Contents

RESEARCH ARTICLES

Contents

Contents

Clinical and Experimental Health Sciences

Knowledge Levels of Medical Students Related to Airway Management in Patients with Maxillofacial Trauma

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ABSTRACT

Objective: Airway management in maxillofacial injuries is quite complex and involves many difficulties. The aim of this study is to evaluate the knowledge level of medical faculty students about airway management in maxillofacial injuries.

Methods: This cross-sectional study was conducted on fifth and sixth-grade students of the Faculty of Medicine, Afyonkarahisar Health Sciences University. After the literature review, a questionnaire was prepared to measure the knowledge level of the students about airway management in maxillofacial trauma. Two hundred eighty students participated in the study.

Results: Thirty percent of the students stated that they performed laryngeal mask airway, 61.1% orotracheal intubation, and 17.8% nasoendotracheal intubation. Thirty-four point forty-four percent of the students stated that they did not do any of these applications. Only 52.2% of the students stated that they used a traditional laryngoscope with Macintosh blades. While 81.1% of the students stated that they did not intubate at all in a patient with maxillofacial trauma, and 74.4% thought that a patient with maxillofacial trauma did not have the education and skills to perform intubation comfortably.

Conclusion: Results of the study reveal that students' knowledge and experience in airway management in emergencies such as maxillofacial trauma were insufficient. This deficiency was especially about the tools and methods used to provide airway patency.

Keywords: Maxillofacial trauma, airway management, emergency aid, medical students.

1. INTRODUCTION

There are severe difficulties for the doctors in patients with maxillofacial trauma, as airway management in these patients can be complicated due to damage to this region. The first difficulty is to safeguard the airway for efficient breathing. The physician should consider those factors when planning to ensure the safety of the airway: a) the severity of the wound and its impact on the airways; b) potential problems with mask breathing or endotracheal intubation; c) potential damage to the cervical spine; d) risk of aspiration of stomach contents; e) severe bleeding that obstructs the vision of the airways and can cause circulatory disturbances; and (f) the form of maxilla-mandibular fixation (MMF) to be carried out at the end of the surgery (1).

There are six particular conditions related to maxillofacial trauma that could be negatively impacting the airway, according to Hutchison et al.(2): a)The nasopharyngeal airway can be obstructed by post-inferior displacement of a broken maxilla parallel to the inclined plane of the base of the skull; b)Bilateral anterior mandible fracture can cause the tongue to slip posteriorly in the supine patient and obstruct the oropharynx; c)Foreign bodies such as dentures and shrapnel, as well as fractured teeth, bone fragments, vomiting, blood, and secretions, may block the airway anywhere in the oropharynx and larynx; d)Bleeding from various vessels in open wounds or serious nasal bleeding can also lead to obstruction of the airways; e)Soft tissue swelling and edema caused by head and neck trauma can cause airway collapse; f)Larynx and trachea injuries could increase the risk of cervical airway obstruction by causing tissues such as epiglottis and vocal cords to swell and displace.

Airway care and cervical spine immobilization and are the highest priority for managing patients with life-threatening injuries, according to Advanced Trauma Life Support(ATLS) suggestions (3). Airway loss can be fatal and more quickly than the loss of breathing capacity or the initiation of circulatory issues. Therefore, life-saving procedures should start with airway management as necessary (4). In particular, the most prevalent crucial care mistakes that contribute to the death of trauma patients are involved in airway and respiratory care(5).Morbidity and mortality are frequently the results

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of critical care faults in in-hospital trauma patients, with airway management arethe most prevalent. Gruen et al.(6) investigated the reason of the death of 2594 trauma patients and found that 16% of inpatient deaths were due to the inability to be intubated or safeguard the airway.

Managing the airway in emergency conditions is an added hassle because the time to complete the mission is limited and the patient's health can get worse rapidly. The performance of emergency intubation is correlated with very high complication rates, which can overlap 20% (7,8). These raised rates are attributed to multiple causes, such as frequent intubation enterprises, the requirement for immediate laryngoscopy without muscle relief, and the operator's inexperience. Hypoxemia, aspiration, esophageal intubation, increases in heart rate, cardiac arrhythmias, and cardiac arrest are the major complications that may occur (1). Care for acute trauma patients in emergencies, is usually ensured by inexperienced people (9).In a multi-center study of 8937 intubations in the emergency room, Walls et al. (10) found that anesthesiologists conducted only 3% of intubations, while the other 97 % were performed by emergency physicians (87%) and other specialties (10%). Unsuccessful attempts at endotracheal intubation by inexperienced people may cause a rapid deterioration in the patient's condition. To improve the clinical outcomes of patients with maxillofacial trauma, it is critical that the personnel managing the airways of such patients be trained and experienced. There is no study in the literature that investigates the knowledge levels of medical faculty students, who will be doctors of the future, about airway management in trauma patients. The purpose of this study is to assess the knowledge level of medical faculty students about airway management in maxillofacial injuries.

2. METHODS

This cross-sectional study was carried out on the students of Afyonkarahisar Health Sciences University Faculty of Medicine. The study was approved by the Clinical Studies Ethics Committee of Afyonkarahisar Health Sciences University (2021/1-26) and was conducted in accordance with the principles of the Helsinki Declaration. A total of 280 students, 135 from the 5th-class and 145 from the 6th-class, were included in the study. After the literature review, a questionnaire form was designed to measure the knowledge level of students about airway management in maxillofacial trauma.

The questionnaire form consisted of two or multiple-choice questions aiming to measure students' knowledge and experience, apart from demographic information such as age and gender. The first three questions consisted of multiple options and students were asked to select one or more options. 25 questions in the questionnaire consisted of statements containing false or correct information about airway management in maxillofacial injury, and students were asked to mark one of the "true" or "false" options for each statement. Correct answers were scored as '1 point' and the total scores of the students ranged from 0-25 points. The five questions of the survey were about the experience of getting to know and using the tools used in airline management. Survey questions in terms of content were checked by an emergency medicine physician. The validity and reliability of the questionnaire were verified with a pretest method in a group of 15 students. The questionnaire sample was sent to the students by e-mail.

2.1. Statistical Analysis

Survey data were analyzed Statistical Package for Social Sciences (SPSS) for Windows software, version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was given using the number and percentage. The compatibility of variables with normal distribution was reviewed by histogram graphics and Kolmogorov-Smirnov tests. When comparing the two groups, the independent samples *t*-test was used in evaluating parametric variables, and the Mann-Whitney U test was used in evaluating nonparametric variables. The confidence interval was set to 95% and $p<0.05$ was considered statistically significant.

3. RESULTS

The questionnaire was answered by a total of 180 students, 124 from the 5th-class and 56 from the 6th-class of the medical faculty (response rate 64.28%). Forty-seven point eight percent of the participants were male (86 student) and 52.2% were female(94 students). The minimum age was 22, the maximum age was 30 (the mean age was 25.61).

The questionnaire asked what the most important priority is in life-threatening injuries according to the ATLS recommendations (3). Sixteen point seven percent of the participants gave the correct answer as airway management and cervical spine immobilization. The answers of the participants regarding the factors that make airway safety difficult in patients with maxillofacial trauma are given in figure 1. Half of the participants correctly marked all of these factors that make airway management difficult in patients with maxillofacial trauma. As hemostasis methods that can be applied in maxillofacial traumas; direct pressure (57.77%), balloon packings (50%), surgical ligation of vessels (41.11%), sutures and staples (33.33%), and intraarterial embolization (17.77%) were stated by the participants. Only 8.8% of the participants stated all hemostasis methods in maxillofacial bleedings correctly.

Table 1 showed the percentage of correct answers given by the participants to the statements containing incorrect or correct information about airway management in maxillofacial

injuries. While the highest response rate (88.9%) would be to "It should be assumed that the stomach of the patient with maxillofacial trauma is completely filled", the lowest response rate (27.8%) would be "Fiber-optic intubation or video laryngoscope is superior in that it works without being affected by blood, vomit and secretions in the airway of the trauma patient ". The response percentage of 9 out

of 25 statements in this category remained below 50%, and most of them were related to the tools and methods used in airway management in maxillofacial injuries.

Table 2 shows the distribution of the average scores obtained by the participants according to their gender and school class. Accordingly, it was observed that the mean scores of women were significantly higher than men ($p<0.05$). In addition, the mean scores of the 5th-class students were found to be significantly higher than the 6th-class students ($p<0.001$). It was observed that the average score of all participants was 15.38 (in the range of 0-25 points). The distribution of the scores obtained by the students is given in figure 2. Here, too, it is seen that student scores are predominantly between 12 and 19 points.

Participants' application of methods to provide airway patency in emergency states is given in figure 3. Accordingly, 30% of the students stated that they performed laryngeal mask airway (LMA), 61.1% orotracheal intubation and 17.8% nasoendotracheal intubation. Thirty-four point forty four percent of the students stated that they did not do any of these applications. The laryngoscope usage situations of the students were shown in figure 4. Accordingly, more than half of the students (%52.2) stated that they used traditional laryngoscopes, and one-third stated that they used video laryngoscopes. Eighteen point nine percent of the students stated that they performed intubation in a patient with maxillofacial trauma. Only 25.6% of the students stated that they thought they had the education and skills to perform intubation in a patient with maxillofacial trauma. In an unconscious patient with maxillofacial trauma, the practices in which students consider themselves competent in airway management are given in figure 5. Accordingly, applying a face mask (%73.3) and oral airway placement(%67.8) were the two applications that students could do most. Besides, %41.1of the students stated that they could perform orotracheal intubation, %26.7of LMA, %6.7of tracheotomy, and %1.1 of cricotomy.

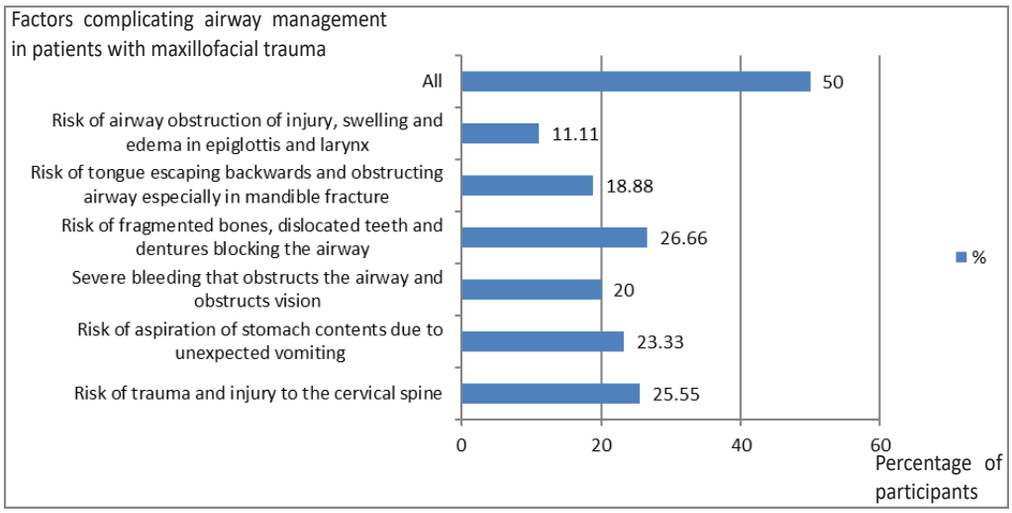

Figure 1. *Students' Opinions About Factors complicating airway management in patients with maxillofacial trauma (%)*

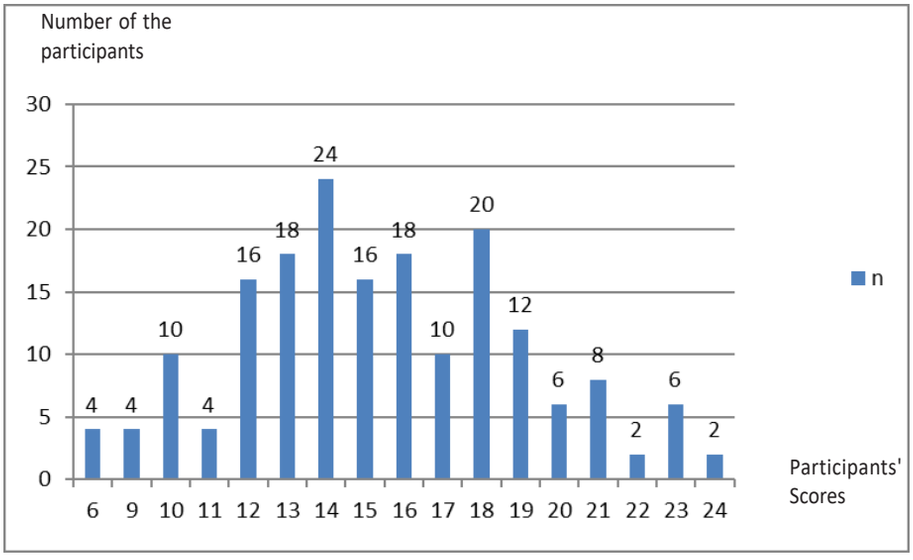

Figure 2. *Distribution of Students' Scores on the Questionnaire Scale(n)*

Table 1. *The ratio of Correct Answers Given by the Students to the Statements About Airway Management in Patients with Maxillofacial Trauma (%)*

	Correct response rate (%)
1. It should be assumed that the stomach of the patient with maxillofacial trauma is completely filled.	88.9
2. In order to empty the stomach of the traumatic patient, the patient must be vomited.*	47.8
3. Alcohol or drug poisoning, brain damage, and pain relieving opioids are potent triggers of nausea and vomiting.	83.3
4. Nasogastric catheter is preferred to empty the stomach in patients with broken nose, mid-face or skull base. *	48.9
5. The Sellick maneuver is a technique in which the esophagus is blocked by applying pressure on the cricoid cartilage and has been used to reduce the risk of lung aspiration.	67.8
6. A patient with a maxillofacial injury should be considered to have a C-spine injury until proven otherwise.	81.1
7. In maxillofacial trauma, the patient's C-spine should be protected with a half-neck collar and spinal immobilization in the supine position, and all neck movements should be avoided.	77.8
8. Macintosh laryngoscope should be preferred instead of video laryngoscope, if possible, to minimize cervical spine movements during intubation. *	41.1
9. If the restricted mouth opening is caused by temporomandibular joint injury, sedation is beneficial in increasing mouth opening. *	43.3
10. Bleeding in the facial area can go to the oropharynx and trigger vomiting while contributing to the obstruction of the respiratory tract.	75.6
11. Intubation is necessary to secure the airway in patients with high risk of pulmonary aspiration.	77.8
12. Mask ventilation and intubation can be applied more easily in patients with maxillofacial trauma compared to other traumas. *	44.4
13. In maxillofacial injuries, it is necessary to pre-oxygenate the patient with a face mask until airway safety is ensured in order to prevent the patient from entering hypoxia.	70
14. If preoxygenation cannot be done in any way, the patient should be ventilated by intubation.	75.6
15. Endotracheal intubation is the gold standard for airway protection in trauma patients.	70
16. Fiber optic intubation or video laryngoscope is superior in that it works without being affected by blood, vomit and secretions in the airway of the trauma patient. *	27.8
17. Nasoendotracheal intubation should be preferred in patients with skull and skull base fractures, midface and nose fragmented. *	56.7
18. Oroendotracheal intubation provides better intraoperative visibility to the surgeon during the operation and allows maxillomandibular fixation. *	34.4
19. Failure should be reported after three attempts at tracheal intubation and oxygenation should be performed after unsuccessful intubation.	66.7
20. Although supraglottic airway devices are not a final airway device, they are rescue devices for ventilating patients until a definite airway is obtained.	76.7
21. Devices such as laryngeal mask airway and combitube are more difficult to administer than intubation, but they provide a more precise and safe airway to the patient.*	35.6
22. Tracheotomy takes less time, is easier to apply and causes less complications than cricotomy. *	30
23. Submental orotracheal intubation has been developed to avoid the need for tracheotomy and to allow unlimited access to the oral area.	70
24. Cricotomy or tracheotomy is a life-saving procedure in "not intubated, non-ventilable" and selected patients.	81.1
25. Extubation should be delayed until normal anatomy is restored and edema subsides.	65.6

**:Wrong expression; LMA: Laryngeal mask airway*

Table 2. *Distribution of Students' Scores According to Their Gender and School Class.*

	N	Mean	Std. Deviation	P value#
Gender				
Male	86	14.79	3.322	0.040*
Female	94	15.93	4.028	
Class				
5	124	16.38	3.902	0.000**
6	56	13.17	2.072	
Total	180	15.38	3.742	

*# : independent samples t-test significance value; *:p<0.05, **: p<0.001*

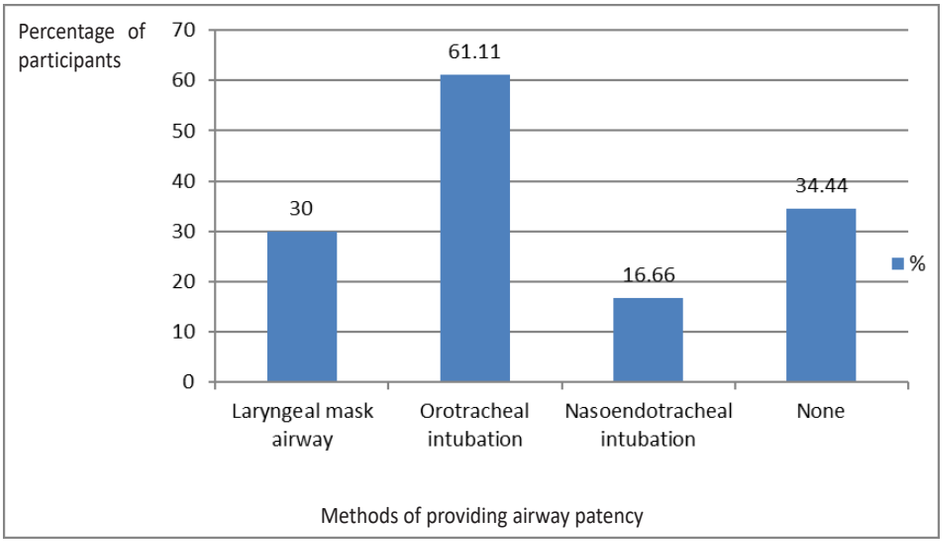

Figure 3. *Students' Use of Airway Patency Methods (%)*

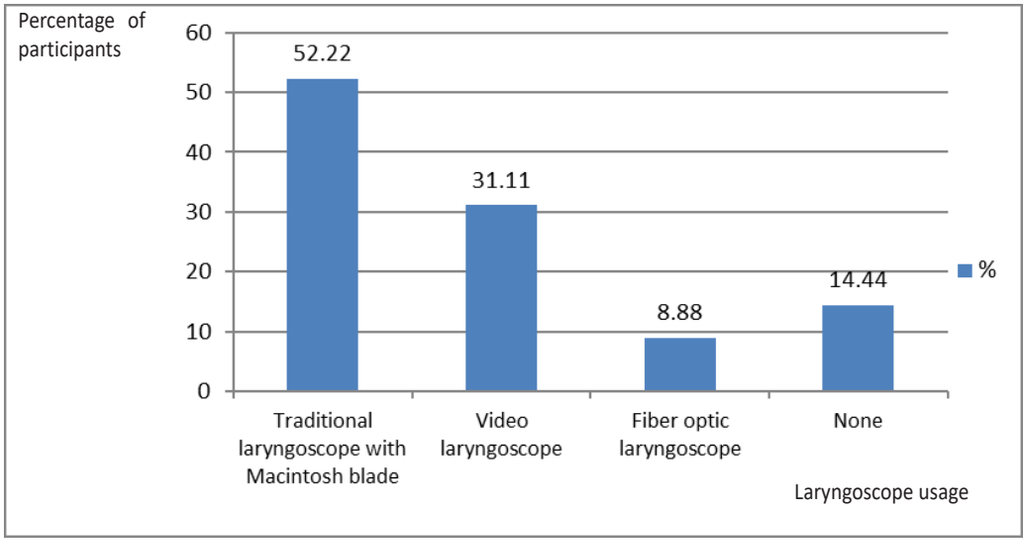

Figure 4. *Laryngoscope Usage Status of Students (%)*

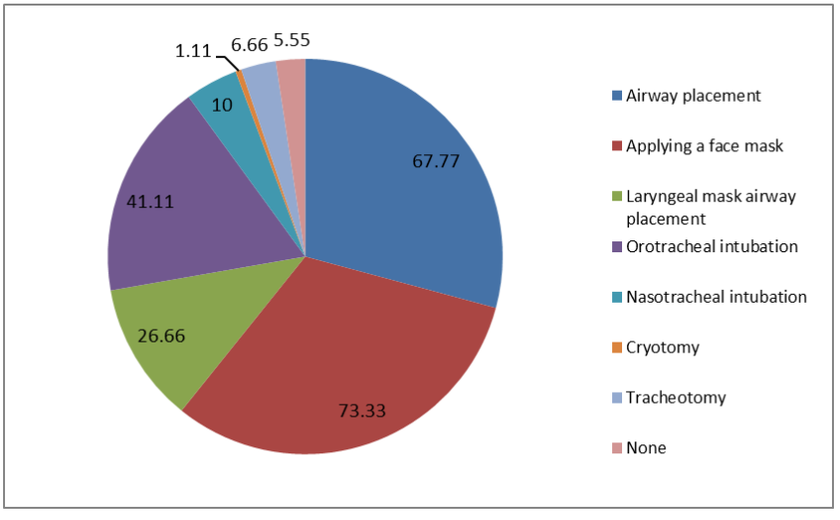

Figure 5. *Students' Perception of Themselves as Sufficient in Methods of Providing Airway Patency in Patients with Maxillo-facial Trauma (%)*

4. DISCUSSION

Maxillofacial injuries endanger patients' airways and can potentially be life-threatening. Airway care is the first priority for managing patients with life-threatening injuries, according to ATLS protocol (3). In this study, attention was drawn to the difficulty of airway management in patients with maxillofacial injury and their knowledge and experience of airway management were investigated. In this study, only 16.7% of the students stated that the first priority in trauma patients was airway management and cervical spine immobilization. Half of the students were aware of all the difficulties in airway management in patients with maxillofacial surgery which in accordance with previousstudy by Hutchinson et al.(2).

Preoxygenation is the first step in the early airway management process and it can prevent the patient from going into hypoxemia. In certain patients, because of the maxillofacial damage itself, preoxygenation is not possible, and hypoxemia may be expected. In trauma patients, endotracheal intubation is the gold standard process for airway safety (1). In the study, 70% of the students stated that preoxygenation is necessary to prevent the patient from entering hypoxemia until airway safety is ensured, and endotracheal intubation is the gold standard for protecting the airway. More than half (52.2%) of the participants in the study think that mask ventilation and intubation are easier in maxillofacial trauma patients compared to other trauma patients. In contrast, there are many challenges for airway management in patients with maxillofacial trauma. The mask cannot be positioned correctly on the face of patients with maxillofacial injuries. A damaged airway can also impede effective air transmission from the mask to the lungs. In a maxillofacial trauma patient, endotracheal intubation is likely to be difficult. During intubation, it may be difficult to see the larynx with a conventional laryngoscope. In a patient with maxillofacial trauma, blood, secretions, and bone fragments may fill the oral cavity, pharynx and larynx (1). In addition, a possible cervical spine injury requires restricted neck movements during intubation. More than half (52.2%) of the participants in the study think that mask ventilation and intubation are easier in maxillofacial patients compared to other traumatic patients. In contrast, there are many challenges for airway management in patients with maxillofacial trauma. The ventilation mask cannot be placed well on the face of patients with maxillofacial injuries. Also, an injured airway can block ventilation from the mask to the lungs. Endotracheal intubation will be more difficult in patients with maxillofacial injury. Because it is very difficult to see the vocal cords in these patients with a conventional direct laryngoscope (1). Gupta et al.(11) determined that the majority of patients with maxillofacial trauma had a mouth opening of fewer than two fingers and a Mallampati score of 3 or 4, and reported that intubation was more difficult in these patients.

Patients with maxillofacial trauma should be considered as "full stomach" as digestion stops when trauma occurs, as in all trauma patients. In addition, the risk of aspiration is high because blood in the upper respiratory tract is swallowed and accumulates in the stomach. It is recommended that the stomach contents be evacuated by a nasogastric tube before improving the patient's breathing. However, the insertion of a nasogastric tube into an uncooperative, sometimes intoxicated patient with a facial injury can trigger vomiting on its own. In addition, it is relatively contraindicated in situations with possible fracture of the skull base (1). In addition, alcohol or drug poisoning, brain injury, and pain-relieving opioids – all common in cases of facial trauma – are potent triggers of nausea and vomiting (12,13). In this study, the majority of the students correctly (88.8%) stated that the trauma patient's stomach should be considered as full. However, about half of them (47.8%) think that the patient should be vomited to empty the stomach. Vomiting may endanger the patient's life by increasing the risk of aspiration. Similarly, 48.9% of them had not know the situations in which nasogastric catheter is contraindicated. Sellick's maneuver is a technique in which the esophagus is blocked by applying pressure to the cricoid cartilage and has historically been used to mitigate the risk of lung aspiration (14). This maneuver can result in significant inhibition of endotracheal intubation because the laryngeal appearance has deteriorated (15). Also, its effectiveness in preventing aspiration is doubtful (16), and in some cases can cause a ruptured esophagus. Therefore, the use of cricoid pressure as aspiration prophylaxis in trauma patients is no more shown (17). In this study, 67.8% of the students answered the question about the Sellick's maneuver correctly.

Cervical spine injuries have been identified in 1-10% of patients with facial fractures (18). Overall, mid-face injuries are related to C5-7 trauma, while lower facial injuries are often related to C1-4 trauma (19). It is assumed that a patient with a supraclavicular injury has a C-spine injury unless confirmed by imaging otherwise (20). The patient's C-spine should be protected by a half-neck collar and spinal immobilization in the supine position, and all neck movements should be avoided. In this study, the majority of the students (81.1%) stated that the possibility of c-spine injury should be considered in patients with maxillofacial trauma, while 77.8% had information about how to protect the c-spine of the traumatic patient. In some studies, video laryngoscopy was recommended instead of a Macintosh blade to minimize neck movements (21). Using a video laryngoscope may be beneficial for patients who require immobilization of the cervical spine, rather than a traditional laryngoscope with a Macintosh blade (22). If the restriction of the opening of the mouth is caused by a temporo-mandibular joint (TMJ) injury, sedation does not enhance the opening of the mouth and can exacerbate the scenario. The study findings showed that only less than half of the students (41%) were aware of the superiority of the video laryngoscope in minimizing neck movements. In addition, half of the students (51.1%) did not have enough knowledge that TMJ injury could limit the mouth opening.

In patients with major trauma, particularly in trauma involving more than two-thirds of the face, "pan facial trauma", uncontrolled heavy bleeding is possible. Since the head and neck area are plenty vascularized, serious life-threatening bleeding may emerge (23). Bleeding affects the patient's condition and prognosis in many ways: a) Blood accumulating in the oral cavity may prevent mask ventilation, b) it may also make intubation difficult by preventing the appearance of anatomical structures c) large hemorrhages may endanger the circulation and cause the death of the patient; (d) coagulation may be impaired due to large blood transfusion, and (e) bleeding complicates surgical procedures. Management of the patient involves volume replacement and local control of bleeding (24,25). Hemostasis can be achieved by external direct pressure, sutures, and staples, filling the oral cavities from the inside, balloon pads, and reduction of facial fractures. When conservative treatment failures, intraarterial embolization or surgical ligation of bleeding vessels may be necessary (26). In the study, 75.6% of the students stated that bleeding in the face area can make airway management difficult. However, it was observed that only 8.8% of the students were knowledgeabout all hemostasis methods in maxillofacial injuries.

The inability to see the vocal cords of a maxillofacial trauma patient is the major impediment to successful endotracheal intubation. To overcome this hurdle, various airline devices and strategies have been improved. Some instruments, such as the flexible fiberoptic bronchoscope (FOB) and video laryngoscope provide an indirect view of the vocal cords and have been recommended in situations where intubation is difficult (27). However, it is very difficult for these devices to give a good image in a trauma patient due to blood and secretions. In this study, only 27.8% of the students stated that fiber optic intubation and video laryngoscope may not provide a good vision when affected by the patient's secretions such as blood and vomit.

Supraglottic airway devices (SAD), such as the LMA and combitube, are a crucial device for managing the difficult airway (28). The SAD is blindly put in the oropharynx for airway treatment of the trauma patient and its efficient insertion involves limited experience (29,30). However, SADs does not ensure a precise airway and can be dislocated when the patient with SAD is transported. It is not the last airway device for the maxillofacial trauma patient, however, SAD is a perfect rescue tool to ventilate patients until a precise airway has been accomplished (31). However, combitube use in a patient with maxillofacial trauma may cause additional damage to the upper respiratory tract (32). In this study, it was observed that 54.4% of the students did not have correct knowledge about devices such as LMA and combitube.

Surgical formation of an airway is a secure procedure of protecting the airway when the operation is performed by a practiced surgeon. However, it includes a 6% risk of complications such as bleeding or pneumothorax (33). This operation can be hard to perform in an emergency (34,35), and the procedure can sometimes be fatal (36). It can be quite stressful for the operator, particularly for the less experienced person (37,38). In this study, it was revealed that 62.2% of the students did not know the difference between tracheostomy and cricotomy. Only 34.4% of the students knew that orotracheal intubation reduced the visibility of the oral cavity during the surgical procedure and made postoperative maxillomandibular fixation impossible. Nasoendotracheal intubation allows maxillomandibular fixation (39) but it is contraindicated in patients with skull fracture or skull base fracture (40). It was revealed that 39.9% of the students in this study were not aware of this contraindication of nasoendotracheal intubation. Maxillofacial surgeons performed submental and retromolar intubation in selected patients with maxillofacial trauma to avoid tracheostomy (41). Extubation should be delayed in intubated patients with maxillofacial trauma until the edema is resolved. Peterson et al. (42) reported that 12% of complications occur during extubation and 5% healing. In the study, it was observed that 70% of the students had information about submental orotracheal intubation, while 65.6% had information about the correct extubation time.

Airway assessment of a patient with maxillofacial trauma should be performed as well and as fast as possible. The doctor must choose the most appropriate method to achieve airway management and the patient should be quickly taken to a well-equipped room. In addition to theoretical knowledge, physicians should also have skills in recognizing and applying instruments used in airway management. In this study, approximately 61.1% of the students stated that they performed orotracheal intubation, while approximately one third (34.4%) stated that they did not perform any of the LMA, orotracheal and nasoendotracheal intubation. In addition, only 41.1% of the students stated that they have sufficient education and skills to perform orotracheal intubation. The rate of students seeing themselves as sufficient in relatively easy procedures such as airway placement (67.7%) and face mask application (73.3%) is not very high. While 81.1% of the students stated that they did not intubate at all in a patient with maxillofacial trauma, 74.4% thought that a patient with maxillofacial trauma did not have the education and skills to perform intubation comfortably. These results reveal that students' knowledge and experience in airway management in emergencies are insufficient.

In the medical school curriculum, airway management education is given in the 5th year in the anesthesia and reanimation course. Training is carried out both theoretically and practically on mannequins. The high average scores of 5th-grade students may be due to the fact that they have just taken this course. In medical education, maxillofacial traumas are narrowly included in emergency traumas within the scope of emergency medicine courses. On the other hand, in dentistry, while maxillofacial traumas are widely included in the 4th grade oral, dental and maxillofacial surgery curriculum, airway management is only theoretically included in the 5th grade first and emergency aid curriculum. Therefore, it is possible that there are some deficiencies

in medical education about maxillofacial traumas and in dentistry education about emergency airway education. Therefore, overcoming these problems with mutual internship programs and close interdisciplinary cooperation seems to be a possible solution in practice.

In recent years, there have been some studies investigating the knowledge and skill level of doctors on airway management in Turkey. In the survey conducted among 27 doctors working in ambulances of 112 emergency services in Turkey, 60 percent of ambulance physicians stated that their training was insufficient. In addition, it was observed that none of the doctors received a special training on airway management after graduation. This high rate shows that physicians need more training on airway management, which is the indispensable first step of basic life support in the medical education process (43). In another study investigating the airway management experiences of research assistants in medical specialization training in Turkey, more than 80% of the participants stated that they received training on airway equipment and its use for the first time in the Anesthesiology and Reanimation internship at the medical faculty. While 34.3% of the participants stated that they did their first endotracheal intubation after graduating from medical school, 13.4% stated that they never used the airway, which is one of the simplest airway devices(44). In present study, which is the first study conducted among medical students in Turkey, the rate of participants who never used airway devices such as orotracheal intubation was found to be 34.44%, similar to the results of the previous study. In addition, 5.55% stated that they consider themselves inadequate in all airway management practices, including airway placement. These findings revealed that there is a significant deficiency in airway management in medical school education.

Airway control is a skill that must be acquired not only for emergency physicians and anesthesiologists but also for all physicians and healthcare professionals. Providing education in this field has become a necessity today. Successful airway management is vital in patients with maxillofacial trauma whose clinical condition requires medical urgency. In order for doctors to know and successfully apply the tools and equipment used to ensure airway patency, the medical school curriculum should be reviewed and the deficiencies should be eliminated. In addition, it is essential for doctors to receive training at regular intervals to improve their knowledge and practice skills. New models of medical education should be developed to allow medical students to acquire technical and non-technical knowledge from the early stages of education through case discussions or real situations. It seems imperative that all components responsible for education, especially anesthesia professional associations, come together and update airway management training with competency-based training instead of a traditional program for physicians to receive qualified training.

This study has some limitations. Since the study was conducted in a medical school, the results do not represent the whole country, although similar training methods are used throughout the country. In addition, in the study, theoretical knowledge of airway management and airway tools in maxillofacial trauma were evaluated. Theoretical and practical evaluations should be made together while evaluating students' knowledge and skills on airway management. However, this study is important in that it is the first study that comprehensively evaluates medical students' knowledge about airway management in maxillofacial trauma.

5. CONCLUSION

According to the findings of this study, students have a lack of knowledge about airway management in patients with maxillofacial trauma. It was observed that the level of knowledge of the students about the tools and methods used in providing airway patency was quite low. Airway management in trauma patients should be comprehensively addressed in the curriculum of emergency medicine and anesthesia – reanimation courses taught at the medical school. Students should be provided with educational opportunities, tools, and equipment so that they can improve both their theoretical knowledge and practical skills on airway management. In addition, applied courses, workshops and congresses, and symposiums should be organized at the national level on airway management in emergencies and the participation of young doctors should be encouraged. Teamwork between maxillofacial surgeons, anesthetists, and emergency medicine and trauma specialists is essential to manage the patient with maxillofacial trauma.

Conflicts of interest: The author declares no conflict of interest

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Clinical and Experimental Health Sciences

Evaluation of the Effectiveness of Distance Learning in Dental Education During COVID-19 Pandemic in Turkey

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ABSTRACT

Objective: COVID-19 pandemic affect dental education as well as everything. Online education become more popular in dentistry nowadays. It was aimed to evaluate the effectiveness of online lessons and satisfaction of the students about distance learning.

Methods: It was asked to respond a 42-items questionnaire at University of Uşak, School of Dentistry. Seven questions were aimed to gather information related to demographic data. Thirty-two lickert and three multiple choice questions to determine the quality of current non-clinical and clinical education and to form a guide for future online dental education. The questionnaire was filled out anonymously. The answers were analyzed on IBM SPSS Statistics for Windows, version 22.0 (IBM Corp, Armonk, NY, USA).

Results: This study aimed to reach 316 dental students, of which 302 (95.5%) responded. The percent of female students was 57.6% and male students was 42.4%. Male students more pleased than female students in term of distance learning ($p=0.032$). Fourth grade students were significantly pleased distance learning than first and second grades students according to the satisfaction questions ($p<0.05$). Most of the students agreed that they would prefer blended education (strongly agree, 43%; agree, 22.2%).

Conclusion: A certain satisfaction about online education was not obtained. Online clinical-based lessons were more difficult to understand for the students. Most of the students agreed that blended education is a good choice. It can be concluded that blended education will be a good alternative system for future dental education.

Keywords: Dental education, distance education, COVID-19

1. INTRODUCTION

The disease that appears firstly in Wuhan state, China lately 2019, is defined as (**CO**rona**VI**rus **D**isease 20**19,** COVID-19) in January 2020 after the investigations on the patients who have respiratory system complains. While the virus isolated just from live animal selling seafood bazaar in Wuhan, it spread to other cities and countries by human-to-human transmission (1,2). After worldwide transmission World Health Organization declared as 'pandemic' on 11 March 2020 (3).

Coronaviruses are the members of a crowded family that can cause diseases from flu to Middle East Respiratory Syndrome, (MERS) or Severe Acute Respiratory Syndrome, (SARS) (1). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV2) transmission couldn't be taken under control and spread rapidly all over the world. Many governments had to make rigid decisions to avoid the virus. It was announced that a quarantine period must begin from 12 March 2020 in our country. People were restricted for going outside, being in covered areas, being so closed to each other. Council of Higher Education announced that all institutions and universities will be closed after the date of 16 March 2020 according to governmental recommendations. Most universities had to continue their education by means of online education modules.

During the pandemic period the dentists were classified in the very-high-risk category due to the potential of exposure to coronavirus through aerosol-containing procedures by Occupational Safety and Health Administration (4,5). Dental institutions concerned great safety risks both for patients and students. Dental schools and hospitals accepted only emergency situations and were mostly closed according to recommendations of Ministry of Health and Council of Higher Education. Dental students turned their home and had to continue distance learning. Academic stuff met with a new 'online' curriculum; delivered virtual lectures on file-sharing platforms. While preclinical simulation procedures proceeded by demonstration videos on the platforms clinical lessons interrupted. Didactic lessons and preclinical

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laboratories' lessons were mostly easy to survive online. Clinical training tried to proceed with case discussions.

Although face-to face education seems to be most effective and acceptable education style by authorities, distance learning was found to be successful in many studies. Online education is an education style that saves time and money, lets students to learn in their own way (6). Didactic lessons constitute the best choice for distance learning. It is easy to teach& learn and effective (7). Distance learning is not a system that commonly used in health-related institutions in Turkey. In dental education, the students get clinical – based education as well as didactic education. It is a big challenge to train online clinical-based lessons. It is still unknown how to continue clinical-based lessons and clinical practice during or after pandemic period. According to the commentary results of a survey that The Association of Dental Education in Europe (ADEE) carried out, the long-term impact on clinical dental education is unclear (8). Meanwhile, the combination of online and face-to-face education 'blended learning' become more popular in dental education (9).

In this study, it was aimed to evaluate the effectiveness of online lessons and satisfaction of the students about distance learning.

2. MATERIAL AND METHODS

Ethical approval was obtained by the Non-Interventional Medicine Ethics Committee of Uşak University Faculty of Medicine on 23.09.2020 (Reference number: 76-08-04). After the pandemic period, all courses had to continue online. Theorical courses were given live and demo videos were filmed for practical courses. Case discussions which evaluating radiological images and clinical photos were done in clinical courses. All online videos were recorded and saved in Google Classroom Platform so students could watch again anytime they want.

This was a cross-sectional descriptive study that included dentistry students as the participants. 42-items questionnaire was conducted to dental students in Uşak University including age, gender, grade, nationality, usage of internet, thoughts and opinions about distance learning and the effect of distance learning to their education performance. The questionnaire consisted simple single answer and multiple-choice questions to determine the quality of current non-clinical and clinical education and to form a guide for future online dental education. Seven questions were aimed to gather information related to demographic data. Thirty-two Lickert questions and 3 multiple choice questions were prepared on Google Forms and sent to students via school e-mail. It was planned to reach all 316 students who were registered to our faculty in 1 to 4 grades. All these students were attended both face-to-face courses and online courses in same year. The questionnaire was filled out anonymously.

2.1. Statistical Analyses

The data obtained through the questionnaire used as a data collection tool analyzed by quantitative methods by using the statistical analysis program of SPSS version 22.0 (IBM, Armonk, NY, USA). Exploratory factor analysis was performed. According to Kolmogorov-Smirnov test for normality of the distribution of variables; Kruskal-Wallis, Mann-Whitney U, and Spearman correlation test were used. Also, descriptive statistics were calculated and for categorical variables chi-square test was used.

3. RESULTS

Cronbach Alfa value for the questionnaire was 0.964. A total of 316 students were participated in this study and 302 of them completed the questionnaire. The response rate was 95.56%. The percent of female students was 57.6% and male students was 42.4%. The average age was 21±1.5 and ranged from 18 to 29. Descriptive statistics were shown in Table 1. All of the students' responses to the questions were presented in Table 2. The detailed results of crucial questions were shown in Figure 1.

Table 1. *Descriptive Statistics of the Present Study*

Variables		Frequency (n) %
Gender	Male	128 (42.4%)
	Female	174 (57.6%)
Grade	First	92 (30.5%)
	Second	84 (27.8%)
	Third	73 (24.2%)
	Fourth	53 (17.5%)
Nationality	Turkish students	291 (96.4%)
	Foreign students	11 (3.6%)
Region	Village	22 (7.3%)
	Town	83 (27.5%)
	City	197 (65.2%)

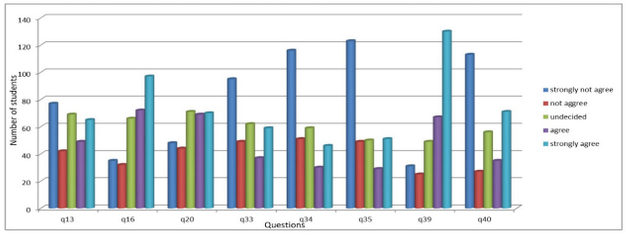

Figure 1. *Graphical Representation of Crucial Questions Via Lickert Scale*

Factor analysis was performed to check the suitability of the data. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.961 and Barlett's test of sphericity was significant ($p<0.001$). According to rotation method (Oblimin with Kaiser Normalization) and the extraction method four factors were extracted. Only 1 item (Q27) had a value below than 0.30 so it wasn't related any factor and it was removed from the questionnaire. These four factors explained the 71.436% of variability and categorized as satisfaction, utilization, benefits of face-to-face, and handicap of face-to-face learning (Table 3).

Table 2. *Students Response to the Questionnaire*

Questions	strongly disagree	disagree	undecided	agree	strongly agree
Q9.I have daily internet access	11 (3.6%)	23 (7.6%)	53 (17.5%)	86 (28.5%)	12 9 (42.7%)
Q10. I know how to open. change. and upload online docs	10 (3.3%)	29 (9.6%)	48 (15.9%)	104 (34.4%)	111 (36.8%)
Q11. I access the internet daily or weekly to check course announcements and online classes	9 (3.0%)	28 (9.3%)	41 (13.6%)	86 (28.5%)	138 (45.7%)
Q12. I feel comfortable exploring / finding online lessons	27 (8.9%)	21 (7.0%)	55 (18.2%)	77 (25.5%)	122 (40.4%)
Q13. I am satisfied with distance education	77 (25.5%)	42 (13.9%)	69 (22.8%)	49 (16.2%)	65 (21.5%)
Q14. I liked the distance education	84 (27.8%)	43 (14.2%)	62 (20.5%)	46 (15.2%)	67 (22.2%)
Q15. I am enthusiastic to attend distance learning courses	79 (26.2%)	45 (14.9%)	68 (22.5%)	43 (14.2%)	67 (22.2%)
Q16. I followed the lessons regularly	35 (11.6%)	32 (10.6%)	66 (21.9%)	72 (23.8%)	97 (32.1%)
Q17. I followed online classes more than traditional/face-to-face training	119 (39.4%)	45 (14.9%)	44 (14.6%)	41 (13.6%)	53 (17.5%)
Q18. I had no problem logging into the system and following the lessons	35 (11.6%)	41 (13.6%)	47(15.6%)	8 2(27.2%)	87 (32.1%)
Q19. I was able to solve the problems that I had while logging into the system	14 (%4.6)	22 (7.3%)	63 (20.9%)	101 (33.4%)	102 (33.8%)
Q20. I was able to understand the subjects taught by distance education	48 (15.9%)	44 (14.6%)	71 (23.5%)	69 (22.8%)	70 (23.2%)
Q21. Where I didn't understand I was able to consult the instructor	28 (9.3%)	35 (11.6%)	75 (24.8%)	73 (24.2%)	91 (30.1%)
Q22. I felt more comfortable participating actively in online classes than face-to-face training.	84 (27.8%)	45 (14.9%)	48 (15.9%)	59 (19.5%)	66 (21.9%)
Q23. I could understand the demonstrations shown by videos in distance education	62 (20.5%)	47 (15.6%)	61 (20.2%)	65 (21.5%)	67 (22.2%)
Q24. Online lessons helped me learn	43 (14.2%)	40 (13.2%)	79 (26.2%)	68 (22.5%)	72 (23.8%)
Q25. The videos helped me learn	37 (12.3%)	41 (13.6%)	71 (23.5%)	70 (23.2%)	83 (27.5%)
Q26. Distance learning has been useful in communicating and interacting with instructors and other students	45 (14.9%)	58 (19.2%)	67 (22.2%)	55 (18.2%)	77 (25.5%)
Q28. I like being able to access distance education at any time	21 (7.0%)	14 (4.6%)	30 (9.9%)	68 (22.5%)	169 (56%)
Q29. I like being able to access distance education from anywhere	22 (7.3%)	15 (5.0%)	34 (11.3%)	70 (23.2%)	16 1(53.3%)
Q30. I am glad to be able to study from anywhere in the world	23 (7.6%)	17 (5.6%)	47 (15.6%)	73 (24.2%)	142 (47%)
Q31. I like how distance education gives students the power to manage their education	40 (13.2%)	31 (10.3%)	47 (15.6%)	71 (23.5%)	113 (37.4%)
Q32. I like that distance education is at my pace/control	32 (10.6%)	29 (9.6%)	48 (15.9%)	72 (23.8%)	121 (40.1%)
Q33. I believe that I will be successful as a result of distance education	95 (31.5%)	49 (16.2%)	62 (20.5%)	37 (12.3%)	59 (19.5%)
Q34. I think distance education is more beneficial than traditional face-to-face education	116 (38.4%)	51 (16.9%)	59 (19.5%)	30 (9.9%)	46 (15.2%)
Q35. I think videos shown in distance education are more useful than live demonstrations	123 (40.7%)	49 (16.2%)	50 (16.6%)	29 (9.6%)	51 (16.9%)
Q36. I think it is a disadvantage that distance education requires a computer and the internet	39 (12.9%)	36 (11.9%)	55 (18.2%)	57 (18.9%)	115 (38.1%)
Q37. Online lectures should replace traditional lectures and live demonstrations	114 (37.7%)	54 (17.9%)	63 (20.9%)	26 (8.6%)	45 (14.9%)
Q38. Online courses and tutorials need to be further developed to support my learning	16 (5.3%)	12 (4.0%)	67 (22.2%)	96 (31.8%)	111 (36.8%)
Q39. I prefer the combination of traditional education and online classes	31 (10.3%)	25 (8.3%)	49 (16.2%)	67 (22.2%)	130 (43.0%)
Q40. I would like the distance education to continue in the new academic year	113 (37.4%)	27 (8.9%)	56 (18.5%)	35 (11.6%)	71 (23.5%)

***Table 3**. Factor Analyses of the Questionnaire*

Questions	Components			
	1	2	3	4
Q9		.909		
Q10		.778		
Q11		.875		
Q12		.851		
Q13	.708			
Q14	.763			
Q15	.710			
Q16	.474			
Q17	.790			
Q18		.574		
Q19		.705		
Q20	.644			
Q21	.339			
Q22	.630			
Q23	.700			
Q24	.612			
Q25	.580			
Q26	.684			
Q27				
Q28			.936	
Q29			.905	
Q30			.788	
Q31			.657	
Q32			.714	
Q33	.846			
Q34	.986			
Q35	.937			
Q36				.594
Q37	.997			
Q38				.808
Q39				.537
Q40	.845			

Male students more pleased than female students in term of distance learning (p=0.032). They felt more enthusiastic to attend online lessons than females(p=0.019). Fourth grade students were significantly pleased distance learning than first and second grade students according to the satisfaction questions (p<0.05). There was no significant difference among grades according to the utilization questions (p>0.05). There were 5 questions about benefits of face-face learning and first grade students were significantly pleased with only Q31 than fourth grade's (p=0.004). There was no significant difference among grades according to the handicap of distance learning questions (p>0.05). There was a positive correlation between grades and Q34, Q37, and Q40 (p<0.05) (Table 4).

Foreign students agreed (54.5% strongly agree and 9.1% agree) that they could understand the subjects with online lessons and could consult with the professor (36.4% strongly agree and 36.4% agree). Besides, they agreed (45.5% strongly agree and 18.2% agree) that they felt more comfortable participating actively in online lessons than face-to-face (p=0.047). Finally, they were glad to study from anywhere in the world (72.7% strongly agree and 9.1% agree).

The students who used computer, followed the lessons regularly and had less problem in joining/following the online lessons, significantly (p<0.05). According to the responses about internet usage (Q6, Q7, Q9, Q10, Q11, Q12, Q36), it had found that students lived in village had trouble using internet, significantly (p<0.05) Thus they followed lessons via mobile phone (p=0.014).

The reason of using internet mostly for checking on social media (78.8%). While 63.9% of students were listening music/watching videos on internet, only 25.8 % of them were trying to reach web-based training programs. Their opinions about difficulties of quarantine period and distance learning were asked in multiple choice questions and the percentages of responses were given in Figure 2.

***Table 4.** P Values of Comparison of the Grades According to 'Satisfaction' Component*

	Q13	Q14	Q15	Q16	Q17	Q20	Q21	Q22	Q23	Q24	Q26	Q33	Q34	Q35	Q37	Q40
1-2	1.000	1.000	1.000	1.000	1.000	1.000	0.505	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1-3	1.000	0.745	1.000	0.052	0.189	1.000	0.219	0.368	1.000	1.000	0.750	1.000	1.000	1.000	1.000	1.000
1-4	0.004	0.000	0.004	0.006	0.000	0.004	0.020	0.001	0.007	0.008	0.019	0.000	0.000	0.164	0.007	0.034
2-3	1.000	1.000	1.000	0.129	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.948
2-4	0.008	0.002	0.042	0.016	0.000	0.005	1.000	0.055	0.013	0.008	0.046	1.000	0.001	0.032	0.045	0.002
3-4	0.062	0.040	0.203	1.000	0.014	0.095	1.000	0.294	0.281	0.307	0.826	0.016	0.026	0.166	0.191	0.153

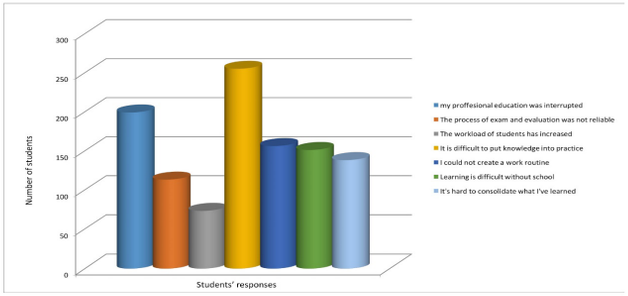

Figure 2. *The Responses to the Multiple-Choice Questions Choice Questions*

4. DISCUSSION

As we all know COVID-19 pandemic affects everyone and everything negatively. Our usual life stopped and everyone had to change daily routines. Institutions also had to change their routine education systems. Online education, distance learning became our new normal. The faculties based on practical training like dental schools were curious about online education all over the world, is it enough or not. Dental students were also curious and stressed. Therefore, our main aim was to evaluate the satisfaction of the students about distance learning in this study.

While online education was popular in some studies, (10-12) the students' opinions were not clear about if they were pleased about distance learning in this study. While 39.4% of them were not agreed, 37.7% of them were agreed. When we evaluate according to the grades, it was found that 4th grade students were more pleased. In our faculty, clinical training begins at the 2nd semester of 3rd grade. So 4th grade students had a clinical experience when quarantine begins. They also have baseline training about didactic lessons. It can be thought that it was easier to intensify the courses than building a new knowledge like in 1st grade. Also, online case discussions were able to affect positively reinforcing courses in 4th grade. 1st grade student who were building a new knowledge about dentistry, were the most unpleased students. They had difficulties in learning without a strong base.

While many studies stated no difference according to gender, contrary to Mubayrik et al (13) study we found that male students were more pleased with online lessons than female students (11,14). It was attributed to that they were more enthusiastic to attend online lessons significantly. It could be related to the socio-cultural difference between countries.

Results of present study indicated that students lived in village had trouble using internet and preferred to follow lessons via mobile phone. The percent of students that followed online lessons regularly was low (18.2% for strongly agree and 13.6% for agree) among the students living in village. Speed and quality of internet connection may be affected by location and it decreases in rural area. Decreasing of speed and quality of internet connection might be reason of choosing mobile phone against computer.

Online education has some disadvantages like requiring computer and internet that most of the students agreed. This could affects joining and following the lessons. Most of the students could follow the lessons regularly. Besides the students who joined the lessons via computer were significantly more comfortable following the lessons regularly. It could be related to sitting in front of computer at a table makes mind more open than watching tablet or mobile phone in a relax mode. Internet is one of the biggest dilemma for this century. It is very useful and dangerous at the same time. When the students were asked the purpose of using internet, 78.8% of the answers were pointing out social media. They spent a lot of time on social media instead of web-based training programs. Social media usage was extremely higher in the current study than the study of Roberts et al (15) (52%); probably by means of the quarantine days' vapidity.

