947
Serum IL-34 (pg/ml)	Baseline	24.65 (22.73–26.9)	24.35 (23.3–25.4)	24.94 (23.9–26)	.519	-	-	-
	1 month		17.59 (15.7–24.4)[d]	15.99 (15.5–19.1)[d]				.134
	3 months		16.44 (15.9–18.2)[d]	17.36 (16–51.4)				.327
	p[c]		**<.001**	**<.001**				
	Δ 0-3		7.35 (5.2–9.4)	7.52 (-27–8.9)				.445

[a]Kruskal Wallis test. [b]Bonferroni-corrected Mann-Whitney U-test or Mann-Whitney U-test. [c]Friedman test. [d]Significant difference compared to baseline (posthoc Bonferroni-corrected Wilcoxon sign-rank test), p<.05 Statistically significant differences are marked in bold. Abbreviations: RANKL, nuclear factor kappa B ligand; OPG, osteoprotegerin; IL, interleukin; pg, picogram; ml, mililiter ; ng, nanogram

Significant improvements in all clinical periodontal parameters at 1 and 3 months compared to baseline (p<.05) were detected in both periodontitis groups. Although reduction in BOP and GI were significantly higher at baseline to 3 months in the non-smoker than smoker periodontitis group (p=.036 and p=.026, respectively), PD and CAL reduction were similar in non-smoker and smoker periodontitis patients (p>.05). The reduction in the mean PD sites with initially PD≥5mm was significantly higher at baseline to 3 months in non-smoker than smoker periodontitis (p<.001).

3.2. Biochemical Findings

All self-defined smoker subjects had cotinine levels ≥8 ng/ml and were confirmed as active smokers (31). The median value of salivary cotinine levels in smoker periodontitis was higher than in healthy or non-smoker periodontitis groups (p<.001) (Table 3).

Salivary levels of OPG, RANKL, RANKL/OPG were similar among groups at baseline (p>.05). In both periodontitis groups, salivary OPG levels decreased, and RANKL/OPG levels increased after NSPT (p<.05). Only IL-34 levels in saliva were lower in the non-smoker periodontally healthy group than both periodontitis groups at all time points (p<.05). Besides, in the non-smoker periodontitis group the salivary level of IL-34 decreased significantly both at 1 and 3 months after NSPT compared to baseline (p<.05) but in the smoker periodontitis group only at 3 months (p<.05).

In serum, OPG and IL-34 levels at baseline were similar between all groups (p>.05), but serum RANKL levels were higher in the smoker periodontitis group than both non-smoker periodontitis and healthy ones at all time points (p<.05). At baseline and 1-month elevated serum RANKL/OPG levels were detected in smoker compared to non-smoker periodontitis group. (p<.05). There were no changes in serum OPG, RANKL, and RANKL/OPG levels in either periodontitis groups after NSPT (p>.05). However, serum IL-34 levels decreased in both groups at all time points compared to baseline (p<.001).

Since only salivary IL-34 and serum RANKL caused significant differences between the study groups, further analyzes were performed on these two molecules. Salivary IL-34 levels were higher in periodontitis patients than in healthy group (p<.001) (Table 4). Salivary IL-34 and serum RANKL levels were lower in the non-smoker individuals than in the smoker individuals (p=.044 and p=.003, respectively).

Correlation analysis revealed that age was positively correlated with all clinical parameters and salivary IL-34 (p<.05) (Table 5). The salivary IL-34 levels were positively correlated with PI, GI, BOP, PD, CAL, cotinine level, smoking duration, and number of smoking (p<.05). Serum RANKL had weak positive correlations with PD and cotinine levels (p<.05) but strong positive correlations with smoking duration and the number of smoking (p<.001).

Significant associations between periodontitis and salivary IL-34 were found both before and after adjusting for age and smoking (p<.05) (Table 6). Moreover, salivary IL-34 levels were associated with smoking (p=.034). Smoking was associated with serum RANKL both before and after adjusting for age and periodontitis (p=.039 and p=.030, respectively).

Table 4. *Comparison of baseline biochemical parameters in relation to periodontal and smoking status.*

Biochemical Parameters	*Periodontitis N=40*	*Periodontally healthy N=20*	*p*[a]	*Smokers N=20*	*Nonsmokers N=40*	*p*[a]
Saliva IL-34 (pg/ml)	425.63 (184.16–1409.6)	99.45 (17.8–346.4)	**<.001**	340.38 (184.1–1543.3)	251.03 (73.5–559.3)	**.044**
Serum RANKL (pg/ml)	21.48 (10.9–43.66)	13.85 (6.94–42.69)	.196	34.55 (15.5–51.7)	12.65 (7.95–35.14)	**.003**

Data are presented as median (Q1-Q3) values. [a]Mann-Whitney U-test. p<.05. Statistically significant differences are marked in bold. Abbreviations: IL, interleukin; RANKL, nuclear factor kappa B ligand; pg, picogram; ml, mililiter

Table 5. *Correlations between selected clinical and biochemical parameters at baseline.*

All groups	Age	Saliva IL-34 (pg/ml)	Serum RANKL (pg/ml)	Cotinine (ng/ml)	Smoking Duration	Number of Smoking Cigarette
PI	**0.459****	**0.449****	0.178	**0.422****	**0.422****	**0.440****
GI	**0.446****	**0.522****	0.134	**0.361****	**0.296***	**0.293***
BOP (%)	**0.408****	**0.561****	0.101	**0.331***	**0.266***	**0.269***
PD (mm)	**0.535****	**0.503****	**0.270***	**0.554****	**0.713****	**0.708****
CAL (mm)	**0.614****	**0.429****	0.182	**0.497****	**0.569****	**0.587****
Saliva IL-34 (pg/ml)	**0.309***	-	-	**0.341****	**0.301***	**0.277***
Serum RANKL (pg/ml)	0.176	-	-	**0.273***	**0.376****	**0.374****

*Correlation coefficient values by Spearman-correlation test. *p<.05, **p<.01. Abbreviations: PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; mm, millimeter; IL, interleukin; RANKL, nuclear factor kappa B ligand; pg, picogram; ml, millilitre*

Table 6. *Multinominal logistic regression analysis for unadjusted and adjusted associations between salivary IL-34, serum RANKL, periodontitis III-C and smoking.*

	Periodontitis III-C Unadjusted OR (95% CI), p	Adjusted (Smoking and Age) OR (95% CI), p	Smoking Unadjusted OR (95% CI), p	Adjusted (Periodontitis and Age) OR (95% CI), p
Saliva IL-34	1.003 (1.001-1.006), **.015**	1.004 (1.000-1.008), **.027**	1.001 (1.000-1.002), **.034**	1.000 (1.000-1.001), .412
Serum RANKL	1.005 (0.986-1.025), .593	0.994 (0.961-1.028), .738	1.022 (1.001-1.043), **.039**	1.042 (1.004-1.082), **.030**

p<.05. Statistical differences are marked in bold. Abbreviations: 95% CI, confidence interval of 95%; OR, odds ratio; IL, interleukin; RANKL, nuclear factor kappa B ligand.

4. DISCUSSION

Smoking is one of the most important risk factors in periodontal disease development and one of periodontitis grade determining factors in the latest periodontal disease classification. The main feature of periodontitis is the alveolar bone loss, which is regulated by the osteoclast and osteoblast activities. The important role of RANKL, OPG and, more recently, IL-34 in osteogenesis was demonstrated (32,33). In the present study, the effect of smoking on clinical parameters and RANKL, OPG, and IL-34 levels in saliva and serum after NSPT in periodontitis-III-C was investigated.

Previous studies suggested that smoking had a negative effect on BOP reduction (1,7). In line with that in the present study, the reductions from baseline to 3 months in GI and BOP were greater in non-smoker periodontitis than in smokers. Considering the reduction in PD and CAL gain, some studies reported that smokers were less responsive to NSPT(4,5), as well as others showed similar results between smoker and non-smoker periodontitis patients (6,7). In this study, smokers and non-smokers with periodontitis patients had similar PD decrease and CAL gain. A recent meta-analysis (34) reported a significant but modest negative effect of smoking in NSPT in periodontitis patients with mean difference 0.33 mm in PD reduction and 0.20 mm in CAL gain. Although these differences are statistically significant, their clinical significance is questionable. Since PD and CAL reflect the average of the whole mouth, including all of the shallow and deep PD, it is more accurate to examine the diseased areas to evaluate the response to NSPT. According to the same meta-analysis, when comparing PD reduction in initially deep pockets (≥5 mm), the difference in PD reduction between smokers and non-smokers periodontitis patients was more pronounced (0.50 mm) (34). Our results indicated that PD reduction baseline to 3-months in sites initially PD≥5 mm was higher in the non-smoker periodontitis patients than in the smoker ones, with a difference of 0.56 mm.

RANKL is the master regulator of osteoclastogenesis and plays an essential role in osteoclast-associated diseases, like periodontitis (35). OPG, on the other hand, is a natural inhibitor of osteoclast differentiation (14). Although the effect of smoking on alveolar bone destruction is well documented, the exact processes by which smoking impacts the periodontium remain unknown. Nicotine and LPS together induce the formation of osteoclast-like cells by increasing M-CSF and PGE2 and decreasing OPG production from osteoblasts. Regardless of the administration of nicotine or LPS, no RANKL expression was found (10).

Although the RANKL-OPG mechanism has been explained in cell and animal studies, the results of the clinical studies in which they were examined in different body fluids could not reach a consensus. Some studies showed elevated levels of RANKL and downregulation of OPG in GCF, saliva or periodontally diseased tissues in periodontitis patients, (20,21) yet others have presented similar RANKL (19,22,36) and OPG (19,37) levels or higher OPG levels (38) in saliva or GCF in periodontitis patients compared with their controls. In addition, the effect of smoking on the RANKL-OPG mechanism has not been clarified. Lappin et al.(16) and Tang et al.(17) reported increased RANKL/OPG ratios in serum and GCF in smokers than in non-smoker periodontitis ones. However, other studies showed no statistical differences in OPG and RANKL levels in serum and GCF between smoker and non-smoker periodontitis patients (15,18,19). While Behfarnia et al. (19) found the salivary RANKL/OPG ratio high in non-smokers, Buduneli et al. (37) found it higher in smokers. Furthermore, in a significant portion of the studies in the literature, salivary RANKL and OPG levels have frequently stayed below the detection limit (36). Although applied laboratory methodologies and selected study populations are often used as justifications for the contradictory results across different studies, RANKL and OPG results, especially in saliva levels, were not consistent.

The recent meta-analysis reported that elevated GCF RANKL levels were significantly detected periodontitis compared to healthy. Nevertheless, no differences in RANKL and OPG levels were found in saliva or serum (39). In line with that, in the present study, salivary RANKL and OPG levels were similar between the healthy and periodontitis groups. However, salivary OPG levels had a trend toward elevation in the periodontitis patients and decreased after NSPT in line with the previous studies (18,40). This could be explained by the increase of osteoclast precursor cells during the periodontal breakdown, and the decrease in release of OPG . However, after NSPT, the need for inhibition decreases, resulting in a reduction in OPG levels.

Another important issue to consider is which form of RANKL contributes to periodontal bone loss. RANKL is an agonistic ligand for RANK in both its soluble and membrane-bound forms. However, the mRANKL is more effective than sRANKL (41). This may be due to the presence of

mRANKL in cell-to-cell contact, but sRANKL is not. The cell-to-cell interaction between osteoclast precursor cells and mesenchymal cells in bone is required to activate osteoclastogenesis. As a result, it's thought that RANKL's cellular origins should be in direct contact with the alveolar bone (35). Indeed, the contribution of sRANKL in periodontal bone resorption has been shown to be negligible in animal studies (42). Thus, salivary and serum sRANKL and OPG levels may not be suitable for detecting active bone resorption in periodontitis.

Smoking may increase matrix metalloproteinase (MMP) expression (43). MMPs appear to be a responsible molecule cleaving the mRANKL to its soluble form, while tissue inhibitors of MMPs (TIMP) prevent this conversion (44). Previous research has indicated that smoker periodontitis patients have significantly higher levels of MMP-9 and lower levels of TIMP-1 in serum than non-smoker periodontitis patients (45,46). This could be the possible explanation for elevated serum RANKL levels in smoker patients in the present study. Moreover, serum RANKL was associated with smoking even adjusted for periodontitis and age. However, more studies are needed to clarify the possible effect of smoking on the relationship between sRANKL and MMPs.

In RANKL-induced osteoclastogenesis, IL-34 operates similarly to M-CSF in osteoclast differentiation. IL-34 in combination with RANKL stimulates osteoclast differentiation, causes bone destruction and decrease bone mass in M-CSF-deficient mouse bone marrow cells (32). The result of present study indicated that salivary IL-34 levels were higher in periodontitis groups than in the healthy group at baseline and associated with periodontitis-III-C in line with a previous study from our group (30). Salivary and serum IL-34 levels were decreased significantly in smoker and non-smoker periodontitis patients after NSPT. These findings were in accordance with previous reports of altered levels of these markers in GCF (21,24,47) and serum (24,47) in periodontitis and decreased after NSPT (21,47). However, some studies reported that healthy controls showed higher IL-34 levels in saliva (48,49) and elevated after NSPT (49). The reason for all these contradictory results may be that IL-34 has both an anti – and pro-inflammatory role (50). However, based on our results we may speculate that IL-34 can be a possible periodontitis and treatment response marker but failed to differentiate the smoking effect. As a result, it's possible to suggest that IL-34 has a pro-inflammatory effect and plays a critical function in the pathology of periodontitis. However, future studies should confirm these results to investigate the potential role of IL-34 in periodontal disease.