Foreign students felt more comfortable participating actively in online lessons than face-to-face. It could be explained that they could contributed and consulted freely by writing

their opinions. However, they did not believe that they could be successful at the end of education like Turkish students. They may have thought that clinic education would not be beneficial enough for them to be carried out online. Besides, the fact that Turkish is not their native language is also a handicap for them.

There are many studies having different results in dental education about blended learning (6,10-12,14,16-18). Especially it became a requirement during the pandemic period. Liu et al (19) declared that online dental continuing education increased when COVID-19 was just an epidemic in China.

Distance learning was found to be more useful in previous studies (10-12). Smith et al (10) reported that video-clips were preferred more than preclinical demonstrations for tooth preparations by students. However, students agreed that it would be better if the supervisor cares individually for improving their skills than watching video clips.

The studies comparing education systems found that blended learning was mostly chosen by the students (6,11,14,16-18). Asiry et al (6) investigated the dental students' perceptions of an online learning and they reported that most of the students preferred a combination of face-to-face learning and online learning. According to the results of Pahinis et al (14) study, students felt comfortable with face-to-face and online-supported learning. Bains et al (18) conducted a study which evaluated the effectiveness of face-to-face, blended, and e-learning for the cephalometric education. They reported that blended learning was the most accepted learning type by the students. The students didn't show much confidence on they would be successful in our study. Most of the students thought that distance learning was not better than face-to-face learning and mostly they were not so closed to continue online lessons next year. They had great

hesitations about their profession, mostly for clinical training like in a Brazilian study (20). It was also commented on the study of Bennardo et al (21) as the clinical training can not be digitalized totally. The students would rather combination of face-to-face lessons and online lessons in accordance with previous studies. It can be related to the nature of dentistry education. Didactic lessons and clinical training combine a successful education. Without practicing on patients, the students can not have self-confidence. It was proved by the response to the multiple-choice questions about their opinions. 84.4 % of the students declared that it is difficult to put into practice that has been learned and 65.9% of them thought that their professional education was interrupted.

This study is one of the few studies in which dental education was evaluated by students during the COVID-19 pandemic in Turkey and it showed that the opinion of professors that it would not be very appropriate to conduct clinical and practical training in dentistry with a distance education model was similar among students. The appropriate education model has been discussed and it is thought that it will shed light on further studies.

There was some limitation in this study. A new survey form was created by examining the survey questions in similar studies and the survey evaluated the students' perspective about the success of distance learning not the real success. In addition, the data were obtained from the students of a single dentistry school, so just one dentistry school education was evaluated in this study.

5. CONCLUSION

COVID-19 pandemic seems to have an influence on dentistry education. Besides online training programmes, education systems begin to change. While dentistry schools having problems with continuing face-to-face education system, they need to move on clinical work within new COVID-19 protocols. It can be thought that blended learning begins to rise as all dental schools' new approach.

Within the limitations of this study; dental students are not so satisfied about online education. They have some hesitations especially on clinical performance. They don't have much self-confidence to face with patients via online training. Only 4th grade students who treated patients before pandemic period think optimistic about distance learning. Most of the students agree that blended education is a good choice.

It can be concluded that blended education will be a good alternative system for future dental education. Further studies are needed to evaluate more data around the world to improve dental education system after COVID-19 pandemic, before other pandemics.

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Clinical and Experimental Health Sciences

Attitudes, Perceptions and Knowledge Regarding the Future of Artificial Intelligence in Oral Radiology Among a Group of Dental Students in Turkey: A Survey

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ABSTRACT

Objective: This study investigated knowledge, attitudes, and perceptions regarding the future of artificial intelligence (AI) for radiological diagnosis among a group of Turkish dental students.

Methods: An online survey was conducted consisting of 11 questions using Google Forms and circulated among 4th and 5th grade students at Marmara University, Faculty of Dentistry. The survey consisted of questions regarding participants' recognition of and attitudes toward AI, their opinions on directions of AI development, and their perceptions about the future of AI in oral radiology. IBM SPSS Statistics 25.0 (IBM SPSS, Turkey) program is used for statistical analysis.

Results: The study group consists of 75 4th and 65 5th grades and a total of 140 students. Of the 140 participating dental students, 60% were already familiar with the concept of AI, 92.9% agreed stated that they would like to use a software/program that can be helpful in radiological diagnosis and 37.9 % reported that AI would have a future in Turkey. Among two grades, there was no statistically significant difference of answers to questions regarding the future and role of artificial intelligence in oral radiology ($p>0.05$).

Conclusion: According to the findings of the study, most dental students were aware of AI, AI systems could be used to improve diagnostic accuracy when reading radiographs, and AI has a promising role in radiological diagnosis.

Keywords: Artificial Intelligence, Dental Students Awareness

1. INTRODUCTION

Artificial intelligence (AI) can be defined in simple terms as using computers or machines to perform tasks that normally require humans (1-5). Machine learning, which is a branch of artificial intelligence, can be used to teach machines and computers how to interpret different kinds of data using various algorithms. AI programs have been designed to interpret data from a variety of sources, and AI applications are commonly used in a variety of fields, including engineering, the stock market, and medicine, among others (3-6). Many individuals, including doctors and physicists, are still unfamiliar with artificial intelligence's principles and true promise, as well as its effect on our personal and professional lives. The medical use of AI systems in medicine has grown in importance in recent years, and their potential uses in dentistry often need careful consideration.

The applications of AI programs in dentistry are very interesting and sustainable, especially in radiology (2-9). In recent years, AI applications in dentistry have attracted attention in areas ranging from the diagnosis of caries to the detection of various pathologies, from planning orthodontic treatment of crowded teeth to robotic surgery and dental implant construction (10-13). Especially adaptation with image processing methods has highlighted the studies of dental radiology. Applications such as classification and segmentation of teeth on 2 and 3 dimensional (2D / 3D) radiological images, determination of dental diseases, determination of gingival diseases and evaluation of risk groups, automatic marking of anatomical structures and cephalometric analysis, diagnosis of some diseases such as osteoporosis that can be detected in jaw radiographs are examples of up-to-date studies (5). It has also been reported

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in the literature that AI is used in the early screening of oral cancer and cervical lymph node metastasis, as well as in the diagnosis and treatment planning of various orofacial diseases (7,8).

Clinicians and dental students, on the other hand, have differing perspectives on AI's future. While many claim that artificial intelligence can open many doors in the fields of medicine and dentistry and will pave the way toward a bright future, some believe that AI is unstable and will never be able to replace radiologists (8).

In an online survey of 250 dentists in India, an online questionnaire with 15 questions was used to determine awareness, behaviors, and opinions about the future of artificial intelligence in oral radiology. The AI definition is common to 68% of the 250 dentists who took part in the study, and 69% plan to use AI to make dental diagnoses. The authors stated that 51% of the participants believe that the key role of AI would be the analysis of complicated radiographic scans, and 63% agree that artificial intelligence has a future in India. The study found that dentists are well aware of artificial intelligence, that artificial intelligence programs can be used by dentists as an auxiliary method to improve diagnostic sensitivity when interpreting radiographs, and that artificial intelligence has a promising role in radiological diagnosis (3).

There is only one study about this particular subject among dental students in Turkey, thus, the aim of this study on the future of artificial intelligence in oral radiology information among a group of dental students in Turkey, is to assess the attitudes and perceptions.

2. METHODS

The study protocol of this study was approved by Marmara University School of Medicine Non-Interventional Clinical Research Ethics Committee on 05/03/2021 with protocol number 09.2021.258. The research group consists of 140 4th and 5th grade dental students studying at Marmara University Faculty of Dentistry and the participants were subjected to an online questionnaire using Google Forms consisting of 11 questions adapted from the study of Sur et al. (3) related to knowledge, attitudes, and perceptions regarding the future of artificial intelligence (AI) for future radiological diagnosis.

2.1 Statistical Analysis

IBM SPSS Statistics 25.0 (IBM SPSS, Turkey) program is used for statistical analysis. Besides descriptive statistical methods (mean, standard deviation, frequency), in comparison of qualitative data, Chi-Square test was used and significance was assessed at $p < 0.05$ level.

3. RESULTS

The study was conducted on 140 students, 55 (39.3%) male and 85 (60.7%) female, with ages ranging from 20 to 28. The avarage age of the students is 22.91 ±1.48 years. 75 (53.6%) of the students are 4th grade, 65 (46.4%) are 5th grade students.

In the study, 84 (60%) of the 140 respondents were already familiar with AI and its software. Despite the fact that 111 dental students (79.3%) agreed that AI has medical uses, only 55 (39.3%) had a basic understanding of how to incorporate AI into their work.

Furthermore, 37 students (26.4%) agreed that AI would speed up the healthcare system, reduce mistakes, and provide a vast quantity of high-quality data in a timely manner without causing emotional or physical exhaustion.

Almost every participant (92.9%) expressed an interest in using applications for radiological diagnosis yet 41.1% of all participants were unsure if AI would make better diagnoses than a human doctor. In our study only 2.1% of participating dentists stated that they would follow the AI's prediction if there is a controversy, while 31.4% were not sure. A total of 114 participants (81.4%) agreed that they would use AI for dental diagnosis and treatment planning and 50% of dental students agreed that the key function of AI is to interpret complicated radiographic scans. Fifty six dental students (40%) were not sure that AI has a future in Turkey, while 91.4% agreed that AI will help dentists in their diagnosis and decision-making (Table 1).

Evaluations of knowledge, attitudes and perception of AI among 4th and 5th grade dental students are shown in Table 2. There was a statistically significant difference for the question "Are you familiar with AI and its applications?" between two grades. The rate of participating to the statement was higher among 5th grade students (p=0.002).

Table 1. *Evaluations by Gender*

		Male	Female	Total	p
Are you familiar with AI and its applications?	Yes	35 (25.0%)	49 (35.0%)	84 (60%)	0.205
	No	8 (5.7%)	7 (5.0%)	15 (10.7%)	
	Not sure	12 (8.6 %)	29 (20.7%)	41 (29.3%)	
Do you agree that AI has useful applications in the medical field?	Yes	46 (32.9%)	65 (46.4%)	111 (79.3%)	0.418
	No	2 (1.4%)	2 (1.4%)	4 (2.9%)	
	Not sure	7 (5.0%)	18 (12.9%)	25 (17.9%)	
Do you have any ideas about how AI might be used in dentistry?	Yes	21 (15.0%)	34 (24.3%)	55 (39.3%)	0.908
	No	10 (7.1%)	7 (12.1%)	27 (19.3%)	
	Not sure	24 (17.1%)	34 (24.3%)	58 (41.4%)	
What are the benefits of using AI, in your opinion?	AI has the potential to improve health-care systems while still reducing medical mistakes.	20 (14.3%)	17 (12.1%)	37 (26.4%)	0.142
	In real time, AI can provide large quantities of scientifically appropriate, high-quality results.	6 (4.3%)	10 (7.1%)	16 (11.4%)	
	AI is resistant to both mental and physical fatigue.	3 (2.1%)	3 (2.1%)	6 (4.3%)	
	All of the above	26 (18.6%)	55 (19.3%)	81 (57.9%)	
Would you like to use a software/program that can be helpful in radiological diagnosis?	Yes	48 (34.3%)	82 (58.6%)	130 (92.9%)	0.096
	No	1 (0.7%)	0 (0.0%)	1 (0.7%)	
	Not sure	6 (4.3%)	3 (2.1%)	9 (6.4%)	
Do you think AI's diagnostic capacity is superior to a human doctor's professional knowledge?	Yes	12 (8.6%)	10 (7.1%)	22 (15.7%)	0.020
	No	25 (17.9%)	27 (19.3%)	52 (37.1%)	
	Not sure	18 (12.9%)	48 (34.3%)	66 (41.1%)	
What decision would you make if your medical opinion and AI's differ?	My own opinion	36 (25.7%)	57 (40.7%)	93 (66.4%)	0.618
	AI's opinion	2 (1.4%)	1 (0.7 %)	3 (2.1%)	
	Not sure	17 (12.1%)	27 (19.3%)	44 (31.4%)	
Do you agree that you may use AI while making dental diagnosis and treatment planning in the future?	Yes	41 (29.3%)	73 (52.1%)	114 (81.4%)	0.215
	No	2 (1.4%)	1 (0.7%)	3 (2.1%)	
	Not sure	12 (8.6%)	11 (7.9%)	23 (16.4%)	
In which field of dentistry do you think AI will be most useful?	Making a diagnosis	14 (10.0%)	18 (12.9%)	32 (22.9%)	0.220
	Making treatment decisions	5 (3.6%)	7 (5.0 %)	12 (8.6 %)	
	Direct treatment (including surgical robots)	14 (10.0%)	12 (8.6%)	26 (18.6%)	
	Interpreting complicated radiographic scans	22 (15.7%)	48 (34.3%)	70 (50.0%)	
Do you think AI has a future in dentistry in Turkey?	Yes	18 (12.9%)	35 (25.0%)	53 (37.9%)	0.017
	No	19 (13.6%)	12 (8.6%)	31 (22.1%)	
	Not sure	18 (12.9%)	38 (27.1%)	56 (40.0%)	
Do you think AI will help dentists in diagnosis and decision-making?	Yes	48 (34.3%)	80 (57.1%)	128 (91.4%)	0.248
	No	1 (0.7%)	0 (0.0%)	1 (0.7%)	
	Not sure	6 (4.3%)	5 (3.6%)	11 (7.9%)	

Chi-square test, p<0.05, AI: Artificial Intelligence

Table 2. *Evaluations by Grades*

		4th grade	5th grade	Total	p
Are you familiar with AI and its applications?	Yes	39 (46.4%)	45 (53.6%)	84 (60.0%)	0.002*
	No	5 (33.3%%)	10 (66.7%)	15 (10.7%)	
	Not sure	31 (75.6%)	10 (25.4%)	41 (29.3%)	
Do you agree that AI has useful applications in the medical field?	Yes	61 (43.6%)	50 (35.7%)	111 (79.3%)	0.811
	No	2 (1.4%)	2 (1.4)	4 (2.9%)	
	Not sure	12 (8.6%)	13 (9.3%)	25 (17.9%)	
Do you have any ideas about how AI might be used in dentistry?	Yes	29 (20.7%)	26 (18.6%)	55 (39.3%)	0.971
	No	15 (10.7%)	12 (8.6%)	27 (19.3%)	
	Not sure	31 (22.1%)	27 (19.3%)	58 (41.4%)	
What are the benefits of using AI, in your opinion?	AI has the potential to improve health-care systems while still reducing medical mistakes.	19 (13.6%)	18 (12.9%)	37 (26.4%)	0.910
	In real time, AI can provide large quantities of scientifically appropriate, high-quality results.	9 (6.4%)	7 (5.0%)	16 (11.4%)	
	AI is resistant to both mental and physical fatigue.	4 (2.9%)	2 (1.4%)	6 (4.3%)	
	All of the above	43 (30.7%)	38 (27.1%)	81 (57.9%)	
Would you like to use a software/ program that can be helpful in radiological diagnosis?	Yes	69 (49.3%)	61 (43.6%)	130 (92.9%)	0.409
	No	0 (0%)	1 (0.7%)	1 (0.7%)	
	Not sure	6 (4.3%)	3 (2.1%)	9 (6.4%)	
Do you think AI's diagnostic capacity is superior to a human doctor's professional knowledge?	Yes	13 (9.3%)	9 (6.4%)	22 (15.7%)	0.587
	No	25 (17.9%)	27 (19.3%)	52 (37.1%)	
	Not sure	37 (26.4%)	29 (20.7%)	66 (47.1%)	
What decision would you make if your medical opinion and AI's differ?	My own opinion	47 (33.6%)	46 (32.9%)	93 (66.4%)	0.209
	AI's opinion	3 (2.1%)	0 (0%)	3 (2.1%)	
	Not sure	25 (17.9%)	19 (13.6%)	44 (31.4%)	
Do you agree that you may use AI while making dental diagnosis and treatment planning in the future?	Yes	61(43.6%)	53 (37.9%)	114 (81.4%)	0.750
	No	1 (0.7%)	2 (1.4%)	3 (2.1%)	
	Not sure	13 (9.3%)	10 (7.1%)	23(16.4%)	
In which field of dentistry do you think AI will be most useful?	Making a diagnosis	14 (10.0%)	18 (12.9%)	32 (22.9%)	0.069
	Making treatment decisions	3 (2.1%)	9 (6.4%)	12 (8.5%)	
	Direct treatment (including surgical robots)	15 (10.7%)	11 (7.9%)	26 (18.6%)	
	Interpreting complicated radiographic scans	43 (30.7%)	27 (19.3%)	70 (50.0%)	
Do you think AI has a future in dentistry in Turkey?	Yes	31 (22.1%)	22 (15.7 %)	53 (37.9 %)	0.497
	No	14 (10.0%)	17 (12.1%)	31 (22.1%)	
	Not sure	30 (21.4%)	26 (18.6 %)	56 (40.0%)	
Do you think AI will help dentists in diagnosis and decision-making?	Yes	67 (47.9 %)	61 (43.6%)	128 (91.4 %)	0.240
	No	0 (0.0%)	1 (0.7%)	1 (0.7%)	
	Not sure	8 (5.7%)	3 (2.1%)	11 (7.9%)	

Chi-square test, p<0.05, AI: Artificial Intelligence

4. DISCUSSION

The applications of artificial intelligence in dentistry are interesting, especially in radiology, and AI can be a valuable resource for new dentists. Merely, a limited number of studies focused on knowledge, attitudes, and perceptions regarding the future of artificial intelligence for radiological diagnosis among dental students.

Oh et al. (1) centralized into how well-informed Korean doctors are about AI and how they feel about it being used in medicine. They used Google Forms to create an online survey of 11 closed-ended questions. The survey included questions about AI awareness and behaviors, AI creation in medicine, and the potential dangers of using AI in medicine. The survey was conducted by 669 participants in total. Just 40 doctors (5.9%) claimed that they were very familiar with artificial intelligence. However, the majority of participants thought AI could be helpful in medicine (83.4% agreement). Disease diagnosis is the field of medicine where respondents decided AI will be most helpful (83.4% agreement). Less than half of the participants (43.9%) agreed that AI is diagnostically superior to human doctors. Merely 237 people (35.4%) accepted that AI will eventually replace them in their employment. Higher rates of agreement was reported

in a study conducted in India, where 68% of dentists are familiar with the definition of AI, 69% believe AI can be used in diagnosis and care preparation, and 63% believe AI has a future in India (3).

In a study of dental students' attitudes and expectations of artificial intelligence, a 22-item questionnaire was administered via Google Forms to dental students from all 9 different Turkish dental schools (14). Of the 1103 students who took part in the study, 48.40% had a basic understanding of AI technology, 85.70% believed dentistry would revolutionize AI, and 74.60% and 79.80 % felt AI-related topics should be included in undergraduate and graduate dental education, respectively. The participants were found to have inadequate knowledge of AI, but were able to learn more about it and believed that artificial intelligence would have a positive effect on prospective dentistry practices. In our survey, 84 (60%) of the 140 participants had prior knowledge of AI and its applications. While 111 dental students (79.3%) accepted that AI has medical applications, only 55 (39.3%) had a basic understanding of how to integrate AI into their practice. In addition, among two grades, there was no statistically significant difference of answers to questions regarding the future and role of artificial intelligence in oral radiology ($p>0.05$).

Dental students were given an online Google forms link to complete a self-administered questionnaire based on their knowledge of artificial intelligence's application in medicine in a study by Ranjana *et al.* (15) According to the findings, about 59% of research participants were aware that artificial intelligence technologies in medicine benefits physicians, and both male and female students were similarly aware of artificial intelligence. When the relationship between gender and their opinion on AI as a method for revolutionizing clinical decision and diagnosis was examined, it was discovered that 28 out of 51 females and 29 out of 59 males firmly agree that clinical decision and diagnosis can be revolutionized with the aid of AI. hough statistically not signficant, female students had a higher agreement rate (46.4%) that AI has useful applications in the medical field than male students in our study.

5. CONCLUSION

AI is a branch of computer science that can analyze large amounts of medical data. In several clinical scenarios, this technology aids in the diagnosis, treatment, and prediction of outcomes. As all AI technology has the ability to evolve into an advanced tool capable of processing more complex data in dentistry, there needs to be a greater knowledge of the technology in order to better understand and analyze it among future dentists.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

Characteristics and Coronavirus Anxiety Levels of Endodontic Patients During the Covid-19 Pandemic

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ABSTRACT

Objective: The aim of this study was to determine the characteristics and coronavirus anxiety levels of patients who applied to an endodontics clinic in Turkey during the Covid-19 pandemic.

Methods: A total of 212 patients who applied to an endodontics clinic between January 1 and 31 2021, were asked to complete a questionnaire that included demographic information, Covid-19 history, anxiety history, and coronavirus anxiety scores. After the patients were examined by an endodontist, their complaint, pain level—using the Verbal Numerical Rating Scale (VNRS)—clinical findings, periapical index score (PAI), and endodontic diagnosis were recorded. Data was analyzed using descriptive statistical methods, while the Chi-square test, Fisher's Exact test, Fisher Freeman Halton test, and Yates Continuity Correction were used for the comparison of the qualitative data (significance level, $P < 0.05$).

Results: About half of the patients (43.4%) were diagnosed with symptomatic irreversible pulpitis. The mean of the VNRS was 4.17 ± 3.14 with a median of 4. Approximately 43% of the patients' PAI scores were 1, which radiologically indicates normal periapical structures. When patients' coronavirus anxiety scores were evaluated, 86.8% were categorized as non-disordered and 13.2% as disordered.

Conclusion: Patients tended to visit an endodontics clinic usually for urgent procedures and postponed other dental procedures due to anxiety related to the Covid-19 pandemic in Turkey.

Keywords: Covid-19 pandemic, coronavirus anxiety scale, endodontic diagnosis, verbal numerical rating scale

1. INTRODUCTION

The Covid-19 epidemic emerged in Wuhan, China, at the end of 2019 and became an important health problem affecting the world in a short period of time (1, 2). The speed and type of reactions to this disease varied according to countries' different health systems, economics, and political ideologies (3). Daily life has changed, and many measures have been taken, including social isolation, closure of schools, and quarantine of societies, to deal with the pandemic (4). After the announcement of the first case in Turkey on March 10, 2020, cases rose rapidly (5). Routine dental practices were suspended in mid-March. Organizations such as the World Health Organization and the American Dental Association recommended that only dental treatments that fall under the definition of emergency be undertaken (2,5). The Turkish Ministry of Health also made this recommendation (6). Once the process of returning to normal life began in June 2020, routine dental procedures commenced once again in Turkey.

Inaccurate information about the pandemic, the rapid increase in the number of infected and deaths, changes in work routines caused stress, anxiety and fear in the population (7-9). It was reported that the Covid-19 pandemic period and the lockdown affected patients' anxiety levels and willingness to make dental appointments. Unconcerned patients were willing to go to dental appointments while anxious patients stated that they preferred to go to the dentist only in the case of emergencies (10). Recently, the Coronavirus Anxiety Scale (CAS)—a brief mental health screener—was developed by Lee (11) to fill a void in the mental health response to the growing public health crisis related to Covid-19. A Turkish validity and reliability study of the CAS was performed by Biçer et al. (12).

In this study, we aimed to examine the characteristics of patients, including symptoms, pain grades, radiological findings, coronavirus anxiety levels, and the diagnoses they received, who applied to an endodontics clinic between certain dates during the pandemic. To the best of our knowledge, there is currently no study on this subject in the literature.

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2. METHODS

This study was approved by the ethical board of Izmir Democracy University (no: 2020/24-2). Patients' participation in this study was voluntary and anonymous. This study was also conducted in compliance with the Helsinki Declaration of 2013. A pilot study was performed on 25 randomly selected patients to validate the questionnaire, and its Cronbach's alpha value was determined to be 87.6%. The pilot study responses were excluded from the final study.

The study population consisted of patients who were referred to the Endodontics Department of Biruni University between January 1 and 31 2021. A total of 212 patients over the age of 13 who applied to the endodontics clinic and agreed to participate in the study, after having passed through the triage area, were included in the investigation. The patient evaluation form consisted of three parts with 25 questions. The first part involved demographic information and questions related to the Covid-19 history of patients (13 questions). The second part questioned the history of anxiety and anxiety levels related to Covid-19 using the CAS (9 questions) (11). In the last part (3 questions), an endodontist recorded the patient complaint, pain level using the Verbal Numerical Rating Scale (VNRS) (13), clinical findings, radiological signs using the periapical index score (PAI) (14), and the endodontic diagnosis. A 5-point Likert scale was used in the CAS, which consisted of five questions and one dimension. Total anxiety level was calculated on the basis of each patients' response. According to this, the highest score obtained from the scale was 20. Scores of 9 and above were interpreted as coronavirus anxiety disordered while scores below 9 were categorized as non-disordered (11). All procedures were performed by a single endodontist.

2.1. Statistical Analysis

The IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for the statistical analysis. Data normality was evaluated using the Shapiro Wilks test. Data were analyzed using descriptive statistical methods (mean, standard deviation, frequency). The Chi-square test, Fisher's Exact test, Fisher Freeman Halton test, and Yates Continuity Correction were used to compare the qualitative data. Cronbach's alpha coefficient was calculated for the scale reliability. Significance was evaluated at the $p<0.05$ level.

3. RESULTS

The demographic information of the patients is given in Table 1. Approximately 24% of participants in the study lived with someone in the Covid-19 risk group, and 13.2% of them stated that they were in close contact with a person who was Covid-19 positive. 10.4% of the patients had been diagnosed with Covid-19, 90.9% had experienced mild symptoms, and 9.1% had experienced severe symptoms. 68.9% reported that they had relatives or friends in their close circle who had tested positive for Covid-19. About 15% had lost a relative or friend (Table 2).

Table 1. *Distribution of demographic information*

		n	%
Age	13-25	66	31,1
	26-35	70	33,0
	36-45	34	16,0
	46-55	26	12,3
	56-64	10	4,7
	65+	6	2,8
Sex	Male	82	38,7
	Female	130	61,3
Education status	Elementary school	32	15,1
	Middle school	32	15,1
	High school	70	33,0
	Undergraduate	60	28,3
	Postgraduate	18	8,5
Maritial Status	Married	126	59,4
	Single	86	40,6
Children	Yes	114	53,8
	No	98	46,2
Chronic diseases	No	156	73,6
	Hypertension	10	4,7
	Diabetes	6	2,8
	KOAH	10	4,7
	Others	30	14,1

Table 2. *The distribution of participants' responses to questions about their Covid-19 history*

		n	%
Is anyone in your household in the risk group for Covid-19?	Yes	50	23,6
	No	162	76,4
Have you had any recent contact with someone who is Covid-19 positive?	Yes	28	13,2
	No	184	86,8
Have you been diagnosed with Covid-19?	Yes	22	10,4
	No	190	89,6
If yes, how badly did you have the disease? (n=22)	Asymptomatic or with mild symptoms	20	90,9
	With severe symptoms	2	9,1
Have anyone in your family or close circle been diagnosed with Covid-19?	Yes	146	68,9
	No	66	31,1
Have you lost a family member or friend due to Covid-19?	Yes	30	14,2
	No	182	85,8

A total of 14.2% of the patients stated that they had previously been diagnosed with an anxiety disorder by a psychologist. The rate of delaying dental appointments due to the pandemic of those who had been previously diagnosed

with an anxiety disorder by a psychiatrist (80%) was found to be statistically significantly higher than those who had not (53.8%) (p:0.013; p<0.05).

The distribution of the CAS questions and the answers given by the patients are summarized in Table 3. The Cronbach's alpha coefficient of the scale was 87.6%. According to the CAS, a total of 184 (86,8%) patients were categorized as non-disordered and 28 (13.2%) as disordered.

Table 3. *Responses given to the CAS*

CAS Questions	Not at all	Rare, less than a day or two	Several days	More than 7 days	Nearly every day over the last 2 weeks
	n (%)	n (%)	n (%)	n (%)	n (%)
I felt dizzy, lightheaded, or faint, when I read or listened to news about the coronavirus.	160 (%75,5)	16 (%7,5)	22 (%10,4)	14 (%6,6)	-
I had trouble falling or staying asleep because I was thinking about the coronavirus.	152 (%71,7)	16 (%7,5)	26 (%12,3)	14 (%6,6)	4 (%1,9)
I felt paralyzed or frozen when I thought about or was exposed to information about the coronavirus..	178 (%84)	10 (%4,7)	12 (%5,7)	6 (%2,8)	6 (%2,8)
I lost interest in eating when I thought about or was exposed to information about the coronavirus.	136 (%64,2)	26 (%12,3)	18 (%8,5)	26 (%12,3)	6 (%2,8)
I felt nauseous or had stomach problems when I thought about or was exposed to information about the coronavirus.	154 (%72,6)	22 (%10,4)	12 (%5,7)	18 (%8,5)	6 (%2,8)

The patient complaints and endodontic diagnoses are shown in Table 4. According to this, patients were diagnosed with symptomatic irreversible pulpitis (43.4%), chronic apical periodontitis (23.6%), other diagnoses (8.5%), pulp necrosis or gangrene (7.5%), reversible pulpitis (5.7%), chronic apical abscess (5.7%), acute apical abscess (4.7%), and trauma-related injury (0.9%). The distribution of clinical findings and PAI scores is shown in Table 5.

Table 4. *The patient complaints and endodontic diagnoses*

Patient Complaint	n	%
Pain at night	50	23,6
Spontaneous pain	50	23,6
Pain with hot and/or cold	68	32,1
Pain when chewing	96	45,3
Routine control and/or follow up	30	14,2
Asymptomatic	28	13,2
Other (trauma etc.)	10	4,7
Diagnosis	**n**	**%**
Reversible pulpitis	12	5,7
Symptomatic irreversible pulpitis	92	43,4
Chronic apical abscess	12	5,7
Chronic apical periodontitis	50	23,6
Acute apical abscess	10	4,7
Traumatic dental injury	2	0,9
Pulp necrosis or gangrene	16	7,5
Other (Endodotic treatment indication for prosthetic reasons etc.)	18	8,5

Table 5. *Clinical Findings and PAI Score*

Clinical Findings	n %
Percussion pain	142 67
Palpation pain	12 5,7
Positive Vitality	108 51
Sinus tract	6 2,8
Intraoral abscess	12 5,7
Extraoral abscess	2 0,9
PAI Score	**n %**
1	90 42,5
2	54 25,5
3	48 22,6
4	10 4,7
5	10 4,7

The VNRS ranged from 0 to 10, with a mean of 4.17 ± 3.14 and a median of 4. The VNRS scores of patients aged 56 and over was found to be significantly lower than those of the 13–25 and 26–35 age groups (p1: 0.012; p2: 0.013; p<0.05). The VNRS scores of patients with acute apical abscess and symptomatic irreversible pulpitis were found to be significantly higher than those with pulp necrosis or gangrene, trauma-related injury, chronic apical abscess, reversible pulpitis, chronic apical periodontitis, and other diagnoses (p<0.05).

The disordered rate of coronavirus anxiety among women (18.5%) was statistically significantly higher than that of men (4.9%) (p:0.008; p<0.05). The coronavirus anxiety disordered rate of undergraduate graduates (26.7%) was found to be statistically significantly higher than that of secondary school graduates (6.3%) and high school (5.7%) graduates (p1: 0.038; p2: 0.002; p<0.05).

Table 6. *Comparison of corona anxiety levels according to Covid-19 history, anxiety disorder and postponing dental treatment appointments*

		Corona anxiety		p
		Non-Disordered	Disordered	
		n (%)	n (%)	
Is there anyone in the covid risk group living in the same household?	Yes	40 (%80)	10 (%20)	[1]0,166
	No	144 (%88,9)	18 (%11,1)	
Have you had contact with someone diagnosed with Covid-19?	Yes	24 (%85,7)	4 (%14,3)	[2]0,525
	No	160 (%87)	24 (%13)	
Have you been diagnosed with Covid-19?	Yes	28 (%68,2)	13 (%31,8)	[3]0,000*
	No	156 (%91,2)	15 (%8,8)	
Is there a person with a positive diagnosis of Covid-19 in the family or close circle?	Yes	120 (%82,2)	26 (%17,8)	
	No	64 (%97)	2 (%3)	[1]0,006*
Have you lost a family member or friend due to Covid-19?	Yes	20 (%66,7)	10 (%33,3)	
	No	164 (%90,1)	18 (%9,9)	[2]0,002*
Have you been previously diagnosed with anxiety disorder by a psychiatrist?	Yes	20 (%66,7)	10 (%33,3)	[1]0,002*
	No	164 (%90,1)	18 (%9,9)	
Have you postponed dental treatments due to pandemic?	Yes	98 (%80,3)	24 (%19,7)	[2]0,002*
	No	86 (%95,6)	4 (%4,4)	
Do you concern about contamination with Covid-19 virus during dental treatment?	Yes	62 (%73,8)	22 (%26,2)	[2]0,000*
	No	122 (%95,3)	6 (%4,7)	

[1]*Continuity (Yates) Correction* [2]*Fisher's Exact Test* [3]*Chi square Test*
**p<0.05*

More than half of patients (57.5%) had postponed their dental treatments due to the pandemic, and 39.6% were concerned about the transmission of Covid-19 during dental treatment. The non-disordered status of those who had not been diagnosed with Covid-19 was found to be significantly higher than those who had been (p1: 0.000; p2: 0.001; p<0.05). The disordered rate of coronavirus anxiety was found to be significantly higher in those who had experienced a positive diagnosis of Covid-19 in their family or close environment (17.8%) than those who had not (3%) (p:0.006; p<0.05). In addition, the coronavirus anxiety disordered rate of patients who had lost a family member or friends due to Covid-19 (33.3%) was found to be significantly higher than those who had not (9.9%) (p: 0.002; p<0.05). The coronavirus anxiety disordered rate of patients who had been previously diagnosed with an anxiety disorder by a psychiatrist (33.3%) was found to be statistically significantly higher than those who had not received such a diagnosis (9.9%) (p:0.002; p<0.05). Moreover, the coronavirus anxiety disordered rate of those who were concerned about the transmission of Covid-19 during dental treatments (26.2%) was found to be significantly higher than those who were not (4.7%) (p:0.000; p<0.05). The coronavirus anxiety disordered rate of patients who had postponed dental treatments due to the pandemic (19.7%) was found to be significantly higher than that of those who had not (4.4%). Approximately 67% of patients who had been previously diagnosed with an anxiety disorder were concerned about the transmission of the virus during dental treatments, which was significantly higher than those who had not been diagnosed with an anxiety disorder (p:0.002; p<0.05) (Table 6).

4. DISCUSSION

Pulpal and periapical lesions were found to be the most common reason for emergency dental visits in China during the Covid-19 pandemic (15). A study that researched the characteristics of endodontic emergencies remarked that symptomatic irreversible pulpitis was the most common disease (16). Similarly, in our study, most of the patients who applied to the endodontics clinic were diagnosed with symptomatic irreversible pulpitis and had normal periapical structures based on their PAI scores. The VNRS scores of patients with symptomatic irreversible pulpitis and acute apical abscess were found to be significantly higher than for other diseases. This result is also in agreement with the study of Yu et al. (16). Patients mostly applied to our clinic with percussion pain (67%), hot-cold sensitivity (32,1%), spontaneous pain (23.6%), and night pain (23.6%). Moreover, in the present study, only 2 out of 212 patients had trauma-related injuries, which might be due to the limitations imposed on outdoor activities during the pandemic. This result is similar to the findings of recent literature (15).

The majority of patients who participated in this study were in the non-disordered category in terms of coronavirus anxiety levels. Based on this finding, it can be said that some dental patients were concerned about Covid-19 in Turkey, but this concern was not at a level that suggested illness or required psychological support. A possible reason for this may be because the study was conducted later in the pandemic, and over time, most people may have become used to living with it. Patients who are already less anxious may also be more comfortable going to dental clinics. Since there are no studies in the literature that examine the coronavirus anxiety levels of patients visiting dental clinics, we cannot compare the results.

In this study, the relationship between the study parameters and patients' coronavirus anxiety status was also examined. According to the gender comparison, the coronavirus anxiety levels of women were higher than those of men. Consistent with our results, Cotrin et al. (17) found that women were more anxious than men about the Covid-19 pandemic. Interestingly, in our study, most patients who visited the endodontics clinic were women. Similarly, Peloso et al. (10) stated in their study that, although women were more anxious about the pandemic than men, they were more willing to go to the dentist. This may be because female patients are more

compatible with dental treatments than men, as reported by Demetriou et al. (18). Considering patients' education levels, it was found that those with bachelor's degrees had a higher coronavirus anxiety disorder status than those with high school and secondary school diplomas. Based on this result, it may be that patients with a higher level of education are more susceptible to anxiety disease due to their greater knowledge about the pandemic.

It is remarkable that the coronavirus anxiety levels of patients who had family members or close relatives who had been diagnosed with Covid-19 were higher than those who had not, even though most of these patients and their relatives had experienced mild symptoms. In our opinion, this may be due to other factors, such as educational status, business conditions, socio-economic situation, or a pre-existing anxiety disorder that triggered coronavirus anxiety. Our results also indicate that some patients had been previously diagnosed with anxiety disorders by a psychologist, and they were likelier to delay their appointments.

In this study, most of the patients who applied to the endodontics clinic declared that they did not have any chronic diseases. This situation can be explained by the fact that patients with chronic diseases do not like to go to the dentist. In other words, patients with chronic illnesses and coronavirus anxiety may delay their dentist appointments.

According to the results of this study, the coronavirus anxiety levels of patients previously diagnosed with an anxiety disorder were found to be higher, which may indicate that patients with a previous history of anxiety experienced increased anxiety associated with the coronavirus pandemic.

Although this study was not conducted at the beginning of the pandemic, when there was relatively greater uncertainty, it was determined that most of the patients who applied to the endodontics clinic postponed their dental appointments, and most of them applied only in the case of emergencies. The main reasons for this included worry about being infected with Covid-19 during dental treatment and/or coronavirus anxiety. Other possible causes may be a prior anxiety history and the socio-economic conditions of patients.

The limitation of the present study is that it included a limited population over a limited time frame. Future studies could be conducted with a larger sample to investigate the characteristics of endodontic patients and their coronavirus anxiety status.

5. CONCLUSION

It was seen that some patients postponed their dental treatments due to anxiety related to the Covid-19 pandemic, applying to endodontics clinics for emergency treatments only. This situation may result in a growth in dental problems after the Covid-19 pandemic ends due to the delay in seeking preventive and conservative treatments. Since it is uncertain how long the pandemic will last, performing both emergency and routine dental treatments are essential for the oral and dental health of individuals. It is important to eliminate the concerns of patients that cause them to delay their dental appointments.

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Clinical and Experimental Health Sciences

CBCT Visualization of Furcation Perforation Repair Materials Using Different Voxel Sizes

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ABSTRACT

Objective: Three-dimensional cone-beam computed tomography is gaining popularity as an imaging modality aiding the performance of difficult endodontic treatment procedures. For this reason, we assessed the visualization of bioactive furcal perforation repair materials in an ex vivo study using CBCT with different voxel sizes and determined which voxel size yielding the best images with least artefacts. Visualization of endodontic restorative material is affected by the appearance of various artifacts. This study was conducted to evaluate the CBCT visualization of three perforation repair materials using five different voxel sizes.

Methods: This study was performed with 84 mandibular molars that had been extracted for various reasons. All samples were measured with a digital caliper (Digimess, São Paulo, Brazil), marked at 3 mm above the cementoenamel junction, and decoronized at this line. The root segments were amputated at 3 mm below the furcation site using diamond discs under water cooling. The openings of the pulp chamber created, following the furcation defects formed. Biodentine, MTA, and EndoSequence were mixed following the manufacturers' instructions and applied to the perforation sites. Five image sets were obtained: 75 micron, 100 micron, 150 micron, 200 micron, and 400 micron. 2 examiners were evaluated the images independently.

Results: The best image quality for all repair materials obtained in 100 micron. Image clarity of repair materials showed no significant difference among voxel sizes of 75 and 150 micron. Image quality was significantly reduced at 200 and 400 micron voxel sizes, and the worst quality was obtained at 400 micron voxels in all groups.

Conclusions: CBCT imaging can be used to examine endodontic repair materials with adjustment of the effective radiation dose rate and selection of the appropriate voxel size.

Keywords: Bioactive aterials, Perforation, Voxel size

1. INTRODUCTION

Perforations and defects in the furcation region affect prognoses following endodontic treatment. Furcation perforations are iatrogenic gaps between the pulp chamber and periodontal ligament; they can cause complications during endodontic treatment and subsequent periodontitis-related lesions (1). Defects on the floor of the pulp chamber lead to periodontal ligament and bone destruction, followed by an inflammatory process that can lead to tooth loss (2). The success of endodontic treatment of a tooth with a furcation perforation depends on furcal defect size, location, the time spent closing it, and the properties of the materials used (3). Many repair materials have been developed and used for perforation closure, including calcium silicate-based cements, such as mineral trioxide aggregate (MTA; Dentsply Maillefer, Ballaigues, Switzerland) (4). MTA is commonly used as a repair material, but it has several limitations (5).

Biodentine (Septodont, Niederkassel, Germany), EndoSequence Materials (Brasseler USA, Savannah, GA, USA) are newly developed bioactive materials. Bioceramics are biocompatible ceramic compounds obtained in situ and in vivo through various chemical processes (6). They are resistant to irrigation and antibacterial, with osteogenetic effects and short application times (7). Biodentine was stated to be improved behaviour properties and takes less time to prepare than MTA. Its physical properties are very similar to dentine and the risk of tooth discoloration is low (8). Recently, EndoSequence was designed for use as root repair material. This premixed material is provided as a condensed mass or

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pre-filled syringe and is easy to manipulate; it has excellent biological and mechanical properties and a high degree of biocompatibility. In addition, it is hydrophilic, radiopaque, osteogenic, and insoluble. It is used for pulp capping and root repair procedures (9). Our understanding of the radiological properties of bioceramic materials is limited by numerous factors (10). Two-dimensional x-rays are inadequate for the analysis and handling of furcation defects. Cone-beam computed tomography (CBCT) is used for endodontic identification, management, prognosis, as it enables the three-dimensional (3D) identification of bony margins and defect sites (11). Voxel size has important effects on CBCT evaluation quality. A voxel is the smallest element of a 3D image. In CBCT imaging, voxels are usually isotropic and range from 75 – 400 micron. The isotropic geometry of the voxels allows for identical image resolution on different planes (2). Visualization of endodontic restorative material is affected by the appearance of various artifacts. Although these image defects cannot be eliminated using current technology, the quality can be improved by changing constraints such as the FOV and voxel sizes (12). Thus, this study was conducted to evaluate the CBCT visualization of three perforation repair materials using five different voxel sizes.

2. METHODS

2.1. Sample Selection

84 mandibular molars were used for this study that had been extracted for various reasons. Ankara University Local Ethical Committee (IRB approval number: 07/10) approved the study. Included teeth were intact or with minimal caries, also had closed apexes and separate roots. Radiographs of all teeth were taken from different directions and examined; teeth with calcified pulp chambers or pulp stones were excluded. Residual debris and soft tissue on the teeth were removed using a periodontal curette, and the teeth were kept in distilled water containing 0.1% thymol crystal at room temperature until use. All samples were measured with a digital caliper (Digimess, São Paulo, Brazil), marked at 3 mm above the cementoenamel junction, and decoronized at this line. The root segments were amputated at 3 mm below the furcation site using diamond discs under water cooling. The openings of the pulp chamber created. Necrotic pulp tissue removed and the cavities were rinsed with sodium hypochlorite. Dentin thickness in the furcation area was measured using a calliper, and teeth with dentine thicknesses of 2.0–2.5 mm were included in the study. The perforations were formed in the furcation areas using a long round drill with water cooling. The perforation areas were irrigated using saline to remove residual dentin. To simulate clinical conditions, the samples were placed in sponges soaked in saline in plastic cylinders. The teeth were then divided randomly into three groups. Biodentine, MTA-Angelus (Angelus Indústria de Produtos Odotontológicos S/A, Londrína, PR, Brazil), and EndoSequence Root Repair Material were mixed according to manufacturer directions and placed over the perforation sites for respective groups using a plugger and dental loupe. The materials were condensed by applying a small amount of pressure. Excess filling material was removed with a thin probe, and a damp cotton pellet was then placed into pulp to harden the material. The samples left at 37°C with 100% moisture for 7 days to allow the materials to harden.

2.2. Scanning Protocol

Each tooth positioned on molar tooth sockets in a dry human mandible. The mandible was properly covered with wax to simulate soft tissue. The mandible then scanned using Planmeca ProMax 3D CBCT (Planmeca Oy, Helsinki, Finland). The scanner offers multiple fields of view, allowing the dentist to select the optimum scan on a case-by-case basis. Images were obtained using a 5 × 5.5 cm FOV (75 micron and 100 micron), a 5 × 5.5 cm FOV (150 micron), a 10 × 5.5 cm FOV (200 micron and 400 micron) with isotropic voxels. All scans were taken at pre-settings of the machine; 90 kVp, 10 mA, 17 s and 90 kV, 10 mA, 15 s for (75, 100, and 150 micron), 96kVp, 12mA, 18 s for (200 micron and 400 micron) voxels.

2.3. CBCT Evaluations

Each scan was evaluated by 2 observers with a mean of 10 years of experience using the unit's software (Planmeca Romexis 4.2). Before evaluations, the observers were pretrained on for the usage of the software in a special session. The observers were calibrated for the study. The images were anonymized during evaluation of the materials. Observers scored clarity of the image of the materials. They were also asked to grade overall image quality on a 1-to-5 point scale based on the visibility of perforation repair materials of different voxel sizes (1: very low, 2: low, 3: moderate, 4: high, 5: very high quality).

The images evalautions were made within one week again, and all images again re-evalueted after 2 months.

2.4. Statistical Analysis

The data analyzed with SPSS (ver. 22; Chicago, IL, USA). Receiver operating characteristic (ROC) curves were used for each observers' evaluations. A p value less than 0.05 was considered as significant. Inter-examiner reliability evalauted with Kappa Cohen's statistical analysis. Weighted kappa coefficients were calculated to assess both intra – and interobserver agreements for each image set. Weighted kappa values were interpreted according to the guidelines of Landis and Koch, as adapted by Altman (k ≤0.20, very low; k = 0.20–0.40, low; k = 0.41–0.60, moderate; k = 0.61–0.80, high; and k = 0.81–1.00, very high quality). The normality of the variables was analysed using the Shapiro-Wilk test. Moreover, three-way ANOVA was used with Bonferroni correction.

3. RESULTS

The inter-examiner reliability showed a high agreement of 90.4% (95% CI 0.773–0.846) between the two observers (KO, BC) which showed no significant difference between observers.

Three-way analysis of variance revealed significant effects of the voxel and material factors. The voxel and material factors had significant effects ($p<0.05$). No interaction between factors was observed.

The best image quality for all repair materials obtained at 100 micron. There was no significant difference among 75 micron and 150 micron for visualization of repair materials.

Image quality of repair materials was significantly reduced at 200 and 400 micron and the worst quality was obtained at 400 micron voxels in all groups (Table 1).

Table 1. *Showing the mean values and standard deviation of the endodontic repair materials according to voxel sizes.*

Restorative Materials / Voxel sizes	Endosequence	Biodentine	MTA-Angelus
75 micron	4,167±0,637^{aA}	4,042±0,624^{aAB}	3,917±0,503^{aA}
100 micron	4,417±0,653^{aB}	4,375±0,575^{aB}	4,000±0,0^{aA}
150 micron	4,000±0,417^{aBC}	3,958±0,690^{aAB}	4,000±0,0^{aA}
200 micron	3,250±0,442^{aC}	3,542±0,883^{aA}	3,583±0,503^{aA}
400 micron	1,833±0,481^{aA}	1,625±1,134^{aC}	1,500±0,510^{aB}

*Different superscript letters indicate statistically difference at 5% significant level ($p<0.05$). (a, b, c for rows and A,B,C for columns)

Comparing the image quality of repair materials, no difference was observed among the repair materials ($p>0.05$). Estimated marginal means are shown in Figure 1.

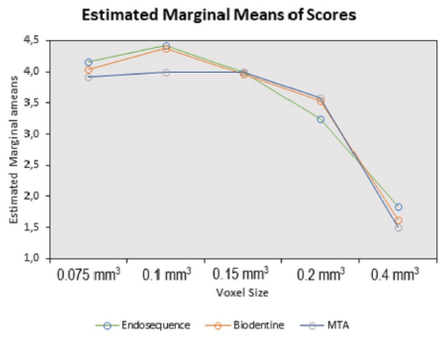

Figure 1. *Estimated marginal means of scores according to voxel sizes.*

4. DISCUSSION

Clinicians should be able to manage the case well when the perforation, which is an undesirable complication occurs. In addition to choosing the most appropriate material to be used, post-treatment radiographic control is also important. Therefore, the present study investigated which voxel setting would be necessary to get the best visual for imaging the material. Conventional periapical radiographs have limitations for evaluation of repair material quality. CBCT is used in many areas in endodontics and has been shown to have the greatest accuracy among imaging modalities for perforation detection (13). Although it has many clinical advantages, including 3D visualization, CBCT has some limitations. The most important limitation is the radiation exposure. The goal is to obtain the best image with a minimum radiation dose. Effective dose differs between CBCT scanners; depending on the size of the FOV, it may be similar to panoramic radiography, but significantly less than medical CT. Image acquisition with a larger voxel size followed by reconstruction at a smaller voxel size may yield similar quality with a reduced radiation dose (14).

Few studies evaluated voxel sizes for furcation perforations. Tomographic image quality is related directly to the acquisition protocol, and particularly the voxel size. Junqueira et al. (15) used voxels of the least thicknesses available (250 and 125 micron) because a lesser slice thickness generates greater spatial resolution, which can directly affect the diagnostic capability. Although they found no significant difference between examinations, the sensitivity and accuracy were less with larger voxels, supporting the results described by Silveira et al. (16), Melo et al. (17) and Brito-Júnior et al. (18). Filling materials were evaluated on images acquired with 200 micron voxels. Properties of all filling materials were similar on images obtained with a 76 micron voxel size. Properties differed among voxel sizes only for Sealer 26 and Endofill, with more artifacts observed with 200 micron voxel resolution (18). Similarly, in our study, image quality was low and artifacts were observed with 200 and 400 micron. Liedke et al. (19) and Silveria et al. (16) assessed the performance for external root resorption, found that the 300 micron voxel size yielded the best outcomes, with less exposures. In our study, the best image quality for furcation defects was achieved with 100 micron voxels. In a previous study in which mandibular molar morphology was examined using CBCT, the best voxel size was determined to be 100 micron (20). Other in vitro studies have demonstrated that CBCT is more reliable than conventional radiography for the diagnosis of endodontic pathologies, such as root fracture, root perforation, and resorption (21). Venskutonis et al. (22) stated in their study for the detection of root perforations the reduction of the voxel size increased the diagnostic value.

Another major disadvantage of CBCT is the appearance of restorative material artifacts, which can be caused by most materials used in endodontics. The restorative materials have specific densities can create artifacts such as blooming, scatter streaks, which can consequence for false-positive results. Thus, the accuracy of CBCT evaluation of root-filled teeth can be compromised by the presence of artifacts such as noise, scattering, missing values due to motion, and rings, as well as beam-hardening and streaking artifacts, which complicate image interpretation (12). Despite these disadvantages, CBCT is the chosen imaging modality for root canal perforations, furcation perforations, and other pathologies. In this study, the visibility of furcation perforations and were evaluated on CBCT images (Figure 2).

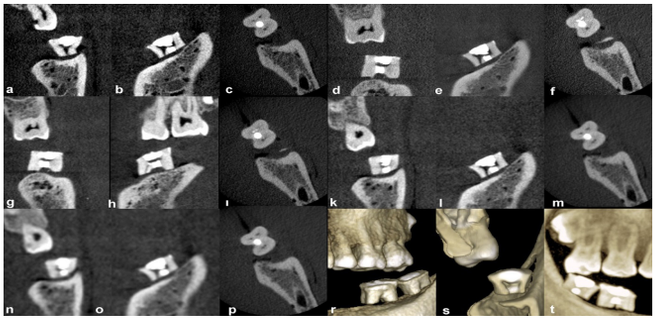

Figure 2. *a, b and c: CBCT images show cross section, sagittal, and axial of 75 micron furcation defects with sealing material. d, e and f: CBCT images show cross section, sagittal, and axial of 100 micron furcation defects with sealing material. g, h and I: CBCT images show cross section, sagittal, and axial of 150 micron furcation defects with sealing material. k, l and m: CBCT images show cross section, sagittal, and axial of 200 micron furcation defects with sealing material. n, o and p: CBCT images show cross section, sagittal, and axial of 400 micron furcation defects with sealing material. r, s and t: CBCT images show 3D representation of furcal defects from buccal cross section and lingual slides.*

A perforation – an iatrogenic, mechanical, or compulsive communication between the root canal system and external tooth surface – should be sealed with a biocompatible material. Jeevani et al. (23) reported that MTA is now considered to be the material of choice for perforation sealing because of its biocompatibility. However, MTA has shortcomings like trouble of handling and slow setting, which may subsidize to leakage, surface disintegration, loss of marginal adaptation, and disruption of the material. As stated before, during image evaluation of various materials, Brito-Júnior et al. (18) assessed streaking artifacts produced by Sealer-26, Endofill, and MTA FillApex. They found Sealer-26 and Endofill produced more artifacts than did MTA FillApex at a voxel size of 200 micron. Biodentine is a calcium silicate–based material that has a polycarboxylate-based hydrosoluble polymer and contains Calcium cilicate. The latter has high compressive and flexural strength. Helvacıoglu et al. (12) reported that Biodentine created the fewest artifacts and showed the best performance among materials evaluated. EndoSequence was developed to overcome the difficulty of handling of MTA. No previous study has explored the CBCT visualization or artifact formation of EndoSequence. No significant difference in image clarity was found among materials in this study. Of five voxel sizes, 100 micron yielded the clearest images.