Well-characterized study groups, follow-up design, and evaluation of cotinine levels are strengths of the present study. Limitations of this study are the lack of GCF to confirm our results and lack of a systemically healthy smoker group and other stages or grades of periodontitis.

5. CONCLUSION

The clinical outcomes of the present study showed that smokers are less responsive to NSPT than non-smoker periodontitis-III-C. In addition, elevated serum RANKL levels are associated with smoking. Furthermore, salivary IL-34 can be a promising marker for periodontitis-III-C, but its role in other stages and grades of periodontitis urges further investigation.

Funding: *This study was supported by grants from Marmara University Scientific Research Projects Coordination Unit #SAG-C-DRP-241.018.0569*

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

Ethics Committee Approval: *This study was approved by Clinical Research Ethics Committee of Faculty of Medicine, Marmara University (Decision date / number: 13.07.2018 / 09.2018.513).*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: V.A., B.D.
Design of the study: B.D.
Acquisition of data for the study: V.A.
Analysis of data for the study: V.A., N.G.G.
Interpretation of data for the study: V.A., N.G.G., B.D.
Drafting the manuscript: V.A., N.G.G.
Revising it critically for important intellectual content: B.D.
Final approval of the version to be published: V.A., N.G.G., B.D.

REFERENCES

[1] Apatzidou D, Riggio M, Kinane D. Impact of smoking on the clinical, microbiological and immunological parameters of adult patients with periodontitis. J Clin Periodontol. 2005;32(9):973-983. DOI: 10.1111/j.1600-051X.2005.00788.x

[2] Johnson GK, Guthmiller JM. The impact of cigarette smoking on periodontal disease and treatment. Periodontol 2000. 2007;44(1):178-194. DOI: 10.1111/j.1600-0757.2007.00212.x

[3] Helal O, Göstemeyer G, Krois J, Fawzy El Sayed K, Graetz C, Schwendicke F. Predictors for tooth loss in periodontitis patients: Systematic review and meta-analysis. J Clin Periodontol. 2019;46(7):699-712. DOI: 10.1111/jcpe.13118

[4] Guru S, Sam SE, Rajan S, Padmanabhan S. Comparative evaluation of salivary hepatocyte growth factor in smokers and non-smokers with chronic periodontitis. J Investig Clin Dent. 2018;9(2):e12306. DOI: 10.1111/jicd.12306

[5] Preshaw PM, Lauffart B, Zak E, Jeffcoat MK, Barton I, Heasman PA. Progression and treatment of chronic adult periodontitis. J Periodontol. 1999;70(10):1209-1220. DOI: 10.1902/jop.1999.70.10.1209

[6] Preshaw P, Holliday R, Law H, Heasman P. Outcomes of non-surgical periodontal treatment by dental hygienists in training: impact of site-and patient-level factors. Int J Dent Hyg. 2013;11(4):273-279. DOI: 10.1111/idh.12032

[7] Ardais R, Mário Tde G, Boligon J, Kantorski KZ, Moreira CH. The effect of smoking on bleeding on probing after nonsurgical periodontal therapy: a quasi-experimental study. Braz Oral Res. 2014;28:1-7. DOI: 10.1590/1807-3107bor-2014.vol28.0058

[8] Buduneli N, Kardeşler L, Işık H, Willis III CS, Hawkins SI, Kinane DF, Scott DA. Effects of smoking and gingival inflammation on salivary antioxidant capacity. J Clin Periodontol. 2006;33(3):159-164. DOI: 10.1111/j.1600-051X.2006.00892.x

[9] Ojima M, Hanioka T. Destructive effects of smoking on molecular and genetic factors of periodontal disease. Tob Induc Dis. 2010;8(1):1-8. DOI: 10.1186/1617-9625-8-4

[10] Tanaka H, Tanabe N, Shoji M, Suzuki N, Katono T, Sato S, Motohashi M, Maeno M. Nicotine and lipopolysaccharide stimulate the formation of osteoclast-like cells by increasing macrophage colony-stimulating factor and prostaglandin E2 production by osteoblasts. Life Sci. 2006;78(15):1733-1740. DOI: 10.1016/j.lfs.2005.08.017

[11] Linden GJ, Lyons A, Scannapieco FA. Periodontal systemic associations: Review of the evidence. J Periodontol. 2013;84 (Suppl 4):8-19. DOI: 10.1902/jop.2013.134.0010.

[12] Kong Y-Y, Yoshida H, Sarosi I, Tan H-L, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature. 1999;397(6717):315-323. DOI: 10.1038/16852

[13] Lacey D, Timms E, Tan H-L, Kelley M, Dunstan C, Burgess T, Elliott R, Colombero A, Elliott G, Scully S. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 1998;93(2):165-176. DOI: 10.1016/S0092-8674(00)81569-X

[14] Simonet W, Lacey D, Dunstan C, Kelley M, Chang M-S, Lüthy R, Nguyen H, Wooden S, Bennett L, Boone T. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell. 1997;89(2):309-319. DOI: 10.1016/S0092-8674(00)80209-3

[15] Özçaka Ö, Nalbantsoy A, Köse T, Buduneli N. Plasma osteoprotegerin levels are decreased in smoker chronic periodontitis patients. Aust Dent J. 2010;55(4):405-410. DOI: 10.1111/j.1834-7819.2010.01261.x

[16] Lappin DF, Sherrabeh S, Jenkins WM, Macpherson LM. Effect of smoking on serum RANKL and OPG in sex, age and clinically matched supportive-therapy periodontitis patients. J Clin Periodontol. 2007;34(4):271-277. DOI: 10.1111/j.1600-051X.2007.01048.x

[17] Tang TH, Fitzsimmons TR, Bartold PM. Effect of smoking on concentrations of receptor activator of nuclear factor κ B ligand and osteoprotegerin in human gingival crevicular fluid. J Clin Periodontol. 2009;36(9):713-718. DOI: 10.1111/j.1600-051X.2009.01444.x

[18] Buduneli N, Buduneli E, Kütükçüler N. Interleukin-17, RANKL, and osteoprotegerin levels in gingival crevicular fluid from smoking and non-smoking patients with chronic periodontitis during initial periodontal treatment. J Clin Periodontol. 2009;80(8):1274-1280. DOI: 10.1902/jop.2009.090106

[19] Behfarnia P, Saied-Moallemi Z, Javanmard SH, Naseri R. Serum, saliva, and GCF concentration of RANKL and osteoprotegerin in smokers versus nonsmokers with chronic periodontitis. Adv Biomed Res. 2016;5:80. DOI: 10.4103/2277-9175.180992.

[20] Yuce HB, Gokturk O, Turkal HA, Inanir A, Benli I, Demir O. Assessment of local and systemic 25-hydroxy-vitamin D, RANKL, OPG, and TNF levels in patients with rheumatoid arthritis and periodontitis. J Oral Sci. 2017;59(3):397-404. DOI: 10.2334/josnusd.16-0677

[21] Doğan SB, Dede FÖ, Balli U, Sertoglu E. Emerging roles of Interleukin-34 together with receptor activator of nuclear factor-kB ligand and osteoprotegerin levels in periodontal disease. Cytokine. 2021;144:155584. DOI: 10.1016/j.cyto.2021.155584

[22] Shetty B, Divakar DD, Al-Kheraif AA, Alharbi AO, Almutairi MST, Alanazi MM. Role of PDT as an adjunct to SRP on whole salivary RANKL and OPG ratio in type-2 diabetic and normoglycemic individuals with chronic periodontitis. Cytokine. 2021;34:102220. DOI: 10.1016/j.pdpdt.2021.102220

[23] Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science. 2008;320(5877):807-811. DOI: 10.1126/science.1154370

[24] Guruprasad C, Pradeep A. Influence of smoking on interleukin-34 levels in gingival crevicular fluid and plasma in periodontal health and disease: A clinico-biochemical study. Bull Tokyo Dent Coll. 2018;59(4):247-255. DOI: 10.2209/tdcpublication.2017-0051

[25] Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol. 2018;89(Suppl 1):S159-S172. DOI: 10.1002/jper.18-0006

[26] Lang NP, Bartold PM. Periodontal health. J Periodontol. 2018;89 Suppl 1:S9-s16. DOI: 10.1002/jper.16-0517

[27] Türkoğlu O, Eren G, Emingil G, Azarsız E, Kutukculer N, Atilla G. Does smoking affect gingival crevicular fluid LL-37 levels following non-surgical periodontal treatment in chronic periodontitis? Arch Oral Biol. 2016;61:98-105. DOI: 10.1016/j.archoralbio.2015.10.018

[28] Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964;22(1):121-135. DOI: 10.3109/000.163.56408993968.

[29] Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. Acta Odontol Scand. 1963;21(6):533-551. DOI: 10.3109/000.163.56309011240

[30] Görgülü NG, Doğan B. Effect of non-surgical periodontal treatment on salivary and serum biomarkers in stage III grade B and C periodontitis. J Periodontol. 2022. DOI: 10.1002/JPER.21-0536

[31] Yamamoto Y, Nishida N, Tanaka M, Hayashi N, Matsuse R, Nakayama K, Morimoto K, Shizukuishi S. Association between passive and active smoking evaluated by salivary cotinine and periodontitis. J Clin Periodontol. 2005;32(10):1041-1046. DOI: 10.1111/j.1600-051X.2005.00819.x

[32] Chen Z, Buki K, Vääräniemi J, Gu G, Väänänen HK. The critical role of IL-34 in osteoclastogenesis. PloS one. 2011;6(4):e18689. DOI: 10.1371/journal.pone.0018689

[33] Yasuda H. Discovery of the RANKL/RANK/OPG system. J Bone Miner Metab. 2021;39(1):2-11. DOI: 10.1007/s00774.020.01175-1

[34] Chang J, Meng HW, Lalla E, Lee CT. The impact of smoking on non-surgical periodontal therapy: A systematic review and meta-analysis. J Clin Periodontol. 2021;48(1):60-75. DOI: 10.1111/jcpe.13384

[35] Tsukasaki M. RANKL and osteoimmunology in periodontitis. J Bone Miner Metab. 2021;39(1):82-90. DOI: 10.1007/s00774.020.01165-3

[36] Frodge BD, Ebersole JL, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. J Periodontol. 2008;79(10):1913-1919. DOI: 10.1902/jop.2008.080070

[37] Buduneli N, Bıyıkoğlu B, Sherrabeh S, Lappin DF. Saliva concentrations of RANKL and osteoprotegerin in smoker

versus non-smoker chronic periodontitis patients. J Clin Periodontol. 2008;35(10):846-852. DOI: 10.1111/j.1600-051X.2008.01310.x

[38] Al-Sabbagh M, Alladah A, Lin Y, Kryscio RJ, Thomas MV, Ebersole JL, Miller CS. Bone remodeling-associated salivary biomarker MIP-1α distinguishes periodontal disease from health. J Periodontal Res. 2012;47(3):389-395. DOI: 10.1111/j.1600-0765.2011.01445.x

[39] Caldeira FID, Hidalgo MAR, Dias MLDC, Scarel-Caminaga RM, Pigossi SC. Systematic review of ratios between disease/health periodontitis modulators and meta-analysis of their levels in gingival tissue and biological fluids. Arch Oral Biol. 2021;127:105147. DOI: 10.1016/j.archoralbio.2021.105147

[40] Tabari ZA, Azadmehr A, Tabrizi MAA, Hamissi J, Ghaedi FB. Salivary soluble receptor activator of nuclear factor kappa B ligand/osteoprotegerin ratio in periodontal disease and health. J Periodontal Implant Sci. 2013;43(5):227. DOI: 10.5051/jpis.2013.43.5.227

[41] Hikita A, Yana I, Wakeyama H, Nakamura M, Kadono Y, Oshima Y, Nakamura K, Seiki M, Tanaka S. Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-kappaB ligand. J Biol Chem. 2006;281(48):36846-36855. DOI: 10.1074/jbc.M606656200

[42] Tsukasaki M, Komatsu N, Nagashima K, Nitta T, Pluemsakunthai W, Shukunami C, Iwakura Y, Nakashima T, Okamoto K, Takayanagi H. Host defense against oral microbiota by bone-damaging T cells. Nat Commun. 2018;9(1):701. DOI: 10.1038/s41467.018.03147-6

[43] Perlstein TS, Lee RT. Smoking, metalloproteinases, and vascular disease. Arterioscler Thromb Vasc Biol. 2006;26(2):250-256. DOI: 10.1161/01.ATV.000.019.9268.27395.4f

[44] Lum L, Wong BR, Josien R, Becherer JD, Erdjument-Bromage H, Schlöndorff J, Tempst P, Choi Y, Blobel CP. Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. J Biol Chem. 1999;274(19):13613-13618. DOI: 10.1074/jbc.274.19.13613

[45] Ozçaka O, Biçakci N, Pussinen P, Sorsa T, Köse T, Buduneli N. Smoking and matrix metalloproteinases, neutrophil elastase and myeloperoxidase in chronic periodontitis. Oral Dis. 2011;17(1):68-76. DOI: 10.1111/j.1601-0825.2010.01705.x

[46] He C-Y, Gao X-Q, Jiang L-P. The impact of smoking on levels of chronic periodontitis-associated biomarkers. Exp Mol Pathol. 2016;101(1):110-115. DOI: 10.1016/j.yexmp.2016.07.004