The limitation of this study was in clinical practice detection of furcal defects in CBCT machines may be less accurate owing metallic or other artifacts. Moreover, patient movement should also be taken into consideration which in this study did not taken into account because of the in vitro nature of the study. Thus, further studies should be done with CBCT optimization filters.

5. CONCLUSION

As a consequence of this study CBCT imaging can be used to examine endodontic repair materials. A voxel size of 150 micron was recognized as the cut-off point for exposure of furcal defects repairing materials.

Conflict of interest

The authors declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

Evaluation of Healthy Nutrition Index-2015, Dental Health and Oral Flora Relationship in School-Age Children

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ABSTRACT

Objective: The aim of the study is to evaluate the role of diet quality and oral flora on the dental health in the childhood period.

Methods: A cross-sectional study was carried out with the participation of 98 children aged 9-14 and their parents who applied to the Pedodontics Clinic Dental Faculty of Marmara University. The decayed, missing, filled tooth and surfaces and DMFT, DMFS indexes were determined through visual dental examination. Anthropometric measurements and saliva samples, as well as 24 h food consumption records, were taken from each child. Saliva samples were analysed microbiologically. To evaluate dietary quality from intakes Healthy Eating Index-2015 (HEI-2015) scores were calculated.

Results: The mean age of children was 10.8±1.5 and study sample compromised 44.9% males and 55.1% females. 75% of the children had normal height and 58.2% of them had normal BMI according to World Health Organisation (WHO) references. The mean number of decayed tooth and surface was 3.07±2.35; 4.34±3.78 respectively and the mean number of DMFT and DMFS indexes were 5.88±2.31; 8.05±4.58 respectively. The mean number of decayed, missing, filled teeth and DMFT, DMFS scores were found to be higher in children with poor diets (according to HEI-2015) but the difference was not statistically significant ($p>0.05$). The presence of lactobacilli in saliva samples of 5.5% of the children was observed and children have lactobacilli in their saliva samples had more decayed teeth ($p<0.05$).

Conclusion: The study findings illustrate the relationship between decay development and lactobacilli. Further studies with high sample sizes are needed to examine the diet quality and oral health.

Keywords: Healthy eating index, Dental caries, Diet quality, Childhood, Oral flora.

1.INTRODUCTION

In parallel with the definition of the general health status of the World Health Organization (WHO), it is emphasized that oral and dental health is the starting and basic point of body health (1). Despite improvements in oral disease prevention and treatment over the past decade incidence of dental caries, the most common oral disease, remains high in pediatric age. A systematic review conducted in 2015 reveals that 2.4 billion people are affected by caries in permanent teeth, while 621 million children are affected by caries in deciduous teeth, demonstrating the 10th most prevalent condition worldwide (2). Childhood dental caries have unfavorable effects on the life quality of children due to disturbance, pain, altered sleeping habits, poor nutrition, and even a decrease in school attendance and performance (3, 4).

Oral health and nutrition are interdependent and have multi-directional relationship (5). Depend on previous research evidence, diet is still an important factor associated with dental caries and considered to be a main cause of dental erosion (6, 7). A good quality diet, which is one of the most important factors contributing to the health and quality of life of children and adolescents, is the only and valid way to prevent dental caries (6) with proper oral hygiene practices (5).

Until today various tools have been developed to assess diet quality. The Healthy Eating Index-2015 (HEI-2015), the most current version, is a dietary assessment tool prepared for this purpose and designed to measure specifically the degree to which a set of foods aligns with the Dietary Guidelines for Americans (8). In HEI-2015 diet quality is evaluated from two perspectives: the first part is adequacy (dietary components to increase) and the second part is moderation (dietary components to decrease) (9). Analyses demonstrated that HEI-2015 is a valid and reliable source in other aspects also

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can be used to evaluate the diet quality of children older than two years as in different populations (8, 10). Thirteen components of the HEI-2015 show consistency with Dietary Guidelines for Americans, recommendations of World Health Organisation as well as dietary guidelines of Turkey (8, 11, 12).

The role of the host microbiota in the health of the individual has been an ongoing and distinct concept for many years (13). The oral cavity is densely colonized with bacteria, diet-like environmental changes may alter the composition of the oral microbiome, and as a result of dysbiosis the risk for oral diseases especially dental caries can increase (14). Streptococcus mutans and Lactobacillus species, which produce acid from bacteria that colonize the oral cavity, are associated with dental caries and cause various oral health problems (15).

The present study aims to evaluate the relationship of dietary intake, as measured by healthy eating index 2015, and oral health indicators in school-age children and the presence of lactobacilli in the oral flora.

2.METHODS

A cross-sectional study was carried out with the participation of children between the ages of 9-14 who applied to Pediatric Dentistry Department of Marmara University Faculty of Dentistry, Turkey. Exclusion criteria were; the children who had any mental or physical conditions, chronic diseases, and those who were taking any antibiotics 2 weeks before the examination. The families of the children were informed about the content and duration of the study in written and verbal by the researcher. A voluntary consent form was signed by the families who accepted to participate in the study. The approval of the research was obtained from Marmara University Faculty of Dentistry Clinical Research Ethics Committee. (The date, 20.12.2016; the number, 2016-66). The questionnaire created to be used in the study consists of two parts: demographic and general information about parents and children, and information on oral and dental health. In addition, food consumption records were taken with the 24-hour recall method to determine the nutritional status of the children. The answers given to the questionnaire and the 24-h recalls were recorded in line with the answers given by the children and their families.

2.1.Sample size estimation

The sample of the study was chosen by random sampling method, and the sample size was aimed to include 88 children with a minimum of 5% sampling error, 0,5 confidence interval, 95% confidence level, and 10% margin of error. The study was finished with the participation of 44 boys and 54 girls, a total of 98 children. Saliva samples were taken from 91 children due to reasons such as not being able to provide enough saliva or/and not want to give saliva.

2.2.Dental examination

Oral and dental examinations of the children were performed by a qualified dentist using a sterile dental mirror at the clinic. Decayed, missing, and filled teeth in permanent teeth were processed into pre-prepared forms according to WHO criteria (16). Soft tissue evaluation and dental anomalies were recorded. The total number of decayed, missing, and filled teeth (Decayed, Missing, And Filled Teeth: DMFT) and tooth surfaces (Decayed, Missing, And Filled Surfaces: DMFS) were calculated, and the mean number of DMFT or DMFS were calculated. The level of caries experience in permanent dentition was determined by the WHO severity criteria for the age groups of children (12 years), levels of DMFT; low < 2.6, moderate 2.7–4.4, high 4.5–6.5, very high >6.5 (17).

2.3.Anthropometric measurements

Anthropometric measurements of the participants were taken by a nutrition and dietetics specialist. The height measurements of the participants were taken with a portable height meter with the feet side by side and the head in the Frankfurt plane and body weights were taken by removing the thick clothes and shoes with a portable body scale. BMI of the children were evaluated according to age and gender by using WHO Anthro Plus Program according to the WHO 2007 reference for ages 5-19 (18).

2.4.Evaluation of food consumption

In order to identify the nutritional status of children, 24-hour food records were taken based on the child's family and child's responses. And evaluated by using the Healthy Eating Index 2015. HEI-2015 consist of thirteen components, while increasing levels of intake receive increasingly higher scores for components adequacy (Total Fruits, Whole Fruits, Total Vegetables, Greens and Beans, vegetables or beans and peas, Whole Grains, Dairy, Total Protein Foods, Seafood and Plant Proteins, Fatty Acids), increasing levels of intake receive decreasingly lower scores for components moderation (Refined Grains, Sodium, Added Sugars, Saturated Fats) (8). The scores of the 13 components were summed up to obtain the final score of the HEI-2015 for each child, which ranged from 0 to 100. For final scores; HEI-2015 <50 "poor diet", 50 <HEI-2015 <80 "diet needs improvement", HEI-2015 >80 signify "good diet" (19).

2.5.Collection of saliva samples and microbiological analysis

Unstimulated mixed saliva was collected from participants in the study two hours after breakfast. The children were asked to spit on the saliva accumulated in the mouth into a wide-mouthed sterile disposable box. Each of the samples was recorded with the same number as the questionnaire and delivered to the laboratory as soon as possible and taken for microbiological analysis.

For the lactobacilli analysis from saliva, the study of Ravindran et al. was followed (20). Loopful saliva was taken and streaked

on a plate with Rogosa SL agar. Samples were incubated at 37°C for 48 hours under an anaerobic environment. After the incubation period, transparent white colonies growing in petri dishes were obtained as suspicious Lactobacillus spp. And gram stained. A pure single colony was obtained by passaging the suspected samples and gram (+) lactobacilli identified after the biochemical testing with a negative oxidase reaction to Catalase/Oxy Test (Liofilchem).

2.6.Statistical Analysis

Data were transferred to the electronic environment, tabulated, and analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). In independent groups, the difference and significance between averages were examined according to whether they met the parametric conditions. Since the difference between the means in two independent groups did not conform to the normal distribution, the Mann-Whitney U test was used to analyze the data, while the Kruskal-Wallis test was used for more than two independent groups. The difference between groups in categorical variables was controlled by the chi-square test. Pearson's correlation test was used to examine the correlations between variables. Significance was assessed at 0.05 levels.

3.RESULTS

The age of the children in the study ranged from 9 to 14 years with a mean of 10.8±1.5. The study sample compromised 44.9% males and 55.1% females. Mean weight, height and body mass index of children are 38.3±10.9, 141.9±11.44 and 18.6±3.08 respectively. When the BMI and height measurements of the children were classified according to gender and WHO references, it was seen that 75% of the children had normal height and 58.2% of them had normal BMI (p>0.05) (Table 1).

Table 1. *Distribution of children's height and BMI measurements by WHO growth references*

		Boys (n:44)		Girls (n:54)		Total (n:98)	
		n	%	n	%	n	%
Height for age	Short	6	13.6	7	12.9	13	13.3
	Normal	32	72.8	42	77.8	74	75.5
	Tall	6	13.6	5	9.3	11	11.2
BMI for age	Underweight	4	9.1	3	5.6	7	7.1
	Healthy weight	28	63.6	29	53.7	57	58.2
	Overweight	9	20.5	16	29.6	25	25.5
	Obese	3	6.8	6	11.1	9	9.2

BMI: Body Mass Index

The number of decayed, missing, filled teeth and tooth surfaces and the mean, minimum and maximum values of DMFT and DMFS indexes of the children were given in Table 2. The mean of decayed, missing, and filled teeth number was 3.07±2.35; 0.10±0.48; 2.75±2.17, respectively. The mean number of DMFT and DMFS indexes were 5.88±2.31; 8.05±4.58, respectively (Table 2).

Table 2. *The values of decayed, missing, filled teeth and tooth surface and DMFT, DMFS indexes*

	Mean (SD)	Min	Max
Decayed teeth	3.07±2.35	0	10
Decayed tooth surface	4.34±3.78	0	17
Missing teeth	0.10±0.48	0	4
Missing tooth surface	0.51±2.43	0	20
Filled teeth	2.75±2.17	0	12
Filled tooth surface	3.11±2.38	0	12
DMFT	5.88±2.31	1	12
DMFS	8.05±4.58	1	29

DMFT: decayed, missing and filled teeth; DMFS: decayed, missing and filled tooth surfaces

While 63.2% of the children brush their teeth regularly, 24.5% did not brush their teeth regularly. 60.7% of those who brush their teeth brush once a day, 39.3% of them brush twice a day. There was no significant difference between the presence and number of decayed, missing, filled teeth and tooth brushing presence or brushing frequency (p>0.05) (Table 3).

Table 3. *Comparison of the oral health indicators and HEI-2015 group*

	HEI-2015 Group		
	Poor diet	Diet needs improvement	P value
Decayed teeth	3.10±2.40	2.87±2.09	0.83
Missing teeth	0.12±0.53	0.00±0.00	0.26
Filling teeth	2.89±2.28	2.50±1.54	0.86
DMFT	6.02±2.26	5.18±2.53	0.21
DMFS	8.32±4.64	6.62±4.12	0.07

DMFT: decayed, missing and filled teeth; DMFS: decayed, missing and filled surfaces, HEI-2015: Healthy Eating Index 2015 (Mann Whitney U test)

The mean HEI-2015 score of the group was 40.39±9.66 and, it was observed that 83.7% had a "poor diet" and the diet of 16.3% was "needs improvement". When the oral health indicators are evaluated according to HEI-2015 the mean number of decayed, missing, filled teeth and DMFT, DMFS scores were found to be higher in children with poor diets but the difference was not statistically significant (p>0.05) (Table 4).

In Table 5, the relationship between the mean score of thirteen HEI components and DMFT index groups was evaluated, and no significant difference was observed between the groups (Table 5).

The presence of lactobacilli in saliva samples of 5.5% of the children was observed (Not shown in Table). The relationship between the presence of lactobacilli in saliva samples and oral health indicators given in Table 6. It was observed that children have lactobacilli in their saliva samples had more decayed teeth (p<0.05).

Table 4. *Comparison of the HEI-2015 components among the DMFT group*

HEI-2015 components	DMFT group Mean (SD)				
	Low	Moderate	High	Very high	P value
Total Fruits	2.68±2,49	2.30±2,05	1,81±2,13	1,82±2,12	0.69
Whole Fruits	3.00±2.73	1,80±2,40	1,79±2,53	1.84±2.31	0.76
Total Vegetables	1.64±2.1	1,49±1.57	1.30±1.66	0.99±1.06	0.77
Greens and Beans	1.34±1.53	1.80±2.10	1.55±2.13	1.71±2.04	0.92
Whole Grains	2.00±4.47	2.08±4.14	2.36±4.01	2.04±3.82	0.88
Dairy	6.36±3.61	5.58±3.56	5.49±3.92	6.00±.3.57	0.94
Total Protein Foods	4.10±0.84	3.18±1.83	3.09±1.90	3.06±1.87	0.74
Seafood and Plant Proteins	4.06±0.59	2.92±1.37	3.64±1.40	3.14±2.02	0.09
Fatty Acids	1.54±2.37	1.63±2.51	1.51±2.58	1.66±2.90	0.98
Refined Grains	0.24±0.53	0.42±1.46	0.83±2.23	0.67±1.75	0.77
Sodium	4.00±3.82	2.50±3.31	3.70±3.44	4.18±3.51	0.21
Added Sugars	10.00±0.00	9.72±0.93	9.82±0.58	9.68±1.27	0.65
Saturated Fats	4.76±2.24	2.88±2.60	3.87±3.72	3.76±3.46	0.59
Total score	45.72±10.03	38.37±9.42	40.81±9.63	40.61±9.86	0.51

DMFT: decayed, missing and filled teeth, HEI-2015: Healthy Eating Index 2015 (Kruskal Wallis test)

Table 5. *The relationship between the presence of lactobacilli in saliva samples and oral health indicators*

	Yes (n:5)	No (n:86)	P value
Decayed teeth	5,8±3.34	2.81±2.17	**0,042**
Missing teeth	0.00±0.00	0.11±0.51	0,544
Filling teeth	1.60±1.51	2.81±2.24	0,204
DMFT	7.40±2.60	5.70±2.32	0,146
DMFS	11.80±10.05	7.56±3.88	0,415

DMFT: decayed, missing and filled teeth; DMFS: decayed, missing and filled surfaces (Mann Whitney U test)

4. DISCUSSION

Good and favorable oral health is precisely important in childhood and oral health diseases such as dental caries have a considerable impact on self-esteem, school performance, eating ability, and a child's quality of life from early childhood to later period (5, 21, 22). According to WHO it's important to reduce the prevalence of caries, which is known to be an infectious and transmissible multifactorial disease accepted to be "triggered" by improper dietary habits (23). Among the many etiological factors, diet has been determined as a specific type of risk factor that draws attention to the development of caries in children (24). This study examined the relationship between diet quality according to Healthy Eating Index 2015 and oral health and oral flora in school-age children.

Within the scope of the research children between the ages of 9-14, which is the age group of the children during their primary education, were included. Anthropometric measurements used in the evaluation of growth and development in children and interpretation of height for age and BMI values for age are important in terms of disease risk assessment. In the TBSA 2010 study, when the height for age status of the children between the ages of 9-14 was examined, it was seen that 57.5%-61.1% had normal height, while this rate was found to be 75% in our study (25). When the BMI for age (for the 9-14 age group) is examined in the TBSA data for normal weight, it is seen that the range of 55.1%-56.8% is similar to our study (58.2%). The present study population showed similar anthropometric measurements compared with the population of Turkey for the same age groups.

Dental caries, which is still a crucial health problem in developed countries, affects 60-90% of school-age children (26). While it is less common in African countries around the world, it is more common in Asian and Latin American countries (1). According to the research profile of oral and dental health of 12 and 15 years age of children in Turkey, caries prevalence was respectively 61.1% and 61.2%; the prevalence of filling was 6.5% and 12.4%, respectively (27). In previous studies conducted with similar age groups, the prevalence of caries was found between 71.2% and 82.6%, parallel to the prevalence in our study (85.6%) (28, 29). While the mean DMFT index of 5.88 ± 2.31 in this study was similar to the averages obtained from various studies (27,30-32), it was observed that it was higher than the averages obtained from other studies (28, 29, 33, 34).

Regular tooth brushing habits and frequency of tooth brushing are known as two important factors in preventing caries formation. In this study, 24.5% of the children did not brush their teeth regularly and there was no significant difference between the number of decayed, missing, filled teeth and brushing frequency ($p>0.05$) similar to the findings in the previous study (24). The main reason for this situation may be the lack of information about the appropriate brushing method and/or the biased over reporting of the frequency of brushing by the parents (24).

Among different foods, dietary sugars, juices, and starchy foods have been suggested to have high cariogenic potential. Apart from those foods, there are foods such as milk, dairy products, and whole grain foods that show anticariogenic effects (35). Undoubtedly cariogenic and anticariogenic foods have an impact on dental caries, however the effect of nutritional status receives insufficient attention. Adoption of healthy eating habits may have positive effects on oral health. HEI-2015, used as one of the most up-to-date tools for dietary assessment comprises all of the major food groups found in MyPyramid.

The impact and quality of dietary intake on oral health status have been investigated infrequently. In this study, Healthy Eating Index-2015, a valid and reliable screening tool, was used to evaluate diet quality. To our knowledge, HEI-2015 utilization has not been reported to identify the relationship

between nutritional status and dental caries, DMFT index in older children. In our study, the mean number of decayed, missing, filled teeth and DMFT, DMFS scores were found to be higher in children with poor diets (p>0.05). Studies evaluating the relationship between Healthy Nutrition Index and childhood dental caries are limited in the literature; however, they have focused especially on early childhood caries in the younger age group (24, 35-37). Unlike our study, previous studies revealed that children with higher HEI scores were less likely to have risk of early childhood caries (25, 36, 37). A study which comprise National Health and Nutrition Examination Survey population over 20 years old evaluating the HEI-2015 and coronal caries found a relationship between HEI quintiles and DMFT index, although the relationship was not consistent among all racial or ethnic groups (38). Apart from the total diet, food items should also be taken into account for their potential based on different food components for the DMFT index. Similar to the study conducted by Zaki et al., most of the HEI components and between the DMFT index groups a significant relationship was not found in our study (35). These findings show that in the older childhood period no statistically significant difference related to dental caries or DMFT index and diet quality according to HEI-2015. One of the reasons for this condition might be related to the age group of the study population. There are individuals with mixed dentition in the age range examined in this study. The expressive power of DMFT in the mixed dentition is limited. Besides, 24 h recall method depended on the declaration and ability to remember all day consumption for parents and their children. The main reason for this situation might be shown as the children participating in the study could not meet the HEI-2015 “adequacy components” following the recommendations on an individual basis or they remained low in the “moderation components” such as added sugar.

There is a dynamic relationship between plaque formation and oral microflora called “The ecological plaque hypothesis”. Dental plaque-induced oral diseases such as dental caries are not species specific but are caused by changes in the environment that lead to an ecological shift favouring the growth of dental caries (39). From this point of view lactic acid bacteria can produce acid and survive in an acidic environment, caries formation has been associated with high lactobacillus counts in the oral flora (40, 41). In present study, It was observed that children have lactobacilli in their saliva samples had more decayed teeth (p<0,05). This significant difference confirms that in addition to S.mutans, Lactobacillus bacteria also cause caries formation. In parallel with the study performed by Bhayat et al., The DMFT index of children with lactobacilli in saliva samples is higher, but the difference is not statistically significant (41). The presence of caries-causing bacteria depends on oral hygiene practice, snacking habit as well as saliva flow and high bacterial infection gives a sign of poorer oral hygiene status .

The 24-hour recall food intake data gathered from participants confined to only one day and 98 children who applied to Marmara University Faculty of Dentistry, Pedodontics Clinic in a certain time interval were proven to be among the study’s primary limitations.

5.CONCLUSION

Childhood is one of the most important periods of life for children who becoming individuals. A good quality diet and good oral health are important factors contributing to health and quality of life. Further studies are needed on eating indices, which provide an accurate and reliable measurement of the cariogenicity of the daily diet consumed by individuals. Additionally, oral health education should be integrated into the nutrition education included in the school education of children.

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Clinical and Experimental Health Sciences

Achillea goniocephala Extract Loaded into Nanochitosan: In Vitro Cytotoxic and Antioxidant Activity

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ABSTRACT

Introduction: The present study aimed to prepare *Achillea goniocephala* chloroform extract and evaluate antioxidant and cytotoxic effects. Then, the nanoparticles (NPs) were synthesized on the most efficient extracts and the biological activities of the free forms of the extracts were compared with the NPs forms.

Methods: Antioxidant capacities of 14 extracts (A-N) prepared using column chromatography were determined by FRAP, DPPH and CUPRAC methods. Again, the cytotoxic activities of all fractions were evaluated on MCF-7 and HT-29 cell lines using the XTT cell viability assay. Chitosan-tripolyphosphate (TPP) NPs of the extracts with the most active biological properties were formed using the ionic gelation method.

Results: The particle size of the NPs synthesized from the two most efficient extracts was found to be between 274.12 and 296.25 nm. The extract-encapsulation and loading-efficiency of the most active NPs were found 77.6±0.04% and 7.76±0.01% for the F extract and 10.2±0.02 and 1.39±0.07% for the H extract, respectively.

Conclusions: While antioxidant activity results of chitosan NPs were close to unencapsulated extracts, cytotoxic activity results were found to be better than unencapsulated extracts. Based on these results, it is thought that it would be more effective to use the encapsulated forms of *A. goniocephala* instead of the raw extract of the plant in the treatment.

Keywords: Nanoparticles, Cytotoxicity, Antioxidant, Chitosan, Encapsulation efficiency

1. INTRODUCTION

Cancer is known as the formation of undesired tissues by uncontrolled division and proliferation of cells in the organism. There are many factors in the epidemiology of the disease, such as lifestyle and unhealthy diet, stress, radiation, and hereditary factors (1). Recently, natural compounds of plant origin, called phytochemicals, have attracted the attention of researchers in the field of pharmacy and medicine worldwide due to their highly important bioactive properties. Nowadays, studies on phytochemicals are performed in the treatment of various diseases (2). *Achillea* species have proven to have analgesic and anti-inflammatory, erythrocyte, and leukocyte preservative, antispasmodic (3) antioxidant and antimicrobial (4) anthelmintic, antiallergic, cholagogue, antibacterial (5) antiulcer, hepatoprotective and anxiolytic (6) effects. In addition, sesquiterpene lactones, alkaloids (senecio), esters (pyrethrins); Saponosides and coumarins have also been identified in this family. Due to the compounds, they contain, most of the *Achillea* plants are used in pharmacy, food industry and other industrial areas due to their latex (7). *A.goniocephala* Boiss. & Balansa (*A. goniocephala*) is an endemic plant and when looking at the literature, there are only two studies on this species and these studies are also related to essential oil analysis. In a study, the essential oil of *A. goniocephala* was analyzed by GC/MS. Camphor and 1,8-cineole were found to be the main ingredients (8). In another study, the essential oil of this plant was analysed and high amount endo-borneol, eucalyptol and camphor were found (9).

With some new nanotechnologies such as encapsulation, preservation of active substances, reduction of activity loss and increasing bioavailability have been proven in recent years (10). The difficulties in converting the phytochemicals contained in plants into drugs are known due to reasons such as low solubility, stability, and bioavailability. Thanks to the nanostructures formed because of adding plant extracts to the nanoparticles (NPs), it has been revealed that the stability and especially the biological activities of the phytochemicals

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in the plant are increased (11). Therefore, the application of nanotechnology to plant extracts can be considered a good strategy for herbal medicines, as it provides many features such as improving pharmacological activity and especially protection from toxicity. Chitosan, which is the most used and non-toxic polymer among the coating materials used in encapsulation, shows a good encapsulation feature. Chitosan is biodegradable, biocompatible, antibacterial, and non-toxic, and besides these properties, it is an interesting material with its abundance and cheapness. For this reason, it is used in many areas such as medicine, food, pharmacy, cosmetics, textile, agriculture, and the removal of environmental pollutants from wastewater. Due to its unique properties, chitosan is widely used in drug delivery systems (12).

In this study, antioxidant, and cytotoxic activities of 14 fractions obtained using column chromatography from *A. goniocephala* chloroform extract, which was found to have a stronger effect than methanol extract in MCF-7 cell line, were evaluated. Then, NPs were prepared with two most active fractions by combining with chitosan. Thus, the biological activities of the pure forms of the extract/fractions with the biological activities of the NP forms were compared. No study has been found in the literature on the antioxidant and cytotoxic activity of the *A.goniocephala*. In addition, plant extract loaded NP synthesis and characterization study was carried out by us for the first time.

2. METHODS

2.1. Materials

The specimens of *A. goniocephala* were collected from field studies and determined by Prof. Dr Turan Arabacı. A voucher specimen (T.Arabacı 2957) was deposited at the herbarium of the Faculty of Pharmacy, Inönü University, Department of Pharmaceutical Botany for future reference. MCF-7 human breast cancer cells, and HT-29 human colorectal adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC). Dulbecco's modified Eagle's medium (DMEM) (ATCC, USA), phosphate buffer saline (PBS), and fetal bovine serum (FBS) were purchased from PAA Ltd. (France). Chitosan (400 kDa, DD 87) were obtained from Fluka. L-glutamine-penicillin-streptomycin solution were purchased from Sigma-Aldrich. XTT reagent (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was purchased from Roche Diagnostic.

2.2. Extraction procedures

The aerial parts of the plant were dried in the shade (25°C) and ground to a fine powder in a mechanic grinder (Renas, RBT1250). The powdered samples were extracted with organic solvents (methanol, and chloroform) using the maceration method until colourless. The organic phase was evaporated to dryness under decreased pressure. All extracts were maintained at 4°C for future analysis. The cytotoxic activities of chloroform (IC_{50} = 0.019 mg/mL) and methanol (IC_{50} = 20.391 mg/mL) extracts were assessed on the MCF-7 cell line, and it was observed that the chloroform extract was more potent against MCF-7 than methanol extract. In this study, column chromatography was performed to separate the possible effective compounds contained in the effective chloroform extract according to its polarity. The chloroform sub-extract (7 g) was inserted to a silica gel column and eluted with toluene, petroleum ether and methanol mixture of increasing polarity of petroleum ether (100%, 1500 mL), petroleum ether /chloroform (75:25, 1500 mL), petroleum ether /chloroform (1:1, 1500 mL), chloroform (100%, 1500 mL), chloroform/methanol (75:25, 1500 mL), chloroform/methanol (1:1, 1500 mL), chloroform/methanol (25:75, 1500 mL), and methanol (100%, 1000 mL) to obtain fourteen fractions.

2.3. Antioxidant assays

Plant samples were tested with Ferric Reducing Antioxidant Power (FRAP) assay, where an increase in plant extracts' absorbance shows increase in the reducing power of the extracts (13). The FRAP results were expressed as mM Fe^{2+}/mg extract. 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging capacity of the extracts was evaluated the experimental protocol found in the literature (14). Results are expressed as IC_{50} values (mg/mL). In Cupric Reducing Antioxidant Capacity (CUPRAC) assay, 60 µL each of copper (II) solution, neocuproin solution and ammonium acetate buffer (1 M) were mixed. 10 µL ethanol and 60 µL of extract were added and shake the solution. The solutions were kept in room conditions with their mouth closed for 60 minutes. At the end of this period, absorbance values at 450 nm were measured against the reference solution that does not contain a sample (15). CUPRAC values were given as mM TroloxE/mg extract.

2.4. Cell culture conditions

Cell culture studies were performed using modified method of (16). Cytotoxicity of the extracts was measured by the XTT assay, using HT-29 and MCF – 7 cells. Cells were cultured in medium glucose DMEM with 10% FBS, 1% L-glutamine, 100 IU/mL penicillin and 10 mg/mL streptomycin in 25 cm^2 polystyrene flasks. The cells were maintained at 37°C within 5% CO_2 humidified atmosphere and were passaged when they had reached 80-90% confluence. Cells were seeded at $10x10^3$ cells each well in 96-well plates containing DMEM (100 µL) containing 10% FBS and incubated overnight. The 14 extracts were dissolved in 20 µL dimethyl sulfoxide (DMSO) and extracts with concentration 500 µg/mL were suspended with DMEM medium and extracts at the concentration of 50 µg/mL per well were put in the 96-well plates. In addition, the equal amount of DMSO were inserted in the positive control. Incubation of the cells was continued for 24 h. Then, the medium was removed, and wells were washed with PBS. To determine cell viability, 100 µL of transparent (colourless) DMEM and 50 µL of XTT labelling solution were added to wells and the cells were incubated for 4 h. Microplate (ELISA)

reader was used to measure the absorbance of XTT-formazan at 450 nm against the control. Cell viability of extracts were calculated compared to control (100% of viability). After the XTT results were evaluated, NPs of the two extracts showing the highest cytotoxicity in MCF-7 and HT-29 cells were prepared. NPs containing different amounts plant extract were applied to the cells separately, and the differences between their cytotoxic effects were evaluated by calculating the IC_{50} values. To calculate the IC_{50} value, samples and NPs containing 12.5, 25, 50, and 100 µg amount of plant extracts were treated with the cells.

2.5. Preparation of chitosan NPs containing chloroform extract

NPs were prepared using ionic gelation method as stated (17). Briefly, medium molecular weight chitosan solutions at a concentration (0.25% w/v) were prepared using glacial acetic acid (0.05% v/v) as a solvent. The chitosan dissolution process was performed via a magnetic stirrer. TPP solution (0.25% w/v) including extract dropped into chitosan solution under predetermined stirring condition. After the stirring period, NPs were centrifugated at 10.000xg for 30 minutes. The supernatant was removed, and particles were washed with double distilled water. This process was repeated threefold. NPs were maintained at +4 ºC after lyophilisation.

2.6. Characterization of NPs

Zeta potential and particle size: Zeta sizer were used to measure of zeta potential and size of NPs. The samples were suspended in PBS (pH 7.4) and measured. All measurements were performed in triplicate.

Scanning electron microscope (SEM): Sample of NP was inserted in metal grids with double-sided adhesive tape, coated with a gold layer under 0.1 torr at room temperature. The morphological properties of NPs were evaluated using SEM (Carl Zeis-Evo 40, Germany) (18).

2.7. Measurement of EE and LC

Ultraviolet visible spectrophotometer was used to measure the encapsulation efficiency (EE %) and loading capacity (LC %) of the extract in NPs. A standard calibration curve of the extract was established at 340 nm. The amount of extract in the supernatant was calculated from the line equation obtained from this curve. The following equations were used to determine the encapsulation efficiency and loading capacity of the particles.

$$EE\ (\%) = \frac{m_o - m_s}{m_o} x100$$

$$LC\ (\%) = \frac{m_o - m_s}{w_{np}} x100$$

where, mo is the initial mass of natural extracts and ms mass of natural extracts in the supernatant and wnp = total weight of the naturally obtained extract of NPs (19). All measurements were performed in triplicate and were reported as mean ± SD (n = 3).

Total phenolic contents in the extracts and NPs were determined by Folin-Ciocalteu colorimetric method. The percentage of the encapsulated extract into chitosan NP was also determined by using a total phenolic assay according to the following equation (20).

$$EE\ (\%) = \frac{\text{Actual phenolic of the extract entrapped in NPs}}{\text{total phenolic of initial extract}} x100$$

2.8. Statistical analysis

All experiments were performed in triplicate. All data from the study were given as the mean ± SD and analysed using Graphpad Prism 5. Statistical differences between the study groups were analysed using a two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Mean values were considered statistically significant if $p<0.05$.

3. RESULTS

3.1. Antioxidant activity

The DPPH radical scavenging activities of the extracts were determined using DPPH method. The antioxidant activities of the extracts and ascorbic acid used as a standard were evaluated by comparing their IC_{50} values. H extract had stronger DPPH radical scavenging potential than the other extracts. The Copper (II) ion-reducing antioxidant capacity of the extracts was evaluated by the CUPRAC method. When CUPRAC results were compared, it was determined that the J, F G and H extracts exhibited the strongest copper (II) ion-reducing antioxidant capacity. The iron (III) ion reducing power of plants is very important in evaluating their antioxidant potential. The iron reduction power is based on the reduction of the herbal extract Fe^{3+} to Fe^{2+} and measured spectrometrically at 593 nm. In this method, high absorbance indicates high iron reduction potential. In this study, the antioxidant power of iron (III) ion reduction of different extracts obtained from the plant was examined comparatively. It was determined that G extract and F extract had stronger iron (III) ion reducing power than other extracts. All the extracts were found to have lower FRAP values than BHT compound (Table 1).

Table 1. *Antioxidant capacity results of the fractions, references and nanocapsules*

Extracts/ compound	DPPH (IC_{50}: mg/mL)	CUPRAC (mM trolox /mg extract)	FRAP (mM $FeSO_4$ /mg extract)
A	0.611±0.218[a]	0.240±0.045[a]	9.791±0.304[a]
B	0.561±0.077[b]	0.044±0.015[b]	9.750±0.181[b,a]
C	1.032±0.224[c]	0.306±0.008[c]	10.355±0.160[c]
D	1.178±0.280[d]	0.116±0.038[d]	11.585±0.716[d]
E	1.536±0.356[e]	0.212±0.016[e]	10.496±0.070[e,c]
F	**0.895±**0.062[f]	**2.582±**0.158[f]	**11.746±**0.209[f]
G	0.809±0.101[g]	2.006±0.158[g]	**13.540±**0.384[g]
H	**0.264±**0.037[h]	**1.111±**0.038[h]	**10.879±**0.152[h]
I	1.349±0.635[i]	1.050±0.010[i]	10.980±0.244[i,h]
J	1.266±0.278[j]	**3.155±**0.172[j]	11.565±0.218[j]
K	0.890±0.289[k]	0.780±0.047[k]	10.395±0.185[k,c]
L	1.543±0.210[l]	0.702±0.060[l]	10.416±0.060[l]
M	2.012±0.688[m]	0.664±0.095[m]	10.436±0.152[m,l,e]
N	2.499±0.423[n]	0.412±0.017[n]	10.154±0.175[n]
H capsule	1.054±0.163[o]	0.155±0.032[o]	8.780±0.740[o]
F capsule	1.426±0.060[p]	0.759±0.040[p,k]	8.659±0.083[p,o]
Ascorbic acid	0.0028±0.000[4]r		
BHT		4.462±0.074[r]	21.706±0.726[r]

All results were expressed as mean±SD by three independent experiments; Means with different superscripts [(a-r)] are significantly different, p<0.05.

3.2. Characterization of chitosan NPs

Particle size, zeta potential, polydispersity index (PDI) of NPs were evaluated and results shown in Table 2. The size of the NPs obtained between 274.12±2.7 nm and 296.25±3.2 nm. The zeta potential values of NPs were between 2.3±0.04 mV and 3.0±0.03 mV. PDI values were between 0.298±0.03 and 0.342±0.04. These results showed that the samples were homogeneous feature without any aggregate. The morphological properties of the NPs were shown in Figure 1.

Table 2. *Zeta potential, particle size, and PDI values of NPs loading with extract*

Formulations (Chitosan – TPP NPs)	Zeta potential (mV)±SD	Size (nm)±SD	PDI±SD
*NP1	2.3±0.04	274.12±2.7	0.298±0.03
*NP2	2.7±0.02	280.15±3.6	0.313±0.03
*NP3	3.0±0.03	296.25±3.2	0.342±0.04

** NP1, NP2, and NP3 containing chitosan (MW: 400 kD), TPP and A. goniocephala chloroform extract. Nanoparticles: NPs.*

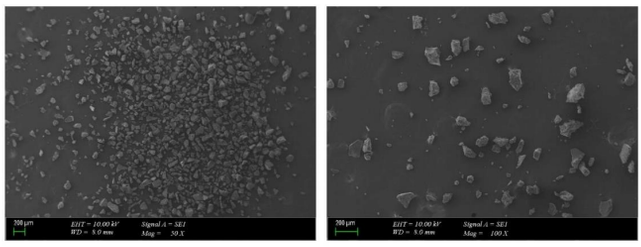

Figure 1. *SEM images of chitosan nanoparticles.*

3.3. Percentage of Encapsulation efficiency from A. goniocephala extracts-chitosan NP

Encapsulation efficiency percentage suggests how much *A. goniocephala* F and H extracts coated in chitosan NP. The percentage of the encapsulated extract into chitosan NP was determined and the results were given in Table 3. As shown in Table 3, the percentage was found 77.6% and 10.2% for F and H fractions, respectively. This showed that as much as 77.6 or 10.2 % of the fractions are successfully encapsulated by chitosan NPs. Also loading capacity (LC) in NPs was found as 7.76 and 1.39 % for F and H fractions, respectively. According to the total phenolic method, the encapsulation efficiency percentages were 64.37% for the F extract and 14.84% for the H extract.

Table 3. *Encapsulation efficiency and loading capacity of the NPs*

Parameter	F extract	H extract
Linear equation	y =0.2808x+0.02339	y =0.4667x-0.002409
Slope±SD	0.2808±0.02	0.4667±0.01
Intercept±SD	0.0234±0.0011	0.002409±0.0007
r	0.9921	0.9925
EE %	77.60±0.04	10.20±0.02
LC %	7.76±0.01	1.39±0.07
TPC*	0.0174±0.0005	0.0566±0.0010
NP	0.0112±0.0003	0.0084±0.0003
EE % (TPC)	64.37±0.06	14.84±0.11

** TPC: Total phenolic contents (mg gallic acid equivalent / mg extract), Nanoparticles: NPs.*

3.4. Cytotoxicity results of extracts, fractions, and NPs

Cancer is the second most common cause of death globally, despite the use of many treatment methods (21). Crucial side effects caused by conventional drugs used for chemotherapy affect patients negatively. Current studies have focused on natural herbal remedies, which are more advantageous than conventional cancer drugs and have anticancer effects (22,23). The cytotoxic activities of chloroform extracts from the plant on MCF-7 and HT-29 cell lines were examined, and results were shown in Figure 2 and 3. Results indicated that F and H extracts possessed more cytotoxic activity on both MCF-7 and HT-29 cell lines compared to other chloroform extracts. On MCF-7 cell line, the cell viability ranged between 51.691±0.566% and 85.019±0.637% (Figure 2). According to the results F and N samples of the extracts of plant showed the highest and lowest cytotoxic effect on MCF-7 cell line, respectively. In addition, H samples showed high cytotoxic activity on MCF-7 cell line (57.502±0.904%).

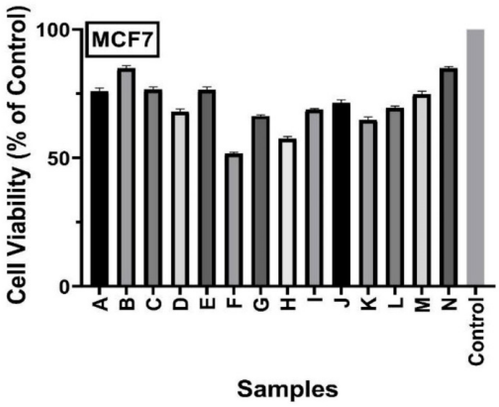

Figure 2. *Cell viability results of chloroform extracts of the plant. Cells were treated with samples at a concentration 50µg/mL. Cell viability of the control group was determined as 100%.*

On HT-29 cell line, the cell viability was between 49.368±0.569 % and 82.642±0.593 % (Figure 3). According to the results F and N samples of the plant extracts showed the highest and lowest cytotoxic effect on HT-29 cell line respectively. In addition, H samples showed high cytotoxic activity on HT-29 cell line (57.502±0.904%). In parallel with the cell viability results of MCF-7 cells, the highest cytotoxic effect was observed in F and H samples in HT-29 cells where all extracts were applied separately (F: 54.504±0.574% and H: 49.368±0.569%). Moreover, N samples showed the least cytotoxic activity (82.642±0.593%).

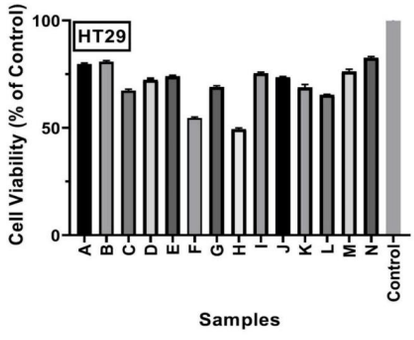

Figure 3. *Cell viability results of chloroform extract of the plant. Cells were treated with samples at a concentration 50µg/mL*

When we evaluated the cytotoxic effects of the extracts on MCF-7 and HT-29 cells, it was observed that F and H chloroform extracts had stronger cytotoxic potential. Based on these results, NPs containing F and H extracts were prepared, and the extracts and NPs containing the extracts were treated with MCF-7 and HT-29 cells at different concentrations. These concentrations were determined to be I:12.5 µg/mL, II: 25 µg/mL, III: 50 µg/mL, and IV: 100 µg/mL. XTT cell viability test was performed in both cell lines at determined concentrations and IC_{50} values were evaluated. When we evaluate the results in Figure 4, the NPs containing the same concentration of extract showed a higher cytotoxic effect in MCF-7 cancer cells compared to the samples containing the extract. In cells treated with different concentrations (I, II, III, and IV) of F extract, the viability of MCF-7 cells was between 76.068±1.704% and 38.586±0.881%. In addition, cells treated with NPs including same concentrations (I, II, III, and IV) of F extract, the viability of MCF-7 cells was between 65.185±0.976% and 32.110±1.747%. In cells treated with different concentrations of H extract, the viability of MCF-7 cells was between 79.997±0.658% and 40.918±0.294%. Moreover, cells treated with NPs including same concentrations of H extract, the viability of MCF-7 cells was between 68.548±1.216% and 35.726±0.655%. According to the results of the XTT cell viability assay, it can be said that depending on the concentration of the extracts applied, it has a higher cytotoxic effect and anticancer effect in MCF-7 cells. IC_{50} values of F extract and NP loading with F extract were calculated as 56.780 µg and 40.860 µg, respectively, on MCF-7 cells. In addition, IC_{50} values of H extract and NP containing H extract were calculated as 61.630 µg and 44.524 µg, consecutively, on MCF-7 cells. FI NP (32.110±1.747%) and HI NP (35.726±0.655%) NP samples indicated the highest cytotoxic effect on MCF-7 cell line. NPs including extracts had lower IC_{50} values than extracts on MCF7 – cell line (Figure 4).

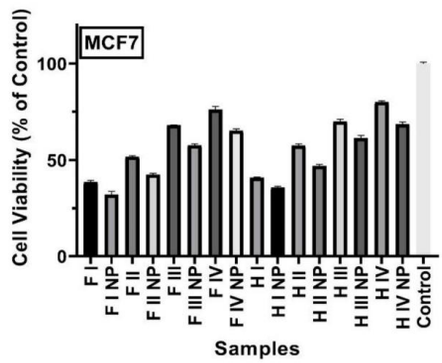

Figure 4. *Cell viability results of F and H chloroform extract and NPs. MCF-7 cells were treated with samples at determined concentration (I:12.5 µg/mL, II: 25 µg/mL, III: 50 µg/mL, and IV: 100 µg/mL). Cell viability of the control group was determined as 100%.*

According to the results of Figure 5, the NPs including the plant extract had a greater cytotoxic activity in HT-29 cancer cells compared to the samples containing the extract. In cells treated with different concentrations (I, II, III, and IV) of F extract, the cell viability of HT-29 cells was between 80.408±0.825% and 43.596±0.882%. In addition, cells treated with NPs containing same concentrations (I, II, III, and IV) of F extract, the cell viability of HT-29 cells was between 66.521±0.976% and 35.440±1.221%. In cells treated with predetermined concentrations of H extract, the cell viability of HT-29 cells was between 77.237±1.155% and 42.580±0.761%. Moreover, cells treated with NPs including same concentrations of H extract, the viability of HT-29 cells was between 69.552±0.891% and 31.560±0.642%. According to the results, IC_{50} values of F extract and NP containing F extract were calculated as 64.460 µg and 45.482 µg, respectively, on HT-29 cells. Moreover, IC_{50}

values of H extract and NP loading with H extract were calculated as 51.280 µg and 40.860 µg, consecutively, on HT-29 cells. In addition, FI NP (35.440±1.222%) and HI NP (31.560±0.642%) NP samples showed the highest cytotoxic effect on HT-29 cell line (Figure 5).

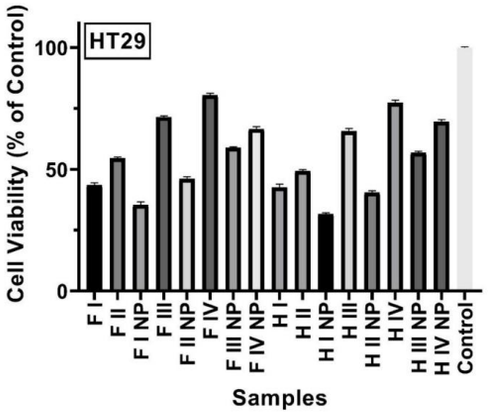

Figure 5. *Cell viability results of F and H chloroform extract and nanoparticles. HT-29 cells were treated with samples at determined concentration (I:12.5 µg/mL, II: 25 µg/mL, III: 50 µg/mL, and IV: 100 µg/mL). Cell viability of the control group was determined as 100%*

4. DISCUSSION

Results showed that chitosan NPs including extracts had lower IC_{50} values than only extracts. In a study, it was proved that *C. casia* extract, which is widely used for antihypercholesterol in Indonesia, can be encapsulated with chitosan NPs and this NP has a cholesterol-lowering effect (19). In another study, chitosan NPs of anticancer effective elagic acid were created. It has been observed that the encapsulated elagic acid gives a lower IC_{50} value for oral cancer treatment compared to pure elagic acid (24). In a study, gelatin-based NPs of anticancer-effective, tannic acid, curcumin, theaflavin and epigallocatechin gallate polyphenols were prepared, and among these molecules, it was found that both free and nanocapsulated forms of epigallocatechin gallate were effective in breast cancer (25). In a study, the efficiency and release of the encapsulation potential of catechins was investigated and for this purpose, it was found that more stable results were obtained in in vitro and in vivo studies with chitosan NPs that they created by adding reducing substances to catechins (26). In a study, PGA-based NP was produced with the double emulsion technique of curcumin. They proved that the drug release rate of the NP was shorter without hydrolytic degradation in the presence of curcumin (27). In another study, NPs loaded with antioxidant hydroxytyrosol, and hydrocortisone were produced. As a result of the study, it was reported that this dual NP structure has an antioxidant and anti-inflammatory effect in the treatment of percutaneous atopic dermatitis (28). In a review study, studies in which nanoencapsulated polyphenols were produced to eliminate the negativities such as weak bioavailability in the therapeutic applications of polyphenols were compiled. As a result of this review, they stated that polyphenols are more effective than their therapeutically pure forms, such as determining their release in nanoencapsulated form, extending the shelf life of active ingredients (29). There is another review in the literature based on the investigation of biopharmaceutical properties of extracts obtained from plants according to lipid and polymer-based NPs. As a result of this review, it was concluded that due to the controlled kinetic release of NPs produced using plant extracts, the effect of increasing the effect against microorganisms such as antioxidants and parasites is increased (30). They found that the IC_{50} value of green tea decreased after the nanoencapsulation created with chitosan, thus increasing the antioxidant effect (31). In another study, they found that a very small dose of *P. dactylifera* loaded chitosan NPs prevented the toxicity of doxorubicin by the development of antioxidant enzymes (32). Biosynthesized AgNPs were found to show a higher antioxidant and antibacterial activity compared to *Prosopis farcta* fruit extract alone (33). Similarly, in our study, chitosan NPs containing the most active extracts were prepared and characterization studies were carried out. In cell culture studies, the anti-proliferative and anticancer effects of the extracts embedded in chitosan, a carrier system, were evaluated. IC_{50} values calculated according to XTT cell viability results explain that NPs containing F and H chloroform extracts of *A. goniocephala* have desirable high anticancer activity.

Encapsulation efficiency is a measure of the ability of the carrier system used to embed the active substance. The encapsulation efficiency of the active substances in the formulations is crucial in terms of bioactivity and pharmacokinetic efficiency. Therefore, for the prepared formulations to show the desired effectiveness in practice, the encapsulation efficiency should be above a certain value. In our study, while the encapsulation efficiency of the F extract was 77.6%, this value was 10.2% in the H extract. When the encapsulation efficiency results were examined, it was observed that especially the F extract was successfully encapsulated by ionic gelation method.

5. CONCLUSION

According to the results of the XTT cytotoxicity and all the antioxidant assays study, among 14 extracts, F and H chloroform extracts of *A. goniocephala* showed better antioxidant and cytotoxic activity. While antioxidant activity results of chitosan NPs were close to unencapsulated extracts, cytotoxic activity results were found to be better than unencapsulated extracts. Based on these findings, it is anticipated that using the encapsulated forms of *A. goniocephala* rather than the raw extract of the plant in the treatment would be more successful.

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Clinical and Experimental Health Sciences

Caregiver Burden in Caregivers of Acute Stroke Patients: From a Biopsychosocial Perspective in a Turkey Sample

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ABSTRACT

Objective: This study aimed to evaluate caregiver burden among caregivers of acute stroke patients with a biopsychosocial perspective in a Turkey sample.

Methods: 72 stroke patients and 72 their caregivers were included the study. The mean age of the stroke patients included in the study was 65±12.39. The mean age of caregivers was 44.5±14 and 66.7% of them were females. Modified Motor Assessment Scale (MMAS), Standardized Mini Mental State Examination (SMMSE) and The Barthel Index (BI) were used to assess the patients with stroke. The caregivers were evaluated by using the Bakas Caregiving Outcomes Scale, Family Sense of Coherence Scale-Short Form (FSOC-S), Hospital Anxiety and Depression Scale (HADS), WHOQOL-Bref-Short Form and Multidimensional Scale of Perceived Social Support (MSPSS).

Results: There were significant positive correlation between the BCOS score and the SMMSE (r=0.36; p=0.002) and BI (r=0.22; p=0.05) scores. A significant positive correlation was found between the BCOS score and MSPSS's family (r=0.31; p=0.007), friend (r=0.41; p<0.01) and special human (r=0.46; p<0.01) sub-parameters. In addition, there were significant positive correlations between BCOS score and the physical (r=0.35; p=0.02) and environmental (r=0.42; p<0.01) sub-dimensions of the WHOQOL-BREF, also HADS Depression sub-score (r=0.93; p=0.01). Correlations between BCOS score and patients' age, MMAS, FSOC-S, scores and HAD Anxiety sub-score were not statistically significant (p>0.05).

Conclusion: The cognitive function and independence level of the patients is associated with care burden. Furthermore, psychosocial features such as poor social functioning, quality of life and emotional health of caregiver have adverse effects on caregiver burden.

Keywords: Acute stroke, Biopsychosocial model, Caregiver burden, Quality of life

1. INTRODUCTION

Stroke is globally the second most common cause of death and a major cause of disability (1). Post-stroke rehabilitation and care have become the most important building blocks in terms of survival and independence of stroke survivors (2). In most cases of stroke, patients generally receive care from their relatives in hospital and at home. Due to the severe disorders and disabilities after stroke, not only the patients but also their caregivers have to struggle with challenging conditions in this process (3).

Caregivers generally deal with a range of care needs and demands such as mobility, self-care, cognitive and mood changes (4). This process causes caregiver burden which is known as the experience of physical, psychological, emotional or social problems due to caring responsibility for ill person (5). Caregiving to a person with disability restricts the work and leisure activities, and negatively affects the family relationship and quality of life of the caregivers. This situation creates a physical and psychological burden on individuals (6). Caregivers also have to cope with chronic stress, especially if there are serious problems about physical / psychological conditions and financial resources (7).

According to current interdisciplinary rehabilitation care of stroke patients suggestions', well-being of caregiver is as important as well-being of the stroke patient in the disease period (8). Also providing support and intervention to caregivers has been emphasized (9). To develop appropriate and effective interventions to meet the specific needs of caregivers, the caregiver burden should be assessed with a broad perspective (10).