[47] Guruprasad C, Pradeep A. Effect of nonsurgical periodontal therapy on interleukin-34 levels in periodontal health and disease. Indian J Dent Res. 2018;29(3):280-285. DOI: 10.4103/ijdr.IJDR_527_17

[48] Martinez G, Majster M, Bjurshammar N, Johannsen A, Figueredo C, Boström E. Salivary colony stimulating factor-1 and interleukin-34 in periodontal disease. J Periodontol. 2017;88(8):e140-e149. DOI: 10.1902/jop.2017.170081

[49] Lira-Junior R, Bissett SM, Preshaw PM, Taylor JJ, Boström EA. Levels of myeloid-related proteins in saliva for screening and monitoring of periodontal disease. J Clin Periodontol. 2021;48(11):1430-1440. DOI: 10.1111/jcpe.13534

[50] Muñoz-Garcia J, Cochonneau D, Télétchéa S, Moranton E, Lanoe D, Brion R, Lézot F, Heymann MF, Heymann D. The twin cytokines interleukin-34 and CSF-1: masterful conductors of macrophage homeostasis. Theranostics. 2021;11(4):1568-1593. DOI: 10.7150/thno.50683

How to cite this article: Arıkan V, Görgülü NG, Doğan B. Clinical and Biochemical Effects of Smoking on Non-Surgical Periodontal Treatment in Grade III Stage C Periodontitis Patients. Clin Exp Health Sci 2023; 13: 218-227. DOI: 10.33808/clinexphealthsci.1128101

Clinical and Experimental Health Sciences

The Effect of Autism Spectrum Disorder on the Processing of Neural Response Metaphors

Bertuğ Sakın[1], Yasemin Kaftar[2], Gaye Albay[2]

[1] University of Health Sciences, Hamidiye Faculty of Health Sciences, Department of Speech and Language Therapy, İstanbul, Türkiye.
[2] İstanbul University, Faculty of Letters, Department of Linguistics, İstanbul, Türkiye.

Correspondence Author: Bertuğ Sakın
E-mail: bertugsakin@gmail.com
Received: 22.12.2021 **Accepted:** 18.04.2022

ABSTRACT

Objective: The aim of this research is to describe the processing of neural response metaphors in individuals with autism spectrum disorder (ASD) speaking Turkish as their native language and to form the basis for further research in this field.

Methods: In this study, a single case study method was used. The data was collected by means of a structured questionnaire. The answers were recorded with the "Systematic Analysis of Language Transcripts" (SALT) program, and the data obtained was summarised using descriptive analysis.

Results: It was found out that the participant had difficulty comprehending, interpreting and using mimics and gestures. He struggled matching emotions and situations with expressions denoting them as well as determining how a person experiencing these emotions and situations might look like. The participant turned out to have reduced ability to interpret idioms and proverbs related to emotions, decide on what actions can be performed at the given place, and form a situation-effect relationship. The participant experienced problems interpreting and using new, formulaic, and malformed metaphors as well metaphors with literal meaning, and could hardly identify and correctly interpret conceptual metaphors within idioms and proverbs.

Conclusion: People with ASD experience difficulty with processing of neural response metaphors by the native speakers of Turkish. The disorders identified within the scope of our research result from the deficiencies of the theory of mind in individuals with ASD, which is supported by other studies on individuals with ASD having different mother tongues.

Keywords: Neurolinguistics, autism spectrum disorder, language disorder, neural theory of language, metaphor

1. INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impaired social interaction and communication, limited attention, and repetitive behaviour. This term was first used by Swiss psychiatrist Paul Eugen Bleuler in 1911 for individuals which completely isolated themselves from the outside world. In 1943, American psychiatrist Leo Kanner made a definition of autism in his study of 11 children displaying patterns of abnormal behaviour. Although Leo Kanner suggested that ASD is seen in children having emotionally cold parents, ASD is now known to be caused by some neurological, genetic, and environmental factors (1). Among these factors there are prenatal infection, metabolic disorders, and the use of anticonvulsant drugs to prevent epileptic seizures during pregnancy. Developmental delay, frequently seen epileptic seizures and increased head circumference indicate that ASD is a neuropsychiatric disorder (2). Studies aiming to reveal the genetic factors of ASD have shown that there is a large difference in comorbidity rates between monozygotic and fraternal twins, and the probability of ASD occurring in both siblings is higher in identical twins than in fraternal twins (3).

Analysis of retrospective reports indicates that the mean age of parents' first concerns about their child's development is 18 months (4). According to participants' reports, motor, sensory and social behaviour abnormalities are frequently observed in the first two years of life, but since they are clinically missed, the diagnosis can only be made between the ages of 2 and 4.

ASD is mainly associated with limited social interaction and repetitive behaviour patterns. However, DSM-5 also includes another symptom known as hypoactivity, a disorder characterized by lethargy, laziness, and slow response to sensory input, as opposed to hyperactivity (5). ASD-induced perception and communication disorders also restrict the development of motor skills. For this reason, individuals with

DOI: 10.33808/clinexphealthsci.1039708

ASD need to develop their motor skills using various therapy methods and special training (6). Motor development is known to have impact on children's communicative, social and cognitive development in the first years of life. In previous studies, delays in motor development have been associated with deficiencies in cognitive development and language (7, 8). Alongside with language delay, ASD is marked by stereotypical and repetitive behaviour, obsession, resistance to change and accompanying anxiety attacks, as well as inability to develop joint attention. Self-care problems, depression and inappropriate sexual behaviour can also be observed in adolescents with ASD (9-12).

Research shows that ASD is often accompanied by language disorders and various behavioural problems. About 50% of individuals with ASD are known to be unable to develop verbal communication until early childhood (13, 14). The difficulties encountered in verbal communication are the inability to use language according to the purpose, various problems in phonological processing, excessive intonation, abnormalities in tone, stress and rhythm, limited vocabulary, errors in advanced syntax production, and semantic mistakes (15). Echolalia, pronoun reversal, excessive use of atypical language and jargon, and difficulties based on pragmatic language use are also frequently observed in individuals with ASD (16, 17). Regarding nonverbal communication, people with ASD experience difficulties in interpreting other people's body language and inferring their intentions, and at the same time they can hardly use their gestures and mimics and express their own intentions. Studies have demonstrated that after non-linguistic barriers to communication are removed, individuals with ASD tend to have fewer perception-related problems than those related to production (18).