Many studies have examined potential factors associated with caregiving burden after stroke, such as the caregivers'

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quality of life, income level, and family integrity. Also, it is known that the patients' age, sex and independence level affects caregiver burden (11-16). However, caregiving burden is related to the combination of multiple and simultaneous factors and the number of studies evaluating these factors as multidimensional (including all bio-psycho-social factors) are limited (17). Moreover, although caregiving burden is known as a chronic period problem, it is also highly prevalent in acute stroke and there are very few studies about caregiving burden in an acute phase of stroke (18). Furthermore, the studies that mention both patient and caregiver features and evaluating biopsychosocial aspects of caregiving in acute period are insufficient in Turkey.

The biopsychosocial approach systematically considers biological (age, gender, acute illness, disability etc.), psychological (mood, personality, behavior, etc.), and social factors (cultural, familial, socioeconomic, medical, etc.) and their complex interactions in understanding health, illness, and health care delivery (19). In health care delivery of stroke patients, caregivers play important role. As caregiving burden can be caused by many factors, assessing caregiver burden with the frame of biopsychosocial model may guide in understanding the caregiving burden of stroke patients and creating strategies on problem-solving interventions of caregivers (10). In this case, while biological factors could belong to both patients' disease characteristics and caregiver demographic characteristics, psychosocial factors could be related to caregivers' features.

Therefore, the aim of our study was to evaluate caregiver burden among caregivers of acute stroke patients with biopsychosocial perspective in a Turkey sample.

2. METHODS

2.1. Recruitment and inclusion of participants

Our descriptive cross-sectional study included individuals with stroke who were being treated at the Neurology Clinic of a Training and Research Hospital and their caregivers between December 2015-July 2017. The study diagram with the individuals included in the study and the evaluation methods are shown in Figure 1.

G Power was used to calculate the sample size of the present study. The sample size was based on the estimates obtained by using Barthel Index scores as a criterion. In order to determine the sample size 0.40 points of effect size, 0.05 type I error and 90% power were accepted The minimum sample size was estimated at 54 participants (20).

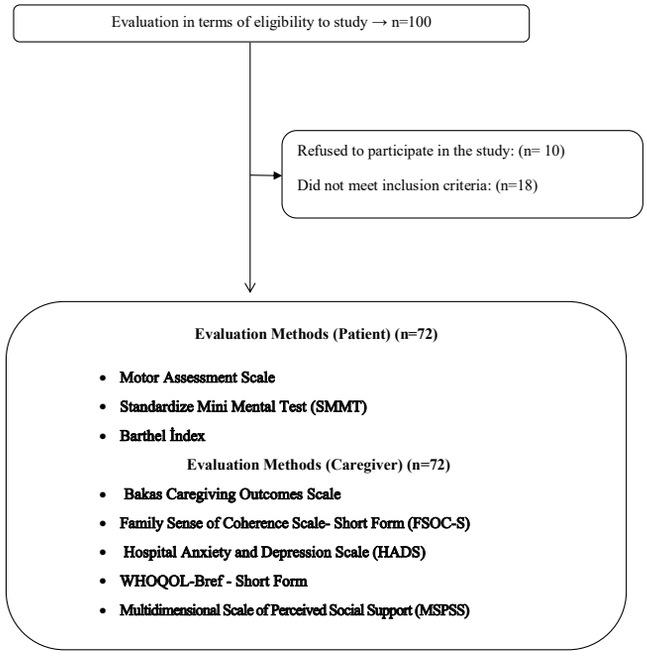
The study was approved by the Clinical Research Ethics Committee. All the subjects were informed about the purpose, duration and evaluations of the study and were included in the study after they approved the Voluntary Informed Consent Form.

Inclusion criteria for patients:

- to have a clinical diagnosis of stroke by computed tomography (CT) or magnetic resonance imaging (MRI),
- to have a Glaskow Score of 9 or higher
- to be 18 years or older,
- Inclusion criteria for caregivers:
- to be 18 years or older,
- to be the main caregiver of the patient

Patients with any other neurological problem and mentally disabled caregivers were excluded from the study.

Figure 1: *Study Diagram*



2.2.Evaluation Methods of Stroke Patients

Demographic and clinical data such as age, gender, affected body part and history of the stroke attack were collected by using the "Case Follow-up Form". "The modified Motor Assessment Scale" was used to evaluate the motor status of the patients. The cognitive levels of the patients were evaluated using "the Standardized Mini Mental Test". Barthel Index was used to evaluate the independence levels of the patients in daily living activities.

2.2.1. The modified Motor Assessment Scale (MMAS)

MMAS is a performance-based scale developed to evaluate daily motor functions in stroke patients. It is a short and practical assessment tool that evaluates eight different motor functions and muscle tone. Each item is scored between 0 and 6. MDS was found to be highly reliable with a mean

correlation of 0.95 and an average test-retest correlation of 0.98 according to a study by Carr JH et al. (21).

2.2.2. Standardised Mini-Mental State Test (SMMSE)

SMMSE is a short screening tool used to quantify the cognitive impairment of the individual and to record the cognitive changes over time. The scale consists of orientation, memory, attention and calculation, recall and language subtitles. The maximum score taken from the scale is 30, and a higher score means better cognitive function (22).

2.2.3.Barthel Index (BI)

The Barthel Index is a short and widely used scale consisting of 10 items, evaluating the level of independence and improvement of the individual's daily living activities. The highest score from the scale is 100, and the highest score is the indicator of best independence level (23).

2.3.Evaluation Methods of Caregivers

Information about caregivers such as age, gender, previous patient care experience was collected by the "Caregiver Follow-up Form". The Bakas Caregiving Outcomes Scale was used to evaluate caregiving burden. The condition of the family relations of participants was measured by the "Family Sense of Coherence Scale – Short Form (FSOC-S)". The anxiety and depression levels of participants were measured by the Hospital Anxiety and Depression Scale Scale (HADS). WHOQOL-Bref – Short Form was used to evaluate the quality of life and the Multidimensional Scale of Perceived Social Support (MSPSS) for social support.

2.3.1. Bakas Caregiving Outcomes Scale (BCOS)

BCOS is a self-report scale that evaluates the care-giving process developed by Bakas et al. The items of the scale are rated on a 7-point scale ranging from –3 (Changed for the worst) to +3 (Changed for the best). The ratings are performed between 1 and 7. The maximum score from the scale is 105 and high scores from the scale indicate better caregiver outcomes (24).

2.3.2 Family Sense of Coherence Scale – Short Form (FSOC-S)

FSOC-S is a 7-point likert scale that was developed by Antonovsky and Sourani. The scale is composed of 12 items and scored from 1 to 7. High scores from the scale indicate high family integrity (25).

2.3.3. Hospital Anxiety and Depression Scale (HADS)

The HAD scale was developed by Zigmond and Snaith to evaluate the level of anxiety and depression. 7 out of 14 questions evaluate anxiety and 7 evaluate depression. Each item is scored between 0 and 3. High scores from the scale indicate high anxiety and depression levels (26).

2.3.4. WHOQOL-BREF

WHOQOL-BREF is a scale developed by the World Health Organization consisting of 4 subscales (Physical Health, Psychological, Social Relations, Environmental). High scores from the scale indicate high quality of life (27). In our study, physical and environmental subscales of the scale were used.

2.3.5. Multidimensional Scale of Perceived Social Support (MSPSS)

The MSPSS is a short, easy-to-use scale that assesses the level of support from family, friends and a special person subjectively. Each subscale consists of 4 questions (3 sub-scales total= 12 questions). High scores from the scale indicate high level of social support (28).

2.4. Data Analysis

All statistical analysis were accomplished by the Statistical Package for the Social Sciences (SPSS) 21.0 (SPSS Inc, Chicago,IL, USA). Descriptive statistics included nominal variables, which were expressed as percentages, and continuous variables, which were expressed as mean and standard deviation. The Kolmogorov-Smirnov test was used to determine for normal distribution of data before the statistical analysis. The distribution of data was found abnormal. Kruskal-Wallis test was used to compare the BCOS score according to demographic and clinical baseline variables. Correlation levels between variables were computed through Spearman's correlation analysis. The strength of correlations was interpreted as: 0.00-0.19 very weak, 0.20-0.39 weak, 0.40-0.59 moderate, 0.60-0.79 strong, 0.80-1.0 very strong (29).

3. RESULTS

A total of 72 stroke patients and their caregivers (n=72) were included the present study. 34.7% of the caregivers participating in the study were found to be in the 45-54 age group, 66.7% were females, 52.8% had a moderate income and 56.9% of caregivers were children of stroke patients. 52.8% of the caregivers participating in the study were living with the patient, 51.4% had not given care to a patient before, 41.7% had been given care for 1-5 years, 61.1% had received help from their family members in the care, and 51.4 % were found to have difficulty in positioning or moving patients (Table 1). The mean score of caregivers was 59.33±15.95, the mean score of the Social Support Scale was 44.12±11.96, the mean score of the WHOOQL-Bref scale physical sub parameter was 23.75±4.08, and the mean score of the environmental parameter was 25.4 ± 5.05, and HAD Scale Anxiety score was 12.00±5.82. , the HAD Scale Depression score was found to be 10.86±5.17.

Table 1. *The demographic and clinical features of caregivers (n=72)*

		Mean ± SD	n	%
Age		44.5±14.8		
Sex	Female		48	66.7
	Male		24	33.3
BCOS Score		59.33 ± 1.95		
MSPSS	Family Support Dimension	15.87± 5.22		
	Friend Support Dimension	14.18± 4.88		
	Special Human Support Dimension	14.06± 5.7		
	Total	44.12 ± 11.96		
WHOQOL-Bref	Physical	23.75 ± 4.08		
	Environmental	25.4 ± 5.05		
HADS Score	Anxiety	12.00 ± 5.82		
	Depression	10.86 ± 5.17		
Income level	Poor		31	43.1
	Moderate		38	52.8
	Good		3	4.2
Relationship with the patient	Partner		18	25.0
	Children		41	56.9
	Relative/ Other		13	18.1
Living together with patient	Yes		38	52.8
	No		34	47.2
Previous Caregiving Experience	Yes		35	48.6
	No		37	51.4
Previous Caregiving Time	Below a year		30	41.7
	1-5 years		30	41.7
	6-11 years and above		12	16.7
Help Status during caregiving	No		25	34.7
	Family/ Relative		47	65.3
Type of received help during caregiving	No		14	19.4
	Information Support		6	8.3
	Care Support		12	16.7
	Financial support		37	51.4
Difficulties during caregiving	No		2	2.8
	Nutrition / Dressing		13	18
	Move / Position Change		37	51.3
	Communication / Other		20	27.8

Mean±SD: Mean±Standart Deviation; n:number; %:percentage BCOS: Bakas Caregiving Outcomes Scale, MSPSS: Multidimensional Scale of Perceived Social Support; WHOQOL-Bref: World Health Organization Quality of Life Bref; HADS: Hospital Anxiety and Depression Scale Anxiety and Depression

The mean age of the stroke patients included in the study was 65±12.39, the mean score of the Mini-Mental State was 15.9±10, the Barthel Index average score was 41.45±38.2 and the mean score of the Motor Rating Scale was 17.95±13.52 (Table 2). According to the BCOS, caregiver burden mean score of caregivers with poor income levels was significantly lower than the other groups (p<0.01). The mean score of the care burden of the caregivers who received information and care support was significantly higher than the other groups (p<0.01) (Table 3).

Table 2. *The demographic and clinical characteristics of stroke patients (n = 72)*

	Mean± SD
Age (years)	65±12.39
Time since stroke (days)	11.4±6.2
SMMT	15.9±10.1
BI	41.45±38.2
MAS	17.95±13.52

Mean±SD: Mean±Standart Deviation; WHOQOL-Bref: World Health Organization Quality of Life Bref; SMMT:Standardized Mini Mental Test; BI:Barthel Index; MMAS: Modified Motor Assessment Scale MAS: Motor Assessment Scale.

Table 3. *Distribution of BCOS Scores according to demographic and clinical characteristics of caregivers of stroke patients*

		Median (min-max)	p
Age Group	Below 18 age	46.5 (45-48)	0.05
	18-24 age	60.0 (45-80)	
	25-34 age	67.5 (51-80)	
	35-44 age	65.0 (48-99)	
	45-54 age	50.0 (32-92)	
	Above 55 age	54.5 (32-91)	
Sex	Female	55.5 (32-92)	0.08
	Male	64.5 (36-99)	
Income Level	Poor	50.0 (32-75)	**0.003**
	Moderate	65.0 (35-99)	
	Good	60.0 (55-92)	
Relationship with the patient	Partner	54.5 (32-92)	0.34
	Children	59.0 (35-99)	
	Relative/ Other	50 (32-92)	
Living together with the patient	Yes	55.0 (32-99)	0.68
	No	58.0 (35-92)	
Previous Caregiving Experience	Yes	57 (35-99)	0.60
	No	58 (32-92)	
Previous Caregiving Time	Below a year	58.5 (35-99)	0.09
	1-5 years	53.5 (33-80)	
	6-11 years and above	85 (82-88)	
Help Status during caregiving	No	65.0 (32-92)	0.22
	Family/ Relative	55.0 (32-99)	
Type of received help during caregiving	No	67.0 (32-92)	**<0.001**
	Information Support	66.0 (45-99)	
	Care Support	76.5 (55-92)	
	Financial support	50 (32-76)	
Difficulty during caregiving	No	63.0 (46-80)	0.10
	Nutrition / Dressing	62.0 (32-78)	
	Move / Position Change	51.0 (32-99)	
	Communication / Other	68.0 (44-92)	

Mean±SD: Mean±Standart Deviation; WHOQOL Bref: World Health Organization Quality of life Bref ,Min max: Minimum-maksimum; p<0,05 significance.

A statistically significant positive correlation was found between the BCOS scores and family (r=0.31; p=0.007), friend (r=0.41; p<0.01) and special human (r=0.46; p<0.01) sub-parameters of the MSPSS Social Support Scale, and the physical (r=0.35; p=0.02) and environmental (r=0.42; p<0.01) sub-dimensions of the WHOQOL-BREF. In addition, there was a statistically significant relationship between the BCOS score and HADS Depression sub-score (r=0.93; p=0.01) Correlations between BCOS score and patients' age and MMAS were not statistically significant (p>0.05) (Table 4).

Table 4. *The relationship between caregiver burden and caregiver characteristics*

		Age	MSPSS Total	MSPSS Family	MSPSS Friend	MSPSS Special Human	WHOQOL-Bref Physical	WHOQOL-Bref Environ.	FSOC-S Score	HADS Anxiety	HADS Depression
BCOS Score	r	0.26	0.53	0.31	0.41	0.46	0.35	0.42	0.01	0.13	0.93
	p*	0.02	0.001	0.007	<0.001	<0.001	0.002	<0.001	0.91	0.26	0.01

**: Spearman Correlation Test; r: Correlation Coefficient; p<0.05 significance, BCOS: Bakas Caregiving Outcomes Scale; MSPSS: Multidimensional Scale of Perceived Social Support;*
WHOQOL-Bref: World Health Organization Quality of Life Bref; FSOC-S: Family Sense of Coherence Scale-Short Form Physical and Environmental; HADS: Hospital Anxiety and Depression Scale Anxiety and Depression

When the relationship between caregiving burden and patient characteristics is examined, there was a statistically significant positive correlation between BCOS and SMMSE scores (r=0.36; p=0.002). In addition, there was a statistically significant positive correlation between BCOS and the Barthel Scores (r=0.22; p=0.05). Correlations between BCOS score and FSOC-S score and HAD Anxiety sub-score were not statistically significant (p>0.05) (Table 5).

Table 5. *The relationship between caregiving burden and patient characteristics*

		Age	SMMT	BI	MMAS
BCOS Score	r	-0.06	0.36	0.34	0.22
	p*	0.59	0.002	0.003	0.05

**: Spearman Correlation Test; r: Correlation Coefficient; p<0.05 significance; BCOS: Bakas Caregiving Outcomes Scale; SMMT: Standardized Mini Mental Test; BI: Barthel Index; MMAS: Modified Motor Assessment Scale*

4. CONCLUSION

Caregiver burden in caregivers of acute phase stroke patients was investigated in terms of biopsychosocial perspective in the present study due to the lack of knowledge about caregiver burden in Turkey. When all factors that may affect the severity of caregiver burden are evaluated together, caregiver burden is found associated with poor social functioning, quality of life and emotional health of caregiver in an acute phase of stroke. In this case, it can be considered that the burden of care is most affected by psychosocial variables. Also, the patients' cognitive and independence level should be considered about the caregiver burden level.

5. DISCUSSION

In a recent meta-analysis about caregiver burden in patients with stroke, it was found that caregivers under higher burden are likely to experience high anxiety and depression. Moreover, patients with lower activity of daily living and anxiety symptoms also lead to more burden to caregivers. In this meta-analysis, the average time after stroke onset was over 6 months in the included studies (30). To our knowledge, there are not sufficient studies in the literature about caregiver burden in caregivers of acute phase stroke patients (30, 31). However, caregivers have to cope with the changes of stroke survivors in many respects such as mobility, mood or communication from the first day of the disease. It is known from previous studies that these changes contribute with increased perceived burden as well as high rate of depressive symptoms, stress and other mental problems in the later years (32). Also, it is associated with poor response to rehabilitation among stroke (31). Therefore, detecting the risk factors and early intervention to decrease the level of burden among stroke caregivers are important clinical implications in the acute stroke rehabilitation field.

Most of the caregivers were women and the children of stroke patients in our study. The previous studies supported that giving care to the parents was an individual duty and responsibility traditionally in Turkish culture (33). A study from Nigeria had similar results about the caregivers' sex and kinship with the patients (18).

The caregivers demonstrated moderate levels of burden, considering the average of 59.33 in the Bakas Caregiving Outcomes scale (BCOS). BCOS score points were found to be lower in lower income levels compared to caregivers with higher income levels. The decrease in mean scores reflected the increment of the burden of care. Similarly, *Tsai Y. H et al (2018)* (34) showed the relationship between caregivers' financial situation and caregivers burden in their study of acute phase stroke patients. Moreover, *Jeong, Y. G. et a.l (2015)* (14) emphasized that lower income negatively affected quality of life of caregivers and this relation was found to increase caregiver burden secondarily among the patients with chronic stroke. Hence, when caregiver burden is examined according to the received support type, we observed that caregiver burden increases in caregivers

who receive financial support. It was also found that the caregivers who need only information and care support from their families or relatives had decreased caregiver burden when compared to the persons who received only financial support. *Yu et al. (2013)* (35) stated that the support of caregivers are generally received from family members as well.

Considering the social component of the biopsychosocial model, our results showed that there was significant correlation between perceived family, friend or special human social support dimensions scores and caregiver burden level. As the social support increased, it was seen that caregiver burden decreased in the present study. These findings were similar with a study which was conducted by Akosile C.O et al. (2018) conducted a study using Cara Giver Strain Index and found similar results with our study (18).

As a point of the psychological component, *Efi, P. et al (2017)* (36) reported the strong correlation between caregiver burden and anxiety, depression and quality of life. In our study there were significant correlations between BCOS caregiver burden and the depression component of HAD scale, WHOQOL-Brief physical and environmental sub scores. It means that the anxiety and depression scores from HAD and the worse quality of life leads to more caregiver burden.

In our study we found a strong relationship between BCOS caregiver burden level and the stroke patients' cognitive status and independency level during activities daily living (ADL). It was seen that as the cognitive level and independence level in ADL increased, the burden of care decreased among caregivers of acute phase stroke patients. The literature shows similar findings. *Caro, C et al (2017)* (37) stated the significant correlation between independence level, cognitive status of stroke patients and caregiver burden.

Lastly, it has been shown that increased caregiver burden in acute stroke is associated with social functioning, quality of life, caregiver's emotional health, patients' cognitive and independence level in accordance with the literature providing information on care burden in chronic strokes (38). Caregiver burden should be considered via a biopsychosocial model and it contributes to more systematic information about health status of stroke survivors and caregivers. More importantly, it is effective in improving the provision of evidence-based recommendations for the design of solution strategies to improve health status.

The study has some strengths. Although there are studies in the literature in which biopsychosocial evaluations and treatments are applied using different assessment parameters (4, 39), to our knowledge, this is the first study which researches caregiver burden in terms of a biopsychosocial perspective among both stroke patients and their caregivers' in a Turkish population. This study has also a guiding feature as it has reflected the neurology service needs beside the clinic interventions to improve the stroke patients' care.

The study has certain limitations. Firstly, long-term findings about caregiver burden and related factors were not collected. Secondly, as this is an observational and cross-sectional study, especially anxiety, depression level of caregivers could not be distinguished from the other reasons except caregiver burden. Lastly, as our results did not show normally distributed data, we could not do any regression analysis to decide which one of the factor has more effects on caregiver burden.

In conclusion, the findings of this study pointed out the significant association between burden and poor social functioning, quality of life and emotional health of caregiver in acute phase of stroke. The burden of care seemed most affected by psychosocial variables; therefore, thinking about the future status of patients, if necessary, caregivers should take support and training about care giving. Therefore, providing education to the caregivers in hospitals about the disease and caregiving could be beneficial. In addition, caregivers should be trained for physically support according to independency level of patient.

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All authors confirm that the patient/person(s) have read this manuscript and given their permission for it to be published.

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Clinical and Experimental Health Sciences

Activation of NLRP1 and NLRP3 Inflammasomes in Multiple Sclerosis and Clinically Isolated Syndrome

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ABSTRACT

Objective: Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative disease characterized with demyelination and axonal damage in central nervous system (CNS). Inflamasomes, which are important part of this inflammatory process, regulate maturation of proinflammatory cytokines. Infamazom complexes are thought to increase in MS attacks. We investigated role of inflammasome complexes (nod-like receptor protein 1 and 3) in serum and cerebrospinal fluid (CSF) levels for MS development.

Methods: Eighteen clinically isolated syndrome (CIS), 19 relapsing remitting multiple sclerosis (RRMS) and 20 healthy control cases were included in the study. Nod-like receptor protein 1 and 3 (NLRP1, NLRP3), inflammasome complex levels and oligoclonal band (OCB) patterns of all the groups were measured in serum and CSF samples using Enzyme-Linked Immuno Sorbent Assay (ELISA) method.

Results: Although NLRP1 and NLRP3 levels in both RRMS and CIS patients measured in serum and CSF were significantly higher than healthy control group, there was no statistically significant difference between RRMS and CIS patients. On the other hand, the levels of NLRP1 and NLRP3 in CSF were significantly higher in OCB pattern positive patients compared to the OCB pattern negative patients.

Conclusion: In this pilot study, it is shown that NLRP1 and NLRP3 inflammasome complexes increased in CSF samples of MS cases and that this tendency occurred during or maybe before the first MS attack. As a result, it was thought that these complexes may have an effect on the formation of the OCB band.

Keywords: Multiple sclerosis, Inflammasome complex, Clinically isolated syndrome

1. INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative disease characterized with demyelination and axonal damage in central nervous system (CNS) (1) Multiple sclerosis promotes especially among young women (women/men:2/1) and it was reported that it affects more than 2 million people in the world. Genetic factors, infectious and other enviromental factors play role in pathogenesis of multiple sclerosis (2). Multiple sclerosis causes neurologic dysfunction and neurodegeneration due to myelin loss and inflamations. Magnetic resonanse, spinal fluid analysis and evoked potantial recordings can be used to support diagnosing multiple sclerosis but they are not spesific for MS(3, 4).

Multiple sclerosis usually begins as a relapsing, episodic disorder. This, relapsing remitting multiple sclerosis (RRMS), evolves into a chronic neurodegenerative disease characterized by progressive neurologic disability(5). CIS occurs in the first episode of neurologic symptoms that lasts at least 24 hours. CIS may or may not go on developing MS. Central nerveus system MRI findings are one or more subclinical white matter lesions(4).

Inflammasomes are multi-oligomeric subunits whose primary duty is the activation of caspase-1 and they regulate maturation of proinflammatory cytokines such as interleukin 1β (IL-1β) and interleukin 18 (IL-18) (6). Inflammasome complex is frequently composed of a pattern recognition receptor (member of nod-like receptor (NLR) family), an adaptor protein and active form of caspase-1. Upon ligand binding, NLR undergoes oligomerization and is bound to adaptor protein with protein region interactions. Thus, it transforms pro-caspase-1 to active caspase-1 biologically. Finally, active caspase-1 breaks down the initial forms of

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IL-1β, IL-18 and IL-33 cytokines and transforms them into their mature forms (6, 7).

Inflammasome complexes are named and function according to the structure of pattern recognition receptor (PRR) they contain. Although over 20 NLR under four different categories were shown, there are still four different inflammasome complexes identified (8).

Inflammasome complexes, which are known to be activated in the early stages of inflammation, are thought to increase in MS attacks (9). This study aims to determine the predictive value of serum and cerebrospinal fluid (CSF) levels of nod – like receptor protein 1 and 3 (NLRP1, NLRP3) molecules in MS development.

2. METHODS

2.1. Study Group

The research included 18 CIS, 19 RRMS and 20 healthy control cases followed between November 2016 and July 2017 in University of Health Sciences Istanbul Haydarpasa Numune Research and Training Hospital. The patients were chosen according to McDonald criteria revised in 2015.

The study was approved by the University of Health Sciences Istanbul Haydarpasa Numune Research and Training Hospital Ethics Committee (dated on 28.11.2016 and with approval KAEK2016 /KK/110)and the participants were enrolled after having signed the written informed consent. Serum and CSF samples of 18 CIS, 19 RRMS and 20 healthy control group patients were used.

Age of onset, number of attacks, number of lesions detected on cranial MRI during serum and CSF samples taking, oligoclonal band positivity (OCB) and expanded disability status scale (EDSS) scores were recorded for all patients.

Healthy CSFs were taken from patients with a complaint of headache, without feature in cranial MR, complete blood count and extensive biochemistry studies, with normal neurological and systemic examinations and normal CSF cells, glucose, and protein values and whose headaches were treated with non-steroidal anti-inflammatory therapy without relapse.

2.2. Serum And CSF Measurement

Serum and CSF samples were taken from all the cases between 8.00-10.00 a.m. while CSF sample was taken, all the cases were in remission and none of them were on corticosteroid treatment. Samples of CIS cases were collected short after their first attacks, when they were in remission period two weeks after completion of steroid treatment. The samples were aliquoted and kept in – 80C freezer until tested. In serum and CSF samples NLRP1 and NLRP3 levels were measured. These measurements were conducted with ELISA method in accordance with the instructions of the producing company (Abcam, Cambridge, England) and for each sample optical density (OD) values were obtained. OD values were converted into concentration values as pg/ml utilizing the standard curve formed with standard protein solutions.

2.3. Statistics

Demographic and clinical characteristics of the cases included in the study were compared with chi-square, Student t-test, ANOVA and Mann-Whitney U test. NLRP1 and NLRP3 levels measured in serum and CSF were compared with ANOVA or t test. Correlation studies were carried out with Pearson and Spearman methods for parametric and non-parametric values, respectively. ($p<0.05$ was considered significant.)

4. RESULTS

4.1. Clinical And Demographic Characteristics

There was no significant difference between the age and gender of the CIS, MS and healthy controls. As expected, disease duration, number of attacks, number of cranial lesions and EDSS scores of MS cases were higher than the CIS cases. There no significant difference between the OCB pattern positivity of CIS and MS groups (Table 1).

Table 1. *Clinical and demographic characteristics of the clinically isolated syndrome (CIS) patients, multiple sclerosis (MS) patients and healthy controls (HS)*

	CIS (n=18)	MS (n=19)	HC (n=20)	p value
Gender: Female/Male	10/8	12/7	11/9	0.850*
Mean age±SD	26.4 ± 7.9	29.9 ± 7.1	28.6 ± 6.3	0.512**
Mean disease duration±SD	-	5.9 ± 1.3	-	-
Number of attacks	1.0 ± 0.0	6.4 ± 3.1	-	Non-applicable
Lesion number on MRI	1.8 ± 1.0	7.1 ± 1.9	-	<0.001***
Oligoclonal band (+) patient number pattern 2 or 3)	10	13	-	0.640*
EDSS score	1.7 ± 0.7	3.1 ± 1.0	-	<0.001†

EDSS, expanded disability status scale, SD, standard deviation. Numerical values are defined in mean ±standard deviation., chi-square test; **, ANOVA; ***, Student t-test; †, Mann-Whitney U test*

4.2. Serum And CSF Measurements

As a result of the measurements performed with ELISA method for CIS, MS and healthy control cases, there was no statistically significant difference between the serum NLRP1 (p=0.369) and NLRP3 (p=0.241) values with Student t test. In the measurements done in CSF samples with ANOVA method, NLRP1 (p=0.031) and NLRP3 (p=0.027) values of the CIS and MS cases were significantly higher than the values of the healthy control cases.

There was a significant difference between the healthy control and patient groups in the paired comparisons carried out with Tukey's post hoc test ($p<0.05$ for all parameters).

There was no statistically significant difference between the CIS and MS groups for all parameters (Figure 1a and 1b).

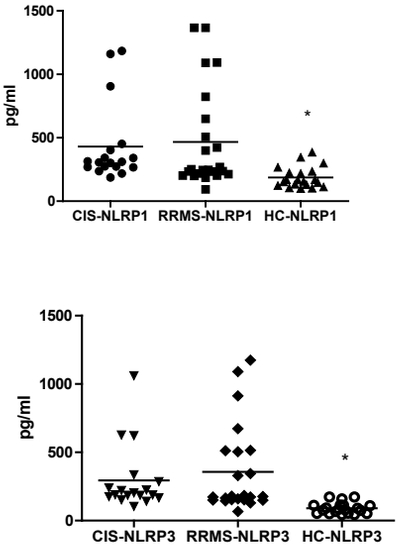

Figure 1a. *Serum NLRP1 – NLRP 3 levels of clinically isolated syndrome (CIS) patients, relapsing remitting multiple sclerosis (RRMS) patients and healthy control cases. Horizontal lines indicate mean values.*

4.3. Correlation Studies

In order to reveal the possible correlations between the NLRP1 and NLRP3 levels measured in serum and CSF samples and age, disease duration, number of attack, number of lesions on MRI and EDSS scores of the CIS and RRMS cases, Pearson (parameters except for EDSS) and Spearman (only for EDSS) tests were applied.

As a result of this assessment, there was no statistically significant difference between serum and cerebrospinal fluid NLRP1 and NLRP3 levels measured in both CIS and RRMS cases, and clinical and demographic characteristics of the patients. ($p>0.05$ for all comparisons).

4.4. Comparison Of OCB Pattern Positive and Negative Cases

In order to investigate the effects of inflamazome complexes on oligoclonal band positivity, statistical analysis was performed with student t test. There was no significant difference between the serum NLRP1 ($p=0.169$) and NLRP3 ($p=0.308$) values of the OCB positive and OCB negative MS cases (Figure 2a) while NLRP1 ($p=0.043$) and NLRP3 ($p=0.017$) values were found significantly high in OCB positive cases in the measurements carried out in CSF samples of RRMS cases (Figure 2b).

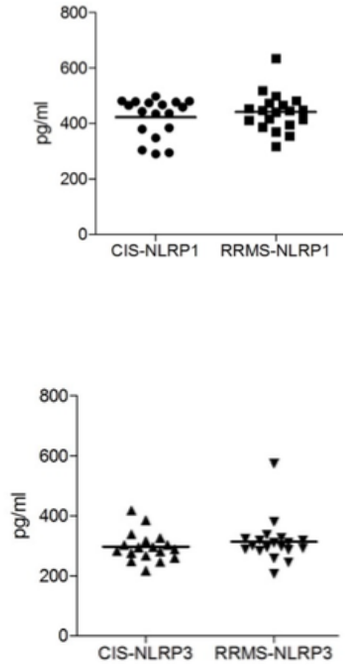

Figure 1b. *Cerebrospinal fluid NLRP1 – NLRP 3 levels of clinically isolated syndrome (CIS) patients and relapsing remitting multiple sclerosis (RRMS) patients. Horizontal lines indicate mean values.*

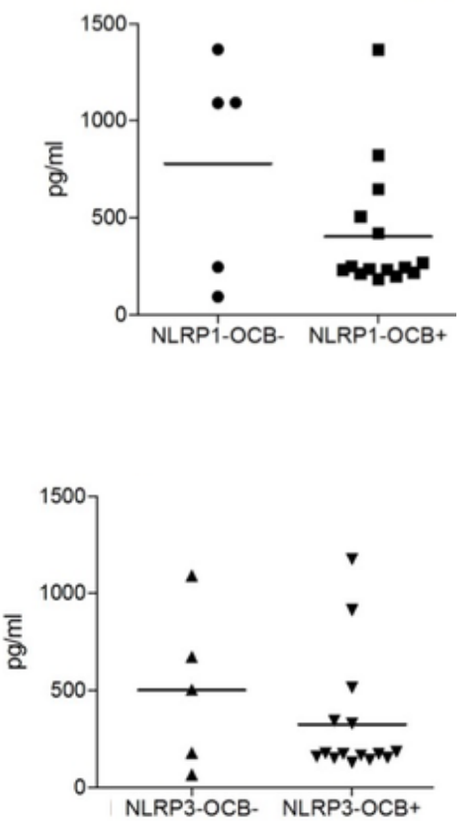

Figure 2a. *Serum NLRP1 – NLRP3 levels of oligoclonal band (OCB) positive (pattern 2 or 3) and OCB negative relapsing remitting multiple sclerosis (MS) patients. Horizontal lines indicate mean values. *p>0.05 by Student's t-test*

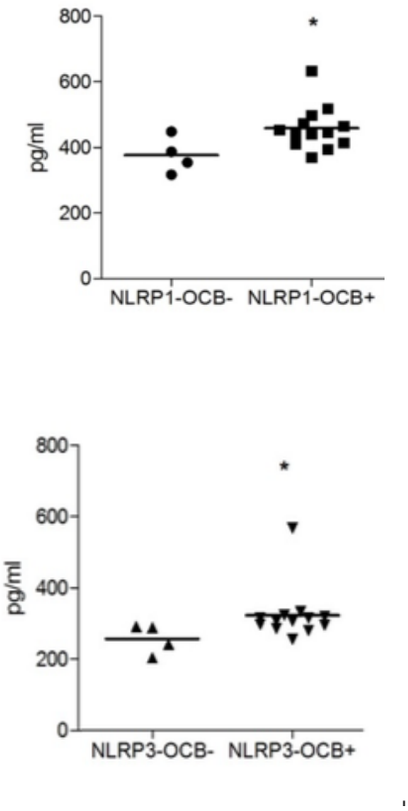

Figure 2b. *Cerebrospinal fluid NLRP1 – NLRP3 levels of oligoclonal band (OCB) positive (pattern 2 or 3) and OCB negative relapsing remitting multiple sclerosis (MS) patients. Horizontal lines indicate mean values. *p<0.05 by Student's t-test*

5. DISCUSSION

Inflammasomes increasingly gain interest in multiple sclerosis and this is shown in experimental autoimmune encephalomyelitis animal model. In experimental autoimmune encephalomyelitis animal model, there was a decrease in disease severity and Th1 ve Th17 cells in peripheral lymphoid tissues and spinal cord (10) of the mice having NLRP3-deficiency. Similarly, in a cuprizone-induced demyelination model, NLRP3 gene expression was significantly upregulated and the mice having NLRP3-deficiency showed delayed demyelination and oligodendrocyte loss (9). It is shown that NLRP1 and NLRP3 inflammasome complexes have a role in MS physiopathology and that they were highly expressed in MS lesions (11-14).

In this study examining the serum and CSF levels of inflammasome complexes it is observed that there was no significant difference between the CIS, RRMS and healthy control cases in terms of serum NLRP1 and NLRP3 levels. This finding suggests that the mentioned serum levels of inflammasome complexes are not important as biomarker. NLRP1 and NLRP3 levels in CSF samples were significantly higher in CIS and MS cases (patient group) compared to the healthy controls. However, there was no significant difference between the CSF levels of the CIS and MS cases. These results support the opinions that intrathecal levels of the inflammasome complexes (NLRP1-3) increase in MS cases. The fact that there was no difference between the CIS and MS cases indicates that the increase in NLRP1 and NLRP3 inflammasome complexes started to occur before the first clinical attack. For this reason, it is not possible to use the inflammasome complexes examined in this study as a biomarker to foresee the CIS-MS transformation.

In our study, no correlation was found between serum and cerebrospinal fluid infamazom complex levels and clinical parameters such as disease duration disability level and number of attacks. This finding suggests that the increasing of the NLRP1 and NLRP3 inflammasome complex levels did not have a significant effect on the clinical course of MS.

An important result of the study is that NLRP1 and NLRP3 levels were higher in the CSF samples of the OCB pattern positive RRMS cases than the OCB pattern negative cases. This finding suggests that inflammasome complexes facilitate the formation of oligoclonal band with an unknown mechanism. It may be related that OCB development depends on the antibodies produced by B lymphocytes accumulated in intrathecal compartment (15). It seems possible that the mentioned inflammasome complexes change the blood-brain barrier (BBB) permeability and facilitate B lymphocyte transmission and thereby increase the accumulation of immunoglobulin G (IgG) molecules forming OCB in CSF.

NLRP1 and NLRP3 basic inflammasomes are pathway factors and it is known that the activation of these two factors lead to the release of various proinflammatory cytokines. In MS cases, NLRP1 and NLRP3 production increases and with immunomodulatory treatment, expression levels of these two molecules decrease (16, 17). It is also indicated that NLRP1 and NLRP3 also release the cytokines that will increase the survival of B lymphocytes in CSF and brain parenchyma (16). Therefore, it is an expected finding that NLRP1 and NLRP3 levels increased in OCB pattern positive patients.

Although OCB pattern positivity is a finding frequently encountered in MS, it is not diagnosed in some MS cases. There are few studies conducted on physiopathological differences of the OCB pattern positive and negative patients. Association of OCB with increased lesion burden and disability is well known (18). The increase of CSF NLRP1 and NLRP3 levels in OCB pattern positive patients is possibly associated with the fact that myeloid cells could easily pass the BBB in these patients. The results suggest that BBB dysfunction developing as a result of this might be one of the underlying factors of OCB pattern positivity.

This study has some limitations; firstly, the number of patients for our research was not sufficient, secondly, we have limited budget for the test materials.

Consequently, in this pilot study, it is shown that NLRP1 and NLRP3 inflammasome complexes increased in CSF samples of MS cases and this tendency occurred in the first MS attack or maybe even before the attack. Some evidence was presented regarding that the mentioned inflammasome complexes may

have a role in OCB development. In case the number of the serum and CSF samples examined is increased in the future studies, whether inflammasome complexes have an effect on CIS-MS transformation will be more clearly understood.

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Clinical and Experimental Health Sciences

Evaluation of The Ethmoid Bone Using by Cone Beam Computed Tomography In Turkish Subpopulation

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ABSTRACT

Objective: The objective of this retrospective study was to evaluated anatomical structures of ethmoid bone on cone beam computed tomography (CBCT) images in a Turkish subpopulation.

Methods: The CBCT images of 200 patients (116 female and 84 male), between the age of 18 and 50 years, who met the study criteria were selected randomly. The medial ethmoid roof height (MERH), cribriform plate height (CP), the height of the lateral lamella of the cribriform plate (LLCP) were measured. All the data were grouped by age, side, and gender.

Results: For MERH; the mean heights for the left and the right side were 25.55 ± 3.00 mm and 25.24 ± 3.01 mm, respectively. There was no significant difference between right MERH and genders ($p>0.05$). The mean LLCP heights on the right and left sides were 4.98±2.12 mm and 4.49 ±1.64 mm, respectively. It was found that LLCP height did not have a statistically significant correlation with gender ($p>0.05$). The average of right and left CPH were calculated 20.55±2.61 mm and 20.82±2.75 mm, respectively. Both the right and left CPH were significantly higher in males ($p<0.05$). Also, there was no correlation between the height of the anatomical structures of the evaluated ethmoid bone and the increase or decrease in age ($p>0.05$).

Conclusion: Morphological differences in the anatomy of ethmoid bone were shown in this study. The anatomical structures showing changes due to factors such as side, age, gender, and race should be examined in three dimensions before the operation.

Keywords: Anatomy, cone beam computed tomography, cribriform plate, ethmoid roof

1. INTRODUCTION

The anatomical form of the paranasal sinuses should be carefully examined to prevent complications before “Functional Endoscopic Sinus Surgery” (FESS), which is used in the treatment of sinonasal pathologies (1). In surgical interventions, a fracture of the cribriform lamina may result in cerebrospinal fluid (CSF) flow, meningitis, and dura mater, and bone defects that may lead to intracranial hypotension. These risks increase in the presence of length increase or deviation in cribriform lamella (2,3). The ethmoid bone roof is composed of the ethmoidal fovea and the lateral lamella of the cribriform plate (LLCP) medially. The LLCP is the thinnest and most indefensible landmark of the skull, so it is the most generally injured part of the skull base during FESS or paranasal sinuses surgery procedures. Therefore, the penetration risk of the anterior cranial fossa in surgical interventions also increases, and complications such as cerebral destruction, hemorrhage, and CSF discharge may develop (4,5).

Cone beam computed tomography (CBCT) is often preferred in all areas of dentistry, as the three-dimensional image can be obtained with a low radiation dose. CBCT, which can be used in many areas such as diagnosis and treatment planning in complicated cases requiring multidisciplinary study, an examination of paranasal sinuses, imaging of osteomeatal structures, has eliminated some disadvantages of conventional two-dimensional radiographs. The complex anatomical form of the maxillofacial region can be evaluated without the superposition of other anatomical structures with high-resolution CBCTs. The advantages of CBCTs are the three-dimensional examination of structures in axial, sagittal, coronal, and cross-sectional planes (6).

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This study, it was aimed to evaluate morphometrically the anatomical structures of the ethmoid bone on CBCT images in a Turkish subpopulation.

2. METHODS

This retrospective study was approved by the Local Ethics Committee of Bolu Abant İzzet Baysal University, (decision date: 20.01.2020, decision number: 34) and the study protocol was conducted following the principles of the Declaration of Helsinki. This study involved the evaluation of CBCTs (images) that were taken previously for various reasons in the dentomaxillofacial radiology clinic. Patients were selected who were over 18 years, had no sinonasal pathology (tumor or cyst, serious rhinosinusitis), no history of trauma and surgical operation that will disrupt the integrity of the paranasal region. Also, inadequate quality CBCT images that could not be diagnosed were excluded from the study.

The CBCT images of 200 patients (116 female and 84 male), between the ages of 18 and 50 years old (mean age 21.57± 4,793 years) who met the study criteria were selected randomly from the digital database of Bolu Abant İzzet Baysal University Faculty of Dentistry.

2.1. CBCT Imaging

CBCT images were obtained with an I-CAT device (Imaging Sciences International, Hatfield, PA) with 16 × 9, 16 × 10, 16 × 11, 16 × 12, or 16 × 13 cm image area, 120 kVp, 5 mA, 8.9-s acquisition time irradiation parameters. All measurements were calculated in 0.3 mm slice thickness in coronal sections via I-CAT Vision software (Imaging Science International) by a single observer.

The following measurements were assessed (7,8):

1. The medial ethmoid roof height (MERH); measured vertical line drawn from the medial ethmoid roof to the horizontal plane in left and right side (The horizontal plane was represents the horizontal line passing through the infraorbital nerves). (Figure 1A).
2. Cribriform plate height (CPH); a measured vertical line drawn from the cribriform plate to the horizontal plane on the left and right side (Figure 1B).
3. The LLCP height; subtracted the CPH from the MERH.

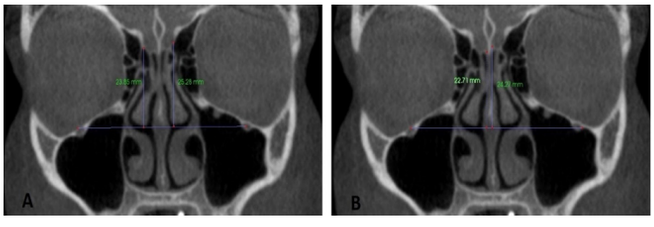

Figure 1. *The measurement of left and right medial ethmoid roof height (A) and left and right cribriform plate height (B) on coronal section. The horizontal line in both images was represents the line passing through the infraorbital nerves.*

All measurements were performed twice by the same observer and recorded in millimeters using a preadsheet (Microsoft Office Excel, Redmond, WA) and made on the same laptop computer (Dell Inc., Round Rock, TX, USA). To test intra-observer agreement, 20% of all measurements were repeated after two weeks. All the data were grouped and compared by age, gender and side.

2.2. Statistical Analysis

SPSS 23.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses, and data are given as mean ± standard deviation. The compatibility of the parameters to normal distribution was calculated by the Kolmogorov-Smirnov test. Wilcoxon sign rank test was used to compare right-left measurements in paranormally distributed data. Differences between genders were evaluated by the Mann-Whitney U test and the correlations between measurements were calculated with the Spearman rank test. The significance level was considered at $p < 0.05$.

3. RESULTS

The intraobserver intraclass correlation (ICC) value was 0.904 (95% CI: 0.743– 0.942). Of the 200 CBCT analyzed, 116 (58%) were female and 84 (42%) were male. The average LLCP heights on the right and left sides were 4.98±2.12 mm and 4.49 ±1.64 mm, respectively. The mean right LLCP height was found to be statistically significantly higher than the left side (p=0.001). In the female, the mean of right and left LLCP height were 5.07±2.07 mm and 4.48±1.63 mm, respectively. In the male, the mean of right and left LLCP height were 4.83±2.21 and 4.50±1.66 mm, respectively. It was found that LLCP height did not have a statistically significant correlation with gender (p>0.05) (Table 1). There was a statistically significant positive correlation among LLCP height sides (p<0.05). There was a statistically significant negative correlation between LLCP and CPH (p<0.05). No association was calculated between LLCP and other parameters (p>0.05) (Table 2).

The average of right and left MERH were calculated 25.55±3.00 mm and 25.24±3.01mm, respectively. Also, there was no significant difference between left and right MERH (p>0.05). There was no significant difference between right MERH and genders (p>0.05) although the left MERH was higher in males (p<0.05) (Table 1). A statistically significant relationship was found between LLCP and MERH (p<0.05). There was a positive relationship between CPH and MERH (p<0.05). No relation was detected between other parameters and MERH (p>0.05) (Table 2).

The average of right and left CPH were calculated 20.55±2.61 mm and 20.82±2.75 mm, respectively. There was a statistically significant positive relationship between right and left CPH (p= 0.001). Both the right and left CPH were significantly higher in males (p<0.05) (Table 1). There was no statistically significant difference between any parameters and CPH (p>0.05) (Table 2). Also, there was no correlation

between the height of the anatomical structures of the evaluated ethmoid bone and the increase or decrease in age (p>0.05) (Table 2).

Table 1. *Comparison of anatomical structures with gender*

		Male (N:84)	Female (N:116)	p
Right MERH (mm)	Mean	25.7646	25.4255	0.514
	SD	2.95527	3.03387	
	Minimum	19.61	19.22	
	Maximum	37.77	37.77	
Left MERH (mm)	Mean	25.8270	24.8952	**0.017***
	SD	2.46627	3.25191	
	Minimum	18.05	5.66	
	Maximum	31.92	31.92	
Right CPH (mm)	Mean	20.9329	20.3362	**0.049***
	SD	2.60014	2.60806	
	Minimum	14.03	14.03	
	Maximum	26.73	28.15	
Left CPH (mm)	Mean	21.3239	20.6110	**0.029***
	SD	2.63431	2.79379	
	Minimum	13.25	13.25	
	Maximum	26.73	28.28	
Right LLCP (mm)	Mean	4.8318	5.0779	0.218
	SD	2.21481	2.07025	
	Minimum	1.58	0.65	
	Maximum	15.83	15.83	
Left LLCP (mm)	Mean	4.5031	4.4875	0.749
	SD	1.66830	1.63370	
	Minimum	0.29	1.29	
	Maximum	8.30	14.33	

** Significant differences (p < 0.05) are indicated in bold. SD: Standart deviation. N: patient. MERH: Medial Etmoit Roof Height. CPH: Cribroform Plate Height. LLCP: Lateral Lamella of Cribroform Plate. mm: millimeter.*

Table 2. *Comparison of anatomical structures with each other*

	p	Right MERH	Left MERH	Right CPH	Left CPH	Right LLCP	Left LLCP
Age		0.558	0.234	0.243	0.754	0.396	0.146
Right MERH			**0.000**	**0.000**	**0.000**	**0.000**	**0.011**
Left MERH				**0.000**	**0.000**	**0.044**	**0.000**
Right CPH					**0.000**	**0.000**	**0.000**
Left CPH						**0.004**	**0.000**
Right LLCP							**0.000**
Left LLCP							

** Significant differences (p < 0.05) are indicated in bold. MERH: Medial Etmoit Roof Height. CPH: Cribroform Plate Height. LLCP: Lateral Lamella of Cribroform Plate.*

4. DISCUSSION

To prevent possible complications such as penetration of the anterior cranial fossa during operation and the occurring cerebral damage, cerebral hemorrhage, and cerebrospinal fluid fistula, the anatomy of the anterior skull base and ethmoid bone should be carefully examined and the lengths of the anatomical structures should be measured. The lateral lamella, which is a thin bone part of CPH, both parts the medial wall of the ethmoid roof and constitutes the upper border of the operative region in FESS operations. This structure is important for the risk of damage in being easily damaged in the operations in the posterior medial concha or posterior ethmoid bone region. (9). In our study, it was proposed to use CBCT images in accordance with the literature due to the complex anatomic of the ethmoid bone we examined and measured. As the depth of the CPH increases, the lateral lamella length increases. This also increases the risk of developing complications during surgery. (6,10-12). In this study, to provide the best CPH measurement, the coronal section that shows this region most clearly is preferred. Using radiological means to determine the ethmoid roof is critical for designing the superior limit of the dissection (13,14).

In the literature, measurements of ethmoid bone components were made in different races. Munoz-Lejia et al. (15) stated that in Mexican, the mean right CPH was 8.94 mm and 10.21 mm in men and women, respectively also the mean left CPH was 8.99 mm and 10.20 mm for men and women, respectively. They found statistical differences between the right and left sides in both genders. Meloni et al. (16) calculated the mean CPH was 5.9 mm (range 1.3-17 mm) in Italians. Similarly, Gera et al. (17) showed that the mean CPH was 5.4 ± 1.7 mm (range, 2.4-10.3 mm). The mean CPH in males was 5.6 ± 1.7 mm (range, 2.4-10.1 mm) while in females it was 5.2 ± 1.7 mm (range, 2.9-10.3 mm). They stated that these differences were found not significant. Erdogan et al. (18) found the right CPH was 25.01 mm (range, 18-32.1 mm), and the left CPH was 25.38 mm (range, 18.5-36.1 mm). When we compare present study with the literature, it is seen that the CPH is higher than the average. The reason for this is thought to be the differences in measurement points. In our study, the measurement of CPH was based on the distance of the cribriform plate to the horizontal line. The reason why take this distance as a basis is to determine the risk of skull base injury during the operation according to the change in this distance in the TMS classification (19). On the other hand, the findings of our study were similar to the study of Erdogan et al. (18) in the Turkish population. This can be interpreted as the fact that the race factor is effective in the morphometric measurements of anatomical structures. This could be the basis for future work. Paber et al. (20) reported that in their study, the mean LLCP height among Filipinos was 2.21mm. They found were no significant difference in the LLCP height among age groups and sides but a significant difference between genders. Similarly, Alazzawi et al. (21) showed that in the Malaysian population, the mean LLCP height was 2.64 mm and the left LLCP height was higher from the right side. Bista et al. (22) found that in their study the mean height of LLCP was 2.8 mm in African.

The LLCP height was calculated greater on the right side in 56% of patients and was greater on the left side in 38% of patients. Abdullah et al. (23) stated that the mean LLCP height was 7.44 ± 1.32 mm in the Asian population (Malays, Chinese, Indian). Erdogan et al. (18) calculated the right LLCP height was 5.78 mm (range 2.1-10.3 mm), and the left LLCP height was 5.98 mm (range, 1-10.9 mm). In present study, we found the mean of right and left LLCP height were 4.98±2.12 mm and 4.49 ±1.64 mm, respectively in the Turkish subpopulation. When we compared the results, it was seen that the LLCP height in the Turkish population was higher than Africans and Filipinos, similarly Turkish population and lower than Asian populations.

5. CONCLUSION

In our study, the relationships between morphometric measurements of ethmoid bone components and, height, age, and gender were shown in the Turkish subpopulation. It was seen that the age factor did not affect the length of these anatomical structures, but race, gender, and the measuring side affected these measurements. It should not be ignored that there may be differences in the operations related to the ethmoid bone in terms of gender and sides. Therefore evaluation of the anatomical structure of ethmoid bone by three-dimensional imaging is recommended to prevent complications that may occur in sinus surgeries.

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Clinical and Experimental Health Sciences

The Effect of High Body Mass Index on Self-Esteem and Sexual Functions in Obese Females Admitted to The Plastic Reconstructive and Aesthetic Surgery Department

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ABSTRACT

Objective: Obesity can be defined as body weight above the desired level. Obesity has gone beyond being a cosmetic problem and is now referred to as a disease, and there is a currently significantly increasing number of studies examining the relationship between the obesity and psychopathology. The psychological and social effects of obesity also affect the self-confidence of the individual and the behavioral orientation at the onset of sexual intercourse and avoidance. The aim of this study was to determine whether high body mass index has an effect on self-esteem and sexual functions in obese women who were referred to an obesity center.