Individuals with ASD have similar vocabulary range and often use speech in idiosyncratic ways. Although individuals with ASD are more or less successful in the production of phrases and their first words, ASD is usually associated with speech retardation (19). When considering monolingualism and bilingualism as another factor affecting vocabulary acquisition and perception by children with ASD, bilingual children turn out to show better results than their monolingual peers, even if the difference is not very significant (20). Alongside with this, the vocabulary breadth of mothers of children with ASD is also known to determine the number of words known by their children. However, mothers' grammar skills don't seem to influence the grammatical competence of their children (21). Semantic methods based on the teacher's emotional response are not very beneficial for the vocabulary acquisition of children with ASD due to the lack of theory of mind (22). At the same time, the problems that children with ASD experience with receptive language and the limitations in fine motor skills cause various difficulties in the acquisition of writing skills. Children with ASD can only overcome these difficulties up to a certain point with the help of their parents and educators. At this point, the severity level of ASD seems to be of great importance. The more the person is affected by ASD, the more difficulties increase in literacy skills as well as in production and perception (16, 19).

The Neural Theory of Language was created by George Lakoff and Jerome Feldman. This theory is based on the view that thought is physical, and that reasoning occurs by activating certain neuronal groups in our brain (23). Metaphorical thinking is a mental function. Lakoff and Johnsen argue that metaphors emerge as a result of neuronal activities in the brain (24). According to structured connectionism developed by Jerome Feldman, all calculations are distributed over a single network, and nothing is built in. The meaning of the function cannot be assigned to a single neuron or a group of neurons. Mirror neurons modulate their activity both when an individual executes a specific motor act and when they observe the same or similar act performed by another individual. Mirror neurons are activated not only in these situations, but also when this action is perceived or imagined to be performed. Feldman claims that meaning is a mental analogy for physical concepts. Following simulation semantics, the neural theory of language proposes that the neural circuitry that defines the meaning of the word "grasp" is the same neural circuitry that is activated in mirror neurons when the act of grasping is performed or imagined (25).

The present study is aimed to examine and describe the way neural response metaphors are processed by an individual with ASD. There are three fundamental types of neural response metaphors: primary, structural, and conceptual. Primary metaphors form the basis for the creation of complex metaphors, the meaning of which can be inferred intuitively without giving the direct analogy. Structural metaphors are a form of metaphor in which an expression is transformed in such a way that evokes other expressions related to it. Conceptual metaphors can be described as a way of making an abstract idea or experience more easily understandable by framing it in terms of another more concrete concept. Conceptual metaphors are divided into two types: orientational metaphors involving spatial relationships and ontological metaphors based on the concretization of abstract metaphors (23, 24).

2. METHODS

Ethical approval was obtained from the Hamidiye Scientific Research Ethical Committee of University of Health Sciences (Approval date and number: 21/604).

In this study, a single case study method was used. The participant of the study was a 10-year-old male child with moderate autism spectrum disorder speaking Turkish as a native language. The participant's family was provided with the necessary information about the study and a consent form was obtained.

The data was collected by means of a structured questionnaire, consisting of ten steps. In the first step, the participant was asked to match the emotion icons with the names of the feelings they express. In the second step, he had to choose the right emotion based on the description of a person's appearance. In the third step, the participant was required to indicate the emotion that a person experiences

in the given situation. In the fourth step, he was given various idioms and asked to name the emotion expressed by them. In the fifth step, a true-false test evaluating the understanding of primary metaphors was applied. In the sixth step, a two-option multiple-choice test was prepared to reveal whether the participant is aware of the difference between wanting to do something and actually doing it. The seventh step was designed to find out if the participant is able to comprehend metaphorical fusions. The eighth and the ninth steps were related to conceptual metaphors and included true or false and multiple-choice questions. In the last step the participant was asked open-ended questions on proverbs/idioms and conceptual metaphors.

The oral answers were recorded by means of the "Systematic Analysis of Language Transcripts" (SALT) program, and the data obtained was summarised using descriptive analysis.

3. RESULTS

At the first stage of the research, the participant was shown emotion icons and asked to match them with the feelings. The participant read aloud the names of the feelings and did the matching task on his own. He matched the scared face expression with the word "happy", the happy face expression with the word "surprised", the surprised and upset face expressions with the word "sad", and the disgusted and angry face expressions with the word "angry". The words "scared" and "disgusted" were left unmatched. Even if the participant named 2 of the 6 face expressions correctly, it's necessary to note that he wrongly matched them with two other emotions as well.

At the second stage of the research, the participant was given a multiple-choice test and asked to choose the correct emotion based on the description of a person's appearance. When asked how a person being nauseous felt, he chose "disgusted" from the options "angry", "surprised" and "disgusted". When asked how a person clenching their teeth felt, the participant chose "happy" from the options "happy", "angry" and "sad". When asked how a crying person felt, the participant chose "disgusted" from the options "disgusted", "sad" and "surprised". When asked how a person whose face has gone pale felt, he chose "happy" from the options "scared", "happy" and "sad". When asked how a person whose eyes and mouth are wide open felt, the participant chose "angry" from the options "disgusted", "angry" and "surprised". When asked how a smiling person felt, the participant chose "surprised" from the options "surprised", "happy" and "sad". In this part of the research, the participant answered 5 of the test questions incorrectly and 1 of them correctly.

At the third stage of the research, the participant was presented a situation and asked to predict what emotions a person would experience in this situation. When asked what a person would feel if something smelt bad, the participant selected "fear" from the options "happiness", "disgust" and "fear". When asked what a person who had received good news would feel, he selected "disgust" from the options "happiness", "sadness" and "disgust". When asked what a person being chased by a dog would feel, the participant selected "fear" from the options "angriness", "sadness" and "fear". When asked what a person who had found themselves in an unexpected situation would feel, the participant selected "angriness" from the options "astonishment", "disgust" and "angriness". When asked what a person would feel if a friend of them had damaged their belongings, the participant selected "angriness" from the options "angriness", "happiness" and "fear".

At the fourth stage of the research, the participant was asked to explain the feelings expressed by the idioms. After being informed that he had given the correct answer to the question what the idiom "ağzı kulaklarına varmak" (grin from ear to ear) meant, the participant gave the same answer (happiness) to the questions concerning other idioms (ağzı açık kalmak – drop one's teeth, yüreği ağzına gelmek – jump of one's skin, ateş püskürmek – breath fire, burnunun direği sızlamak – be down in the mouth, içi almamak – be reluctant to do something).

At the fifth stage of the research, the participant had to match places with the activities done there and state whether the given statements were true (T) or false (F). The participant marked the statement "Someone who wants to sleep goes to the bedroom" as true (T). After the enquirer said that this was the correct answer, the participant marked all other statements (someone who wants to sleep goes to the kitchen, someone who wants to eat goes to the bedroom, someone who wants to get dressed goes to the kitchen, someone who wants to read a book goes to the toilet) as true (T).