Methods: This descriptive study was conducted between 1.11.2018 and 15.01.2020. A total of 202 patients referred from the plastic surgery department to the obesity centre were included in the study. All the patients were assessed twice within a 7-month period with the Rosenberg Self-Esteem Scale, Body Perception Scale and Arizona Sexual Experiences Scale.

Results: Of the 202 women evaluated, 66.3% were in the 51-70 years age range, and 52% had a body mass index in the range of 35-39.9. Evaluation of the two applications of the scales showed a significant positive relationship between the first scale and the second scale scores seven months after weight loss.

Conclusion: The results of this study showed that a decrease in body weight had a positive effect on body perception and on sexual satisfaction.

Keywords: Obesity, sexual satisfaction, self esteem, body perception

1. INTRODUCTION

Nutrition is one of the most important current health problems, because inadequate or unbalanced nutrition can have anegative effect on health. Obesity is generally classified using body mass index (BMI), which is calculated as body weight in kilograms divided by height in meters squared (kg/m2) (1). Obesity can also be defined as body weight above the desired level (2).

Being overweight is defined as a BMI value between 25 and 30 according to the Centers for Disease Control and Prevention of the USA (3).

The human body is mainly composed of active mass (muscle, liver, heart, etc.), adipose tissue (fat), extracellular fluid (blood, lymph, etc.), and connective tissue (skin, bone connective tissue) As a structure, obesity is characterized by relative increment in fat mass compared to other body components (2). Intra-abdominal fat mass in particular is responsible for the obesity condition and could lead to a higher risk of insulin resistance and heart disease (4). Environmental, neurological, physiological, biochemical, cultural and psychological factors in addition to heredity, are effective in relation to each other and make the prevention and treatment of obesity more difficult and complicated (5).

With increasing incidence in recent years, obesity has gone beyond being a cosmetic problem and is now referred to as a disease. Recently, there has been a significant increase in the number of studies examining the relationship between obesity and psychopathology. These studies have suggested that obesity is a complex condition which could lead to anxiety, depression, and consequently deterioration in personal health and quality of life (6,7). In some other studies of body perception in obese individuals, it has been reported that BMI has a significant effect on body dissatisfaction (8) and an obese group had more body dissatisfaction than a non-obese group (9). In addition, increased sexual dysfunction and higher comorbidity rates have been mentioned (6,7). The mechanism of sexual dysfunction secondary to obesity is multifactorial (10). The psychological and social effects of

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obesity also affect the self-confidence of the individual and behavioral orientation at the onset of sexual intercourse and avoidance. The place of sexuality in human life is indisputable and can be said to have significant effects on quality of life for both men and women.

However, few studies have examined the effect of obesity on sexual functions in women. The characteristics, physiology and mechanisms of female sexual dysfunction differ from and are more complex than those of men. Female sexual dysfunction has an impact on quality of life and a decrease in quality of life will cause sexual dysfunction (11). The aim of the current study was to determine whether high body mass index has an effect on self-esteem and sexual functions in obese women who were referred to an obesity center.

2. METHODS

This descriptive study was conducted on 202 patients in the Obesity Center of our hospital between 1.11.2018 and 15.01.2020. The Ethics Committee of Istanbul Okan University approved the study with a number 14 decision by 22.01.2020 date. All the study procedures were applied in accordance with the 2008 Helsinki Declaration.

The study inclusion criteria were as follows:

1 – BMI of ≥30,

2 – Voluntary participation,

3 – Female gender, aged 18-70 years,

4 – Education level of at least primary school,

5 – In an established sexual relationship.

Obese female patients who presented at the Plastic, Reconstructive and Aesthetic Surgery service for body shaping were referred to the Obesity Center to obtain a permanent and appropriate weight range and to prepare for surgery. The obese patients were informed that they were overweight and surgery could result in more complications if performed before reaching a permanent and appropriate weight, and there could be poorer outcomes with long-term weight loss.

Height and weight were measured and body mass index values were calculated according to the index formula suggested by the World Health Organization in 1988 (12). Each subject was interviewed individually for 20 mins under suitable conditions. The evaluation scale questionnaires were completed on first admission to the Obesity Center and after seven months when the study was ended.

2.1. Patient Information and Demographic Data Form

This form included the following data: Age, BMI, education level, occupation, marital status, education of the spouse, occupation, number of children, economic status, smoking, alcohol, drug use, exercise, diet, and eating behavior. The form related to obesity questioned emotional state, family structure, parents, and siblings, age at onset of obesity, the presence of any triggering factor, psychiatric admission due to obesity, use of drugs to prevent obesity, opinions about obesity, and the effects of obesity on social life.

2.2. Rosenberg Self-Esteem Scale

This scale was developed by Morris Rosenberg in 1963, and has been used as a measurement tool in many studies following reliability and validity trials conducted in the USA. In Turkey, the validity and reliability coefficients were calculated as 71 and 75, respectively, by Çuhadaroğlu. The Rosenberg Self-Esteem Scale is a self-report scale consisting of 63 multiple-choice questions. The first ten items of the scale were utilized to measure the self-esteem status in this study. Items 1, 2, 4, 6, and 7 question positive self-assessment, and items 3, 5, 8, 9, and 10 question negative self-assessment, with scores ranging from 0 to 3. Items 2, 5, 6, 8, and 9 are reverse scored. The total score range is between 0-30, with 15-25 points indicating that self-esteem is sufficient, and <15 points indicating low self-esteem (13).

2.3. The Body Perception Scale

This scale was developed in 1953 by Secord and Jourand, and the validity and reliability of the scale were measured in 1989 by Hovardaoğlu. The scale consists of 40 items and each item is related to an organ or part of the body (such as arms, legs, face) or a function (such as the level of sexual activity). Each item is scored from 1 to 5, providing a total score between 40 and 200. A higher score indicates a higher level of satisfaction. The cut-off score of the scale was 135 and those with a score <135 were defined as low body perception (14).

2.4. Arizona Sexual Experiences Scale (ASEX)

This is a Likert-type self-assessment scale consisting of five questions, which meet the current diagnostic criteria of DSM-IV and ICD-10 developed for sexual dysfunction characterization. Validity and reliability studies of the scale for a Turkish population have been conducted. The total scale score varies from 5 to 30 with each item scored from 1 to 6. Sexual dysfunction is considered to be present in patients with a total scale score of ≤ 19, or with any sub-dimension score of ≥5, or with 3 or more sub-dimension scores of 4. Low scores indicate that sexual response is strong, easy and satisfactory, while higher scores indicate the presence of a possible sexual dysfunction (15).

2.5. Statistical Analysis

Data obtained in the study were analyzed statistically using IBM SPSS Statistics vn. 21 software (SPSS IBM, Chicago, IL, USA). Conformity of the data to normal distribution was evaluated with the Kolmogorov-Smirnov test, and the homogeneity was tested. Descriptive statistical methods (mean, standard deviation, frequency), t tests and correlation

analysis were used to evaluate the study data. A value of p<0.05 was accepted as statistically significant.

3. RESULTS

Of the total 202 women evaluated, 66.3% were in the 51-70 age range, 52% had BMI in the range of 35-39.9, and 51.5% had a waist-hip ratio of ≥0.85. Education level of primary school or lower was reported by 43.1% of the women, 68.3% were housewives, 66.8% were married, 55% had three or more children, 37.1% were aged < 20 years at the onset of obesity and 83.7% consumed excessive carbohydrate and fatty food content. When the reason for the onset of obesity was examined, it was found that 63.4% gained weight after stopping smoking and 71.8% when going through the menopause. The income level of 44.6% was low, 47% were taking medication for obesity, 55.9% had presented at the Psychiatry Dept because of obesity, and 80.2% stated that they ate more food when they were sad (Table 1).

Table 1. *Sociodemographic characteristics of Obese Women Applying to an Obesity Center*

	n	%
Age		
18-30	17	8.4
31-50	51	25.2
51-70	134	66.3
BMI		
30-34,9	26	12.9
35-39,9	105	52.0
≥40	71	35.1
Waist Hip Ratio		
<0,8	6	3.0
0,8-0,85	92	45.5
≥0,85	104	51.5
Education Level		
Primary school or lower	87	43.1
High School or lower	92	45.5
Undergraduate or graduate	23	11.4
Job		
Housewife	138	68.3
Civil Servant Or Laborer	9	4.5
Tradesman	36	17.8
Retired	19	9.4
Marital Status		
Single	57	28.2
Married	135	66.8
Widow	10	5.0
Child		
None	11	5.4
1-3	80	39.6
>3	111	55.0
Economic Level		
Income<Expenditure	90	44.6
Income= Expenditure	32	15.8
Income>Expenditure	80	39.6
Beginning Age Of Obesity		
Under 20	75	37.1
20-30	71	35.1
30 or more	56	27.7
Consumed excessive carbohydrate and fatty food content		
Exists	169	83.7
None	33	16.3
Gained weight after stopping smoking		
Exists	128	63.4
None	74	36.6
Gained weight when going through the menopause		
Exists	145	71.8
None	57	28.2
Presented at the Psychiatry Dept because of obesity		
Exists	113	55.9
None	89	44.1
Medication For Obesity		
Exists	95	47.0
None	107	53.0
Eat When You Are Happy		
Exists	109	54.0
None	93	46.0
Eat When You Are Sad		
Exists	162	80.2
None	40	19.8
Affecting social life due to obesity		
I Don't Want To Join Social Life	141	69.8
I Never Join Social Life	61	30.2
Are Mother And Father Together		
Together	68	33.7
Divorced	78	38.6
Parents Dead	56	27.7
Is Mother Obese?		
Yes	185	91.6
No	17	8.4
Is Father Obese?		
Yes	106	52.5
No	96	47.5
Is Sister Obese?		
Yes	175	86.6
No	27	13.4

In evaluation of the data obtained from the second applications of the questionnaires, there was seen to be a significant positive relationship between the first Rosenberg Self-Esteem Scale score and the second scale score applied after seven months after weight loss. According to the results of the Paired Samples t-test, a decrease in BMI had a positive effect on self-esteem (p <0.001). According to the two applications of the Arizona Sexual Satisfaction Scale, a decrease in BMI had a positive effect on sexual satisfaction (p < 0.001). When these two body perception scales were

compared, a decrease in BMI was determined to have a positive effect on body perception in obese women (p < 0.001). A statistically significant positive correlation was determined between BMI and eating when unhappy (p<0.001) (Table 2). A statistically significant positive correlation was found between the BMI of women and admission to obesity-related psychiatry (p<0.001) (Table 3).

The analyses applied showed a statistically significant positive relationship between low income level of the obese women and the Rosenberg Self-Esteem Scale scores, and between low income and the Body Perception Scale scores (p<0.001, p<0.001). No statistically significant relationship was determined between low income level and the Arizona Sexual Satisfaction Scale points.

Table 2. *The Relationship Between the Sad Eating Behavior of Obese Women and High Body Mass Index (BMI)*

	n	r	p
Eating when unhappy BMI	202	0,551	<0,001

A statistically significant positive correlation was determined between BMI and eating when unhappy (p<0.001).

Table 3. *The Relationship Between Obesity-Related Psychiatry Clinic Apply Status and High Body Mass Index (BMI) of Obese Women*

	n	r	p
Obese which has psychiatry clinic apply BMI	202	0,646	<0,001

A statistically significant positive correlation was found between the BMI of women and admission to obesity-related psychiatry (p<0.001).

4. DISCUSSION

In recent years, the prevalence of obesity has greatly increased in all regions of the world, in both rich western countries and poorer countries. There is a need for collaboration between people, governments, the media and the food industry to change the societal environment to be able to reduce weight gain, and encourage healthy lifestyles in all age groups, but especially in children and adolescents (16).

In the current study, the age at onset of obesity was 18 ± 3.4 years, which supports the findings of Tuzun et al (17). However, there are conflicting data in literature about the age at onset of obesity. It has been reported that less than a third of obese patients were obese in childhood, and although it can start at any age, obesity is mostly reported to develop after puberty (18).

In the current study, the mean rate of obesity in parents and siblings of obese participants was significantly higher than in non-obese individuals. This suggests that having obese parents can increase the tendency to obesity during childhood. It has been mentioned in the literature that obesity in close relatives was considered a genetic risk factor for obstetric obesity (19).

The responses given by the obese women to the question of "What is the most important factor causing your obesity?" were "Eating too much carbohydrate and fatty foods" at the rate of 83.7%, followed by entering menopause and quitting sports. Previous papers, have reported physical inactivity as the most important reason for the onset of obesity (18, 20, 21). In a study by Bray, it was reported that physical inactivity contributed significantly to the onset of obesity at an approximate rate of 67.5% (18). Wilson et al. reported that obesity was most common in men with a sedentary lifestyle, while pregnancy was a major factor for women (22).

In the current study, "emotion-induced eating" (sad, cheerful, anxious, angry, etc) was significantly higher in obese group (p < 0.001), and it was observed that obese participants ate more when upset. Other similar studies have shown that emotional eating is related to weight gain (23). Canetti et al, reported that increased eating in conditions of distress, depression, and fatigue, resulted in a decrease in fear, tension and pain (24). Ganley et al. stated that emotional eating behavior was related to negative emotions such as anger, depression, distress, anxiety and loneliness (23).

In the current study, it was observed that the self-esteem of obese subjects was remarkably lower than that of non-obese subjects (p = 0.001). The vast majority of research papers have shown a linear relationship between obesity and decreased self-esteem (25, 26). In a previous study conducted to examine the levels of depression and self-esteem in obese women, 42.5% were depressed and 58.6% had low self-esteem (27). In a study by Ogden and Evans, it was found that depression was higher and self-esteem was lower in obese cases (28), and Kartal also reported lower self-esteem in obese participants (29). In a prospective study conducted with 64 women by Galletly et al., a decrease in depression rate and increase in self-esteem were recorded in correlation with weight loss (30). In the current study, after 7 months of losing weight, the evaluation scales were applied again and there was found to be a significant increase in the total scores of the self-esteem scale second application.

The current study obese group gave negative feedback that they were dissatisfied with their body at a statistically significantly higher rate than the non-obese group (p< 0.001). In a study by Caldwell et al., it was suggested that BMI had a significant effect on body dissatisfaction status (8). Sarwer et al. reported that obese individuals had a higher level of body dissatisfaction compared to a non-obese group (9). In another study conducted using the Body Dysmorphic Disorder Scale and Body Shape Questionnaire, it was found that more than 80% of female participants had negative body perception (31). Cash reported that obese people who reduced their weight by 24% using a very low-calorie diet experienced significant improvements in all aspects of body perception (32). In the current study, the weight loss of patients was found to contribute to a positive change in body perception according to the results of the the second body perception scale applied at the end of the 7th month.

It was also observed that obese patients had impairments in sexual functions, and sexual satisfaction was negatively affected compared to the non-obese group. The causes of the formation of sexual dysfunction accompanying obesity are multifactorial, and there are high comorbidity rates that will clearly cause sexual dysfunction especially in morbid obesity. The effect of some common diseases such as DM and HT (33), psychiatric problems such as depression and anxiety, and the drugs used for sexual functions are well-known (10). Several studies have focused on defining the characteristics of the sexual life of obese women with sexual dysfunction. The literature shows that sexual desire and orgasm disorders are common in women (34). In the current study, obese women had low sexual satisfaction. In a study by Kaneshiro et al. conducted with 6690 women in the USA, it was reported that an increase in obesity negatively affected female sexuality (35). Kolotkin et al. evaluated the quality of life and sexual satisfaction of subjects with mean BMI level over 40 kg/m^2, and found that more than half of the overweight women had low sexual satisfaction and avoided having sexual intercourse because of their low body perception (36). In the current study, there was observed to be an increase in the scores obtained from the sexual satisfaction dimension of the ASEX scale with the decrease in BMI in the second application of the scale after seven months. The sexual satisfaction level and desire were seen to increase in obese women as they lost weight.

When the income level was low in obese women, it was determined that BMI increased and self-esteem decreased. The fact that this study was conducted in a public hospital on subjects with a low socio-economic level could have affected this result. A low socioeconomic level and a greater number of people in the household can cause obesity by lowering the amount spent on sufficient and balanced nutrition. In another study that examined the frequency of obesity and the factors affecting obesity in married women living in slum areas, there was determined to be a significantly positive relationship between BMI and the number of people in the household. Similarly, Blumel et al reported that an increase in the number of people living in the home increased the risk of obesity 1.31-fold (37).

5. CONCLUSION

Obesity causes many health problems, both mental and physical. It can be seen that the general quality of life of obese individuals is diminished as a result of both health problems and social difficulties. Therefore, factors causing obesity and their consequences should be considered in multi-dimensional way. Investigation of biological, psychological and social factors that contribute to obesity can be a guide in preventing or reducing obesity. There is a need for collaboration of society, government, the media, and the food industry to change the environment to be able to reduce weight gain, and to promote healthy lifestyles in all age groups, especially in children and adolescents.

There is increasing interest in studies on the psychosocial aspects of obesity. Previously conducted research trials have shown that childhood and adolescent traumas are an important factor in the development of obesity as was also reported in this study. In addition, negative body perception and low self-esteem seen in obese individuals are regarded as an important condition that should be addressed in the treatment of obesity.

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Clinical and Experimental Health Sciences

Determination of Energy and Nutrient Intakes in Pediatric Burned Patients

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ABSTRACT

Objective: The prevalence of malnutrition defined by nutritional insufficiency is substantially high in children admitted to the burn center. This study aimed to evaluate the energy and nutrient intakes of pediatric burned patients.

Methods: This prospective study was conducted between May 2016 and January 2017, involving 45 (2 to 18 years old) children and their caregivers. Data were collected via three 24 h dietary recall, socio-demographic questionnaire, and assessment of clinical characteristics was done in the Hospital Burns Centre. The energy and nutrient intakes of pediatric burned patients were calculated using computer software and compared with the nutritional recommendations. Statistical tests included analyses of t-test, ANOVA, Pearson Correlation test, and $\chi 2$ tests.

Results: None of the children reached the requirements for energy and macronutrients. There was a negative correlation between the meeting of requirements and the age ($P < 0.05$). Oral enteral nutrition increased the meeting energy requirements ($P < 0.05$). Depending on the presence of a perioperative fasting period, the percentage of children who met the energy and protein requirements was significantly lower ($P < 0.05$). The burned area did not affect the percentage of children meeting their energy and protein intakes ($P > 0.05$).

Conclusions: Oral enteral nutrition facilitates the ability of children to reach requirements; however, perioperative fasting periods and age are barriers to ensure adequate energy and nutrient intake in pediatric burned patients. Therefore, individualized nutritional support and education is vital for burned children to meet increased energy and nutrient requirements.

Keywords: Nutritional status, child, burns, energy intake, nutrient intake.

1. INTRODUCTION

Burns are acute and preventable injuries caused by exposure to heat, electricity, chemical substances, and radioactive or caustic agents that affect the skin and/or subcutaneous tissues (1). Burns affect an average of 2.5 million people every year worldwide (2). Because burns result in a loss of skin, burned persons may develop fluid–electrolyte disorders and severe catabolism, leading to energy, protein, and micronutrient deficiencies. Burns that negatively affect the entire organ system are one of the most important causes of morbidity and mortality, particularly in children. Burns are among the second – or third-ranked causes of mortality in children depending on the age, burn area, and degree. In addition, burn-related mortality rates increase up to 4 years old (3-6). Inhalation burns and sepsis are also common causes of mortality from burns (3).

Risk factors that cause burns vary depending on the country, region, education level, culture, socioeconomic status, habits, living conditions, and environmental conditions (7). The most common burns in children are caused by scalding, flames, and electrical and chemical burns; the upper and lower extremities are the most commonly burned areas, and second-degree burns are the most frequent type (8). In a study conducted in Turkey, scalding was the most common type of pediatric burns and the most common cause of scald injuries were hot water or hot tea and coffee. In the same study, the upper and lower limbs were involved in 41.2 and 43.0 % of the patients (9).

Pediatric burns require a multidisciplinary approach and are difficult to treat (1). Nutritional support is also considered an important component of burn treatment. In moderate to severe burns, providing adequate nutritional support is important for reducing mortality and morbidity. The negative clinical outcomes lead to a hypermetabolic condition in which burned children are at risk of malnutrition (6). In a study conducted in the USA, malnutrition was detected in 23.8% patients in the pediatric burn unit and the highest rate was found in the youngest age (0-3 years) (95%) (10).

It is important to ensure adequate nutrition and weight tracking to prevent weight loss because late wound healing, muscle

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atrophy, growth retardation, decreased immuno-competence, and protein and energy malnutrition become apparent with weight loss during the treatment process (6). Burn traumas have more effects than adults due to the fact that children are in the age of growth and their physical activities are high (11). Pediatric burned patients must remain in the normal growth range according to their age and gender (12).

Given the importance of nutritional support, the purpose of this study was to evaluate the energy and nutrient intake of pediatric burned patients and compare with requirements.

2. MATERIALS AND METHODS

2.1. Subjects and Study Design

This prospective study was conducted in the Dr. Lutfi Kirdar Education and Research Hospital Burn Centre in Istanbul/ Turkey, between May 2016 and January 2017. The study comprised 51 pediatric burned inpatients (25 females and 26 males) aged 2–18 years who were admitted to the burn centre. Enterally and orally fed children were included in this study. Five children and caregivers who didn't accept to participate in the study and one parenterally fed child were excluded. There was no specific routine nutrition support program that the hospital applies to the pediatric burned patients in the burn centre. High energy (1 ml = 1.5 kcal) and 8% protein products were used as oral enteral products in the burn centre.

A total of 45 children were included in this study. Permission to conduct the study was granted by the Ethics Committee of the Marmara University School of Medicine (Approval date: 01.04.2016 – No: 09.2016.255). All children and caregivers who volunteered to participate in the study provided written informed consent. All questionnaires were administered by researchers in face-to-face interviews with the children and caregivers. Before the interview, the participants received an oral explanation of the study purpose.

2.2. Data Analysis and Dietary Assessment

Data were collected via a socio-demographic questionnaire (age, height, weight, etc.), an assessment of clinical characteristics (Total body surface area (%TBSA) of burn, burn degree, burn area, and burn cause), and three 24 h dietary recalls (24-HDRs) administered by dietitians. The weight percentile for age was calculated from standard pediatric growth charts (13). The questionnaire consisted of 4 sections and 20 questions about the children, family, hospital food, and food records. Classification of burn severity was evaluated on the basis of Murray (2007) (14). Daily energy and nutrient intake was calculated by using computer software (Ebispro, Stuttgart, Germany; Turkish version: BeBiS, Vers. 6.1). The daily average energy requirement was determined with the Curreri formula, macronutrient and micronutrient requirements were evaluated on the basis of Chan et al. (6), Clark et al. (15) and Rodriguez et al. (16) (Table 1).

The daily average energy and nutrient intake were also evaluated according to the perioperative fasting period (applying before routine practices such as washout, dressing changes, and surgery for burns). The American Society of Anesthesiologists (ASA) practice guideline was used to determine the perioperative fasting period (17). The ASA practice guideline (2011) valid at the time of the study was suggested the following to reduce the severity of complications related to perioperative pulmonary aspiration of gastric contents; restriction of clear fluids for two hours, breast milk for four hours, infant formula for six hours, a light meal or non-human milk for six hours or more, and a meal that include fried or fatty foods or meat for eight hours (17).

Table 1. *Energy and nutrient requirements*

Energy and nutrients	Daily requirements
Energy (kcal)	*
Carbohydrate (g)	**
Protein (g)	**
Fat (g)	**
Vitamin A (µg)	750-1500 µg (0-13 age) 3000 µg (>13 age)
Vitamin D (µg)	15 µg
Vitamin E (mg)	4.0-10.7 µg
Vitamin C (mg)	250-500 mg
Vitamin K (µg)	2-60 µg (0-13 age) 75-120 µg (>13 age)
Folic acid (µg)	1000 µg (Three times a week)
Iron (mg)	0.3-8 mg (0-13 age) 8-18 mg (>13 age)
Copper (mg)	0.8-2.8 µg (0-13 age) 4 µg (>13 age)
Zinc (mg)	2-8 mg (0-13 age) 25-40 mg (>13 age)

** Daily average energy intake was calculated using the Curreri formula,** Macronutrient requirements were evaluated on the basis of Clark et al. (15). Micronutrient requirements were evaluated on the basis of Chan et al. (6), and Rodriguez et al. (16). The percentages of children meeting the micronutrient requirements were calculated according to the sub-limits mentioned above.*

2.3. Statistical Analysis

The Kolmogorov–Smirnov test was used to assess the normality of the distributions. Independent Sample T-Test was used to a statistical comparison between two independent groups, and the One-way Anova test for between more than two independent groups. Pearson Correlation test was used to evaluate the relationship between the mean age of children and the percentages of meeting energy and macronutrient requirements. Data were shown as the mean and standard deviation. The nominal variables were analyzed using the χ^2 test. $P < 0.05$ was considered significant. All statistical analyses were performed using the SPSS 22.0 (Statistical Package for Social Sciences) program.

3. RESULTS

The participants included 19 (42.2%) females and 26 (57.8%) males with a mean age of 6.0 ± 4.4 years. Most of the children (55.5%) were between 2 and 3 years old. The mean body weight reported at admission was 22.7 ± 13.7 kg. Four percent of the children fell below the 3rd percentile, 85% were between the 3rd and 97th percentiles, and 11% were over the 97th percentile according to weight for age standards at admission. The most common cause of burns was scalding in 34 (75.6%) children, followed by flame burns in 10 (22.2%) children and electrical burns in 1 (2.2%) child. The mean %TBSA was 14.5 ± 7.4% (range of 5–34%). Thirty-five (77.8%) children had second-degree burns, seven (15.6%) had third-degree burns, and three (6.7%) had first-degree burns. In 17 children (37.8%), the burns covered less than <10% of TBSA, in 21 children (46.6%) the burns covered 10-20% of TBSA, whereas in 7 children (15.6%), the burns covered >20% of TBSA.

Particularly in 4-6 and 15-18 age groups, energy and macronutrient intakes were higher than other age groups. In line with energy and macronutrient intakes, except for vitamin C and vitamin D, vitamin and mineral intakes were higher in 4-6 and 15-18 age groups (Table 2).

Table 2. *Mean Daily Intakes for Energy and Nutrients According to Age Groups*

Energy and nutrients	2-3 age (n=19)	4-6 age (n=7)	7-10 age (n=12)	11-14 age (n=4)	15-18 age (n=3)
Energy (kcal)	696.5 ± 255.9	1053.2 ± 310.2	768.4 ± 336.3	732.2 ± 345.4	1103.8 ± 574.6
Carbohydrate (g)	80.5 ± 34.2	115.4 ± 35.0	82.3 ± 32.2	84.7 ± 32.5	144.2 ± 87.23
Protein (g)	23.4 ± 9.2	39.7 ± 12.8	31.3 ± 16.0	25.4 ± 13.4	40.3 ± 24.9
Fat (g)	30.0 ± 12.5	46.7 ± 14.4	33.5 ± 17.3	30.9 ± 18.7	38.4 ± 20.6
Vitamin A (µg)	315.2 ± 134.8	495.8 ± 199.6	380.9 ± 177.6	449.8 ± 330.1	1557.0 ± 1621
Vitamin D (µg)	1.1 ± 1.2	0.9 ± 0.6	0.6 ± 0.7	0.4 ± 0.6	0.3 ± 0.4
Vitamin E (mg)	3.4 ± 2.1	7.7 ± 3.7	5.3 ± 3.1	5.3 ± 2.9	8.2 ± 7.9
Vitamin C (mg)	37.2 ± 16.2	51.1 ± 25.5	57.2 ± 28.1	59.7 ± 42.4	144.8 ± 160.1
Vitamin K (µg)	61.5 ± 38.47	119.6 ± 34.9	99.3 ± 52.8	99.2 ± 47.3	156.4 ± 95.1
Folic acid (µg)	64.7 ± 32.1	120.7 ± 18.8	105.0 ± 46.5	102. ± 46.8	229.9 ± 165.8
Iron (mg)	3.6 ± 1.2	5.4 ± 1.4	4.8 ± 2.2	4.1 ± 1.9	6.5 ± 3.7
Copper (mg)	0.6± 0.2	1.1 ± 0.3	0.9 ± 0.3	0.8 ± 0.3	1.2 ±0.7
Zinc (mg)	3.4 ± 1.5	6.3 ± 1.9	4.8 ± 2.4	4.0 ± 2.3	7.0 ± 4.4

The status of children's energy and nutrient intake to meet the requirements was shown in Table 3. None of the children reached the energy, carbohydrate, protein, folic acid, and vitamin D intake requirements.

There was no statistically difference between genders in terms of meeting energy and nutrient intakes ($P > 0.05$).

When the energy and macronutrient intakes of pediatric burned patients were evaluated, it was found that 67.7% of the children met 20-50% of energy, while 71.1% met 20-50% of both protein and carbohydrate. In addition, it was determined that 46.7% of the children meet the fat above 70%.

Table 3. *The meeting status of the requirements for energy and nutrients by age groups*

Energy and nutrients	2-3 age (n=19)	4-6 age (n=7)	7-10 age (n=12)	11-14 age (n=4)	15-18 age (n=3)	Total (n=45)
	n-%	n-%	n-%	n-%	n-%	n (%)
Energy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Carbohydrate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Protein	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fat	7 (36.8)	3 (42.9)	12 (100.0)	4 (100.0)	3 (100.0)	29 (64.4)
Vitamin A	0 (0.0)	1 (14.3)	0 (0.0)	1 (25.0)	1 (33.3)	3 (6.6)
Vitamin D	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vitamin E	7 (36.8)	7 (100.0)	8 (66.7)	3 (75.0)	1 (33.3)	26 (57.8)
Vitamin C	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (2.2)
Vitamin K	19 (100.0)	7 (100.0)	12 (100.0)	4 (100.0)	2 (66.7)	44 (97.8)
Folic acid	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Iron	19 (100.0)	7 (100.0)	12 (100.0)	4 (100.0)	3 (100.0)	45 (100.0)
Copper	3 (15.8)	6 (85.7)	7 (58.3)	1 (25.0)	0 (0.0)	17 (37.7)
Zinc	15 (78.9)	7 (100.0)	10 (83.3)	3 (75.0)	0 (0.0)	35 (77.7)

The percentages of meeting the energy and nutrient requirements decreased by age ($P < 0.05$). A modest but statistically significant negative correlation was found between the mean age of the children and the percentages of meeting energy requirement ($P = 0.002$, $r = -0.443$) and fat requirements ($P = 0.002$, $r = -0.459$), whereas a weak but statistically significant negative correlation was found for carbohydrate ($P = 0.009$, $r = -0.383$) and protein requirements ($P = 0.032$, $r = -0.321$).

The cause, degree, and burn percentages were not significantly associated with meeting daily energy and nutrient requirements ($P > 0.05$). Burn areas such as the head, neck, and arm also did not influence the percentage

of children meeting energy and macronutrient requirements ($P > 0.05$).

In this study, 8 (17.8%) children received oral enteral nutrition supplements. Oral enteral nutrition was shown to contribute to 26.3% of the daily energy intake in these children. When daily intakes were compared with who did not receive oral enteral nutrition (n=37), the percentages of children who met energy, carbohydrate, and fat requirements were significantly higher in children who received oral enteral nutrition ($P < 0.05$). However, these results did not hold for protein and micronutrients ($P > 0.05$).

The mean energy and nutrient intakes on days with and without a perioperative fasting period was shown in Table 4. The percentage of children who met protein requirements was significantly lower on the days with perioperative fasting periods ($P < 0.05$), whereas this difference was found not statistically significant for energy, carbohydrates and fat ($P > 0.05$). For micronutrient intakes, only the percentages of children meeting the vitamin E, folic acid, copper, and zinc requirements were significantly lower on days with perioperative fasting periods ($P < 0.05$).

Table 4. *Comparison of energy and nutrients intakes according to perioperative fasting period*

Energy and nutrients	Days with a perioperative fasting period	Days without a perioperative fasting period	*p*
Energy (kcal)	661.5 ± 350.5	904.3 ± 440.0	0.068
Carbohydrate (g)	81.2 ± 47.4	97.3 ± 54.0	0.769
Protein (g)	23.4 ± 14.8	34.8 ± 20.5	0.045*
Fat (g)	29.9 ± 19.5	38.1 ± 21.7	0.453
Vitamin A (µg)	360.8 ± 319.3	495.6 ± 313.2	0.312
Vitamin D (µg)	0.7 ± 1.2	1.1 ± 1.3	0.061
Vitamin E (mg)	4.2 ± 3.4	6.1 ± 5.3	0.034*
Vitamin C (mg)	45.2 ± 29.3	55.3 ± 40.1	0.786
Vitamin K (µg)	75.0 ± 56.3	111.3 ± 91.1	0.082
Folic acid (µg)	70.3 ± 40.3	115.6 ± 77.6	0.048*
Iron (mg)	3.7 ± 1.9	5.1 ± 3.3	0.176
Copper (mg)	0.7 ± 0.4	0.9 ± 0.5	0.004*
Zinc (mg)	3.8 ± 2.5	5.2 ± 3.4	0.020*

** P < 0.05*

4. DISCUSSION

Several researchers have investigated the nutritional requirements in children with thermal injuries. It is well documented that the maintenance of adequate nutrition is vital in pediatric burned patients because they have inadequate energy reserves and greater energy and nutrient requirements than adults. However, no children reached the energy, carbohydrate, and protein intake requirements; these results were not valid for fat intake. These findings may be a result of children and their caregivers choosing fast food, snacks, etc., instead of hospital and home foods and focusing on food consumption rather than adequate and balanced nutrition. These results indicate that children and their caregivers must receive nutritional education because the diets of pediatric burned patients are substantially inadequate. It has been observed that most children can meet only their daily vitamin E, vitamin K, zinc, and iron requirements. The results in regard to vitamin and mineral requirements in pediatric burned patients remain controversial. Before considering a vitamin and mineral supplement, the nutritional status of the child and bioavailability of a nutritional intervention should be determined (18).

Acute nutritional losses occur because of burn-related catabolism, which makes it very difficult to meet requirements in a short period (19). Enteral nutrition is the preferred option to reach adequate nutrition, prevent metabolic complications in pediatric burned patients who require more nutritional intake because it decreases the risk of infectious complications (20-22). Shahi et. al. recommend early initiation of oral feeding within four hours of presentation in neurologically appropriate pediatric burned patients (23). Oral route with high protein foods and small, frequent feedings is always preferred, if the patient is able to consume adequate according to guidelines. For instance, when the patient is unable to eat or consume adequate intake (defined as at least 60% of energy requirement), enteral and/or parenteral nutrition should be considered (24). In our study, it was found that enteral nutrition helps to meet the increased energy and macronutrient needs. In addition, it was found that 82.2% of the children had an energy intake percentage below 50%. Despite all these reasons, patients were not given a standard enteral nutritional support in the burn centre.

Dylewski et al. noted that the average age of malnourished burned children was older than that of well-nourished burned children (25). Similarly in this study, the percentages who met daily energy and nutrient requirements decreased with age. This may have been due to increased needs and the food that older children prefer.

There are several factors that affect food consumption, such as perioperative fasting periods. These factors trigger nutritional depletion (20). Therefore, nutritional support is absolutely necessary to regulate preoperative nutritional deficiency and to modulate the transition from catabolism to anabolism during the postoperative period (26). Allowing the intake of clear fluids before anesthesia improves child and parental satisfaction, increases gastric pH, increases the ingestion of calories, decreases the risk of hypoglycemia and lipolysis, and improves fluid homeostasis. The European Society of Anesthesiology has reported that children should

be encouraged to drink clear fluids up to 2 h before elective surgery (27,28). The American Society of Anesthesiologists (ASA) recommends restriction of clear fluids for two hours, breast milk for four hours, infant formula for six hours, a light meal or non-human milk for six hours and a large fatty meal for eight hours (17,29). However, currently, most burn treatment centers maintain patients under the protocol NPO, or nothing through the mouth, for at least 8 h or overnight fasting before any surgical procedure (30).

The study was conducted on the declared weight of the children, and it is a limitation that their weight tracking was not assessed. In previous studies, only protein and energy intake were considered when the nutritional status of pediatric burned patients is determined. In our study, the energy, micronutrient and macronutrient intake of children were assessed.

In conclusion, it is difficult for pediatric burned patients to meet nutritional requirements. To provide adequate and balanced nutrition, a dietitian should determine a nutrition plan for each child based on individual factors. Pediatric burned patients should be closely monitored for deterioration in nutritional status because burns are dynamic. In addition, enteral nutritional support should be initiated to optimize burn care. Nutrition education for caregivers who assist in feeding and pediatric burned patients should be a part of the burn unit routine.

It will be needed to create a specific, flexible feeding schedule in the multidisciplinary approach. It should be both practical, suitable for the hospital environment and provide the nutritional needs of the burn patient.

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Clinical and Experimental Health Sciences

The Effect of the Public Health Nursing Course on Students' Healthy Lifestyle Behaviors

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ABSTRACT

Objective: This study was conducted to examine the effect of the public health nursing course given to nursing students on their healthy lifestyle behaviors.

Methods: A one-group pretest-posttest quasi-experimental design was used in the study, which was carried out with 80 students studying at the nursing department of a foundation university and volunteering to participate in the study. The data were collected twice before and after the course using the Personal Information Form and the Health-Promoting Lifestyle Profile (HPLP). Descriptive statistics, Mann-Whitney U and Kruskal-Wallis tests, the paired t-test, were used to evaluate the data.

Results: After the public health nursing course, a significant increase was determined in the students' HPLP total score and the "exercise", "nutrition", "self-realization", "health responsibility", "stress management", and "interpersonal support" subscale scores ($p<0.05$). A significant difference was found in the "HPLP" scale total scores according to the students' physical activity level variable, "interpersonal support" subscale according to the gender and body mass index variables, in the "exercise" subscale according to the smoking status and physical activity level variable ($p<0.05$).

Conclusion: The public health nursing course was observed to affect nursing students' healthy lifestyle behaviors. For a healthy lifestyle of nursing students, it is recommended that programs for gaining these behaviors be developed in light of the current literature and advanced studies should be carried out to determine the transformation status of the provided education into behavior.

Keywords: Healthy lifestyle behaviors, public health nursing, nursing students

1. INTRODUCTION

Chronic diseases, which are a significant public health problem, are affected by lifestyle choices. Healthy lifestyle behaviors such as healthy nutrition, exercise, regular and adequate sleep, and avoiding excessive alcohol consumption help prevent and control chronic diseases (1). Nurses, who take an essential place in providing healthcare and education to individuals, are the key in promoting healthy lifestyle behaviors in society. The nurse, who expects behavior change from an individual, must primarily lead the individual and be a role model by exhibiting the same behavior (2). Personal health practices of healthcare professionals affect the health education and guidance they provide to patients (3). A study found that among nurses providing health education to society, those with a normal body mass index (BMI) created more confidence in individuals (4). Furthermore, it is known that developing and adopting preventive health behaviors from an early age increase compliance with behavior. Since changing lifestyle behaviors becomes more difficult with the increasing age, it is essential to encourage healthy behaviors at the beginning of nursing education (5). Moreover, the university period is among the risky periods for gaining risky behaviors such as unhealthy nutrition, sedentary lifestyle, smoking, alcohol or substance use, unprotected sexual intercourse, self-harming behaviors, risky (drunk, fast, unbelted, unlicensed) driving, skipping school, dropping out of school, and indifference to lessons (6). In the literature, it is recommended that courses for nursing students to gain these behaviors should be included in the curriculum for them to have a healthy lifestyle (7-9). Group training is the most common intervention to improve students' healthy lifestyle behaviors. Studies examining the effect of short-term group education programs on improving the health of

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university students have shown that students improve their healthy lifestyle behaviors, increase their physical activity (PA) levels, increase the consumption of vegetables and fruits, and the program causes weight loss and changes in body composition (10-15). Moreover, there are subjects related to health behaviors in courses such as public health nursing and health promotion in nursing undergraduate education. There are many descriptive studies on the health behaviors of students in Turkey. However, studies explaining the effect of courses involving healthy lifestyle behaviors on students' behaviors are limited.

The hypothesis to be tested in the study is as follows:

- "The public health nursing course affects students' healthy lifestyle behaviors".

2. METHODS

2.1. Study Design

This study was conducted using a one-group pretest-posttest quasi-experimental design to examine the effect of the public health nursing course given to nursing students on their healthy lifestyle behaviors.

2.2. Research Participants

The study was carried out with 80 students who were enrolled in the third year of the nursing department of a foundation university and who took the public health nursing course in the spring term of the 2018-2019 academic year. Eighty students who responded to the data collection tools completely were included in the study using the full counting sampling method.

2.3. Data Collection Tools

The study data were collected using the "Personal Information Form" and the "Health-Promoting Lifestyle Profile (HPLP)."

Personal Information Form: It is a form prepared in line with the literature and consisting of seven questions about age, gender, marital status, weight, BMI, smoking, and PA level.

Health-Promoting Lifestyle Profile (HPLP): It is a 4-point Likert-type scale, the validity and reliability studies of which were conducted by Esin (1999) and which measures health promotion behaviors with six subscales "self-realization, health responsibility, exercise, nutrition, interpersonal support, and stress management" and 48 items. The lowest score that can be obtained from the scale is 48, and the highest score is 192. There are no negative items on the scale (16). Cronbach's alpha (α) coefficient of the scale is 0.91, and it was calculated to be 0.87 in this study. The high reliability limit was above 0.80, and the measurement results were reliable (17).

2.4. Intervention

The public health nursing course is taught as a 4-hour theoretical course for 14 weeks in the spring semester of the third-year curriculum of the nursing department. Subjects such as "the definition of health, preventive health services, the concept of health promotion, healthy nutrition for the prevention of chronic health problems, exercise, stress management, smoking and alcohol use and immunization for the prevention of infectious diseases, personal hygiene and cleaning, safe sex life" are included in the content of the course to ensure that students gain healthy lifestyle behaviors. The researchers provided education to students through direct instruction, question-answer, discussion, and video watching methods. Moreover, the Healthy Lifestyle Behaviors Booklet prepared by the researchers was given to students at the beginning of the course. The students did not take any other course to develop healthy lifestyle behaviors before the public health nursing course in the nursing undergraduate program.

2.5. Data Collection

The data were collected by the researchers using the face-to-face interview method before the beginning of the public health nursing course and at the end of the 14-week course period. After explanations about the study were made, the students were asked to find a nickname for themselves and write their nicknames instead of the name and surname in the data collection tools. The students filled out the data collection tools in approximately 15-20 minutes. At the end of the course period, the students filled out the data collection tools again using the same nicknames.

2.6. Ethical Considerations

The written consent was obtained from the ethics committee of a foundation university (10840098-604.01.01-E.98), and the necessary written permission was obtained from the relevant institution before starting the study. The students participating in the study were asked to write a nickname instead of their name and surname, and the data were anonymized. Verbal and written consent was obtained from the students who agreed to participate in the study voluntarily after the study data were announced not to be used elsewhere other than the research report.

2.7. Data Analysis

The data were analyzed in the computer environment, and descriptive statistical methods of number, percentage, mean and standard deviation were used. The pretest-posttest comparison of the scale and subscale mean scores was made using the paired t-test for dependent samples. The distribution of numerical variables was tested using the Kolmogorov-Smirnov test, and it was identified that the data were not normally distributed ($p<0.05$). Therefore, the differences between individual characteristic variables and

the questionnaire scores were analyzed using the Mann-Whitney U and Kruskal-Wallis tests. The statistical significance was considered as p<0.05.

3. RESULTS

The mean age of the students in the study was 21.60 ± 1.19 years, and 87.5% of the students were female, and 97.5% were single. Of the students, 12.5% smoked. The mean weight was 56.99±11.81, the mean BMI was 21.22±3.10, and when BMI was evaluated, 12.5% were observed to be overweight. Of the students, 35% stated that they had insufficient PA, 57.5% stated that they did "moderate to vigorous PA for at least 20 minutes" 1 day a week, and 7.5% stated that they did "moderate to vigorous PA for at least 20 minutes" 1-2 times a week (Table 1).

A statistically significant difference was revealed between the pretest and posttest HPLP and subscale scores of the students participating in the study (p<0.05). It was determined that the posttest scores of the HPLP and its subscales were significantly higher than the pretest scores (Table 2).

The pretest and posttest comparisons of the HPLP and subscale scores according to the students' descriptive characteristics are given in Table 3. The interpersonal support subscale posttest score of female students was positively higher than that of male students, and the difference was significant (Z=-2.109, p=0.035). The interpersonal support subscale posttest score of students with normal weight was higher than that of overweight students, and the difference was significant (Z=-2.251, p=0.024). The exercise subscale posttest score of non-smokers was found to be higher than that of smokers, and the difference was significant (Z=-2.204, p=0.028). The exercise subscale pretest and posttest scores of the students who did "at least 20 min of moderate to vigorous PA 1–2 times/week" were found to be higher than other students, and the difference was significant (p<0.05). The HPLP posttest score of the students who did "at least 20 min of moderate to vigorous PA 1–2 times/week" was found to be higher than other students, and the difference was significant (KW=7.397, p=0.025).

Table 1. *Characteristics of nursing students (n=80)*

Characteristics	Mean	SD
Age (years)	21.60	1.19
Weight (kg)	56.99	11.81
BMI (kg/m²)	21.22	3.10
	n	**%**
Sex		
Female	70	87.5
Male	10	12.5
Marital status		
Married	2	2.5
Single	78	97.5
Smoking Status		
Smoker	70	87.5
Non-smoker	10	12.5
BMI Classification		
Normal	70	87.5
Overweight	10	12.5
PA Level		
Insufficient PA	28	35
At least 20 min of moderate to vigorous PA 1 day/week	46	57.5
At least 20 min of moderate to vigorous PA 1–2 times/week	6	7.5

BMI= body mass index; PA= physical activity.

Table 2. *Pre – and post-assessment of the HPLP scores (n=80)*

HPLP and subscale	Pretest	Posttest		
	Mean±SD	Mean±SD	t	p
Self-realization	32.88±4.46	36.35±4.70	-4.930	0.000*
Health responsibility	24.18±3.71	27.70±4.07	-6.255	0.000*
Nutrition	14.97±2.52	16.61±2.20	-4.833	0.000*
Exercise	12.53±2.04	13.39±1.88	-3.212	0.002*
Stress Management	15.05±2.59	18.36±3.19	-4.745	0.000*
Interpersonal support	18.61±2.93	20.53±2.81	-6.895	0.000*
Total	117.77±14.38	133.80±15.42	-6.482	0.000*

*HPLP= Health-Promoting Lifestyle Profile, t: Paired Sample t-Test, *p< 0.05*

Table 3. *Comparison of the HPLP and subscale scores according to the students' characteristics (n=80)*

Descriptive Characteristics		Self-realization Pretest	Self-realization Posttest	Health responsibility Pretest	Health Responsibility Posttest	Nutrition Pretest	Nutrition Posttest	Exercise Pretest	Exercise Posttest	Stress management Pretest	Stress management Posttest	Interpersonal support Pretest	Interpersonal support Posttest	HPLP Total Pretest	HPLP Total Posttest
Sex	Female (70)	40.60	40.69	39.67	39.86	40.37	40.05	40.99	41.66	39.91	40.42	40.81	40.81	37.05	37.26
	Male (10)	39.80	24.44	30.80	26.94	37.11	29.94	37.10	27.06	36.70	32.44	38.30	23.38	36.67	22.33
	Z	-.102	-1.952	-1.188	-1.593	-.405	-1.227	-.503	-1.824	-1.001	-.422	32.44	-2.109	-.050	-1.697
	P	.919	.051	.235	.111	.686	.220	.615	.068	.317	.673	.747	**.035***	.960	.090
Smoking Status	Smoker (10)	35.50	53.25	23.60	43.40	40.50	50.60	30.40	56.90	25.30	49.40	39.70	40.70	31.40	49.88
	Non-smoker (70)	37.64	34.97	36.42	34.89	36.74	34.89	38.01	35.54	37.34	36.09	37.34	35.64	34.21	32.44
	Z	-.216	-1.728	-1.362	-.917	-.386	-1.661	-.779	-2.204	-1.366	-1.251	-.238	-.534	-.311	-1.763
	P	.829	.084	.173	.359	.700	.097	.436	**.028***	.172	.211	.812	.594	.756	.078
BMI	Overweight(10)	34.45	29.19	33.33	33.19	42.89	31.44	35.40	34.50	33.15	30.61	29.35	23.39	31.75	27.79
	Normal (70)	41.36	40.14	39.19	39.13	39.63	40.00	41.23	40.71	40.43	40.66	42.09	41.07	37.65	36.90
	Z	-.884	-1.315	-.750	-.732	-.405	-1.093	-.755	-.775	-1.261	-.957	-1.633	-2.251	-.743	-1.111
	P	.377	.188	.453	.464	.686	.274	.450	.438	.207	.338	.103	**.024***	.458	.267
PA Level	Insufficient PA	39.59	34.73	32.02	32.28	35.88	34.36	28.02	30.50	39.80	35.88	37.73	35.38	31.92	29.43
	At least 20 min of moderate to vigorous PA 1 day/week	41.00	39.65	40.83	40.19	41.60	39.70	45.86	43.47	39.48	39.09	41.43	39.93	39.69	37.91
	At least 20 min of moderate to vigorous PA 1–2 times/week	40.92	54.25	49.50	54.42	47.25	55.67	57.67	58.33	38.10	59.42	46.25	49.25	39.30	53.50
	KW	.067	3.871	4.249	5.709	1.753	4.704	14.298	9.936	.024	5.449	.850	2.112	2.188	7.397
	P	.967	.144	.119	.058	.416	.095	**.001****	**.007****	.988	.066	.654	.348	.335	**.025***

*HPLP= Health-Promoting Lifestyle Profile, BMI= body mass index, PA= physical activity, Z= Mann-Whitney U test, KW= Kruskal-Wallis test, *p<.05, **p<.01*

4. DISCUSSION

Although it is thought that nurses and other healthcare professionals may have better lifestyle behaviors than society, this is not always true. Some studies indicate that the health status of nurses and other healthcare professionals may actually be lower than that of the general population (18). The current studies identify nursing students to be at risk for unhealthy lifestyle behaviors and mental health problems. Moreover, while there are many descriptive studies on the healthy lifestyle behaviors of nursing students, there are very few interventional studies on improving the health of these students (19). Therefore, it is necessary to organize training programs to improve the health of nursing students. In this study, the effect of the public health nursing course on healthy lifestyle behaviors was examined with the HPLP (all points and subscales). After the public health nursing course, there was an increase in the HPLP and all subscales. Thus, it was determined that the public health nursing course affected healthy lifestyle behaviors.

Subjects such as the concept of health, preventive health services, health promotion, healthy nutrition for preventing chronic health problems, exercise, stress management, smoking and alcohol use behaviors, immunization for preventing infectious diseases, personal hygiene and cleaning, and safe sex life were included in the content of the public health nursing course to ensure that healthy lifestyle behaviors were gained. It is thought that the inclusion of these subjects contributes to the increase in students' HPLP and subscale scores. Similarly to the current research findings, Coskun et al. (2019) and Alpar et al. (2008) found that the HPLP scores increased statistically significantly after the training provided to nursing students (3,7). In a study conducted in Taiwan by Hsiao et al. (2005), an increase was observed in students' HPLP scores after the health promotion course given to nursing students (18). In a study conducted

by Yeh et al. (2005) with 42 nursing students taking the public health nursing course, a significant increase was observed in students' healthy nutrition and PA at the end of the course compared to the period before the course (20).

A study carried out by Melynk et al. (2014) to evaluate the effect of the 15-week Healthy Lifestyle Behaviors course given to 36 university students on their healthy lifestyle choices, PA, cognitive beliefs, knowledge, and mental health found that students were more willing to apply healthy lifestyle behaviors and their physical activities increased significantly compared to the baseline (12). Contrary to the research findings, the 8-week health promotion and enhancement program, prepared by Erenoglu et al. (2019) for university students and including the subjects of balanced nutrition, stress management, effective communication, health responsibility, and spiritual development, was observed to affect healthy lifestyle behaviors (21).

In this study, the posttest interpersonal support subscale scores of female students and group of students with normal weight were higher. Likewise, Gill et al. (2017) reported that the perceived family support of female students was higher than that of male students (22). Social support refers to the functions of emotional, informational, and instrumental assistance from family, friends, teachers, and significant others. Thus, social support is an umbrella term that includes various supports from all relationships that are significant for the individual (23). Previous studies have shown that women are more likely than men to report more social support and be more socially involved in well-being (24,25). Gender has always been an important factor in these studies on interpersonel support, and there have been results reporting that females receive more interpersonal support from their environment and perceive this social support better.

Similar to our study findings, Göger et al. (2019) and Yılmazel (2016) also determined that the interpersonal support subscale score values of females with normal weight were higher than those of obese women (26,27). In the study carried out by Kocaman and Telatar (2020), it was stated that overweight individuals were under social pressure from family, spouses, and friends to lose weight (28). It can be said that the low interpersonal support score of overweight individuals is due to the effect of social pressure on them. There are positive correlations between social relationships and well-being. Diener and Ryan (2009) reported that "individuals with numerous friends and family members were likely to have higher subjective well-being" (29).