At the sixth stage of the research, the participant was asked to choose the statements that could be inferred from the given situations. The participant answered 5 of the questions incorrectly and 1 of them correctly. When asked which one would have a fish, someone who likes fishing or someone who is fishing, the participant answered, "Someone who likes fishing". When asked which one would finish a book, someone who is reading a book or someone who wants to read a book, the participant answered, "Someone who wants to read a book". When asked which one would finish their homework, someone who is doing a homework or someone who has a homework, the participant answered, "Someone who has a homework". When asked which one can catch a ball, someone who is looking at the ball or someone who is trying to catch it, the participant answered, "Someone who is looking at the ball". When asked which one can touch a cat, someone who has a cat or someone who is thinking about a cat, the participant answered, "Someone who has a cat". When asked which one can hold a glass, someone who is trying to reach the glass or someone who is pointing at the glass, the participant answered, "Someone who is pointing at the glass".

At the seventh stage of the research, the participant had to determine whether two people which have something in common are the same person. Being given the statements

"My mother is a woman", "My father's sister is a woman", and "My mother's sister is a woman", the participant marked the statement "My mother, my father's sister, and my mother's sister are different people" as true (T) and the statements "My mother's sister is my mother" and "My father's sister is my mother's sister" as false (F). Being given the statements "The policemen is married", "The doctor is married", and "The teacher is married", the participant marked the statements "The policemen is a doctor", "The doctor is a teacher", and "The policemen, the doctor, and the teacher are the same person" as true (T). The participant was able to differentiate between the concepts of "mother" and "aunt". However, when asked to make the same distinction between the professions, he failed the task and marked all the options incorrectly.

At the eighth stage of the research, the participant was introduced to the conceptual metaphor "bad things are dark" illustrated by the examples of "kara baht" ("dark fate") and "kara bulutlar" ("dark clouds"), after which he was asked to mark other metaphors containing the word "dark" as true (T) or false (F). The participant marked the novel metaphor "kara zamanlardan geçtik" ("we have passed through dark times") as false, the conventional metaphor "kara bulutlar dolaşıyor dört yanında" ("dark clouds are hovering all around") as true, and the statement "ekran ne kadar karanlık" ("how dark is the screen") where "dark" is used in the literal meaning as false. However, when asked if the statement "I have dark luck" was wrong, the participant gave the correct answer.

At the nineth stage of the research, the participant had to choose the right meaning of the conceptual metaphors "kör talihini yenemedi" (he/she couldn't beat his/her blind luck) and "karanlık günler çok yakın" ("dark days are close"). He incorrectly matched the meaning of the first one with "good luck" instead of "bad luck". However, the participant correctly identified the meaning of the second metaphor and chose the "bad days" option.

At the tenth stage of the research, the participant was given a number of sentences containing conceptual metaphors and asked to answer the questions. When asked if the person described by the words "tencere dibin kara, seninki benden kara" (the bottom of the pot is black, yours is blacker than me) is good or bad, he replied, "The pot is black". When asked how a person "who has not seen the light of day" ("gün yüzü görmemiş") might have spent their day, the participant repeated the words of the enquirer by saying, "He has not seen the light of day". However, after being given a clue by the enquirer who asked whether the person might have had a good or a bad day, the participant chose the "bad day" option. When the participant was asked which side of another person's character a person who said, "I won't see him/her again after I saw his/her dark side" might have faced, with the help of additional questions such as "Was it his/her good or bad side?" and "Has he/she done something good or something evil?", the participant responded that it was the bad side.

4. DISCUSSION

The aim of this research was to reveal the effect of ASD on the processing of neural response metaphors. It was determined that the participant with ASD, who was a native speaker of Turkish, had problems understanding, expressing and interpreting situations based on facial expressions, appearance and emotions caused by certain events. The theory of mind of healthy children develops by the end of early childhood, while children with ASD appear unable to build the mental model of other minds (26). The research findings show that individuals with ASD find it hard to interpret nonverbal images and their main and connotative meanings due to the lack of theory of mind (27). In this respect, the results of our study involving a native speaker of Turkish are supported by studies conducted on speakers of other languages.

The participant gave correct answers to the first questions in tasks that required interpreting idioms and the feelings they express and matching the place with the action that can be performed at that place. After being told that his answer was correct, the participant made a hasty generalization and gave the same answer to all the subsequent questions regardless of whether it was right. Similar results have been obtained in other studies involving participants with ASD having different mother tongues (5, 28).

In another part of the study, the participant was asked to predict the consequences that the given situations may lead to and to identify the names of relatives and professions that have something in common. The participant proved to be unsuccessful fulfilling these tasks being able to match only those relatives that he is likely to frequently spend time within daily life. The inability of individuals with ASD to estimate the situation and the results related to that situation is also discussed by Frith and Happe (1994) (27). As for the findings concerning matching the names of relatives and professions, similar results were obtained by De Fossé (2004) (15).

The participant also scored low in that part of the study that dealt with conceptual metaphors. However, the participant turned out to show better results after being provided some clues and scaffolding. This finding is supported by other studies on the perception and interpretation of metaphors by individuals with ASD (24, 29, 30).

5. CONCLUSION

All individuals with ASD, which is a lifelong neurodevelopmental disorder, have a certain set of characteristics that distinguish them from others belonging to this group. These features vary depending on the severity of ASD and are affected by individual differences. For this reason, the aim of this case study was to examine the effect of ASD on the processing of neural response metaphors by a single subject, to reveal individual differences, and to compare the results obtained with other studies on individuals with ASD having different mother tongues.

The results of the study show that individuals with ASD have difficulty comprehending, interpreting and using mimics and gestures, which are an important part of nonverbal communication. They struggle matching emotions and situations with expressions denoting them as well as determining how a person experiencing these emotions and situations might look like. Individuals with ASD are not good at interpreting idioms and proverbs related to emotions, deciding on what actions can be performed at the given place, and forming a situation-effect relationship. They have problems interpreting and using new, formulaic, and malformed metaphors as well metaphors with literal meaning, and can hardly identify and interpret conceptual metaphors within idioms and proverbs.

The most important reason for these disorders is the lack of theory of mind seen in children with ASD. Healthy children's theory of mind develops by the end of early childhood and enables them to correctly interpret all the components of language as well as the imaginary structure of abstract language at the moment of communication. Theory of mind enables individuals to imagine themselves outside the situation they are currently in. The disorders identified within the scope of our research result from the deficiencies of the theory of mind in individuals with ASD, which is supported by other studies on individuals with ASD having different mother tongues.

Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *Ethical approval was obtained from the Hamidiye Scientific Research Ethical Committee of University of Health Sciences (Approval year and number: 21/604).*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: B.S.
Design of the study: B.S.
Acquisition of data for the study: B.S., Y.K., G.A.
Analysis of data for the study: B.S., Y.K., G.A.
Interpretation of data for the study: B.S., Y.K., G.A.
Drafting the manuscript: B.S., Y.K., G.A.
Revising it critically for important intellectual content: B.S., Y.K., G.A.
Final approval of the version to be published: B.S., Y.K., G.A.

REFERENCES

[1] Kanner L. Autistic disturbances of affective contact. Nervous Child 1943;2:217-250.

[2] Szatmari P. The causes of autism spectrum disorders. BMJ 2003;326(7382):173-174. DOI: 10.1136/bmj.326.7382.173

[3] Folstein S, Rutter M. Infantile autism: A study of 21 twin pairs. Journal of Child Psychology and Psychiatry 1977;18(4):297-321. DOI: 10.1111/j.1469-7610.1977.tb00443.x

[4] Baird G, Cass H, Slonims V. Diagnosis of autism. BMJ 2003;327(7413):488-493. DOI: 10.1136/bmj.327.7413.488

[5] American Psychiatric Association (DSM-5). Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Washington, DC: American Psychiatric Association Press, Inc; 2013. DOI: 10.1176/appi.books.978.089.0425596

[6] Rinner L. Sensory assessment for children and youth with autism spectrum disorders. Assessment for Effective Intervention 2002;27(1-2):37-46. DOI:10.1177/073.724.770202700106

[7] Dziuk MA, Larson JCG, Apostu A, Mahone EM, Denckla MB, Mostofsky SH. Dyspraxia in autism: association with motor, social, and communicative deficits. Developmental Medicine & Child Neurology 2007;49(10):734-739. DOI: 10.1111/j.1469-8749.2007.00734.x

[8] Iverson JM. Developing language in a developing body: the relationship between motor development and language development. Journal of Child Language 2010;37(02):229-261. DOI: 10.1017/S030.500.0909990432

[9] Hoch J, Spofford L, Dimian A, Tervo R, MacLean WE, Symons, FJ. A direct comparison of self-injurious and stereotyped motor behavior between preschool-aged children with and without developmental delays. Journal of Pediatric Psychology 2015;41(5):566-572. DOI: 10.1093/jpepsy/jsv102

[10] Bono MA, Daley T, Sigman M. Relations among joint attention, amount of intervention and language gain in autism. Journal of Autism and Developmental Disorders 2004;34(5):495-505. DOI: 10.1007/s10803.004.2545-x

[11] MacLean W.E, Ellis DN. Galbreath HN, Halpern LF, Baumeister AA. Rhythmic motor behavior of preambulatory motor impaired, down syndrome and nondisabled children: A comparative analysis. Journal of Abnormal Child Psychology 1991;19(3):319-330. DOI: 10.1007/BF00911234

[12] Kravitz H, Boehm, JJ. Rhythmic Habit patterns in infancy: their sequence, age of onset, and frequency. Child Development 1971;42(2):399-453. DOI: 10.2307/1127475

[13] Brock J, Norbury C, Einav S, Nation K. Do individuals with autism process words in context? Evidence from language-mediated eye-movements. Cognition 2008;108(3):896-904. DOI: 10.1016/j.cognition.2008.06.007

[14] Ökcün-Akçamuş M. Otizm spektrum bozukluğu olan çocukların sosyal iletişim becerileri ve dil gelişim özellikleri. Özel Eğitim Dergisi 2016;17(2):163-190. DOI: 10.21565/ozelegitimdergisi.246293

[15] De Fossé L, Hodge SM, Makris N, Kennedy DN, Caviness VS, McGrath L., … Harris G. J. Language-association cortex asymmetry in autism and specific language impairment. Annals of Neurology 2004;56(6):757-766. DOI: 10.1002/ana.20275

[16] Kuo LJ. Language and literacy development among children with autism spectrum disorder. Journal of Childhood & Developmental Disorders 2017;03(03):1-4. DOI: 10.4172/2472-1786.100052

[17] Gernsbacher MA, Morson EM, Grace EJ. Language and speech in autism. Annual Review of Linguistics 2016;2(1):413-425. DOI: 10.1146/annurev-linguist-030.514.124824

[18] Hilvert E, Davidson D, Gámez PB. Assessment of personal narrative writing in children with and without autism spectrum disorder. Research in Autism Spectrum Disorders, 2020;69:101453. DOI: 10.1016/j.rasd.2019.101453

[19] Hilvert E, Sterling A. Vocabulary and grammar development in children with autism spectrum disorder, fragile X syndrome, and Down syndrome. International Review of Research in Developmental Disabilities 2019;57:119-169. DOI: 10.1016/bs.irrdd.2019.07.003

[20] Lund EM, Kohlmeier TL, Durán LK. Comparative language development in bilingual and monolingual children

with autism spectrum disorder: A systematic review. Journal of Early Intervention 2017;39(2):1-19. DOI: 10.1177/105.381.5117690871

[21] Naigles L. Input and language development in children with autism. Seminars in Speech and Language 2013;34(04):237-248. DOI: 10.1055/s-0033.135.3446

[22] McGregor KK, Bean A. How children with autism extend new words. Journal of Speech Language and Hearing Research 2012;55(1):70-83. DOI: 10.1044/1092-4388(2011/11-0024)

[23] Lakoff G. The neural theory of metaphor. R. W. Gibbs, editor. The Cambridge Handbook of Metaphor and Thought. Santa Cruz: Cambridge University Press; 2008.p.17-38. DOI: 10.1017/CBO978.051.1816802

[24] Lakoff G, Johnson M. Metaphors We Live By. London: The University of Chicago Press; 1980. DOI: 10.2307/414069

[25] Feldman, J. From Molecule to Metaphor: A Neural Theory of Language. Cambridge: Massachusetts Institute Press; 2006. DOI: 10.7551/mitpress/3135.001.0001

[26] Wellman HM, Estes D. Early understanding of mental entities: a reexamination of childhood realism. Child Development 1986;57(4):910-923. DOI: 10.1111/j.1467-8624.1986.tb00254.x

[27] Frith U, Happé F. Autism: beyond "theory of mind." Cognition 1994;50(1-3):115-132. DOI: 10.1016/0010-0277(94)90024-8

[28] Whyte EM, Nelson KE, Khan KS. Learning of idiomatic language expressions in a group intervention for children with autism. Autism 2011;17(4):449-464. DOI: 10.1177/136.236.1311422530

[29] Nippold MA. Evaluating and enhancing idiom comprehension in language-disordered students. Language, Speech, and Hearing Services in Schools 1991;22:100-106. DOI: 10.1044/0161-1461.2203.100

[30] Levorato MC, Cacciari C. Children's comprehension and production of idioms: the role of context and familiarity. Journal of Child Language 1992;19:415-433. DOI: 10.1017/S030.500.0900011478

How to cite this article: Sakın B, Kaftar Y, Albay G. The Effect of Autism Spectrum Disorder on the Processing of Neural Response Metaphors. Clin Exp Health Sci 2023; 13: 228-233. DOI: 10.33808/clinexphealthsci.1039708