The exercise subscale posttest score of non-smoking students was higher than that of smoking students. A negative and significant relationship was found between physical exercise and smoking in many studies (30). Previous studies have found a significant relationship between physical exercise performance and lung function capacity (31-33). The most important factor explaining this relationship is that smoking significantly affects lung functions and consequently affects physical exercise performance.

The HPLP posttest score, exercise subscale pretest and posttest score of the students who stated that they did "at least 20 minutes of moderate to vigorous PA 1-2 times a week" were found to be higher than the other students. PA is defined as "activities that occur with energy consumption using our muscles and joints in daily life, increase heart and respiratory rate and result in fatigue at different intensities." The activities we define as physical activity can be activities that we choose for entertainment purposes, or they can be activities done out of necessity or necessity (such as housework). On the other hand, exercise is "a subcategory of PA, is planned, structured, repetitive, and purposeful" (34). Therefore, a person who does "moderate to vigorous PA" for at least 20 minutes 1-2 times a week means exercising. This supports the present finding.

5. CONCLUSION

In this study, a contribution was made to developing healthy lifestyle behaviors by applying a classroom-based education program for nursing students. The HPLP and subscale scores of students increased statistically significantly after the public health nursing course. The target behavioral interventions involving health and life skills should be compulsorily provided to nursing students to ensure the health of future healthcare professionals. At the same time, it can be said that the education received by nursing students will affect the health services they will provide to society in the future and that today's students will be an important role model for the healthy society of tomorrow. For a healthy lifestyle of nursing students, it is recommended that programs for gaining these behaviors should be developed in light of the current literature and advanced studies should be carried out to determine the transformation status of the provided education into behavior.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Clinical and Experimental Health Sciences

Hygiene Behavior in Middle School Students in Ankara, Turkey

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ABSTRACT

Objective: Hygiene is important as the first line of defence to mitigate the spread of pathogens in people's everyday environments. The objective of the present study was to investigate the hygiene behaviors of Turkish adolescents living in Ankara.

Methods: The participants were 822 boys and 834 girls, in total 1656 Turkish adolescents aged between 12–18 years. We conducted a cross-sectional study using a sociodemographic form and the Hygiene Behaviour Inventory (H123) including the questions about personal hygiene habits. Data from the study were evaluated statistically by the chi-square test. Statistical analyses were performed using the SPSS software (version 22.0).

Results: Our results showed that the frequency of washing hands on an average day was significantly higher in girls than boys ($p<0.001$). Besides, handwashing scores were significantly higher in girls after coming home from outside, after touching an animal and before preparing food ($p <0.001$). 56.5% of girls usually and 43.1% of boys occasionally washing their hands after handling raw foods and before handling cooked foods ($p<0.05$). Girls compared to boys tended to wash their hands for 21 seconds or more ($p<0.001$). We also found that 87.1% of boys and 12.9% of girls preferred to wear the same underclothes two days in a row ($p<0.001$).

Conclusion: Girl adolescents have been found to show more rigorous behavior about hygiene compared to boys.

Keywords: Hygiene behaviour inventory, adolescents, healthcare, hygiene practices

1. INTRODUCTION

Hygiene is important as the first line of defence to mitigate the spread of pathogens in people's everyday environments. Access to adequate sanitation infrastructure, including toilets, showers and handwashing facilities, has long been identified as a precursor for hygiene and good health. The word *hygiene* originates with Hygieia, the Greek goddess of health. According to the World Health Organization (WHO), "Hygiene refers to conditions and practices that help to maintain health and prevent the spread of diseases". Hygienic behavior is the regular practice and behaviors related to the preservation of healthy living. Good hygienic behavior improves the quality of life by preventing the spread of communicable diseases. Hygiene behavior includes hand hygiene, personal grooming, household cleanliness, and food-related hygiene. Hygienic practices have helped prevent and control many infectious diseases in both community and hospital environments. This has reduced morbidity and mortality across the world (1-4).

In chronological terms, adolescence is defined as the age between 10 and 19 years. During this transition period from childhood to adulthood, intense cognitive, emotional, social, physical and hormonal transformations take place. During adolescence, young people are assuming responsibility for their health attitudes and behaviors (5,6).

All over the world, 3.4 million people, mostly children, die annually due to water-related diseases and poor environmental conditions. According to Address Based Population Registration System (ABPRS) results; while Turkey's total population was 80 million 810 thousand and 525 by the end of 2017, child population was 22 million 883 thousand and 288. While the child population which includes the 0-17 age group according to the definition of the United Nations constituted 48.5% of the total population in 1970, this proportion was 41.8% in 1990 and 28.3% in 2017. In Turkey, adolescents are doubled in the absence of proper guidance and care of a responsible adult. Many of them are from rural families who have moved in search of a better quality of life in urban centres only to find opportunities for well-paid jobs. Low levels of education, entrenched social

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attitudes and customs also affect the protective environment for adolescents of both sexes. Therefore, adolescents are a priority group for health promotion, due to the behaviors that expose them to different situations that represent health risks. For the health professionals to emphasize effective intervention measures, they need to get to know the adolescents' health-related knowledge and practices, as their habits interfere directly with their quality of life in adulthood (7,8).

Pengpid and Peltzer (2010) examined the hygiene behavior of 25,760 school children aged 13-15 years from nine African countries. They reported sub-optimal hygiene behaviors, the proportions of school children reporting optimal (>once a day) tooth brushing (77.3%), was higher than the proportions reported for washing their hands regularly before meals (62.2%), after toileting (58.4%) and washing their hands with soap (35.0%) (9). Arikan et al. (2014) investigated the personal hygiene status in 1521 primary school students. They found that 27.7% of students had inadequate personal hygiene. The number of students with poor hygiene increased with the decreasing maternal education level and household income level. Also, the frequency of having poor hygiene was higher among boys (10). The study was aimed to evaluate the hygiene behavior of middle school students in Ankara – Turkey.

2. METHODS

2.1. Participants and Setting

A cross-sectional study was carried out among adolescents from randomly selected, five public middle schools in the capital city of Turkey, Ankara. Data were collected in March 2016. The study population included 1656 adolescents, 822 boys, and 834 girls were selected through simple random sampling. All participants ranged from 12 to14 years (B=822, 49.7%, G=834, 50.4%). Inclusion criteria were; able to comprehend and communicate using Turkish, having no psychiatric history and being willing to volunteer to complete the questionnaires.

2.2. Instruments

The questionnaires were administered to the students during class hours. Researchers explained the nature of the study to all students and showed them how to complete the questionnaires. With the assistance of the teachers, students were invited to participate in the study and asked to fill out the questionnaires as honestly and accurately as possible. Students were assured of the confidentiality of their responses. In the questionnaire, the students were asked about age, gender, sleeping in the separate bedroom and the educational status of their parents. To consider the information on students' hygiene behavior, the hygiene inventory (HI23) was used.

The HI23 tool assists health professionals to assess the necessary practice populations place on themselves in preventing the spread of healthcare-associated infections. The original HI23 was developed by Stevenson et al. (2009) (11). The scale was adapted to Turkish in 2010 by Altun et al. The coefficient alpha was 0.79 for the HI23. The finding indicates that HI23 is a valid and reliable tool for measuring hygiene management practices in the Turkish population The HI23 comprised 5 sub-scales: general, household, food-related, hand-washing technique, and personal hygiene. The HI23 scale consists of 23 items assessed on a 5-point Likert scale. Higher scores indicating more frequent or higher levels of these practices and representing higher hygiene behavior (12).

Questions 2 to 8 and 12 to 16 had common response options: ''always'' (scored as 4), ''usually'' (3), ''occasionally'' (2), and ''never'' (1). Additional response options were provided for questions 3 (''don't touch them''), 4 (''never eat with hands'') and 9 (''never use public toilets''), all scored as 4 (ie, hygiene-related response). Additional response options also were included for questions 5 (''never prepare food''), 6 (''never prepare food''), 12 (''never handle raw food''), 16 (''never handle raw food''), 14 (''don't use them''), and 14 (''unsure''). If an additional response was endorsed, then an average score (ie, 2.5) was given. Question 1 had the following response options: ''never'' (scored as 1), ''1 to 5'' (2), ''6 to 10'' (3) and ''11'' (4). For question 8, response options were ''less than once a day'' (scored as 4), ''once a day'' (3), ''twice a day'' (2), and ''more than twice a day'' (1). For questions 9 to 11, response options were ''never'' (scored as 1) ''once'' (2) ''twice'' (3) ''three times or more'' (4), and ''unsure.'' ''Unsure'' responses were coded with an average score (ie, 2.5). For question 22, the response options were ''under 5 seconds'' (scored as 1), ''6 to 10 seconds'' (2), ''11 to 20 seconds'' (3) and (21 seconds or more'' (4). Question 19 had the response options ''often'' (scored as 4), ''sometimes'' (3), ''rarely'' (2), and ''never'' (1). Finally, questions 20 to 23 had response options ''never'' (scored as 4), ''rarely'' (3), ''sometimes'' (2) and ''often'' (1).

2.3. Ethical Aspects of the Study

Students were assured of the confidentiality of their responses. The study was conducted in compliance with the Helsinki Declaration. All the materials used in the study were reviewed and approved by the Executive Boards of the Schools. All data collection protocols and research design were approved by the Ethical Commission of the Gazi University, (18/06/2015-26636).

2.4. Statistical Analyses

Statistical analyses were performed with SPSS version 22.0 (Statistical Package for Social Sciences, Chicago, Illinois, United States). Normality was tested using the Shapiro-Wilk test, followed by the chi-square test for comparison between boys and girls. *P* values < 0.05 and < 0.001 were considered statistically significant.

3. RESULTS

The study was carried out on a total of 1656 students consisting of 822 boys (49.7%) and 834 girls (50.4%) adolescents (range, 12-14 years). Of the adolescents included in the study, 30.6 % were 12, 31.8 % were 13 and 37.5% were 14 years old.

In Table 1, maternal education levels of adolescents were secondary (25.9%) and high school (40.3%). Paternal education levels of adolescents were high school (32.6%) and university (26.6%).

According to Table 2, the frequency of washing hands on an average day was significantly higher in girls than boys ($p<0.001$). In addition, handwashing scores were significantly higher in girls after coming home from outside, after touching an animal and before preparing food ($p <0.001$). As shown in Table 2, the girls usually preferred to cover the seat of a public toilet with paper ($p <0.001$).

In the evaluation of food hygiene scores in Table 3, 56.5% of girls usually and 43.1% of boys occasionally washing their hands after handling raw foods and before handling cooked foods ($p<0.05$). There were no significant differences between the scores of boys and girls for washing utensils after handling raw foods and before handling cooked foods. 46.1% of boys and 31.7% of girls stated that they could not be sure of the use of separate chopping boards for raw and cooked foods ($p<0.05$).

Table 4 shows that there was not a significant difference between hand hygiene scores of both sexes. However, the time duration of handwashing (54% of girls and 11.9% of boys tended to wash their hands for 21 seconds or more) scores were significantly higher in girls ($p<0.001$). Also, the habit of using antibacterial gel or wipes scores (while14.9% of girls and 0.5% of boys responded “often”, 39.8% of girls and 25.8% of boys responded “sometimes” to the survey question) were significantly higher in girls ($p<0.05$).

Table 5 shows that 87.1% of boys and 12.9% of girls preferred to wear the same underclothes two days in a row ($p<0.001$). Parents’ educational levels positively influence the hygiene attitude of adolescents.

Table 1. *Distribution of demographic characteristics of adolescents (n:1656)*

Gender	**n**				**%**	
Boy	822				49.7	
Girl	834				50.4	
	Boy (n:822)		**Girl (n:834)**		**Sum**	
Age groups	**n**	**%**	**n**	**%**	**n**	**%**
12	223	27.1	284	34.1	507	30.6
13	271	33.0	257	30.9	528	31.8
14	328	39.9	293	35.0	621	37.5
Maternal Education						
Primary	92	11.2	104	12.5	196	11.8
Secondary	231	28.1	198	23.7	429	25.9
High School	321	39.1	346	41.5	667	40.3
University	178	21.7	186	22.3	364	22.8
Paternal Education						
Primary	77	9.4	93	11.2	170	10.2
Secondary	305	37.0	201	24.1	506	30.6
High School	238	29.0	302	36.2	540	32.6
University	202	24.6	238	28.5	440	26.6

Table 2. *General Hygiene Behaviours of the Adolescents According to H123 Scale (Boy: 822; Girl:834) n: 1656*

General hygiene	Gender	Score 'never'(1)		Score '1 to 5' (2)		Score '6 to 10' (3)		Score '11 +' (4)		Score 'Unsure' (ie,2.5)		χ^2	p
Questions		**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**		
1. On an average day, approximately how many times do you wash your hands?	B	-	-	96	11.7	214	26.0	271	33.0	24	3.3	20.614	<0.001**
	G	-	-	57	6.8	394	47.3	301	36.1	82	9.8		
		Score 'never'(1)		**Score 'occasionally' (2)**		**Score 'usually' (3)**		**Score 'always' (4)**		**Score 'unsure' (ie2.5)**			
2. Upon getting home, do you wash your hands?	B	17	2.0	107	13.1	288	35.0	199	24.2	211	25.7	32.233	<0.001**
	G	9	1.2	84	10.1	344	41.2	301	36.0	96	11.5		
3. After touching a pet or other animal, do you wash your hands?	B	8	1.0	279	33.9	361	43.9	144	17.5	30	3.7	47.066	<0.001**
	G	-	-	69	8.3	402	48.2	345	41.4	18	2.1		
4. Before eating food with your hands, do you wash your hands?	B	12	1.5	296	30.0	182	22.1	278	33.8	54	6.6	49.211	0.039*
	G	4	0.5	102	12.2	296	35.5	355	42.6	77	9.2		
5. Before preparing food, do you wash your hands?	B	-	-	111	13.5	410	49.9	271	33.0	30	3.6	5.477	0.341
	G	-	-	128	15.3	429	51.4	257	30.8	20	2.5		
6. If you need to touch your face or body (eg, to scratch) while preparing food, do you wash your hands?	B	482	58.6	216	26.3	92	11.2	-	-	32	3.9	6.801	0.257
	G	491	58.9	244	29.3	57	6.8	1	0.1	41	4.9		
7. Do you wash fruit and vegetables before you eat them?	B	13	1.5	401	48.8	164	20.0	12	1.5	232	28.2	15.021	0.004**
	G	138	16.5	216	25.9	281	33.7	160	19.2	39	4.7		
8. When you use a public toilet, do you cover the seat with paper?	B	617	75.1	19	2.3	8	1.0	12	1.5	166	20.1	71.119	<0.001**
	G	44	5.3	332	39.8	127	15.2	155	18.6	176	21.1		

(p<0.05), (p<0.001)***

Table 3. *Household Hygiene and Food-Related Hygiene Behaviours of the Adolescents According to H123 Scale (Boy: 822; Girl:834) n: 1656*

Household hygiene Questions	Gender	Score 'never'(1)		Score 'once'(2)		Score 'twice'(3)		Score 'three times or more' (4)		Score 'unsure'(ie2.5)		χ^2	p
		n	%	S	%	S	%	S	%	S	%		
9. How often, in the last month, has your bathroom at home been cleaned?	B	17	2.1	419	51.0	29	3.5	-	-	357	43.4	52.496	0.029*
	G	-	-	107	12.8	441	52.9	9	1.1	277	33.2		
10. How often, in the last month, has your toilet at home been cleaned?	B	3	0.4	442	53.8	218	26.5	6	0.7	153	18.6	47.124	0.024*
	G	-	-	17	2.3	436	52.2	296	35.4	85	10.1		
11. How often, in the last month, has your kitchen at home been cleaned?	B	11	1.4	408	49.6	218	26.5	66	8.0	119	14.5	23.931	0.031*
	G	3	0.4	151	18.1	505	60.6	169	20.2	6	0.7		

Food-related hygiene Questions		score 'never' (1)		score 'occasionally (2)		score 'usually' (3)		score 'always' (5)		score 'unsure' (6)		χ^2	p
		n	%	n	%	n	%	n	%	n	%		
12. After handling raw foods and before handling cooked foods, do you wash your hands?	B	245	29.8	354	43.1	73	8.9	29	3.5	121	14.7	38.516	<0.001**
	G	24	2.9	162	19.4	471	56.5	112	13.4	65	7.8		
13. After handling raw foods and before handling cooked foods, do you wash the utensils used?	B	314	38.2	108	13.1	226	27.5	11	1.4	163	19.8	7.769	0.517
	G	296	35.5	118	14.1	299	35.9	22	2.6	99	11.9		
14. Do you use separate chopping boards for raw and cooked foods?	B	416	50.6	18	2.2	9	1.1	-	-	379	46.1	51.916	0.032*
	G	531	63.7	32	3.8	7	0.8	-	-	264	31.7		

(p<0.05), (p<0.001)***

Table 4. *Hand Hygiene Behaviours of the Adolescents According to H123 Scale (Boy: 822; Girl:834) n: 1656*

Hand hygiene technique	Gender	Score 'never'(1)		Score 'occasionally'(2)		Score 'usually'(3)		Score 'always'(4)		Score 'unsure' (ie 2.5)		χ^2	p
Questions		n	%	n	%	n	%	n	%	n	%		
15. When warm water is available, do you wash your hands with warm water?	B	2	0.2	129	15.7	191	23.2	294	35.8	206	25.1	8.891	0.254
	G	-	-	131	15.7	193	23.1	309	37.1	201	24.1		
16. After washing your hands, do you dry your hands completely?	B	-	-	241	29.3	319	38.8	164	20.0	98	11.9	9.414	0.261
	G	-	-	218	26.1	331	39.7	184	22.1	101	12.1		
17. When soap is available, do you wash your hands with soap?	B	-	-	-	-	341	41.5	468	56.9	13	1.6	4.716	0.672
	G	-	-	-	-	319	38.2	491	58.9	24	2.9		

		'under 5 seconds' Score (1)		'6 to10 seconds' Score (2)		'11 to 20 seconds' Score (3)		'21 seconds or more' Score (4)					
		S	%	S	%	S	%	S	%				
18. When you wash your hands, approximately how long do you wash them for?	B	26	3.2	327	39.8	371	45.1	98	11.9			40.319	<0.001**
	G	3	0.3	172	20.6	209	25.1	450	54.0				
		'Often' Score (4)		'Sometimes' Score (3)		'Rarely' Score (2)		'Never' Score (1)					
		S	%	S	%	S	%	S	%				
19. Do you use antibacterial gel or wipes to clean your hands?	B	4	0.5	77	9.4	212	25.8	84	10.2			48.412	0.041*
	G	124	14.9	332	39.8	324	38.8	54	6.5				

(p<0.05), (p<0.001)***

Table 5. *Personal Hygiene Behaviours of the Adolescents According to H123 Scale (Boy: 822; Girl:834) n: 1656*

Personal hygiene	Gender	Score 'Never'(4)		Score 'Rarely'(3)		Score 'Sometimes'(2)		Score 'Often'(1)		χ^2	p
Questions		S	%	S	%	S	%	S	%		
20. Do you wear the same top or shirt two days in a row?	B	1	0.1	22	2.7	158	19.2	641	78.0	4.578	0.252
	G	-	-	29	3.5	123	14.7	682	81.8		
21. Do you wear the same skirt or pants two days in a row?	B	-	-	-	-	9	1.1	813	98.9	4.680	0.294
	G	-	-	2	0.2	9	1.1	823	98.7		
22. Do you wear the same underclothes two days in a row?	B	18	2.2	69	8.4	19	2.3	716	87.1	21.490	<0.001**
	G	147	17.6	23	2.8	556	66.7	108	12.9		
23. Do you go without a wash, shower or bath two days in a row?	B	-	-	167	20.3	642	78.1	13	1.6	6.741	0.266
	G	1	0.1	146	17.5	669	80.2	18	2.2		

(p<0.05), (p<0.001)***

4. DISCUSSION

Hygiene is generally discussed in the context of preventing the transmission of infection. Hygiene behavior is the regular practices and behaviors associated with the preservation of healthy living (13,14). An estimated one million annual infectious diseases worldwide could be averted by improved hygiene practices (15). Adolescence represents a period of transition when children are establishing autonomy over their behaviors (16). Additionally, it is a stage when teens do not always appreciate help from adults, despite the strong influence of the family on hygiene issues (17). In 2011, Miko et al. examined personal and household hygiene among 299 undergraduate college students In New York. They found that girls noted better personal hygiene than boys (18). Kaya et al. (2006), planned to determine the personal hygiene behaviors of 236 secondary and high students in Ankara. They stated that the average hygiene score of boys was lower than girls (19). Another study among 146,462 middle school students who participated in the Global School-based Student Health Survey from 44 low and middle-income countries presented some differences between the genders (20). According to this study, girls took sufficiently care on their bathing, washing, and tooth brushing. Dorri et al. (2009) investigated the relationship between oral hygiene behaviors and general hygiene behaviors among 1054 Iranian adolescents living in Mashhad. The results showed that girls were significantly more likely than boys to practice oral hygiene and general hygiene behaviors (21). Consistent with previous researches, in our study, we found that most of the hygiene scores of the girls were significantly higher than the boys. It can be said that girl adolescents have been found to show more rigorous behavior about hygiene compared to boys. Possible factors associated with sex differences in hygienic behaviors. For example, the traditional social role may also have a role in influencing hygiene behaviors. Women and girls are often regarded as primary care providers in a family. Also, girls' higher compliance is associated with their tendency to practice socially acceptable behaviors.

Health promotion has been defined as the process of enabling people to increase control over and improve their health. One of the intervention strategies in health promotion is health education. The earliest critical period is believed to be the transition from primary to secondary school. It has been shown that relatively stable patterns of health-related behaviors are established during adolescence (22). Kim et al. (2012) studied to determine the effects of educational intervention on 400 adolescents' hand hygiene behavior. They showed a significant increase in proper handwashing and food safety knowledge after education. Education is very important in raising awareness about hygiene behavior in adolescents (23). Zhou et al. (2015) examined the effectiveness of a self-regulation intervention among 307 Chinese adolescents to identify the effects of planning on hand hygiene. They demonstrated that teaching planning strategies constituted an approach to improve regular hand hygiene practice in adolescents (24).

Some limitations should be taken into account in interpreting our results. Firstly, the self-reported nature of all outcomes means that our data may be influenced by this technique. Secondly, this study did not include an evaluation of lifestyle factors such as smoking, alcohol consumption, eating habits, stressful situations at home.

5. CONCLUSION

In conclusion, our findings reinforce previous studies reported about the hygiene behavior of adolescents, we also illuminate certain subpopulations of middle school students who may benefit from targeted health education. Based on our data, hygiene-promoting programs can be effective for improving hygiene practices and the motivation of adolescents. Visual prompts in addition to educational campaigns may promote better hygienic behavior among adolescents. Besides, expanding the number of participants and applying other analysis techniques can contribute to enhance the range of the results and reduce the study limitations.

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Clinical and Experimental Health Sciences

Surgery Nurses' Awareness on SBAR Communication Model and SBAR Training

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ABSTRACT

Objective: The study has been carried out to analyze surgery nurses' awareness on SBAR communication model and to measure the effectiveness of SBAR training on it. SBAR communication model, which abbreviates "situation, background, assessment and recommendation", undertakes a significant role to provide patient safety.

Methods: It was undertaken between 1 March 2019 – 30 April 2019 with a sample consisting of 138 nurses serving in surgical units of the hospital involved in the study. A session of 45-minute training, which has been delivered to surgery nurses, consisted of example scenarios and practices based on SBAR communication model as well as the user guide and some video shots related to communication settings in compliance with SBAR Model. The data were collected during pre-training, immediately after post-training and one-month after the post-training sessions via face-to face interviews conducted with surgery nurses.

Results: Mean values of nurses' SBAR communication model awareness have been calculated for all 18 questions in the form. Those mean values have been displayed as 62.76±28.52 during pre-training, 95.05±7.56 during immediate post-training and 90.90±13.89 during a-month-later session of post-training. Comparisons of pre-training and post-training scores have displayed a statistically significant increase in mean values, both after the training and one month later ($p<0.05$).

Conclusion: The findings observed in the study show that trainings have a positive effect on raising awareness with respect to SBAR communication model to measure the effectiveness of SBAR training on it.

Keywords: Patient Safety, Health Communication, Patient Care Planning, Nursing

1. INTRODUCTION

Communication is a core element of healthcare services and has a significant impact both on patient safety and clinical results. Healthcare organizations focus on promoting effective communication to prevent negative situations possible to result from communication failures (1). Patient safety is crucial for the delivery of effective, high-quality healthcare and is defined by the World Alliance for Patient Safety of World Health Organization (WHO) as follows: ¨The reduction of risk of unnecessary harm associated with healthcare to an acceptable minimum¨ (2). WHO states that every year, circa 10 million people encounter problems originated from avoidable medical practices, that's why WHO recommends making more research on patient safety (3). The objective of the concept is to maintain safety for patients, patient relatives and healthcare professionals by excluding potential factors that may physically and psychologically affect the healthcare environment in a negative manner (4). Regarding the report published by the Institute of Medicine in 1999, Joint Commission on Accreditation of Healthcare Organizations (JCAHO) has initiated implementations of patient safety all over the world, which have been collaborated by the Joint Commission International (JCI). Being one of International Patient Safety Goals, which was announced by JCI in 2006 and updated in 2014, improvement of effective communication becomes a key factor for sustainable patient care and ensures safe care practices (5,6). Effective communication failures in healthcare result in unexpected and critical outcomes for patients; retarded patient care, surgical mistakes, falling off, extended hospitalization, serious injuries and even death are some examples of those critical outcomes (7). Among effective communication

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techniques, the most widely used one, which covers a place of 69.6%, is SBAR (Situation-Background-Assessment-Recommendation) Communication Model (8). It is an evidence-based professional communication model which creates a framework for how to convey patient-related information through short-cut, clear, quick and explicit ways to other health professionals, especially in emergency situations (9, 10). Communication among healthcare professionals is required for high-quality care and patient safety particularly when critical cases occur. Nevertheless, SBAR awareness and utilization of this communication model in clinical units are considered to realize less commonly. In that context, this research, which has been planned as a descriptive study, aims to analyze SBAR communication model awareness of surgery nurses before and after SBAR training, considering pre-training, immediate post-training and the first subsequent month results.

2. METHOD

2.1. Research Design and Sample

The research has been carried out as a descriptive study to identify SBAR communication model awareness of nurses who work at surgery units of a university hospital. The study universe comprised 220 surgery nurses working at the surgery service, surgical ambulatory care and surgery room of the hospital during 1st March 2019 – 30th April 2019. The sampling has consisted of 138 surgery nurses who were selected by simple random sampling method and who were up to research criteria and confirmed their participation in the survey.

2.2. Data Collection

The research data have been acquired through face-to-face interviews of the researcher. As a starting step, "Personal Info Sheet" and "SBAR Communication Model Assessment Form" have been completed by participants as a pre-test, prior to the training. The 45-minute training session has been initiated by a Power Point slideshow delivering information to surgery nurses about SBAR communication model. In video shots on communication practices, the use of SBAR communication model has been explained through example scenarios. In order to guide further, SBAR Communication Model brochure has been delivered to nurses before they leave the training room. Post-test was applied to participants, again during face-to-face interviews, just after the training and one month later.

"Personal Info Sheet" and "SBAR Communication Model Assessment Form" have been utilized for data collection. Both tools have been prepared by researchers after consulting the field experts and referring to the literature (11-13).

Personal Info Sheet consists of 5 questions concerning personal features of nurses (age, gender, education, department where they are employed and duration of their employment).

SBAR Communication Model Assessment Form consists of questions determining surgery nurses' awareness on SBAR communication model and previous training experience about the concept. In addition, the frequency of their use of SBAR model and its effectiveness has also been checked through questions. In the form, there are 18 True-False questions to assess surgery nurses' SBAR communication model awareness levels. The scale varies from 0 to 1 as the former representing false and the latter representing true responses. Thus, the minimum score becomes 0 and maximum score reaches to 18. The calculation results have been standardized by converting them to 100 point scaling. Kuder-Richardson-21 test has been used to measure the internal consistency value of SBAR Communication Model Assessment Form through its 18 true-false statements. Considering validity results, the form has been found to be highly valid and reliable (Table 1).

Table 1. *SBAR Communication Model Assessment Form Validity and Reliability*

SBAR Communication Model Assessment Form	Kuder-Richardson-21 Info Model
Pre-Training (18 questions)	0.896
Post-Training (Immediate) (18 questions)	0.791
Post-Training (1st Month) (18 questions)	0.821

Research question

How high is SBAR communication model awareness of nurses?

How high is the impact of SBAR training on surgery nurses?

2.3. Ethical Consent

In order to carry out the research, written consent of the Non-initiative Clinical Research Ethical Committee (No: 2019.23.02.07), which is affiliated to the Faculty of Medicine, has been taken as well as the written permission of Health Practices and Research Centre, where the study would be implemented. Surgery nurses who would take part in the survey have been informed about the research procedures before getting their oral and written consent. Regarding the privacy policy, the identities of participants have not been disclosed in the article.

2.4. Statistical Analysis

The institutional statistics program has been utilized to process the data. Descriptive statistical methods such as mean, standard deviation, median, frequency, percent,

minimum and maximum scores have all been performed during analyses. Shapiro-Wilk test and graphical tools have been used to measure the normality of the population. In addition, Mann-Whitney U test has been used to compare two groups of not normally distributed variables and Kruskall Walls test has been conducted to compare three or more groups derived from not normally distributed variables. Pair-wise adjustments have been done through Bonferroni-Dunn post hoc test. The validity and reliability of 18 items in Nurses' SBAR Communication Model Assessment Form has been tested by Kuder Richardson-21, whereas Friedman test measured the effects of not normally distributed variables. Bonferroni-Dunn test has been again performed to reach pair-wise comparisons of those variables. For the interpretations of analyses, $p<0.05$ has been accepted as the level of statistical significance.

3. RESULTS

The study has been conducted with 138 surgery nurses in total; 86.2% (n=119) of whom have been females and 13.8% (n=19) has been composed of males. The age average of participants has been 29.52±5.75 years. 71.8% of participants were graduates with Bachelor's degree. Among the participants, 71% has been employed in surgery service units whereas 37.7% of them having professional background of 1-5 years.

Table 2. *Distribution of Knowledge and Training Background about SBAR Communication Model*

		Pre-Training		Post-Training Immediate		Post-Training 1st. Month	
		n	%	n	%	n	%
Knowledge about SBAR	Yes	28	20,3	138	100	138	100
	No	110	79,7	-	-	-	-
Training Background about SBAR	Yes	15	10,9	138	100	138	100
	No	123	89,1	-	-	-	-
Training Setting	Formal	2	13,3	2	1,4	2	1,4
	In-service	13	86,7	136	98,6	136	98,6

According to pre-training results, participants' knowledge about SBAR communication model and their eagerness to use the model seems at very low percentages though the rates keep inclining just after the training and one month later (Table 2).

The responses by nurses to the questions ¨Do you think SBAR communication model is effective on patient safety?¨ and ¨Would you like to practice SBAR communication model in your professional life?¨ display low rates for pre-training stage. However, the rates referring to its effectiveness and nurses' willingness to practice in clinic settings show an increase both at immediate post-training and at the-first-month stages (Table 3).

Table 3. *Distribution of the Effectiveness of SBAR on Patient Safety and Willingness to Use in Professional Settings*

	Pre-training		Post-training (Immediate)		Post-training (1st month)	
	n	%	n	%	n	%
Do you think SBAR communication model is effective on patient safety?						
Never effective	6	4,3	1	0,7	1	0,7
Not really effective	3	2,2	1	0,7	0	0
Partially effective	33	23,9	4	2,9	12	8,7
Effective	43	31,2	20	14,5	28	20,3
Very effective	53	38,4	112	81,2	97	70,3
Would you like to practice SBAR communication model in your professional life?						
I never use it	41	29,7	11	8,0	3	2,2
I don't use it	16	11,6	10	7,2	2	1,4
I sometimes use it	53	38,4	23	16,7	30	21,7
I use it	17	12,3	26	18,8	35	25,4
I always use it	11	8	68	49,3	68	49,3

Regarding the distribution of pre-training responses to questions measuring SBAR awareness among nurses, the lowest accuracy score goes to the statement "I believe that SBAR communication model urges me to questioning after a stressful situation" whereas the highest accuracy score goes to "I believe communication problems in patient care may lead to serious medical errors". Compared to those pre-training results, all the accurate responses given by nurses tend to increase just after the training and one month later (Table 4).

Table 4. *Distribution of SBAR Communication Model Awareness Levels*

SBAR Communication Model	Pre-Training		Post-Training (Immediate)		Post-Training (1st month)	
	True n %	False n %	True n %	False n %	True n %	False n %
1.I believe that communication problems in patient care may lead to serious medical errors.	109(79)	29(21)	134(97.1)	4(2.9)	127(92)	11(8)
2.SBAR enhances communication with physician in the clinic.	85(61.6)	53(38.4)	129(93.5)	9(6.5)	124(89.9)	14(10.1)
3.SBAR is not a reliable and approved communication tool that reduces adverse cases.	80(58)	58(42)	132(95.7)	6(4.3)	122(88.4)	16(11.6)
4.SBAR is a professional communication technique that facilitates short-cut, clear, thorough and standardized communication among healthcare professionals in order to achieve prevalent exchange of information.	97(70.3)	41(29.7)	134(97.1)	4(2.9)	135(97.8)	3(2.2)
5.SBAR communication model denotes the abbreviation of 'Situation, Background, Assessment, Recommendation'.	95(68.8)	43(31.2)	138(100)	0(0)	134(97.1)	4(2.9)
6.When referring to part ¨S¨ of SBAR communication model, the question ¨What is the current situation of the patient?¨is responded.	90(65.2)	48(34.8)	137(99.3)	1(0.7)	136(98.6)	2(1.4)
7.When referring to part ¨B¨ of SBAR communication model, the question ¨Why does the patient come? / What is his/her clinical background?¨is responded.	92(66.7)	46(33.3)	134(97.1)	4(2.9)	132(95.7)	6(4.3)
8.When referring to part ¨A¨ of SBAR communication model, the question ¨In my opinion, what is the problem?¨ is responded.	80(58)	58(42)	135(97.8)	3(2.2)	127(92)	11(8)
9.When referring to part ¨R¨ of SBAR communication model the question ¨What is my recommendation?¨ is responded.	92(66.7)	46(33.3)	135(97.8)	3(2.2)	135(97.8)	3(2.2)
10.SBAR creates a lingua franca (common language) for healthcare professionals.	103(74.6)	35(25.2)	133(96.4)	5(3.6)	115(83.3)	23(16.7)
11.SBAR Communication Model is not helpful for easy recognition and encouraging prioritized preparations for communication**.**	76(55.1)	62(44.9)	123(89.1)	15(10.9)	96(69.9)	42(30.4)
12. In the Recommendation section, an advice can be given to the physician.	58(42)	80(58)	120(87)	18(13)	129(93.5)	9(6.5)
13.SBAR improves communication, increases patient safety.	103(74.6)	35(25.4)	136(98.6)	2(1.4)	134(97.1)	4(2.9)
14.SBAR Communication Model is a structured communication tool that displays reduction of negative cases in hospital settings.	88(63.8)	50(36.2)	136(98.6)	2(1.4)	127(92)	11(8)
15. The rationale of a patient's problem should be dwelled on during the Situation section.	80(58)	58(42)	129(93.5)	9(6.5)	127(92)	11(8)
16.SBAR can be used in all occasions where patient's data is conveyed.	81(58.7)	57(41.3)	126(91.3)	12(8.7)	128(92.8)	10(7.2)
17.I believe that bedside delivery via SBAR Communication Model helps patient care.	97(70.3)	41(29.7)	135(97.8)	3(2.2)	123(89.1)	15(10.9)
18.I believe that SBAR communication model urges me to questioning after a stressful situation	53(38.4)	85(61.6)	115(83.3)	23(16.7)	107(77.5)	31(22.5)

Nurses' pre-training awareness scores for SBAR communication model vary on a scale between 0 and 100. However, both post-training scores and the ones received in the first month are found to be higher than the pre-training results ($p<0.01$) (Table 5).

Considering the distribution of SBAR awareness scores in units, a statistically significant difference emerges from the comparison of post-training and pre-training scores of various groups ($p<0.05$). The results exhibit higher difference for nurses working in the operation room than their counterparts working in the ambulatory care unit ($p=0.049$; $p<0.05$) (Table 6).

Table 5. *Assessment of SBAR Communication Model Awareness Scores*

	SBAR Communication Model Awareness Scores	
	Min.-Max. (Median)	Mean±Std. Dv.
Pre-Training	0-100 (66,7)	62,76±28,52
Post-Training (Immediate)	66,7-100 (100)	95,05±7,56
Post-Training (1st. Month)	11,1-100 (94,4)	90,90±13,89
Test value	χ^2:145,591	
[a]***p***	***0,001****	
Difference (Pre-T. – Post-T. Im.)	-16,7/100 (27,8)	32,29±29,07
Difference (Post-T. 1st M. – Pre-T.)	-44,5/100 (22,2)	28,14±30,32
Difference (Post-T. 1st M. – Post-T. Im.)	-83,3/22,2 (0)	-4,15±14,60

Table 6. *Assessment of SBAR Communication Model Awareness Scores Regarding the Unit of Employment*

		Unit of Employment			*Test value* [b]*p*
		Surgery Service (n=98)	Ambulatory Care (n=24)	Operation Room (n=16)	
Pre-Training	*Min.-Max. (Median)*	0-100 (66,7)	27,8-100 (75)	0-100 (50)	χ^2:2,445
	Mean±Std. Dv.	61,91±28,48	71,99±19,90	54,17±36,82	***0,294***
Post-Training (Immediate)	*Min.-Max. (Median)*	66,7-100 (100)	72,2-100 (94,4)	88,9-100 (100)	χ^2:13,165
	Mean±Std. Dv.	95,29±7,28	91,20±9,11	99,31±2,78	***0,001****
Post-Training(1st. Month)	*Min.-Max. (Median)*	11,1-100 (94,4)	61,1-100 (94,4)	77,8-100 (94,4)	χ^2:0,286
	Mean±Std. Dv.	90,48±15,28	90,74±11,31	93,75±6,69	***0,867***
	Test value	χ^2:109,009	χ^2:20,076	χ^2:17,760	
	[a]***p***	***0,001****	***0,001****	***0,001****	
Difference (Pre-T. / Post-T. Im.)	*Min.-Max. (Median)*	-16,7/100 (27,8)	0/61,1 (13,9)	0/100 (50)	χ^2:6,678
	Mean±Std. Dv.	33,39±28,51	19,21±19,59	45,14±37,62	***0,035****
Difference (Post-T. 1st. M. / Pre-T.)	*Min.-Max. (Median)*	-44,5/100 (22,2)	-11,1/61,1 (8,3)	-11,1/88,9 (44)	χ^2:3,395
	Mean±Std. Dv.	28,57±30,48	18,75±23,04	39,58±35,94	***0,183***
Difference (Post-T. 1st. M. / Post-T. Im.)	*Min.-Max. (Median)*	-83,3/22,2 (0)	-22,2/22,2 (0)	-22,2/5,6 (-5,6)	χ^2:2,491
	Mean±Std. Dv.	-4,82±16,34	-0,46±9,54	-5,56±7,31	***0,288***
[a]*Friedman Test*	[b]*Kruskal Wallis Test*	**p<0,05*	***p<0,01*		

4. DISCUSSION

Current nursing care focuses on therapeutic communication with patients and general human communication aspect (14). Surgery services, ambulatory care units and operation rooms are departments where emergency responses and initiatives frequently occur, critical check-outs and quick decision making emerges and communication-based teamwork is required (15, 16). Our research has been conducted with nurses employed at surgical service units, ambulatory care units and operation rooms. Pre-training results have shown that participants' knowledge of SBAR communication model was at low percents but the training has created awareness of nurses and this awareness has been triggered by training repetitions. Chetwood et al [12] has stated that only 15% of the participants had some knowledge about SBAR communication model prior to their training (12). Practicing out inter-professional communication in an evidence-based manner is considered is vital for nursing and especially for patient safety. Contradicting to that importance, study results have revealed both the knowledge and the use of SBAR have been scarce before the training, just to verify the previous literature (1, 12, 17). The low rate of surgical nurses' SBAR knowledge deficiency prior to training is considered to emerge from the limited coverage of SBAR modules in both formal education and in-service programmes. In our study, prior to the training, very few nurses have mentioned the effectiveness of SBAR and their willingness to use the model in the clinic whereas this number has increased after the training. An earlier study shows the improvement of team work, safety climate and job satisfaction among healthcare professionals via the use of SBAR model (17). Another research informs that after using SBAR, nurses have encountered fewer errors and felt higher self-confidence due to the facilitating power of the model on inter-professional communication (1). Raymond and Harrison's study, which is about the use of SBAR Communication Model in neonatology, reports SBAR use before the training at 29 % though with a rising rate to 70 % after the training (18). According to Achrekar et al. who analyze the impact of SBAR on nursing practices, 63% of nurses state that SBAR communication model will totally increase patient safety while 37% saying the increase will be partial (11). Bloom at al.'s research, which compares pre-intervention and post-intervention opinions of surgeons and surgery nurses, reveals that after the training, the majority of surgeons and nurses agree with the statement suggesting ¨SBAR communication model ensures patient safety by improving communication¨ (19). De Meester et al. figure out the effectiveness of SBAR on early diagnosis of the changes in patients' general situations and the planning of nursing care (1). The significance of SBAR communication model for patient safety is also emphasized by previous literature (1, 11, 17-19). Patient's information to be conveyed becomes standardized in a framework by SBAR communication model. Thus, SBAR model enhances team-wise work by avoiding the loss of relevant information and that ensures its vitality for patient safety. The study reveals the need for the recognition and practice of SBAR communication model by healthcare professionals.

Limitation

The study has been carried out with surgery nurses only in one centre. It has not been tested whether SBAR was being used by nurses before or not, only their statements have been analyzed through the form. Therefore, it is not possible to generalize the study results to all nurses and other departments. It is recommended to perform more researches with larger sampling groups to comment on the use of SBAR as well.

5. CONCLUSION

SBAR training delivered to nurses has enhanced the perception of effective communication and cooperation by healthcare professionals. Compared to pre-training results, it has been detected that nurses' SBAR awareness after the training has increased and a rising trend has appeared about the use of the model in clinics. It is recommended to deliver trainings on communication models in formal education and its sustainability should be allowed via in-service training programmes.

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Clinical and Experimental Health Sciences

The Effects of Different Pre-operative Information Timings on Patients' Anxiety Level and Pain Perception

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ABSTRACT

Objective: The purpose of this study was to investigate the impact of different timings of pre-operative information on patients' anxiety level and pain perception after open flap debridement.

Methods: 45 individuals with chronic periodontitis were equally allocated to the following groups: Group 1: Informed 7 days before surgery; Group 2: Informed 1 month before the surgery and Group 3: Informed by other dentists. All patients received open flap debridement. The anxiety level before surgery was evaluated by State-Trait Anxiety Inventory (STAI) and the pain perception by Visual Analogue Scale (VAS) on the days 1, 3, and 7 after surgery.

Results: The state anxiety levels significantly increased immediately before surgery in all groups ($P<0.05$). No statistically significant difference was found in terms of STAI and VAS between the groups ($P>0.05$).

VAS 1st and 3rd day values were significantly correlated with STAI-Trait values (r=0.311, $P=0.038$ and r=0.422, $P=0.004$, respectively).

Conclusion: Pre-operative information provided by dentists at different times has no effect on post-operative pain and anxiety of the patients. Post-operative pain is associated with pre-operative anxiety. Determining the anxiety levels of patients before the operation acts an important part in the success of periodontal surgical treatments and pain management.

Keywords: Anxiety, Chronic Periodontitis, Postoperative Pain, Pain Assessment, Surgical Flap.

1. INTRODUCTION

Most of the dental treatments cause a certain level of anxiety affecting the course of the treatment in a negative way in patients. Bad experiences or complicated treatments in the past may affect the psychology of the patients, and in some cases, this occurs subconsciously resulting in fears called dental phobia (1). The fear and anxiety resulting from the previous experiences are difficult to overcome and can cause them to be stressed and feel tense during the treatment. In parallel with this, patients who are more stressed in their daily lives have higher levels of anxiety during the treatment. Moreover, periodontal disease can become more severe with increased stress levels, which is a risk factor, and periodontal destruction can progress gradually. Increased anxiety may cause the dentist to become more nervous during the treatment and may decrease the success of the procedure. Therefore, reducing stress levels in patients is one of the important impacts that may increase the success rate in all dental treatments (2,3).

The effect of anxiety level on pain perception has been shown previously (4). Pain levels have been shown to be higher in patients with high levels of anxiety, excitement and fear (5,6). Among the patients with high anxiety levels, those with low pain thresholds have also higher pain expectations after stressful dental treatments (4). When patients experience excessive pain after the surgery, they tend to disrupt oral care, ending in complications of wound healing with the increase in post-operative analgesic use due to anxiety (5). Patients who undergo emergency surgery needed to use more post-operative analgesics due to the lack of pre-operative information and psychological preparation (7). Therefore, minimizing stress level may well be beneficial in

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terms of post-treatment patient comfort and post-operative complications for the success of periodontal treatments.

Patients who require surgical periodontal treatment usually obtain information about the necessity of surgery from dentists, oral medicine specialists or periodontists. In particular, the patients who are advised to see a periodontist by another dentist with the knowledge that they should have periodontal surgery can investigate this operation from different sources until the surgery. Thus, some doubts arise in their minds and anxiety levels may increase. On the other hand, it may be beneficial to inform patients about the procedure in advance and prepare them for the treatment. Information about dental procedures is usually given to the patients in detail immediately before the procedure. However, the stress level of the patient is at the maximum level in this period. Therefore, it may be recommended to inform the patients in detail in the previous sessions, not immediately before the procedure, in order to reduce their concerns (8).

VAS has been used previously in many studies to assess pain after periodontal surgery (9–12). The State-Trait Anxiety Inventory (STAI) (13) was used in this present study to measure state anxiety and trait anxiety. The validity and reliability of the Turkish version of STAI were confirmed by Le Compte and Öner (14,15). The inventory has two separate scales of 20 items each which are rated on a 4-point Likert-type scale. The first questionnaire, STAI-State (STAI-S), measures the level of state anxiety and determines how an individual feels at a given moment and under certain conditions. The second questionnaire, STAI-Trait (STAI-T) determines how patients feel about their general life, regardless of the circumstances and conditions in which they are concerned, in order to measure the trait anxiety level.

To the best of our knowledge, there is no study conducted on the effect of optimum timing of pre-operative information for periodontal surgical procedures in the literature. The purpose of this study was to investigate the impact of different timings of pre-operative information on patients' anxiety levels and pain perception. In our study which was performed on patients who had not undergone periodontal surgery before, we also aimed to analyze the correlation between anxiety and post-operative pain scores after periodontal flap surgery. The tested hypothesis was that timing of pre-operative information would affect patients' anxiety levels and pain perception.

2. MATERIALS AND METHODS

2.1. Patient Selection

The study protocol was approved by the Ethical Committee of Istanbul Aydın University, Faculty of Dentistry (Approval No: 480.2/011) and was carried out in accordance with the Helsinki protocols and the ethical requirements (16). After explaining the nature, risks, benefits, and procedures of research, informed written consent was obtained from all participants. The enrollment period was from March 2015 to December 2016.

The study was conducted on a total of 45 participants diagnosed clinically and radiographically as chronic periodontitis (CP) according to the International Classification of Periodontal Diseases and Conditions in 1999 (17). Patients requiring open flap debridement surgery in at least one quadrant with at least three teeth after non-surgical periodontal therapy were seen eligible to participate.

2.2. Sample Size Calculation

The sample size was calculated according to a previous study employing the same method (18). Power analysis calculations showed that each group requires at least 15 patients, with a confidence interval of 95% (α=0.05).

2.3. Inclusion Criteria

1. Age ≥18 years,
2. Diagnosis with chronic periodontitis requiring open flap debridement surgery,
3. No contraindication for periodontal surgery,
4. Not receiving periodontal treatment in the past 6 months from the date of enrollment,
5. Presence of at least 20 natural teeth.

2.4. Exclusion Criteria

1. Any evidence of severe or uncontrolled systemic diseases that may affect the outcome of periodontal treatment,
2. Pregnancy or lactation,
3. Taking any medication that interferes with the health of the periodontal tissues or their healing,
4. Psychiatric disorders and the use of psychotropic agents,
5. Taking anti-inflammatory drugs or systemic antibiotics in the past 6 months from the date of enrollment,
6. Alcohol or drug addiction,
7. History of aggressive periodontitis,
8. Previous history of periodontal or dental surgery,
9. Periodontal regenerative procedures.

2.5. Study Design and Clinical Procedures

The demographic data of patients, including gender, age, and smoking status were recorded. The panoramic radiographs were taken, and periodontal clinical parameters were recorded as a part of routine diagnosis. All patients received initial periodontal therapy (IPT). Open flap debridement was planned for residual pockets with probing pocket depths of 5-8 mm and clinical attachment loss of 3-4 mm exhibiting clinical signs of inflammation (Bleeding on Probing (BoP)

and/or suppuration) following IPT. If the patient could not maintain an adequate standard of plaque control (The average individual scores for Plaque Index (19) and Gingival Index (20) were less than 1), no surgical treatment was scheduled. The patients were allocated to one of the following three groups:

Group 1: Informed 7 days prior to periodontal surgery (n = 15).

Group 2: Informed 1 month prior to periodontal surgery (n = 15).

Group 3: Regardless of information timing, informed by other dentists before referred to the Periodontology clinic (n = 15).

The patients in Group 1 and 2 were randomly assigned to each group by tossing of coin by an independent researcher who did not collect the data or perform the procedures. Individuals were given structured and standardized information about the operations at different times and their anxiety levels were compared. The potential association between timing of information, patients' demographic data and anxiety levels was also investigated.

2.6. Periodontal Clinical Parameters

The following clinical parameters were recorded in a sequential order at baseline and 4 weeks after IPT by blinded experienced periodontist (N.T.) who did not perform the surgical procedures: Plaque Index (19) (PI), Gingival Index (20) (GI), probing pocket depth (PPD), BoP (21), gingival recession (GR) and clinical attachment level (CAL). The periodontal clinical parameters were used only for the diagnosis, and thus, no statistical evaluation on these parameters was performed.

2.7. Initial Periodontal Therapy

After completing the baseline monitoring, the participants received IPT including oral hygiene instructions, full mouth scaling and root planning under local anesthesia and occlusal therapy if necessary. Throughout the research process, patients were provided proper oral hygiene instructions and maintained self-performed oral hygiene. Scaling and root planning were performed once a week for 2 weeks by a blinded experienced periodontist (B.K.). IPT did not include any medications such as non-steroidal anti-inflammatory drugs or antibiotics.

2.8. Surgical Procedure

The surgical procedures were performed under local anesthesia by an experienced periodontist (B.K.) who also performed IPT. Following the intracrevicular incision, a mucoperiosteal periodontal flap was elevated. Only when necessary, vertical releasing incisions were performed to obtain a better access or closure of the surgical site. Periodontal defects were carefully debrided and the defects and the adjacent mucoperiosteal flaps were rinsed thoroughly with sterile saline. The root surfaces were thoroughly scaled and planned. The flaps were repositioned and primarily sutured.

2.9. Post-operative Care

The surgical procedures were performed under local anesthesia by an experienced periodontist (B.K.) who also performed IPT. Verbal and written post-operative instructions were given to all patients. Analgesics (2x275 mg non-steroidal anti-inflammatory drug i.e. NSAID/day) were prescribed when needed. The number of analgesics taken was recorded for 7 days. In addition, all patients were instructed to rinse with a 0.2% chlorhexidine gluconate solution for 60 seconds twice daily for 1 week after surgery for post-operative plaque control. The patients were advised not to smoke during the healing period. The sutures were removed 1 week after the surgery.

2.10. Pain Evaluation

The pain levels of the patients were recorded at post-operative days 1, 3, and 7 using a visual analogue scale (VAS) (22). The VAS used in this study consisted of a horizontal 10 cm line with no other sign, with descriptors 'no pain' at the beginning and 'worst pain ever' at the end. There were no other markings on the line. The patient was asked to place a mark on this line to indicate the level of intensity of pain. The distance from 'no pain' (0) to the point marked by the patient was measured and recorded in centimeters.

2.11. State-Trait Anxiety Inventory (STAI)

In answering the STAI-S, patients were asked to choose one of the following responses: 1) not at all, 2) somewhat, 3) moderately so, and 4) very much so; while answering the STAI-T, patients were asked to choose one of the following responses: 1) almost never, 2) sometimes, 3) often, and 4) almost always, respectively. The total score for each subscale varies between 20 and 80. High levels of anxiety are associated with higher scores.

STAI-S and STAI-T questionnaires were applied respectively at the beginning of the study (t1). All patients completed the questionnaire by themselves. Unclear questions were explained by the researcher. The STAI-S questionnaire was repeated immediately after the patients were informed about the surgery (t2) and immediately before the operation (t3). The patients in group 3 were previously referred to the periodontology clinic after receiving information about the surgery. Therefore, STAI-S was not applied immediately after the patients were informed about the surgery in this group.

2.12. Statistical Analyses

All statistical analyses in this study were performed using Number Cruncher Statistical System (NCSS 2007 Statistical Software, Utah, USA). Continuous variables are expressed as mean and standard deviation (SD) values. The Friedman

test for repeated measures of multiple groups, the Kruskal Wallis test for comparisons between the groups, the Dunn's multiple comparison test for subgroup comparisons, the Wilcoxon test for two-way repeated measures of groups, the Mann-Whitney U test for comparison of paired groups, the Chi-square test for comparison of the qualitative data, and the Pearson Correlation test to determine the strength of association between the variables were used. A *P* value less than 0.05 was considered statistically significant.

3. RESULTS

No postoperative complication was observed in any participants. A total of 45 patients (21 males, 24 females) with a mean age of 44.2±12.16 participated in the study. In any demographic data, there was no statistically significant difference between the groups (*P*>0.05) (Table 1). The differences in the amount of analgesic consumed and the amount of smoking were not statistically significant between the groups (*P*>0.05) (Table 1).

Table 1. *The demographic data of the participants in the groups.*

		Group 1 (n=15)		Group 2 (n=15)		Group 3 (n=15)		P*
Age (mean±SD)		47.47±12.88		43.13±10.88		44.2±12.16		0.592
Gender (n, %)	Male	6	40.00%	7	46.67%	8	53.33%	0.765
	Female	9	60.00%	8	53.33%	7	46.67%	
Smoking status (n, %)	Smoker	7	46.67%	9	60.00%	9	60.00%	0.677
	Never smoker	6	40.00%	3	20.00%	1	6.67%	
	Former smoker	2	13.33%	3	20.00%	5	33.33%	
The number of cigarettes (mean±SD)		13.40±7.09		7.33±2.52		8.6±4.72		0.399
Use of analgesics (n, %)	+	5	33.33%	4	26.67%	3	20.00%	0.711
	–	10	66.67%	11	73.33%	12	80.00%	
The number of analgesics used (mean±SD)		1.9±1.2		3.27±1.95		3.67±2.31		0.101

*SD: Standard Deviation, n: Number. * P<0.05 was considered statistically significant (The Kruskal Wallis test and the Chi-square test).*

STAI-S and STAI-T

The anxiety levels of the groups at different times are shown in Table 2. Group 1 exhibited a statistically significant increase in STAI-S between t1, t2 and t3 times (*P*=0.006). STAI-S at t3 time was found to be significantly higher than t1 and t2 (*P*=0.006 and *P*=0.021, respectively), although there was no statistically significant difference between t1 and t2 times (*P*=0.176).

In group 2, there was a statistically significant increase in STAI-S between t1, t2 and t3 times (*P*=0.003). At the time t1, STAI-S was significantly lower than t2 and t3 (*P*=0.036 and *P*=0.003, respectively) and a statistically significant difference was also found between t2 and t3 (*P*=0.202).

In Group 3, STAI-S was found to be statistically significantly higher at t3 time than t1 (*P*=0.019).

The STAI-S of Group 3 was higher than Group 1 and Group 2 at t1, although this difference was not statistically significant (*P*=0.052). There were no statistically significant differences between groups at any time point in STAI-S and at t1 in STAI-T (*P*>0.05).

Table 2. *STAI-S and STAI-T values of the study groups at different times.*

STAI-S (mean±SD)	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	P*
t1	29.47±08.77	29.47±7.09	38.00±14.74	0.052
t2	31.93±10.69	33.93±8.61	NA	0.577
t3	37.00±11.92	36.47±8.37	41.93±13.00	0.351
P†	**0.006**†	**0.003**†	**0.019**†	
STAI-T (mean±SD)	42.87±05.22	42.80±04.81	42.27±07.92	0.958

*STAI: State-Trait Anxiety Inventory, STAI-S: STAI-State, STAI-T: STAI-Trait, SD: Standard Deviation, n: Number, NA: Non-available, t: Time. * P<0.05 was considered statistically significant (The Kruskal Wallis test and the Chi-square test). † P<0.05 was considered statistically significant (The Friedman test and the Wilcoxon test).*

VAS

The pain perceptions of the groups at different times are shown in Table 3. There was a statistically significant decrease in VAS of Group 1 between days 1, 3, and 7 (*P*=0.0001). The VAS of day 1 was found to be significantly higher than days 3 and 7 (*P*=0.003), although there was no statistically significant difference between the VAS on day 3 and day 7 (*P*=0.238).

There was a statistically significant decrease in VAS of Group 2 between days 1, 3 and 7 (*P*=0.0001). The VAS of day 1 was found to be significantly higher than days 3 and 7 (*P*=0.0001). The VAS on day 1 was significantly higher than VAS on day 3 and day 7 (*P*=0.0001), and the VAS on day 3 were significantly higher than VAS on day 7 (*P*=0.015).

There was a statistically significant decrease in VAS of Group 3 between days 1, 3, and 7 (*P*=0.0001). The mean VAS of day 1 was found to be statistically significantly higher than the mean of day 3 and 7 (*P*=0.001 and *P*=0.0001, respectively), although there was no statistically significant difference between the VAS on day 3 and day 7 (*P*=0.179).

There was no statistically significant difference between the groups at any time point (*P*>0.05). VAS 1st and 3rd day values were significantly correlated with STAI-T values (r=0.311, *P*=0.038 and r=0.422, *P*=0.004, respectively).

Table 3. *Pain perception of the groups at different times.*

VAS	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	P*
Day 1 (mean±SD)	1.500±1.603	2.753±1.623	2.647±2.403	0.151
Day 3 (mean±SD)	0.133±0.419	0.867±1.175	0.720±1.880	0.275
Day 7 (mean±SD)	0.000±0.000	0.067±0.209	0.040±0.155	0.480
P†	**0.0001†**	**0.0001†**	**0.0001†**	

*VAS: Visual Analogue Scale, SD: Standard Deviation, n: Number. * P<0.05 was considered statistically significant (The Kruskal Wallis test and the Chi-square test). † P<0.05 was considered statistically significant (The Friedman test and the Wilcoxon test).*

4. DISCUSSION

The main purpose of periodontal treatment is to stop or prevent periodontal infection by removing pathogenic periodontal microflora and trying to eliminate risk factors, but also to provide regeneration in periodontal supporting tissues (23). Surgical treatment techniques such as open flap debridement are frequently applied for this purpose, particularly in the management of severe periodontitis, especially with severe clinical attachment loss (11). In the treatment and long-term maintenance of patients with chronic periodontitis who require open flap debridement, stress and anxiety can affect the severity of the disease, the course of the treatment, and also the postoperative pain level (2,3). Therefore, stress and anxiety levels should be minimized in surgical procedures with the aim of providing maximum periodontal recovery and minimum complications (2). Different and unknown therapies can cause a certain level of anxiety in patients, and the anxiety level of patients who receive information from other sources, such as other patients who receive treatment and the internet, may increase during the time until the operation (4). In our study, we sought an answer to the question of when the pre-operative information should be transmitted, which may affect the treatment process by affecting the stress and anxiety levels in periodontal flap operations. To the best of our knowledge, there is no article in the literature that investigates the effect of timing of pre-operative information on these parameters.

In our study, state anxiety levels significantly increased immediately before surgery in all groups, as expected. However, no statistically significant difference was found in terms of anxiety between different timings of pre-operative information. The levels of anxiety state at baseline were found to be higher in patients who were referred by another dentist with the knowledge of prospective surgery. However, the anxiety levels of patients in this group immediately before surgery were found to be similar to the other groups. Contrary to our findings, there are limited studies showing that individuals informed before the day of surgery have higher pre-operative anxiety (24). In the current study, no relationship between informing period and pain perception was found, although higher VAS values were seen in individuals informed 1 month prior to periodontal surgery. This finding can be attributed to the fact that the patients in this group had higher pain expectancy with the increase in anxiety and fear levels related to surgery for 1 month period until the time of operation. In a recent systematic review, it was revealed that different timings of pre-operative information had no effect on pre-operative anxiety or post-operative pain, in line with the findings of our study (25). However, our study is the first report describing the effect of informing period on post-operative pain and anxiety for periodontal surgical procedures. Since there are no previous reports, we could not compare our findings.

Post-operative pain is an acute pathological pain that starts with surgery, gradually decreases during the recovery phase and ends with tissue healing. The post-operative pain levels of the patients were evaluated by a visual analog scale in the present study, since it's a simple, sensitive, and reliable method that takes a short time (26,27). In addition, it has been reported that the VAS scale which is 10 cm in length is the most appropriate scale for the measurement of dental pain compared to the other dimensions (28). In our study, post-operative pain perception was observed to decrease over time in all groups. The VAS scores were close to zero in all groups at the end of the study. Consistent with previous studies, this study demonstrated that a majority of patients exhibited mild pain perception during the first week of recovery following open flap debridement and pain was generally well tolerated (29–31). However, in the study of Canakci et al. (23), which was inconsistent with the findings of our study, a higher pain perception was reported in patients receiving open flap debridement. The reason for the discrepancy between studies may be due to the inhomogeneity of variables that affect the perception of pain.

The findings of this study have to be seen in the light of some limitations. In addition to the factors related to the patient (gender, socio-economic status, age, and education level), many factors such as the location and size of the surgical site, the type of incision, and the surgery duration affect the duration and severity of the post-surgical pain. However, anxiety, fear, and individual differences in pain perception are considered to be important determinants of post-operative pain. In our study, analyses of other factors affecting anxiety or pain were not conducted, and the relationship between state and trait anxiety and pain in the post-operative period was investigated. Furthermore, this study was initiated and conducted in groups with similar demographic data including age, gender, smoking status and socioeconomic status.

In the present study, pre-operative anxiety levels of the patients were also compared to their pain perception, and a significant correlation was observed between STAI-T and the 3rd and 7th-day VAS scores. Similar to our findings, it has been observed in many studies that higher anxiety levels before periodontal surgical procedures were positively correlated with postoperative pain level (9,11,23). Trait anxiety level has been suggested as a predictor for pre – and post-operative pain (32).

Pre-operative anxiety is associated with increased post-operative pain and thus, increased analgesic consumption during this period. In our study, the amount of analgesic used was not different between the groups. This finding seems to be related to the other findings of our study, which showed no difference between different timings of pre-operative information in terms of pre-operative anxiety and therefore post-operative pain perception.

5. CONCLUSION

Pre-operative information provided by dentists at different times has no effect on post-operative pain and anxiety of the patients. Post-operative pain is associated with pre-operative anxiety. Determining the anxiety levels of patients before the operation acts an important part in the success of periodontal surgical treatments and pain management. Further studies including other factors influencing pain and anxiety and incorporating a greater number of participants are needed to confirm these results.

Conflicts of interest: The authors have declared that there are no conflicts of interest.

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Authors' contributions: NT and BK: Conception and design of the study, acquisition of data, analysis and interpretation of data. Drafting of manuscript: NT and BK. All authors revised the article critically for important intellectual content, and all authors approved the final version of the manuscript to be submitted.

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Clinical and Experimental Health Sciences

The Effect of Helfer Skin Tap Technique and ShotBlocker Application on Pain in Deltoid Muscle Injection

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ABSTRACT

Objective: This study was performed experimentally and single-blind randomized controlled trial to compare the effects of Helfer Skin Tap technique and Shot Blocker application on pain in the intramuscular injectıon application to the deltoid muscle.

Methods: The study was designed as a prospective, single-blind randomized controlled trial. The research was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) Checklist guide. The study was conducted in a family health center in Sivas Province between 05.10.2020-30.12.2020. Individuals who received Hepatitis B vaccine injection were included in the sample of the study. All intramuscular injections were made by the same nurse, and the approaches to reducing pain were made by the researcher. A total of 120 patients including the Helfer Skin Tap Technique (*n*=40), the standard application (*n*=40) and the ShotBlocker technique (*n*=40) were included. Mann Whitney U and Kruskal Wallis tests were used to evaluate the data.

Results: In our study, 75% (*n*=30) of the Helfer Skin Tap group, 82.50% (*n*=33) of the standard administration group and 75% (*n*=30) of the ShotBlocker group reported that they had no fear of injection. When the distribution of post-injection pain scores of the individuals according to different injection methods was examined, the difference between the average pain scores of the different methods used to provide pain control was found to be significant and statistically significant (*p*=0.001).

Conclusion: In line with the findings of our study, it was concluded that the use of ShotBlocker in intramuscular injection application of individuals was more effective in reducing pain due to injection compared to Helfer Skin Tap and standard application, and the Helfer Skin Tap technique was more successful in pain control than standard practice. All health professionals', especially nurses', awareness of the effectiveness of different methods in reducing pain during IM injection should be raised. To ensure pain control during IM injection, Shotblocker and Helfer Skın Tap Technıque should be preferred since they are easy to apply and cost effective,

Keywords: Intramuscular Injection, Pain Control, Helfer Skin Tap, Shot Blocker

1. INTRODUCTION

Intramuscular (IM) injection administration is an important part of parenteral drug administration and is a common nursing function that is frequently used in clinical practice (1,2). Although considered as a simple intervention, IM injections cause very serious complications if they are not done with appropriate methods (2-4). In the studies conducted, it was determined that IM injections were among the most painful applications among invasive procedures performed in hospitals (5,6). It has been reported that the pain that develops due to IM injection is due to the mechanical trauma caused by the needle insertion and the sudden pressure created when the drug is administered into the muscle (7). Because the injection area is small among the IM injection sites, the most painful area is the deltoid area (8). In this context, it is extremely important to use pain control approaches in IM injection applied to this area.

In the literature, it is emphasized that IM injection, which is applied based on guidelines and scientific knowledge, may experience less pain and prevent injection-related complications (5,6,9). In this context, when the literature was examined, it was seen that different methods, techniques and approaches such as cold application during IM injection, internal rotation of the extremities, use of ShotBlocker, Helfer Skin Tap technique, acupuncture application, relaxation exercises and the Z technique reduce injection pain. (7,10,11) Helfer Skin Tap technique, one of these techniques, was developed by nurse Joanne Kieffer Helfer to reduce pain associated with IM injection (12). This technique is based on providing pain control by providing mechanical stimulation with rhythmic touching to the injection area, and it is an easy-to-apply and time-consuming pain relief approach (13). IM injection applied to the dorsogluteal region using the Helfer

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Skin Tap technique has also been stated that this technique is an effective approach to relieve injection pain (11,14).

Another method used to reduce injection pain is ShotBlocker application. ShotBlocker is a plastic tool that prevents the perception of pain in intramuscular injection and its transmission to the central nervous system by temporarily blocking the peripheral nerve endings. ShotBlocker is a small, flat, non-invasive, horseshoe (U-shaped) plastic tool that can be used for all age groups to reduce the pain associated with intramuscular injection, it can be used quickly and easily, inexpensive, does not require prior material preparation, It has properties used by keeping it on its surface (15).The proposed mechanism of action of ShotBlocker is that the pressure exerted on the skin by the protrusions in this tool stimulates smaller diameter and faster nerve endings (15,16). This stimulation reduce spain by temporarily blocking the slower pain signals during injection, closing the gates to the central nervous system. Most of the published studies examining the effect of ShotBlocker in reducing intramuscular injection pain have been conducted with children (16,17) and its use in adults has been very limited (18,19). Nurses are responsible for preventing injection pain or relieving the patient with the techniques they use in drug administration. Pain due to intramuscular injection, which has physical and emotional effects, should be reduced in order to maintain patient-nurse relations, patient care quality and patient satisfaction (16, 18,19).

When the studies using Helfer Skin Tap and ShotBlocker techniques were examined, no study was found that evaluated the effectiveness of these two techniques in the application of deltoid muscle injection together. It is important to use evidence-based approaches in order to highlight the concept of quality in the delivery of nursing care. In this context, the aim of our research is to determine the effect of Helfer Skin Tap technique and ShotBlocker application on pain in the application of IM injection to the deltoid muscle.

Research Hypotheses;

H1: Standard injection practice in IM injection is effective on injection pain.

H2: The use of ShotBlocker in IM injection is effective on injection pain.

H3: Helfer Skin Tap application in IIM injection is effective on injection pain.

2. METHODS

Our study, which is a randomized controlled experimental study, was included 120 individuals who met then clusion criteria for IM Hepatitis B injection at Family Health Center, affiliated to Sivas Public Health Services Presidency, between 05.10.2020 and 30.12.2020. Patients were randomized by age, gender and BKİ. The study was designed as a prospective, single-blind randomized controlled trial. The research was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) Checklist guide (Fiqure 1). When α =0.05, d=0.48, power (1 – β)= 0.80 were taken to meet the parametric test assumptions, the power of the test (p=0.821364) was found, and the sample was calculated as n1 =33, n2=33, n3=33. In case of loss of sample during the research, 10% more of the calculated sample was taken and 40 individuals were included in each group. While the groups were randomized, “application group-I”, “application group-II and” control group-I “were written on a card. A total of 120 patients including the Helfer Skin Tap Technique *(n* = 40), the standard application (*n* = 40) and the ShotBlocker technique (*n* = 40) were included in the study to meet the parametric test assumptions.

Randomization: Individuals who applied to Toki Family Health Center during the study and met the inclusion and exclusion criteria were included in the study. In the literature, it is stated that injection pain may differ according to age, gender and BMI (10, 18). Therefore, in order to control the factors that may affect the homogeneity of the study, patients who met the inclusion criteria were stratified according to age, gender and BMI value and randomized into blocks. The number of people was determined according to the number of samples determined in each stratum of the four strata. Since we do not have an existing pool of individuals and individuals who applied to the Family Health Center were included in the sample, the individuals in each stratum were assigned to the research groups by block randomization method using a computer-generated list (www.randomizer.org). Group assignment was made by the Family Health Center nurse, who would not participate in data collection or statistical analysis, in sequential numbering, and group assignments were kept confidential in closed-opaque envelopes. It was determined whether the person would be in the groups during the block, according to which strata group characteristics the new individual who applied to the Family Health Center met the inclusion and exclusion criteria and whose consent was obtained. To confirm the homogeneity of the groups after randomization, the groups were compared according to age, gender and BMI using the chi-square test. Randomization was provided so that there was no statistically significant difference between the groups.

Blinding: While it was not possible for the researcher and the patients to be blinded to the intervention, the research data were coded as A, B and C, and entered into the database by someone other than the researcher, and statistical analyzes were carried out with coding by a biostatistics expert (statistician blinded).

The criteria for inclusion in the study were determined to be the following: *(a)* Speaking and understanding Turkish, *(b)* volunteering to participate in the study and obtaining a written consent, *(c)* aged 18-65, *(d)* when the patient’s file was examined, sensory-motor deficit, *(e)* Hepatitis B vaccine to be administered, *(f)* not receiving oral or parenteral analgesic treatment prior to administration, *(g)* not having a general VAS score above 0 before injection, i.e. no general pain Patients with *(h)* orientation of place and time, *(I)* without vision and hearing problems were included in the

study. Research Personal Information Form and Visual Analog Scala (VAS) were used to collect data of the study.

The exclusion criteria from the study were determined as follows: *(a)* who are not willing to participate in the research, *(b)*Infection, scar tissue, wound, burn, incision, etc. at the IM injection site. found patients were not included in the study.

2.1. Personal Information Form

This form was prepared by the researchers in line with the literature and consists of 4 items: age, gender, body mass index and fear of injection (2, 3, 8, 9, 10).

2.2. The Visual Analogue Scale (VAS)

The scale was first used in the 1970s. It was defined by Selby et al in the 1980s to assess the quality of life in cancer patients (20). VAS has been used in many studies evaluating different parameters after the 1990s, and it has recently been used to measure special conditions such as pain. The test has proven itself for a long time, and it is widely accepted and easily applicable in the world literature. It is a 10 cm-long scale, the left end of which is for "no pain" and the right end of which is for "severe pain", on which the individual can mark the level of their pain VAS is used to convert some non-quantifiable values into numeric values(21,22).Two end definitions of the parameter to be evaluated are written on both ends of a 100 mm line, and the individual is asked to indicate their pain status to the appropriate point on this line by drawing a line, marking a point, or marking a sign. The length of the distance from the point showing "no pain" to the point which the individual marked indicates the individual's pain. The most important advantage of the scale is that it does not use a language and is easy to apply. Whether the alignment of the line on which the test is applied is vertical or horizontal, or its length does not affect the result of the measurement. VAS is reported to be more sensitive and reliable than other one-dimensional scales for measuring pain severity (21,22).

2.3. Administration of The Data Collection Tools

Before starting the study, approval was obtained from Clinical Research Ethics Committee and then from the center where the application was performed. In order to apply the study, the researcher was in the Family Health Center for 5 days during the week between 08.00-17.00 in accordance with the working hours of the Family Health Center. In the study, all injections were used by the same ASM nurse in order not to affect the decision of the individuals, while the approaches to reduce pain were taken by researcher carried out by (Figure 1). VAS was applied to the individuals included in the study before injection and the individual with a score above 0, that is, experiencing a general pain, was not included in the study on the grounds that individual could not evaluate the pain due to injection correctly. Patients were randomized according to their age, gender and BKİ. While the groups were randomized, "application group-I", "application group-II and" control group-I "were written on a card. A total of 120 patients including the Helfer Skin Tap Technique (n = 40), the standard application (n = 40) and the ShotBlocker technique (n = 40) were included in the study to meet the parametric test assumptions. The individuals in the first group were injected with Hepatitis B vaccine by the same ASM nurse using the Helfer Skin Tap Technique, and then the pain level was determined using VAS. Hepatitis B vaccine was injected to the individuals in the second group by the same ASM nurse using the standard technique in line with the injection administration protocol, and then the pain and level were determined using VAS. Individuals in the third group were injected with Hepatitis B vaccine by the same ASM nurse using Shot Blocker, and then the pain level was determined using VAS (Figure 2).

The application protocols used in the research were prepared by the researchers in line with the guidelines in the literature (4, 7, 8 ,10) (Figure 1).

Medicine	HBVAXPRO 10 mcg/1 ml
Injector Volume	5 ml
Needle number	21
Needle point replacement	Every injection
Air lock technique	0.2 ml
Injection Area	Deltoid area
Injection site cleaning	70% ethyl alcohol
Needle entry and exit angle	90 derece
Injection time	1 ml /10 sn
After injection	Light pressure on the injection area, no massage
The person who recorded the data	Researcher

Figure 1. *Intramuscular Injection Protocol*

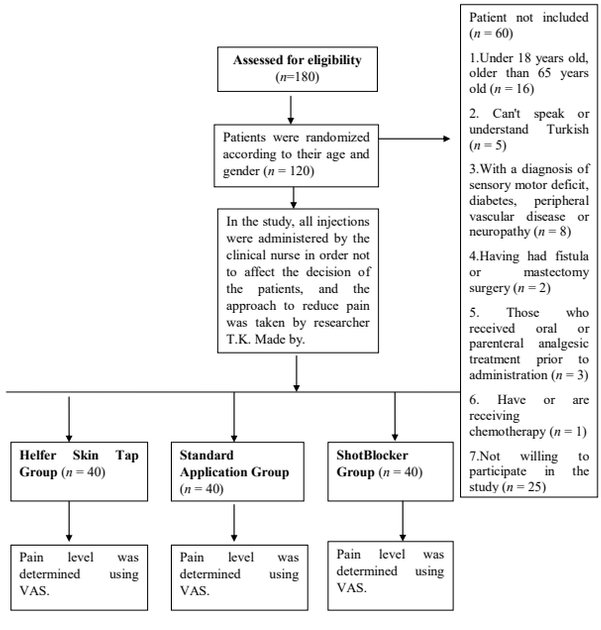

Figure 2. *Flow diagram*

2.4. Ethical Consideration

The study was carried out in accordance with the Declaration of Helsinki 2008 Principles and at the outset, the approval of Sivas Cumhuriyet University Faculty of Medicine Clinical Research Ethics Committee (Decision No: 2020-06/05) and the institutional permission were obtained. After the individuals included in the study were informed and their informed consent was obtained, the researcher started collecting the study data. The individuals were made sure that that the decision on whether or not to participate in the study totally belonged to them, the data obtained from this study would be used only within the scope of the research, and that confidentiality would definitely be ensured.

2.5. Evaluation of Data

Analysis of the study data was performed using IBM SPSS Statistics Version 20.0 (IBM, Inc., Armonk, NY, USA) and tables were created.In statistical analysis; Descriptive statistics and frequency distributions of demographic data are given. Since scale is used while determining our data, nonparametric statistical methods are used. In statistical analysis, Kruskal Wallis test and Mann Whitney U test were used and the level of error was taken as 0.05.

3. RESULTS

In Table 1, the distribution of the individuals included in the study according to some descriptive characteristics is given. When the distribution of individuals according to their gender is examined; 60% of the Helfer skin tap group, 62.5% of the standard application group and 60% of the ShotBlocker group consists of female individuals. 90% of individuals in the helfer skin tap group, 80% in the standard application group, and 90% in the shotblocker group are between the ages of 18-34. Again, 75% of the individuals' helfer skin tap group, 72.5% of the standard application group, and 70% of the ShotBlocker group's BMI is 18.50-24.99kg/m2. In our study, 75% (n = 30) of the Helfer Skin Tap group, 82.50% (n = 33) of the standard application group, and 75% (n = 30) of the Shot Blocker group reported that they had no fear of needles (Table 1).

When the demographic characteristics of the individuals and the distribution of the mean pain scores after IM injection were examined according to different injection methods, there was no statistically significant difference between the gender, BMI values and injection fear conditions of the individuals (p> 0.05), the individuals aged 41-64 were Mean pain scores were found to be statistically significantly lower than those in the 18-40 age group (p <0.05) (Table 2).

When the distribution of post-injection pain score averages of individuals according to different injection methods were examined, it was calculated that the average pain score of the Helfer Skin Tap group was 4.00 (2.00, 6.00) and 7.00 (4.00, 9.00) in the standard application group and 3.00 (2.00, 4.00) in the ShotBlocker group. The difference between the average pain scores of the different methods used for pain control in IM injection application was found to be significant and statistically significant (p = 0.001) (Table 3).

Table 1. *Distribution of Individuals Received Intramuscular Injection by Some Descriptive Features*

Characteristic	Helfer Skin Tap Group *n %*	Standard Application Group *n %*	ShotBlocker Group *n %*
Gender			
Female	24 (60%)	25 (62.5%)	24 (60%)
Male	16 (40%)	15 (37.5%)	16 (40%)
Age			
18-40	36 (90%)	32(80%)	36(90%)
41-64	4 (10%)	8 (20%)	4(10%)
BMI			
18.50-24.99 kg/m^2	30(75%)	29 (72.5%)	28(70%)
25.00-29.99 kg/m^2	10(25%)	11(27.5%)	12(30%)
Fear of needles			
Evet	10(25%)	7 (17.5%)	10(25%)
Hayır	30(75%)	33(82.5%)	30(75%)

Table 2. *Demographic Characteristics of Individuals and Distribution of Post-Injection Pain Scores According to Different Injection Methods*

Characteristic	Helfer Skin Tap Group *(n=40)*	Standard Application Group *(n=40)*	ShotBlocker Group *(n=40)*
Gender	**Median (min-max)**		
Female	4.00 (2.00,6.00)	7.00 (4.00, 9.00)	3.00 (2.00, 4.00)
Male	3.00 (2.00, 6.00)	6.00 (4.00, 9.00)	3.00 (2.00, 4.00)
Statistical Result (*p*)	*p*=0.315	*p*=0.224	*p*=0.976
Age			
18-40	4.00 (2.00, 6.00)	7.00 (4.00, 9.00)	2.00 (2.00, 2.00)
40-64	4.00 (3.00, 5.00)	8.50 (7.00, 9.00)	3.00 (2.00, 4.00)
Statistical Result (*p*)	*p*=0.690	*p*=0.020	*p*=0.025
BMI			
18.50-24.99kg/m^2	4.00 (2.00, 6.00)	7.00 (4.00, 9.00)	3.00 (2.00, 4.00)
25.00-29.99 kg/m^2	4.00 (3.00, 5.00)	7.00 (4.00, 9.00)	3.00 (2.00, 4.00)
Statistical Result (*p*)	*p*=0.863	*p*=0.530	*p*=0.640
Fear of needles			
Yes	4.00 (2.00,6.00)	8.00 (4.00,9.00)	2.00 (2.00,3.00)
No	3.50 (2.00,6.00)	7.00 (4.00,9.00)	3.00 (2.00,4.00)
Statistical Result (*p*)	*p*=0.559	*p*=0.073	*p*=0.098

Table 3. *Distribution of the Individuals Mean Pain Points Post-Injection According to Different Injection Methods*

Pain scores	Helfer Skin Tap Group (n=40)	Standard Application Group (n=40)	ShotBlocker Group (n=40)
Median (min-max)			
Pain scores post IM Injection	4.00 (2.00, 6.00)	7.00 (4.00, 9.00)	3.00 (2.00, 4.00)
Statistical Result *p*=0.001			

4. DISCUSSION

Pain is a complex and multidimensional condition associated with actual or potential tissue damage, leading to unpleasant sensory and emotional experiences. It is also an individual, unique, and subjective experience that can be difficult to describe and describe (11). Prevention and relief of pain is the most fundamental requirement of human rights. Therefore, using the best approach in pain management is one of the primary responsibilities of the nurse (23,24).

Nurses are responsible for relieving individuals with the methods and techniques they apply in care (11,25). In the literature, it is stated that gender is important among the factors affecting pain behavior in individuals, and men can tolerate pain better with the effect of universal and social reasons (11,26). However, in our study, the difference between the pain levels of men and women due to IM injection application was found to be statistically insignificant ($p>$ 0.05), and there was no difference between genders in responses to acute pain in IM injection application (Table 2).

The investigation of the causes of intramuscular injection-induced pain demonstrated that the length and thickness of the needle the injection site the technique used the amount and the physical and chemical properties (osmolarity, pH, concentration and auxiliary chemicals) of the drug injected were effective (10, 27). Within this context, all the IM injections in our study were administered in accordance with the standard injection protocol consistent with the guidelines in this field (Figure 1).

In our study, it was found that the average pain scores of individuals aged 41-64 in standard and Shot Blocker application were statistically significantly lower than those in the 18-40 age group (Table 2). Considering the physiological changes and regressions in the transmission and perception of pain with the advancement of age, it may be an expected result that elderly individuals experience less pain after injection. Different results were obtained in the studies conducted on this subject, and it was reported that the pain experienced after injection in older ages was higher than in other age groups (23,28).

When the mean post-injection pain scores of the individuals were examined according to their BMI values, the difference between the pain levels of the individuals in all three groups due to IM injection applications was found to be statistically insignificant (p>0.05) (Table 4).

However, when the literature is examined, it has been stated that the perceived pain associated with injection is less in individuals with thick subcutaneous adipose tissue (18, 29). Our research does not show parallelism with the literature in this aspect.

Considered as an integral part of healthcare services, IM injections are widely used in treatment processes, and as an invasive procedure, they often cause pain (10,16). Because the injection area is small among the IM injection sites, the most painful area is the deltoid area (8). In this context, it is extremely important to use pain control approaches in IM injection applied to this area (11,18). Various methods and techniques are used to reduce the pain caused by injection applications and to increase the comfort of the individual (11). In our study, the mean pain scores of the Helfer Skin Tap technique, which is used to provide pain control in IM injection application, compared to the standard application, and the use of Shot Blocker was found to be statistically significantly lower than the Helfer Skin Tap and standard application. When the studies using the Helfer Skin Tap technique in the literature were examined, Jyoti et all., (2018) found that the use of the Helfer Skin Tap technique to provide pain control in IM injection application was found to be significantly effective in reducing pain due to injection (26). Again, Soliman and Hassnein (2016) found that IM injections using the Helfer Skin Tap technique were an effective method in reducing IM injection pain compared to the standard technique (27). When the use of ShotBlocker was examined in the literature, Aydın &Avşar (2019) found that the administration ShotBlocker on reduced injection pain (23). Çağlar et al., (2017) determined that the use of ShotBlocker during hepatitis B vaccine administration to newborns is effective in reducing injection pain (29). Çelik and Khorshid (2015) found that pain in IM injection using ShotBlocker was significantly less than the control and placebo groups in their study with individuals over the age of 18. (18). Our research supports the literature in this respect. In our study, when the relationship between the pain levels experienced after IM injection using different non-pharmacological methods was examined, it was determined that the pain experienced due to injection could be reduced with the use of non-pharmacological methods. In this context, the application of different methods for pain control and the evaluation of their effectiveness are very important and necessary in terms of evidence-based applications.

5. CONCLUSION

In line with the findings obtained in our study, it was concluded that the use of ShotBlocker was more effective than the Helfer Skin Tap and the standard application group in reducing the pain due to injection in IM injection application, and the Helfer Skin Tap technique was more successful than the standard application.

In this context, it is recommended to conduct similar studies in different populations for the Helfer Skin Tap and ShotBlocker application in order to standardize the

applications and create evidence in IM injection application. In line with these results, it is recommended that health professionals, especially nurses, use non-pharmacological methods with proven efficacy more frequently to relieve pain in İM injection, follow the developments in this field and put them into practice

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Clinical and Experimental Health Sciences

Determination of Quality of Life of Individuals Before and After Bariatric Surgery: Prospective Study with 1 Year Follow-Up

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ABSTRACT

Objective: This study was conducted to investigate the effect of bariatric surgery on quality of life.

Methods: This study used a quantitative, descriptive, cross-sectional and prospective study design. This study was conducted between 1 January 2016 and 1 June 2017 at an university hospital in Turkey. The questionnaire form that developed by the researchers and the Short Form-36 quality of life scale before bariatric surgery were applied to the individuals. Participants were evaluated with the same scale at 1 month, 3 months and 1 year after bariatric surgery.

Results: The mean age of the individuals was 37.37 ± 9.18.While the mean of body mass index was 48.18±5.8 kg/m2 before bariatric surgery, it decreased to 27.18±3.21 kg/m2 in one year after bariatric surgery. Simple linear regression analysis was given between the subscale of SF-36 and the descriptive characteristics of the participants according to the follow-up times and there was a moderate positive relationship between preoperative physical health and gender (β = 0.450, p <0.05). In the lineer regression analyses, a statistically significant relationship was found between the body mass index in the 3rd month and the quality of life before bariatric surgery (β=1.446, p<0.05), between pre – body mass index and quality of life in post – bariatric surgery in 1st month (β=1,474, p<0.05), between body mass index in post – bariatric surgery in 1st month and quality of life in post – bariatric surgery in 1st month (β =2.173, p<0.05), between body mass index in post – bariatric surgery in 3rd month and quality of life in post – bariatric surgery in 1st year (β =-0.955, p<0.05).

Conclusion: It is obvious that the nurse's counseling role is very important in the long-term follow-up of patients, including improving the quality of life. Within 1 year, bariatric surgery found a positive effect on quality of life as a result of weight loss.

Keywords: Bariatric surgery, quality of life, nursing.

1. INTRODUCTION

Quality of life (QoL) is a subjective concept that does not have a standard indication, reflects a person's ability to perform the activities of daily living on their own, to the point of people's personal and social satisfaction (1). Health-related quality of life (HRQoL) is a complex and multidimensional structure of a person's health and well-being perception, directly or indirectly from a bio-psychosocial perspective (1,2). Today, improving the QoL has become a universal target adopted by societies. Indeed, HRQoL is now considered, worldwide, to be a vital parameter after medical treatment (3). In the literature, there are many factors that affect HRQoL (4). One of them is obesity, and especially morbid obesity (body mass index (BMI)> 40 kg/m^2) which is reported to have a significant negative impact on HRQoL (5,6) because obesity prevents a person from performing his/her daily activities and prepares the basis of obesity-related comorbidity and early mortality (7). As the concept of HRQoL is also associated with survival and accompanying diseases, obesity is seen as a preventable vital problem. In this context, many treatment methods have been developed worldwide to control obesity and to maximize the QoL and physical and mental health (8). It is bariatric surgery (*obesity surgery* −OS) that gives the most positive results among these methods (9). In many different countries across the world, OS has been practised for more than 50 years (10). The first surgical procedure, aimed in particular at weight loss, was end-to-endjejuno-ileostomy by Varco of the University of Minnesota in 1953. Gastric bypass surgery was performed in 1960 for the first time by Mason. In 1976, Scopinaro introduced the first bio-pancreatic diversion and this started to be applied (10). And firstly, the adjustable gastric band was applied by Kuzmak in 1986 and laparoscopic OS has became popular in 1993. The first sleevegastrectomy was described in 1993. Since 1994, it has been successfully applied laparoscopically (10). Today,

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all of these interventions are performed laparoscopically with technological developments (10). Because laparoscopic surgery has less negative impact on QoL than open surgery, OS is preferred by many people as a weight loss method (11). In a study that includes data from Turkey, conducted by Angrisani et al. (12) a total of 468,609 people had OS in 2013; with the highest number being the figures for the US/ Canada (n=154,276). In Turkey, this number of underwent OS was 3,250.

Besides obesity, comorbid diseases also increase. The presence of morbid obesity-related diseases negatively affects the QoL (13). After OS, patients' QoL improves with the reduction of BMI and also diseases caused by obesity as a result of general health development (13,14,15). Although there are evidences that there is a relationship between improved QoL and weight loss (16,17). An improvement in QoL cannot be said to be related to weight loss alone, because psychological factors (for example, hope, happiness, love-respect, improvement of the financial condition) are also reported to contribute to the recovery rate (5). It is reported that the success after OS affects not only the weight loss but also the mental health and psychosocial status and positively affects the QoL (18).

Many studies can be found in the world literature, examining the effect of OS on QoL, (13, 18, 19, 20, 21) but the only one study (22) in Turkey. This study is thought to be important in terms of weight loss after OS and the improvement in the physical and mental functions of individuals and their awareness of QoL. The fact that there is a limited number of studies examining the effect of OS on QoL in Turkey was instrumental in the planning of this work. In addition, as it is known, nurses play an important role in improving the QoL of patients and adapting to life in the postoperative period. Therefore, this study also shows how important the nurses' follow-up is after surgery. The aim of this study was to evaluate, pre-operatively, in the 1st month, 3rd month and 1st year after surgery, the changes in the QoL of individuals who had undergone OS because of obesity.

2. METHODS

2.1.Study Design and Sample

This study used a quantitative, descriptive, cross-sectional and prospective study design. The population of the study consisted of 63 individuals who have had OS in the general surgery outpatient clinic of hospital between 1 January 2016 and 1 January 2017. The sample of the study consisted of 40 individuals who were conscious, with no orientation problems, who could communicate, were 18 years and over and who agreed to participate in the study.

2.2.Procedure

The purpose of the study was explained to the individuals. Informed consent was obtained from all individual participants included in the study. Individuals' phone numbers were recorded when applying preoperative forms to them. In the 1st month, 3rd month and 1st year after the operation, every person was telephoned again and an interview was arranged in the general surgery outpatient clinic of the hospital. In Turkey, patients are invited to the clinic at certain periods. If the patient prefers, comes for control after OS. However, in this study, the situation was evaluated by the first researcher (the nurse academician) after OS, by calling the patients every 10 days or by meeting them face to face. Patients' QoL scores were measured with SF-36 during routine hospital controls. In these periods, the forms were applied to the individuals in the face-to-face interview method in the general surgery clinic pre-OS and it took about 30-35 minutes to complete the forms. Although the sample size was low in our study, the study was completed with 40 people during the long follow-up (1 year) without any loss of participants.

2.3.Measurements

The data were obtained using a questionnaire developed by the researchers and the Short Form-36 Quality of Life Scale (SF-36).

The Questionnaire Form: The questionnaire developed by the researchers in accordance with the literature (20, 21, 22) consists of seven questions on age, gender, marital status, educational status, presence of chronic disease, how many years of obesity and type of surgery.

The Short Form 36: The SF-36 was developed by the Rand Corparation in 1992. The SF-36 includes one multi-item scale measuring each of the eight health concepts: physical health (PH) (10 items); role limitations because of physical health problems (RP) (4 items); bodily pain (BP) (2 items); social functioning (SF) (2 items); general mental health (MH) (5 items); role limitations because of emotional problems (RE) (3 items); vitality (VT) (energy/fatigue) (4 items) and general health perceptions (GH) (5 items) (23). The SF-36 items and scales were constructed for scoring using the Likert method of summated ratings, excluding the fourth and fifth items, which are answered in a yes/no format. For some of the Likert-type questions, inverse scoring was used. Instead of giving a single total score, the scale gives a total score for each subscale. The subscales assess health between 0 and 100 points, 0 indicating poor health status, and 100 indicating good health status. The SF-36 is divided into eight subscales to generate two summary scores. The reliability and validity of the Turkish version of the SF-36 was assessed by Koçyiğit et al. in 1999 (24). Cronbach's-α coefficient was calculated for each subscale and was found between 0.73 – 0.76.

Before the data was collected, the ethical approvals (decision number: 2017-07/15 Date: 26.07.2017) were got permission of the institution where the application was performed and written permission was obtained from the general surgery department institution official. This study was carried out with the 1964 Helsinki declaration and later changes or comparable ethical standards.

2.4.Statistical Analysis

Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS, version 22.0). Descriptive statistics (e.g. mean, standard deviation and frequency distributions) were generated for all variables. With the Kolmogorov-Smirnov (K-S) test, it was determined that the data showed normal distribution and parametric tests were used while making the analyzes. T test and one way ANOVA test were used in the study when comparing whether there was a difference between the averages obtained in terms of a variable obtained by measurement in independent groups (two or more). Durbin–Watson test was used to determine if there was a relationship between dependent variables. The Pearson product–moment correlation were tested to determine whether there was a relationship between follow-up times of individuals and total QoL scores. Simple linear regression analysis was used to determine whether there is a relationship between independent variables and SF-36 subscale according to follow-up times. And we carried out simple linear regression analyzes to discuss the relationship between two outcome variables, total QoL and BMI, according to the time of follow-up. The level of significance for all analyses was set at $p<0.05$.

3. RESULTS

The basic characteristics of the participants and total score of QoL of individuals according to the follow-up times in the study sample are shown in Table 1. A total of 40 participants completed the questionnaire. The average age of participants in the study was 37.37 ±9.18 years old. The majority of the participants in the study were female, married, were high school graduates, had not chronic disease, had been obese for 5−10 years and had undergone a Roux-en-Ygastric bypass. As seen in Table 1, it was found that there was a significant relationship between the participants' total QoL preoperatively ($p = 0.007$) and this difference was in favor of the male gender. Again, a statistical difference was determined between preoperative QoL and how many years participants were obese ($p = 0.042$). This difference was found to be caused by obese people over 10 years. No statistically significant relationship was found between other descriptive features and total QoL at follow-up. ($p>0.05$).

The results showed that, in our sample, the mean preoperative body mass index (BMI) was 48.18 ±5.8 kg / m^2 (min: 37, max: 59), at the postoperative 1st month BMI was 42.92 ±5.31 kg / m^2 (min: 33, max: 52), in the 3rd month BMI was 35.65 ±5.14) kg / m^2 (min: 25, max: 45) and after the 1st year, BMI was 27.18±3.21 kg/ m^2 (min: 22, max: 35). The relationship between BMI and QoL according to the follow-up time of individuals is shown in Graph 1. As seen in the graph, QoL increases as the BMI rate decreases.

Table 2 shows the correlation analysis between the follow-up times of individuals and the total scores of QoL. When the table is examined; the QoL before OS was found to be positively related to the QoL in the 1st month after OS ($r=0.313$, $p=0.025$) and the Qol after OS 1st year was found to be positively related to the QoL in the 3rd month after OS ($r=.427$, $p=0.003$).

Table 3 shows the simple lineer regression analysis between BMI and QoL total score according to follow-up times. As shown in the table, a significant relationship was found between the BMI in the 3rd month and the QoL before OS ($\beta=1.446$, $p<0.05$), and between the preoperative BMI and QoL in the 1st month after OS ($\beta=1.474$, $p<0.05$), and between the BMI in the 1st month and the QoL in the 1st month after OS ($\beta=2.173$, $p<0.05$). In addition, a significant relationship was found between the BMI at the 3rdmonth after OS and the QoL in the 1st year after OS ($\beta = – 0.955$, $p <0.05$).

In Table 4, the average score of subscale of SF-36 according to tho follow-up times was given. When the table is examined, it is seen that there is a statistically significant relationship between each subscale of SF-36 and the follow-up times ($p=.000$). In other words, the follow-up periods have statistical significance in all subclae of SF-36 in the evaluation of QoL.

As shown in Table 5, simple linear regression analysis was given between the subscale of SF-36 and the descriptive characteristics of the participants according to the follow-up times. In the table, according to the results of the analysis, the results with statistically significant relationship between the subscale of SF-36 and the descriptive features according to the follow-up times are given ($p <0.05$). As seen in the table, there was a moderate positive relationship between preoperative PH and gender($\beta = 0.450$, $p <0.05$). A low positive correlation was determined between RP at the 3rd month after surgery and how many years the participants were obese ($\beta = 0.143$, $p <0.05$). A low negative correlation was found between BP in the first year after surgery and how many years the participants were obese ($\beta=-0.325$, $p<0.05$) A moderately negative correlation was determined between the preoperative GH and the type of surgery ($\beta= – 0.434$, $p<0.05$). A low positive correlation was found between SF at first month and those with chronic disease ($\beta=0.382$, $p<0.05$). Finally, in Table 5, a highly negative correlation was determined between the preoperative RE and the presence of chronic disease ($\beta= – 0.978$, $p<0.05$).

4. DISCUSSION

Obesity has become the biggest preventable health problem affecting the QoL in recent years and OS is the most effective treatment method (25). In this study, the changein QoL of the individuals who underwent OS was evaluated preoperatively and in the 1st month, 3rd month and 1st year after OS, and there was an increase in QoL after OS over time. This is in agreement with studies that found OS not only improved some of the disease symptoms but also improved the QoL by prolonging the life of the individuals (26, 27).

In the present study, it was found that there was a statistically significant relationship in favor of male gender before surgery. A study in Sweden found that there is a similarity

between men and women in PH, the subscale of SF-36 (28). Tsai et al. (29) reported that overweight men were less likely than overweight women to have accurate weight perception, weight dissatisfaction, and attempted weight loss. A study (30) reported that men were less likely than women to perceive they were overweight. This can be explained, in part, by gender-based differences in perceptions of body weight and obesity-related QoL, weight-related attitudes, behaviors, and cultural perspectives. However, there was no significant relationship between post-operative sex and the PH subscale of SF-36 and it can be said that the operation may have physically affected both sex groups equally.

In the present study, it was found that QoL increased as a result of BMI reduction with weight loss. In previous studies (25, 31, 32, 33), a positive correlation was found between weight loss after OS and improvement in QoL. As it is known, the association between BMI and QoL is an inverse linear trend (34). It is also likely that pre-existing and/or post-surgical physical, psychological and social factors interact with weight loss to influence the improvement in QoL following OS. Therefore, the results of this study and previous studies on obesity and QoL can be interpreted as individuals' weight loss after OS, increased physical functions as a result of improved general health, and strengthened social relationships make them feel better emotionally.

As is already known, obesity causes comorbidities and reduced QoL (19, 35). The QoL of individuals improves as a result of the decrease in the diseases caused by obesity after OS (13). In the current study, contrary to the literature knowledge, it was found that the presence of chronic disease did not affect the QoL. The reason for this is that the number of samples is limited. However, in this study, a low positive correlation was found between the SF subscale of SF-36 and those with chronic disease at the first postoperative month. This may be due to the fact that those with chronic diseases feel better socially in the early postoperative period compared to the preoperative period.

There are studies in the literature showing that the type of surgery has no effect on QoL (36, 37). In this study, a moderately negative correlation was found between the GH subscale of the preoperative SF-36 and the type of surgery. So, regardless of the type of surgery, the general health of individuals before surgery was already low. This may have been caused by adverse health conditions caused by obesity on the person.

Although there is evidence of a relationship between improvement in QoL and weight loss, (37) it cannot be said that improvement in QoL is related to weight loss only; there are also psychological factors (eg. hope, happiness, love-respect, improvement of financial situation) that contribute to the rate of recovery (5). Some studies suggest that after OS, QoL is improved forup to 1 year (17) and then there is a decrease in QoL. In contrast, some studies suggest that QoL continues to improve for 2–4 years after OS (38). In the present study, the QoL of the individuals was determined to be at its highest in the 1st year after OS. This can be interpreted as being due to the fact that the fastest weight loss happens in the first 1 year. After 1 year, weight loss may stop and subjects may even gainweight again. Therefore, it is important to follow long-term individuals with a multidisciplinary team approach.

5. CONCLUSION

For a long-term and successful outcome of OS, a person needs to make lifelong nutrition and lifestyle changes. In this case, the support of health professionals is important. In our study, when evaluated within the scope of the Short Form-36, the problems of the patients in many sub-dimensions were determined by the nurse. After the surgical procedure, the lifestyle changes that the patients should make at home after discharge should be followed up by the nurse. This follow-up is important both for early detection of problems and early intervention. Today, the concept of tele-nursing has become popular and has taken an important role in patient follow-up. In this study, 1-year patient results were also obtained as a result of nurse follow-up. In line with the results of this study, the importance of nurse follow-up in prospective studies has been revealed. Patients needed help to both improve their quality of life and maintain healthy weight loss. As a result, in one-year follow-up of individuals who had undergone OS, the procedure was found to be associated with weight loss and an increase in QoL. Results of this study may be generalisable to other surgery areas, states and countries.

Limitations of The Study

This study has several limitations. Unfortunately, the small sample size and the loss of half of the total number of patients to follow-up (50.0%), and reflects the results in only one region. Neverthelessthis study data would provide a more comprehensive insight into the long-term outcomes of the evaluated OS.

In fact, this is more important than a few examples to understand the seriousness of significance. If these studies are conducted with the cohort technique and individuals are followed for a long time, they will make significant contributions to both Turkish and world literature. Therefore, it may be suggested to repeat the study with more samples and longer follow-up periods. Also, more research is needed to see if patients are followed by a nurse after OS.

Conflict of Interest

The authors do not have any conflict of interest to disclose.

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Clinical and Experimental Health Sciences

Evaluation of the Effects of Donepezil, Memantine and α-lipoic Acid Combined Administration in Amnesia Rats on Impaired Cognitive Functions in Terms of Behavioural, Apoptotic, Cholinergic and Glutamatergic Systems

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ABSTRACT

Objective: The aim of the study was to evaluate the possible protective effect of donepezil, memantine and alpha-lipoic acid (α-LA) combined therapy in the scopolamine-induced amnesia rat model.

Methods: In this study, the effect of combined therapy used in the treatment of scopolamine-induced amnesia on behavioural parameters was evaluated using Y-maze and new object recognition (NOR) test. In addition, muscarinic acetylcholine receptor subtype M1, N-methyl-D-aspartate receptor NR2B subunit, brain-derived neurotrophic factor (BDNF) and mitochondrial apoptosis-related proteins [B-cell lymphoma-2 (Bcl-2) / Bcl-2 associated X (Bax) ratio, caspase (casp) – 3, and – 9] expression levels were evaluated using the western blot method in the frontal cortex and hippocampus regions.

Results: The main findings of this study demonstrated that in scopolamine-induced amnesia rats, cognitive dysfunction determined by both the Y-maze and the NOR test were reversed with the combined treatment of memantine, donepezil and α-LA. According to immunoblotting results in both brain regions, scopolamine-induced decreased M1, BDNF, Bcl-2 / Bax ratio and increased NR2B, casp-3 and – 9 expression levels were found to be reversed to almost control values with combined treatment.

Conclusion: Consistent with the literature, our study results suggest that the positive contribution of α-LA to the combined treatment of donepezil and memantine, which is used in the routine treatment of neurodegenerative diseases, may be a treatment option in the future.

Keywords: Alpha-lipoic acid, amnesia, apoptosis, scopolamine, Y-maze.

1. INTRODUCTION

The incidence of Alzheimer's disease (AD), which is a neurodegenerative disease and one of the most common causes of amnesia, is increasing due to the prolongation of the average life expectancy. While a number of pathophysiological events such as the degeneration of cholinergic and glutaminergic neurons stand out in the pathophysiology of AD, the etiology of AD has not been fully elucidated yet (1).

Cognitive disorders seen in AD can be mimicked by creating amnesia caused by Scopolamine, a muscarinic acetylcholine receptor (mAChR) antagonist, in rodents (2). Scopolamine-induced amnesia, showing the possible interaction between mAChR and N-methyl-D-aspartate receptor (NMDAR) in the cognitive functions in the hippocampus and frontal cortex (3, 4). Brain-derived neurotrophic factor (BDNF) is an important component in the preservation of synaptic plasticity and synaptogenesis in the hippocampus region, which is the basis of memory and consolidation. While there are studies emphasizing the decrease of BDNF level and protein expression occurring in AD process, which reveals the importance of BDNF in AD pathogenesis, there are also conflicting results in the literature (5,6).

NMDARs carry out learning and memory activities in the brain, consist of NMDAR 1 and 2 (NR1, NR2) subunits. It has been reported that AD causes degeneration of the hippocampus, cell loss, extensive pathological changes and increases in NR2 density due to cell death in the hippocampus and frontal cortex regions (7). While glutamate ligands can function as a general marker of NR2 subunits, it is almost impossible to conclude whether specific combinations of an NR2 subunit or all subunits have changed in AD. In the literature by using subunit-specific

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antibodies, it is reported that protein levels decrease for NR2B, not NR2A, throughout the hippocampus of AD subjects with moderate and severe pathology (8). Studies show that the blockade of NMDAR function causes neuronal apoptosis and degeneration. On the other hand, excessive NMDAR activity in terms of the magnitude of activation has deleterious effects on neuronal structures. In the literature, memantine, (1-amino-3, 5-dimethyladamantane hydrochloride), a non-competitive NMDAR antagonist, has been shown to reduce oxidative damage and prevent cellular aging in neuronal cells (9). The acetylcholinesterase inhibitor donepezil was developed to prevent a decrease in cholinergic conduction due to the display of cholinergic neuron degeneration of AD (10). The existence of evidence for functional impairment of neurotransmitter systems in AD is emphasized cholinergic activity findings especially in cortical projections in AD patients' cognitive deficiency. This case constitutes the basic rationale of cholinergic replacement therapy in the treatment approach of AD patients. Despite the reported symptomatic and cognitive benefits of donepezil and memantine in AD patients, these drugs cannot stop or prevent AD progression (11-13).

The disruption/damage occurring in the mitochondria, which plays a key role in the bioenergetic modulation of the cell, can lead to activation of the caspase cascade and thus cell death. In post-mortem research on AD patients' brains, it was found that there was an increase in mitochondrial DNA and proteins (14,15). α-LA is a natural antioxidant synthesized in the human body and a naturally occurring free radical scavenger that has been displayed to increase glutathione, regenerates antioxidants (16,17). α-LA is a natural antioxidant synthesized in the human body and is an ideal neuroprotective antioxidant that increases acetylcholine production in the pathogenesis of AD (14, 18). Supplementation of α-LA (which plays a key role in mitochondrial energy production), improves cognitive and learning-remembering functions by increasing total antioxidant capacity and reducing neurodegeneration in the hippocampal region (18, 19).

The aim of this study was to evaluate the possible protective effect of donepezil, memantine and α-LA combination therapy on the scopolamine-induced rat amnesia model. For this aim, immunoblotting method was used to determine the changes in the expression level of mitochondrial apoptotic proteins, BDNF, M1 and NR2B in the hippocampal and frontal cortex regions.

2. METHODS

2.1. Drugs and Chemicals

Scopolamine, alpha lipoic acid, donepezil and memantine were purchased from Sigma (St. Louis, USA). Scopolamine and memantine were dissolved in physiological saline (PS) to create a final injection volume of 1 ml/kg. Scopolamine, donepezil, memantine or saline was injected intraperitoneally (i.p) 3h before the onset of the cognitive function test (11). All antibodies were supplied from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA).

2.2. Animals and Conditions

In the present study, 48 healthy Wistar albino male rats, 12-14 weeks old, weighing 250-300 g, supplied from the XXX were used. All procedures related to animal experiments were approved by the Ethics Commission from Experimental Animals Research and Implementation Centre, DEHAMER (App. No: 31.2018.Mar).

All animals were conditioned with the reverse of the circadian light cycle for ten days (12 h light/dark) where they were housed in rooms with controlled temperature and humidity 22 ± 2° C and 40–60%, respectively) before the onset of the behaviour experiments. The rats kept in polypropylene cages (four animals per cage) were accessed to unlimited access to standard rat diet and drinking water.

2.3. Experimental Design

Rats were randomly divided into two groups as control and amnesia groups. The rats in the control group were given saline (saline group: 0.9% Saline) treatment, a-lipoic acid (LA group: 100mg/kg) (20), donepezil + memantine (D+M group: 0.1mg/kg + 1 mg/kg; as a positive control) (17) or donepezil + memantine + a-lipoic acid (D+M+LA group) treatment, while all of the amnesia groups either did not receive treatment (amnesia group: Scopolamine 1mg/kg, as a negative control) (21, 22) or were treated with LA (LA amnesia group: Scopolamine 1mg/kg + a-lipoic acid 100 mg/kg), D+M (D+M amnesia group: Scopolamine 1mg/kg + donepezil 0.1mg/kg + memantine 1 mg/kg) or D+M+LA (D+M+LA amnesia group: Scopolamine 1mg/kg+ donepezil 0.1mg/kg + memantine 1 mg/kg + a-lipoic acid 100mg/kg). Forty-eight rats were used, six in each group. All treatments were given as i.p. for 14 days. (Fig. 1).

On the 15th day the rats were anesthetized and killed by cervical dislocation. The brain was quickly removed and cleaned with ice-cold 0.1 M phosphate buffer saline (PBS; pH 7.4). The hippocampus and frontal cortex regions were immediately dissected according to Paxinos rat brain atlas (23) and then all dissected brain regions were stored at −80 °C using in immunoblotting assay.

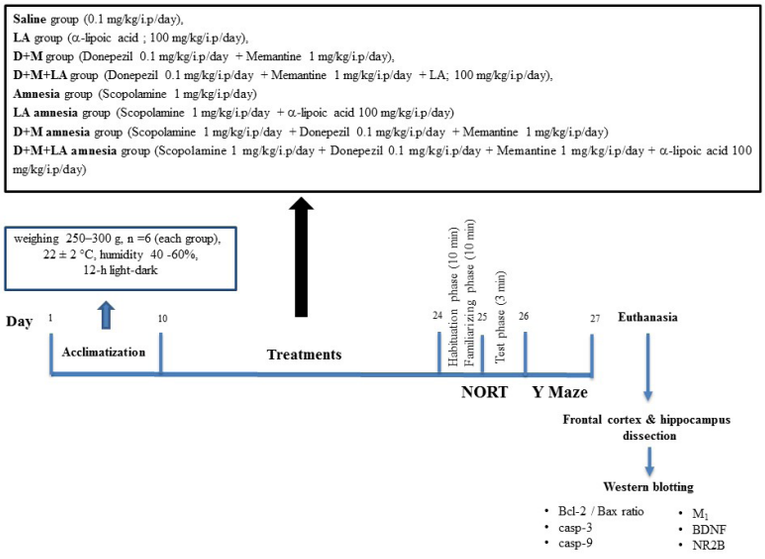

Figure 1. *An overview of experimental design*

2.4. The Evaluation of Cognition Functions

NOR test was used as a measure of short-term/working memory was used to evaluate the cognitive status of rats and the effect of treatments. NORT steps were carried out as follows: Acclimation, Trial 1 and 2. In the acclimation phase that the test equipment does not contain any objects, the rats were accustomed to the test set up for 3 min. In the first trial phase, two identical objects were presented to the rats for 3/10 min. In the second trial phase, which was applied 24 h after the first trial phase, one of the objects (a familiar object from 1st trial phase) presented was replaced with a new object (a novel object) and presented to the rats for 3 min. The formula described previously was used in the expression of the discrimination index created by measuring the time that the rats spent on investigated each object during Trial 2 (24).

The Y-maze test, a behavioural test that meant to quantify working memory and measures the willingness to discover new environments in rats, was used to evaluate the short-term spatial memory performance and cognitive deficiencies of the rats and the effects of treatments on cognition (25). Rats in the own cage were placed in the test room 1 h before the single session Y maze study to minimize the effects of the stress factor on behaviour during the test. The Y maze made of plexiglass material, 15 cm wide, 40 cm long, 30 cm high (three-arm maze with equal angles (120°) between all arms). The rat was placed in the centre of the maze and allowed to move freely through three arms during the test section (8 min). In the estimating short-term memory, consecutive selections such as ACB, CAB, or BAC were recorded. Meanwhile, triad (set of three letters) containing all three letters is scored as alternation. Total number of entries should not include first recorded arm (always B). The sequence and the number of arm entries were recorded using a video camera for each rat. The four limbs of the rat in the arm were counted as an entry. Successive entry in each arm was considered as alteration in behavior (26). The alternation score (%) for each rat was indicated as the total arm entries minus two and multiplied by 100 (26).

2.5. Immunoblotting

Before transferred onto the membrane (nitrocellulose, Schleicher and Schuell, Germany), all homogenized brain regions were separated into proteins with 12 % SDS-PAGE. The membranes were blocked by 1 % BSA in Tris-buffered saline with 0.1% Tween 20 (pH 7.6) for 2 h, and then incubated 14 h at 4 °C with the related antibody [Bcl-2 (1:100; sc-7382; @ 26 kD), Bax (1:100; sc-20067; @ 23 kD), casp-3 (1:200; sc-56053; @ 20 kD), casp-9 (1:200; sc-56076; @ 35 kD), M_1 (1:100; sc-365966; @ 52kD), BDNF (1:200; ab9794; @ 15 kD), or NR2B (1:100; sc-365597; @ 180 kD). Then, all membranes were incubated with a secondary antibody (sc-2354, 1:5000) for 1 h at 21 ± 3 °C temperature. Densitometric analysis of labelled proteins were performed using Bio-Rad Molecular Analyzer software (free edition, www.totallab.com). All values were normalized to b-actin (1:200 dilution; Santa Cruz, CA, USA).

2.6. Statistical Analysis

GraphPad software (Prism 3.0; GraphPad Software, San Diego, CA, USA) was used for statistical analysis. All data are represented as mean ± SEM. The groups of data were compared with analysis repeated measures two-way ANOVA followed by Bonferroni multiple comparison post hoc tests. $p < 0.05$ was considered a statistically significant difference between groups.

3. RESULTS

3.1. Behavioural Parameters

There was no significant difference in the difference score obtained from the NORT results of the LA, D+M and D+M+LA groups compared to the saline group. According to NOR test results, the difference score was found to be significantly decreased in the amnesia group as compared to the saline group ($t = 4.777$, $p < 0.001$; $dF = 56$). While the difference score increased in the amnesia groups treated with D+M and D+M+LA compared to the untreated amnesia group ($t = 4.143$, $p < 0.01$; $t = 6.610$, $p < 0.001$, respectively; Fig. 2A), there was no significant change in the amnesia group treated with LA.

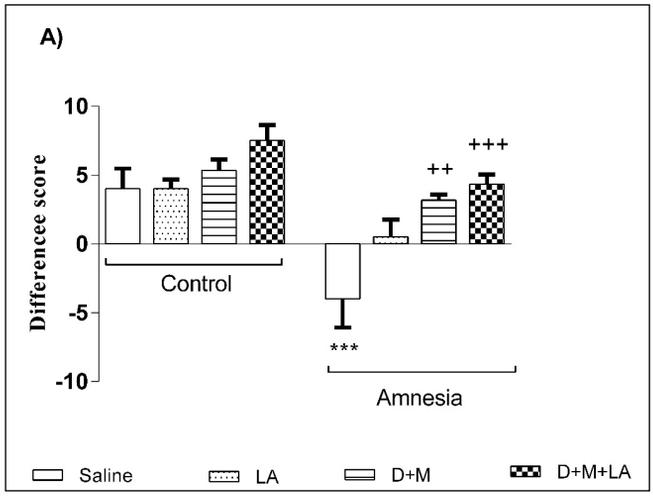

Figure 2. *According to a) NORT and b) Y-maze tests, the effect of saline, α-lipoic acid (LA), donepezil (D) + Memantine (M) (D+M), D+M+LA combine treatment on the scopolamine-induced amnesia rat cognitive deficit.*

*All data are represented as mean ± SEM. ***p<0.001, compared to saline treated-control group; ++p<0.01 and +++p<0.001, compared to only scopolamine group, p<0.05 compared to D+M-treated scop group (n = 8).*

There was not detected differently in the mean change behaviour scores for the saline, LA, D+M, and D+M+LA groups were 82.3 ± 5.4, 76.6 ± 6.9, 85.0 ± 5.8, and 83.0 ± 5.3 %, respectively. The amnesia group showed a significant decrease in the percent of alteration in the behaviour compared to the saline group ($t = 5.30$, $p < 0.001$, $dF = 56$; Fig. 2B). It was determined that the percentage of change in behaviour in the D+M and D+M+LA amnesia groups improved significantly compared to the amnesia group ($t = 3.49$, $p < 0.05$; $t = 6.73$, $p < 0.001$; Fig. 2B).

3.2. Immunoblotting Assay

Change's expression in the Bcl-2 (anti-apoptotic protein) / Bax (pro-apoptotic protein) ratio, casp-3 and casp-9 levels in the frontal cortex and hippocampus regions were used to evaluate apoptosis in the mitochondrial pathway (n = 4). The BDNF and NR2B expression level, contributing to the survival of the central nervous system was used to evaluate neuroplasticity in both brain regions.

There was no statistical difference between the saline group and the treatments administrated to the control group in terms of Bcl-2 / Bax ratio, casp-3, and casp-9 expression levels.

In hippocampal region analysis, it was determined that the Bcl-2 / Bax ratio decreased in the amnesia group compared to the saline group ($t = 6.344$, $p < 0.001$, Fig. 3A), and the expression levels of casp-3 and casp-9 increased ($t = 3.489$, $p < 0.05$, Fig. 3C; $t = 5.409$, $p<0.001$, Fig. 3E). It was found that the decrease in Bcl-2 / Bax ratio and the increase in casp-9 almost returned to control (saline group) values with D+M ($t=3.749$, $p < 0.05$; $t=7.707$, $p<0.001$, respectively) and D+M+LA ($t=7.497$, $p<0.001$; $t=8.856$, $p<0.001$, respectively) treatments administrated to the amnesia group. While casp-3 expression level decreased in amnesia group treated with D+M+LA compared to untreated amnesia group ($t=4.178$, $p< 0.01$), D+M treatment applied to amnesia group had no effect on casp-3 expression level. While D+M treatment alone could not reverse the increase in casp-3, adding LA to the combined treatment reversed this situation. In addition, LA did not change the Bcl-2 / Bax ratio and casp-3 expression level in amnesia rats. Differently, it was determined that LA treatment given to the amnesia group caused a decrease in casp-9 expression level in the hippocampus ($t = 5.648$, $p < 0.001$).

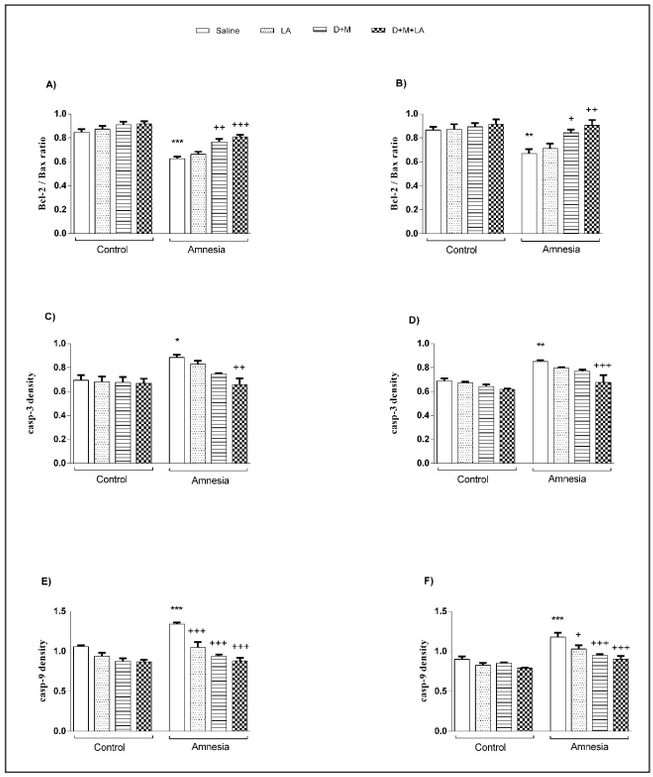

Figure 3. *The density of immunoblotting of a-b) Bcl-2 / Bax ratio, c-d) caspase-3, e-f) caspase-9 expression levels of the hippocampus and frontal cortex regions (respectively) of rats in the scopolamine-induced amnesia rat model.*

*All data are represented as mean ± SEM. *p<0.05, **p<0.01 and ***p<0.001, compared to saline treated-control group; +p<0.05, ++p<0.01 and +++p<0.001, compared to only scopolamine group; p<0.05 compared to D+M-treated scop group.*

When the amnesia group was compared with the saline group, Bcl-2 / Bax ratio was decreased in the frontal cortex region of the amnesia group compared to the saline group (t=3.98, p < 0.01, Fig. 3B) but casp-3 and – 9 levels were increased (t= 4.503, p < 0.01, Fig. 3D; t=5.873, p<0.001, Fig. 3F). In the D+M amnesia group, Bcl-2 / Bax ratio increased in frontal cortex region, unlike casp-9 expressions (t=3.23, p < 0.05; t=4.815, p < 0.001, respectively). There was no significant difference in the expression of casp-3 in the D+M amnesia group. When the amnesia group treated with D+M+LA was compared with the amnesia group, it was determined that the Bcl-2 / Bax ratio decreased and casp-3 and – 9 expressions increased (t=5.24, p < 0.001; t=4.925, p < 0.001; t=5.873, p < 0.001; respectively). In addition, in the hippocampus region of the amnesia group, D+M+LA treatment increased Bcl-2 / Bax ratio compared to D+M treatment group (t=3.749, p < 0.05). LA treatment in amnesia rats did not change the Bcl-2 / Bax ratio and casp-3 level as in the frontal cortex region. It was determined that the treatment given to amnesia group treated with LA caused a decrease in the expression level of casp-9 (t =3.174, p < 0.05).

There was no statistical difference between the saline group and the treatments administrated to the control group in terms of M1, BDNF and NR2B expression levels in both regions. It was determined that M_1 expression levels decreased in the hippocampus and frontal cortex regions in the amnesia group compared to the saline treated-control group (t=5.725, Fig. 4A; t=6.390, Fig. 4B; p < 0.001 in both regions). Among the treatments administrated to amnesia groups, D+M and D+M+LA treatments were determined to significantly increase M_1 expression in the hippocampal region compared to the amnesia group (t=4.425, p < 0.01; t=8.242, p < 0.001, respectively). Similar to the hippocampus, it was determined that the D+M and D+M+LA treatments administrated to the amnesia group showed the same effect in the frontal cortex region (t=3.238, p < 0.05; t=7.080, p < 0.001, respectively).

When the BDNF expression level was examined in the hippocampus region, it was determined that the BDNF level of the amnesia group decreased significantly compared to the saline group (t=5.676, p<0.001, Fig. 4C). In all groups treated for scopolamine-induced amnesia, BDNF level was found to increase significantly compared to the amnesia group (t= 3.997, p<0.01 for D+M-treatment; t= 7.182, p < 0.001 for D+M+LA-treatment). It was determined that the BDNF expression level of the amnesia group in the frontal cortex region decreased compared to the saline group (t=5.522, p<0.001, Fig. 4D). It was found that the decrease in BDNF expression was reversed and reached control values with D+M, and D+M+LA treatments administered to scopolamine-induced amnesia group (t=3.654, p < 0.05; t=6.902, p < 0.001).

In both regions, NR2B expression level was increased in the amnesia group compared to the saline group (t= 6.810, p < 0.001, Fig. 4E; t= 3.824, p < 0.01, Fig. 4F). In addition, NR2B expression was decreased in the D+M amnesia group compared to the amnesia group (t=3.190, p<0.05; t=3.364, p<0.05) in the both regions. In the hippocampus and frontal cortex regions, NR2B expression was decreased in the D+M+LA amnesia group compared to the amnesia group (t=6.781, p < 0.001; t=6.098, p < 0.01). While LA treatment administrated to the amnesia group did not cause any change in BDNF and NR2B expressions in both regions, it increased the M_1 expression level significantly compared to the amnesia group (p < 0.05).

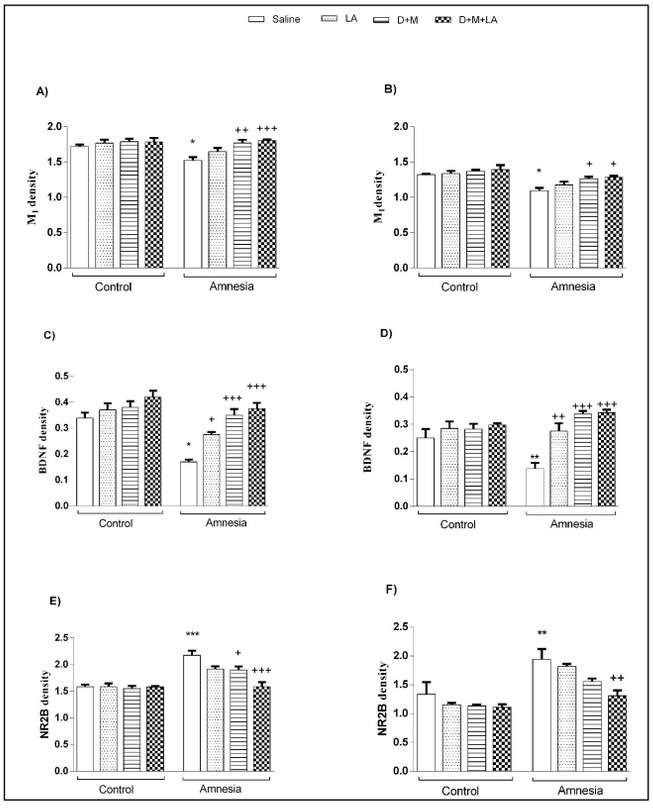

Figure 4. *The density of immunoblotting of a-b) M_1, c-d) BDNF, e-f) NR2B expression levels of the hippocampus and frontal cortex regions (respectively) of rats in the scopolamine-induced amnesia rat model.*

*All data are represented as mean ± SEM. *p<0.05, **p<0.01 and ***p<0.001, compared to saline treated-control group; +p<0.05, ++p<0.01 and +++p<0.001, compared to only scopolamine group; p<0.05 compared to D+M-treated scop group.*

D+M+LA treatment in the hippocampus region of the amnesia groups increased M_1, BDNF and decreased NR2B expression level compared to D+M treatment, while the difference between these two treatments in the cortex region was determined only in the BDNF expression level. Representative immunoblotting membranes obtained from different brain tissues for all antibodies used in the experiments were shown in Fig. 5.

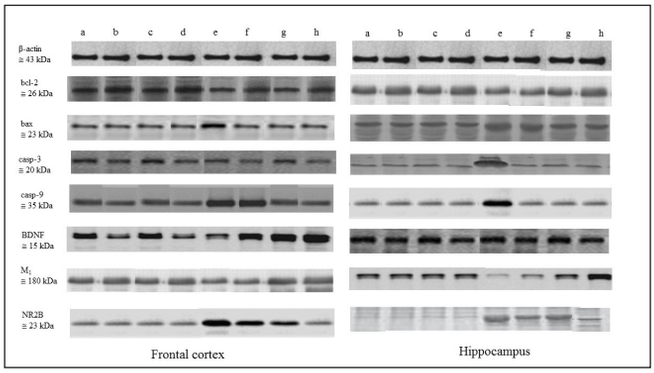

Figure 5. *The representative image of membranes obtained from western blotting experiments in the scopolamine-induced amnesia rat model.*
a. saline group; b. α-lipoic acid group; c. Donepezil + Memantine group; d. Donepezil + Memantine + α-lipoic acid group; e. amnesia (scopolamine-induced) group; f. α-lipoic acid amnesia group, g. Donepezil + Memantine amnesia group; h. Donepezil + Memantine + α-lipoic acid amnesia group.

4. DISCUSSION

The main findings of the current study demonstrated that combined treatment of memantine, donepezil and α-LA ameliorated behavioural and memory impairment in rats in the scopolamine-induced amnesia model. We have found that scopolamine-treated rats showed cognitive dysfunction in both the Y-maze and NOR test, while these decreased cognitive functions were reversed with memantine, donepezil and a-LA combined treatment. In addition, it was determined that the decreased Bcl-2 / Bax ratio and increased casp-3 and – 9 expression levels caused by scopolamine via the combined treatment in the hippocampus and frontal cortex regions were also improved. Another finding of the study is that decreased BDNF, M_1 and increased NR2B expressions due to amnesia in both cortex and hippocampus regions were reversed with combined treatment.

Previous studies reporting that *α*-LA plays a role in the biosynthesis of mitochondrial matrix proteins highlight its positive effect on mitochondria. *α*-LA performs indirect effect on mitochondria, which also has a protective role in the context of neuronal damage in the process of apoptosis, by stimulating mitochondrial biogenesis (14). In a study evaluating the effect of the expression of the Bcl-2 apoptosis regulator in the hippocampal region of rats, it was determined that Bcl-2 expression was increased due to α-LA administration (27). Since AD and amnesia are known to be related to mitochondrial damage occurring in neurons, some of the researches for the treatment of AD have turned to the search for a strong mitochondrial therapeutic agent. In the study that illuminated the neuroprotective molecular mechanism of *α*-LA to H2O2-induced cell death, H_2O_2 exposure has been reported to cause a decrease in the ratio of Bcl-2 / Bax that supports apoptosis and this is prevented by a-LA therapy. The results of this study also reported that suppression of casp-3 activation suppressed the formation of apoptosome (19). The improvements in cognitive and behavioural performances by *α*-LA observed here are in agreement with previous reports and our results suggest that *α*-LA might play a role in regulating the development of apoptosis. Therefore, *α*-LA may act as a potential therapeutic agent for its beneficial effect on the pathogenesis of amnesia. Our study results indicate that the combined treatment of memantine, donepezil and *α*-LA may be effective in mitochondria-mediated apoptosis. We suggest that α-LA may be effective in the treatment of amnesia due to its potential to restore mitochondrial damage, in addition to the treatment of memantine and donepezil in the clinic from our study findings.

There are studies in the literature that use *α*-LA therapy with various agents acting on central or peripheral nerve cells. In the schizophrenia model induced by ketamine, in the study in which the effect of α-LA and clozapine combined therapy was evaluated with Y-maze, it was found that combined therapy could restore impaired behavioural parameters. In the same study results, it has been reported that BDNF deficiency in frontal cortex caused by ketamine-induced is significantly reversed by α-LA and clozapine combined treatment (28). Although the relationship between AD and BDNF is still controversial, many data indicate that there is a negative correlation. Studies suggest that the decrease of BDNF mRNA in the hippocampus of individuals with AD suggests that BDNF may contribute to the progression of cell loss in AD. Peng et al. (2009) found that significant deficiency in BDNF levels in patients with AD showed a positive correlation with the degree of cognitive impairment of AD (29). In human and animal studies using donepezil in the treatment of AD, the drug has been shown to increase the level and expression of BDNF (30).

In addition to their vital role in synaptic transmission and plasticity, NMDARs also play a critical role in the neuronal survival pathway. Especially, synaptic NMDAR activation increases cell survival, while extra-synaptic activation triggers cell death. The impaired balance between these two NMDA population groups causes chronic neurodegenerative diseases (31). Current studies provide documents on NMDA receptor binding in AD, but details of changes in specific NMDA receptor subunits are not sufficient. Although studies in the literature show that NMDA receptors play a role in the pathogenesis of AD, the change in the expression levels of NMDA receptors and their relationship with apoptosis has not been clearly determined in this process. As in the results of the study by Liu et al., (2012) in our results high NR2B expression and apoptosis showed remarkable harmony in the hippocampus and frontal cortex regions of AD-like rats. The effects of acute donepezil or memantine or the combined application of both treatments on memory performances were tested in studies conducted to evaluate the treatments used to prevent the stimulant effect of NMDAR and to increase cholinergic function (32,33). Memantine, a widely accepted NMDA antagonist for treating AD, has also been proven to have an enhancing effect on the BDNF level (33). While some data do not fully support the positive correlation

between the BDNF and AD, it is unlikely to reject that higher BDNF levels actually improve cognitive dysfunction in AD. There are still problems in the development of BDNF-based therapies as BDNF increases in the early stage of AD and decreases in the final stage of AD (6). Our results have been shown to increase BDNF expression in both hippocampus and frontal cortex with combined therapy in accordance with the literature. Moreover, in our findings, the increase in NR2B expression and the decrease in bcl-2/bax ratio due to scopolamine-induced was reversed with α-LA treatment.

The association of acetylcholine with memory and cognitive function is well documented in the literature (34,35). Most of this information is about that the various improvements in cognitive function can be observed after cholinergic receptor activation. A role for mAChR has been hypothesized in many neurodegenerative disorders such as post-traumatic stress disorder. The most common types of the mAChRs that are found in the brain are M_1 type receptors and they are localized in the different brain regions such as hippocampus, cortex, and striatum (36). mAChRs are known to play a role in learning and memory, but the clarification of the precise roles of these receptors remains. It has been reported that M_1 blockage with different antagonists causes cognitive reductions in attention, learning, and memory. Muscarinic antagonists are an interesting option for inducing cognitive impairment and finding new pharmacological alternatives (37). Especially, the effect of scopolamine is interpreted as a cholinergic deficiency, which emphasizes the effect and importance of acetylcholine on memory functions (5). As a result of the study conducted by Araújo et al. (2017), it was emphasized that a-LA increases muscarinic M_1 receptor affinity in the hippocampus and frontal cortex regions (38). Therefore, in this study, the change in the expression of the M_1 subtype of mAChRs, which has an important role in cognitive functions and behavioural parameters, was evaluated by adding α-LA to the combination of donepezil and memantine used in the routine treatment of AD. In the study in which *α*-LA therapy was evaluated in the memory deficits in mice, it was determined that the treatment increased muscarinic M_1 receptor expression in the hippocampus (39). According to our findings, it was determined that the administration of *α*-LA to combined donepezil and memantine therapy in the hippocampus and frontal cortex regions increased the decreased M_1 receptor expression in scopolamine-induced amnesia rat model. In both our behavioural tests and molecular experimental results, the addition of *α*-LA to combined therapy showed that it significantly improved memory and the level of M_1 protein expression. As emphasized at the end of the related study, our results suggest that a-LA may have therapeutic potential in improving cholinergic functions. The down regulation of M_1 receptors may be regarded as a natural consequence of the plasticity. Therefore, this result may suggest that cholinergic receptors may mediate the improvement of cognitive functions in the AD process induced by scopolamine as a result of the addition of α-LA to AD routine treatment. Our study results suggested administration of α-LA to the combination of donepezil and memantine therapy as an excellent therapeutic candidate for cholinergic hypofunction in neurodegenerative disorders such as amnesia.

5. CONCLUSION

According to the results of our study, it has been shown that muscarinic (M_1) and glutamatergic (NR2B) systems play an important role in Alzheimer's pathogenesis. α-LA acid is a powerful antioxidant. Although it corrects apoptosis accompanied by oxidative stress, its effect alone is insufficient since it does not have as strong effects on the muscarinic and glutamatergic systems as other drugs. At present, donepezil and memantine are often preferred for treatment of AD based on cognitive deficits. We determined that the antiapoptotic effect of adding α-LA to donepezil and memantine treatment improves cognitive functions by regulating changes in M_1, NR2B and BDNF protein expressions in AD. In further studies, different studies and clinical evaluations can be pioneered by evaluating the effects of the drugs used in the treatment on the side effect profiles.

Conflict of interest: We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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Clinical and Experimental Health Sciences

Preliminary Findings of the Distribution of Alpha-2-Adrenergic Receptor (*ADRA2A*) rs1800544 Polymorphisms in Kickboxing Players

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ABSTRACT

Objective: Adrenergic receptors play an important role in providing neurotransmitter release from the sympathetic nervous system and adrenergic neurons. The rs1800544 polymorphism in 3'-untranslated region of Alpha-2-Adrenergic Receptor (*ADRA2A*) is if caused by C>G tranversion at position –1291. In this study, we objective to analyze the rs1800544 distribution in kickboxing players.

Methods: A total of 12 male players and 101 sedentary individuals (as a control group) participated in the study. Following DNA isolation, rs1800544 polymorphism of *ADRA2A* gene was determined by real-time polymerase chain reaction (Rt-PCR). Statistical analysis was carried out by chi-square test.

Results: CC, CG and GG genotypes and percentages for rs1800544 polymorphism were analyzed as 5 (41,7%), 4 (33,3%) and 3 (25%), respectively. C allele was counted as 14 (58,3%) and G allele as 10 (41,7%). In the control group CC, CG and GG were found as 34 (33,6%), 53 (52,5%), 14(13,9%), respectively. C allele was counted as 121 (60%) and G allele as 81 (40%). When we compare athletes and controls in the terms of genotypes, we detected no statistically significant difference (p>0,3940). For the allele comparison, we detected no significant difference between groups (p>0,0871).

Conclusion: Determination of gene combinations in athletes is of great importance for determination of the effect of genetic factors in athletic performance. In our cohort, CC genotype was more frequently found, and C allele was also higher in players. We think that the effect of *ADR2A2* gene on athletic performance might be determined more clearly by the fact that this study is an example of similar studies involving higher numbers of subjects.

Keywords: *ADRA2A*, Polymorphism, Sports, Kickboxing players

1. INTRODUCTION

Sport is a physical and cognitive contest that pursues rules for a specific purpose such as war or hunting preparations to determine the best, which comes from past to present activity. In both individual or team sports, athletic performance is limited by player's sporting ability or capacity that is influenced by several factors including physical, physiological, environmental and mental (1,2). All factors are directly or indirectly related with genetic/hereditary background even for diet type or exercise characteristics (3,4). This is why, the intrigue of association between sports and genes or continues to fascinate both athletes and researchers, despite current findings.

Kickboxing has historically been developed from karate, Thai Boxing and western boxing, and is a fighting sport that is generally used for standing and self-defense based on kicking and punching. Worldwide interest in kickboxing is increasing due to its beneficial effects such as personal protection, increasing muscle strength and keeping the body in shape (5). However, heavy blows to the head, neck and abdomen of the athletes during the competitions cause serious injuries and negatively affect the athlete's health in the short and long term.

Adrenergic receptors (adreno receptors) are members of the G protein-coupled receptor family class. It is a catecholamine linked to norepinephrine (noradrenaline) and epinephrine (6). It plays a major role in the release activity of the adrenaline hormone and the neurotransmitter of noradrenaline. There are 3 adrenergic receptor variants; α1, α2 and β (7). These receptors play an important role in the release activity of neurotransmitters from sympathetic

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nerves and adrenergic neurons in our central nervous system. Studies in mice revealed that both the α2A and α2C subforms of the receptors are required for presynaptic control of transmitter release from sympathetic nerves in the heart and central noradrenergic neurons. In the study, the α2A subtype was found to inhibit transmitter release at high stimulation frequencies, while the α2C subtype modulates neurotransmission at lower nerve activity levels (8).

Studies have shown that methylphenidate improves attention by acting on alpha2-adrenergic receptors found in dopamine-containing neurons. The functional rs1800544 polymorphism, which is formed by C>G tranversion at a position –1291, is located in the promoter region of *ADRA2A* gene. Another functional rs553668 polymorphism is located in the 3'-UTR region of the gene and occurs as a result of A>G transition. These are the two main polymorphisms investigated in Attention-Deficit/Hyperactivity Disorder (ADHD) patients. In pharmacogenetic studies conducted to date, the role of rs1800544 in the *ADRA2A* gene promoter region was investigated and the relationship of the G allele with MPH was evaluated (9). Also, attention deficit symptoms are associated with rs1800544. The *ADRA2A* gene is a small gene with a genomic size of <4000 bp. The a-2A-adrenergic receptor (*ADRA2A*) also plays a role in the regulation of sympathetic nervous system activity.

The aim of our study is to evaluate the distribution of *ADRA2A* (rs1800544) polymorphism in kickboxing players. To date, there are no studies on Turkish kickboxing players in the terms of *ADRA2A* polymorphism yet. Therefore, this report will be the first to determine the allelic distribution of rs1800544 polymorphism of kickboxing players.

2. METHODS

2.1. The Participants

A total of 12 male professional kickboxing players and 101 sedentary controls, all with Turkish ancestry, were recruited for the study. Our study protocol were prepared in accordance with the Helsinki Declaration-2 (2015) guidelines and approved by Uskudar University Non-Interventional Ethics Committee. (Protocol number 61351342/2019-550). The volunteers participating in the study were given detailed information about the analyzes and outputs before the study and their consent forms were obtained from them.

2.2. ADRA2A Genotyping

DNA Isolation

Oral epithelium cells were collected by DNA collection sticks from the volunteers who participated in the study, and DNA isolation was completed by using a PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, CA, USA). Briefly, 20µL proteinase K was vortexed by adding 10µL of RNAase to 200µL of DNA isolation. After 2 min at room temperature, 200µL of binding buffer was added and homogenized with stirring. After incubation for 10 minutes in a 55° C water bath, 200µL of ethanol was added and vortexed for 5 seconds. It was taken to the filtered tube and centrifuged at 10000g for 1 minute. The supernatant was discarded and 500µL of washing buffer was added to the pellet and centrifuged at 10000 g for 1.15 seconds. 80µL of elution buffer was added and incubated and centrifuged at maximum speed for 1 minute. An average of 20ng of DNA was isolated from each sample and the isolated DNAs were evaluated according to the OD260/280 spectrophotometric ratio. The DNA samples obtained were stored at – 20°C until the analysis of the relevant gene regions was completed.

Genotyping of *ADRA2A* rs1800544

Genotyping of *ADRA2A* rs1800544 was performed from the isolated DNA by using 7500 Fast Real-Time PCR System (Applied Biosystems). TaqMan Genotyping Assays (Applied Biosystems Foster City, CA, USA) genotyping kit was used for allelic determination. C and G alleles were determined using VIC and FAM primers, respectively (Table 1). Genotyping was completed using 5µL master mix, 3.75µL H_2O, 0.25µL assay and 1µL (10ng) DNA.

Table 1. *VIC/FAM labelled times primer use to ADRA2A rs1800544 polymorphisim*

qPZR	Sequence (5^3)
VIC/FAM	CCGTTGCGTTCTGCTCCGTCGGCCC**[C/G]**GAGCTGCATGGCCAACTCCCAGCAG

2.3. Statistics

All data were analyzed by using SPSS 20, 0 for windows (SPSS Inc., Chicago, IL, USA). Statistical analysis was conducted by using the chi-square test. Values less than P <0.05 were considered significant.

3. RESULTS

12 kickboxing and 101 control group participated in the study. Genotypes and alleles for the *ADRA2A* gene region are summarized in the Table 2. CC, CG and GG genotypes and percentages for rs1800544 polymorphism were analyzed as 5 (41,7%), 4 (33,3%) and 3 (25%); respectively. C allele was counted as 14 (58,3%) and G allele as 10 (41,7%). CC, CG and GG genotypes and percentages for rs1800544 polymorphism in controls were as 34 (33,6%), 53 (52,5%) and 14 (13,9%); respectively. For allelic distributions, C allele was counted as 121 (60%) and G allele as 81 (40%) (Table 2). When we compare athletes and controls in the terms of genotypes, we detected no statistically significant difference (p> 0,3940). For the allele comparison, we detected no significant difference between groups (p> 0,0871).

Table 2. *Genotype and allele distributions of ADRA2A rs1800544 polymorphism of kickboxing players.*

	ADRA2A Genotype			P value	Allele Frequency		P Value
	CC	CG	GG		C	G	
Players (12)	5	4	3		14	10	
Percentage	41,7%	33,3%	25%		58,3%	41,7%	
Control (101)	34	53	14	0,3940	121	81	0,0871
Percentage	33,6%	52,5%	13,9%		60%	40%	

**Significance was assessed at least at the p<0,05 level. Comparison with the control group was made using the χ^2 test.*

4. DISCUSSION

Kickboxing is a sport in which two competitors compete to get the most score points with the technique and speed in accordance with the rules. While punches and kicks are strictly controlled, points are awarded by hitting the hands or feet in accordance with the rules, with proper techniques on the permitted areas.

In the formation and development of athletic performance, muscle metabolism is expected to be optimal. The effect of genetic variations on metabolism on and cellular basis of activities, such as exercise, is important a lot through article crucial, therefore optimal to more favorable forms are expected to be included in the genotypes of athletes (10).

Recent studies have shown that genetic parameters are important in athletic performance and physical health. In studies to date, more than 200 gene variants have been associated with athletic performance, some with structural and some with functional factors such as enzymes (11). Recent studies that aimed to determine the athletic performance in the terms of environmental (epigenetic) and genetic factors had not been fully identified the association of genetic and epigenetic factors and warrants further research in sports genetics (12).

CC genotype was more dominant compared with other genotypes and C allele in the terms of *ADRA2A* rs1800544 polymorphism in our cohort. According to the best of our knowledge, this report is the first which investigate reflects the genotype distribution of rs1800544 polymorphism in Turkish kickboxing players.

Studies have reported that individuals with the C allele of *ADRA2A* rs1800544 polymorphism are higher than the G allele. It is stated that adrenergic receptors play a role in the regulation of adipose tissue lipolysis, which is one of the most important steps in meeting the energy needs of athletes during endurance sports training (13).

Wolfarth et al. (14) examined the *ADRA2A* and *ADRB2* gene polymorphisms in the study comparing 148 Caucasian elite endurance athletes and 149 the control group. Professional endurance athletes and a control group were compared. A significantly higher frequency of the 6.7-kb allele was observed in athletes, suggesting that this genetic variation in the *ADRA2A* gene may play a role in sustaining the endurance training necessary for enhanced maximal aerobic power. It is stated that genetic variation in the *ADRA2A* gene or in a locus in the immediate vicinity can be effective in sports that require endurance to achieve high levels of maximum aerobic power.

5. CONCLUSION

Currently, genetic factors are gaining importance importance in determining athletic performance. As athletic performance involves multifactorial elements, it is thought that analyzing gene groups instead of single gene analyzes may provide precise results in the studies. Determination of gene combinations in Turkish kickboxing is of increasing importance whilst carrying out these studies. Therefore, instead of comparing the alleles between athletes' sedentary individuals, we aimed to determine their distributions on successful athletes.

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Clinical and Experimental Health Sciences

The Effect of Foot Massage on Pain and Anxiety Levels after Laparoscopic Cholecystectomy: A Randomized-Controlled Trial

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ABSTRACT

Objective: This study was conducted to determine the effect of foot reflexology massage performed on patients after laparoscopic cholecystectomy on pain levels, analgesic use and anxiety levels.

Methods: This prospective, experimental and randomized-controlled study was conducted with patients who underwent cholecystectomy. Ninety patients in total were divided into the experiment (n = 30), control (n = 30) and placebo (n = 30) groups. Foot reflexology massage was performed on the patients in the experiment group two times after surgery. Placebo massage was performed on the patients in the placebo group at the same times. Only routine treatment and care were given to the patients in the control group. The patients' pain and anxiety levels and their amounts of analgesic use within 24 hours were monitored.

Results: The patients' state anxiety scores decreased significantly from the preoperative to the postoperative measurements, and the degree of decrease did not differ between the groups ($p>0.05$). The pain scores of the patients decreased after the first and second massage applications in all groups in comparison to their pre-massage scores, and the pain in the experiment group felt after the second massage was lower than that in the patients in the placebo group ($p<0.01$). It was found that analgesic administration for the patients within 24 hours after the operation did not differ in terms of quantity or time of administration ($p>0.05$).

Conclusion: As a result, it may be stated that using reflexology with pharmacological methods to reduce postoperative pain will increase the effectiveness of pain treatment and the quality of nursing care.

Keywords: Pain, anxiety, reflexology, foot massage, cholecystectomy

1. INTRODUCTION

Laparoscopic cholecystectomy is the most preferred method in the treatment of gallbladder stones, one of the most common digestive system problems worldwide (1-3). After laparoscopic cholecystectomy procedures, pain may be observed due to surgical manipulation, irritation from the carbon dioxide given to the intraperitoneal area, intraabdominal pressure increase, peritonitis caused by bile and trauma caused by trocars (4, 5). It was reported that 13% of patients who underwent laparoscopic cholecystectomy experienced severe pain in the first week after surgery, and 17 – 41% were hospitalized due to pain (5). In a large-scale study with over 50,000 patients, Joshi and Kehlet evaluated pain severity levels. They demonstrated that patients experienced more severe pain in comparison to 'major' procedures after 'minor' surgical interventions such as appendectomy, tonsillectomy and cholecystectomy (6). Additionally, the majority of patients undergoing surgical interventions experienced different levels of anxiety due to the form of anesthesia, difficulty in waking up after surgery, disability, post-operative pain, difficulty in working life, loss of body control, and fear of loss of sexual function (7-10). It is a known fact that a high anxiety level in the patient affects their pain levels in the postoperative period (11). Considering the complications of pharmacological methods such as respiratory depression, itching, nausea, vomiting and decreased gastrointestinal motility in the treatment of postoperative pain and anxiety, complementary and alternative therapy (CAT) methods have gained importance (12).

In the literature, it is stated that one of the methods of complementary and alternative medicine for relieving pain and anxiety in the postoperative period is reflexology massage

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(12-27). The International Reflexology Institute defines reflexology as a technique that is applied manually to the reflex points in the hands, feet and ears, which are associated with all glands, organs and body parts, and helps normalize body functions (28). Reflexology may often be used as a supplement to relieve the negative effects of chemotherapy and contribute to quality of life, regulate autonomic nervous system functions, strengthen the immune system and reduce stress, anxiety, agitation, tension, depression, fatigue, insomnia, as well as pain severity in acute and chronic painful conditions (22, 29-34).

However, there is a lack of studies in the literature that clearly demonstrate the effects of placebo-controlled reflexology massage. This study was planned and performed to determine the effect of foot reflexology massage on pain and anxiety levels in patients undergoing laparoscopic cholecystectomy surgery.

2. METHODS

2.1. Study Design and Setting

The research was conducted in a general surgery clinic of a university hospital in Istanbul between September 2017 and November 2018 with a prospective, single-center, experimental and randomized-controlled design. The study was started after receiving the approval of the clinical research ethics committee of the hospital (Date: 20.03.2017, Decision No: 001). Before starting the research process, clinical nurses were informed about the research. Patients who met the research criteria were interviewed and informed about the research, their questions were answered, and their consent was obtained verbally and in writing.

2.2. Participants

The population of the study included all patients undergoing laparoscopic cholecystectomy surgery within the specified time interval. Power analysis was performed using the G*Power 3.1 program to determine the minimum number of participants, and the power of the test was calculated. For the power of the study to exceed 80%, it was calculated that a total of 84 people, including 28 people per group, should be reached with a 5% significance level and a 0.25 effect size (t = 1.404; effect size d = 0.25). Considering that there could be losses during the research period, it was decided to admit a total of 90 patients, including 30 in each group.

The randomization of the patients who met the sampling criteria was performed using the closed envelope method, and the patients were randomly divided into three groups as foot reflexology massage (experiment group), placebo foot massage (placebo group) and no intervention (control group).

Patients without any health problems related to the foot (local infection, open lesion/wound, scar tissue, edema), who were aged 18 or older, without communication difficulties and mental deficiencies, without any complications (e.g., bleeding, nausea, vomiting, thrombophlebitis) after surgery, without the use of any medication such as analgesics before the first massage planned to be performed after surgery, without patient-controlled analgesia after surgery, with no drain placed in the surgical site, without addiction disorders and with no chronic pain in any part of their body were included in the study. The randomization of the study is shown in CONSORT (Consolidated Standards of Reporting Trials) 2010 (Figure 1)

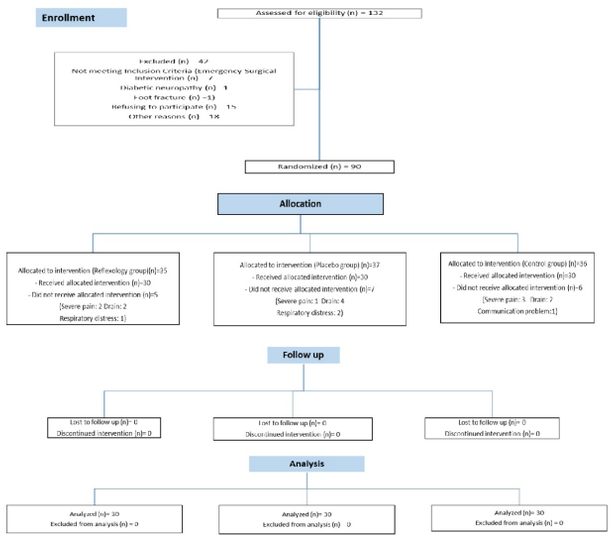

Figure 1: *CONSORT flow diagram*

2.3. Intervention

After the patients were admitted to the ward on the day of their surgical operation, a data collection form containing information about the patient's personal and medical history was completed. The content and method of the study, the scales to be used in the evaluation of the variables and the ethical principles that would be followed during their participation in the study were introduced to the patient. The vital signs of the patient (blood pressure, pulse rate, respiratory rate, oxygen saturation) were measured and recorded.

First Stage: In the early postoperative period, when the patient's location, time and person orientation was achieved, the severity and quality of the pain was evaluated. Their vital signs were measured. Foot massage was performed for the experiment group and placebo group based on their protocols specified above. Pain intensity and vital signs were re-evaluated 10 minutes after the end of the massage. The measurements were repeated at the same time as the other group, and no intervention was performed for the control group.

The verbal expression of the patient was excluded from the scope of the study by administering analgesics at the request of a physician to the patients whose pain severity was unbearable based on the observation of the researcher or the patient's physiological symptoms. From among a total of 132 patients who received laparoscopic cholecystectomy operations throughout

the study period, the study excluded 7 patients as they received emergency surgical interventions, 15 as they did not provide consent to participate in the study, 3 as they experienced respiratory distress after the surgery, 8 as drains were placed into them after the surgery, 5 as they experienced severe pain after the surgery, 3 as it was not possible to communicate with them in the postoperative period, one patient as they had diabetic neuropathy and another as they experienced a fracture in their foot in a recent time.

Second Stage: When the half-life of the analgesics given to the patients in the postoperative recovery unit at the 3rd hour after the surgery had expired, the patients in the experiment and placebo groups received a second foot massage. Pain levels and vital signs were measured in the same way as in the previous stage.

Third Stage: At the 4th hour after surgery, the State Anxiety Inventory was re-applied to all patients. Analgesics and their usage amounts were recorded within 24 hours after surgery.

Experiment group Foot Reflexology Protocol

The patients were informed about the treatment protocol. At the beginning of the massage, foot warming movements were performed for about 5 minutes. Light pressure was applied to the solar plexus area of the feet for one minute. First, reflexology was applied to the gallbladder and digestive and musculoskeletal areas of the right foot.

Reflexology massage was performed only on the areas related to the digestive system and the musculoskeletal system because there is no area belonging to the gallbladder in the left foot. Light pressure was applied to the solar plexus area of the feet for one minute, and the treatment was terminated. The reflexology massage to the right and left feet was administered over three periods, lasting 30-35 minutes in total.

Placebo Group Massage Protocol

The protocol was explained to the patients. Foot heating with light pressure was performed only on the dorsal areas of the patients in this group because the plantar region of the foot is associated with the gallbladder and other reflexology regions of the digestive system. The massage applied on the right and left feet was performed for a total of 10-15 minutes.

The reflexology massage was performed by the researcher who has been trained and certified in this field under the supervision of a physician. The massage protocols that were determined for the experiment and placebo groups were evaluated by two reflexologists who are experts in their field. The vital signs of the patients were monitored five times using a clinical blood pressure measurement device and a pulse oximeter. The specified measurement times were as follows: (Z1) before surgery, (Z2) before 1st massage, (Z3) 10th min after 1st massage, (Z4) before 2nd massage, and (Z5) 10th min after 2nd massage.

2.4. Data Analysis

The data obtained in the study were analyzed using the Statistical Package for the Social Sciences 2018 program**.** Whether or not the data met assumptions of normal distribution was tested, frequencies, percentages and means were used as descriptive statistics, and Chi-squared test, t-test, one-way analysis of variance (ANOVA), regression and correlation analyses or Mann-Whitney U and Kruskal-Wallis tests were used accordingly based on the normality of data distribution.

3. RESULTS

It was determined that each group had similar introductory characteristics in their first measurements (Table 1). In the measurements before the surgery, the mean trait anxiety scores of the experiment group, control group and placebo group were 37.63 ± 6.30, 41.40 ± 7.98 and 36.26 ± 6.37, respectively. There was no statistically significant difference between the trait anxiety scores of the groups ($p>0.05$). Similarly, in the intergroup comparisons, there was no difference between the mean preoperative state anxiety scores of the patients in the groups (experiment: 36.76 ± 10.75; control: 38.40 ± 8.90; placebo; 39.83 ± 10.66) or their mean postoperative state anxiety scores (experiment: 29.10 ± 6.67; control: 31.76 ± 8.35; placebo: 31.16 ± 6.94) ($p>0.05$). In the intragroup comparisons, it was found that the mean postoperative anxiety scores in all groups were significantly lower in comparison to their mean preoperative scores ($p<0.05$), but there was no statistically significant intergroup difference among the groups in terms of their mean postoperative scores ($p<0.05$) (Table 2).

The mean pain scores of the patients in all groups after the 1st massage were significantly lower than their mean scores before the 1st massage ($p<0.05$). Additionally, the mean pain scores expressed by the patients in all groups before the 2nd massage were lower than those they expressed before the 1st massage, and the mean pain scores expressed after the 2nd massage were lower than those before the 2nd massage ($p<0.05$) (Table 3). According to the results of the intergroup comparisons of the mean pain scores of the patients before and after the first and second massages, the patients in the experiment group had lower pain scores after the 2nd massage than those in the placebo group ($p<0.01$). However, it was not possible to precisely reveal between which groups the pain score after the 1st massage significantly differed in the statistical sense ($p = 0.01$) (Bonferroni test) (Table 4).

When the amounts of analgesics administered to the patients in the experiment, control and placebo groups in the first 24 hours after the operation were examined according to the drug types, it was determined that, based on the mean values, 18 mg tenoxicam, 2200 mg paracetamol and 35.33 mg tramadol were administered to the patients in the experiment group; 16 mg tenoxicam, 1666.80 mg paracetamol and 65.84 mg tramadol were administered to the patients in the control group, and 18.14 mg tenoxicam, 1166.58 mg paracetamol and 54.66 mg tramadol were administered in the placebo group. There was no statistically significant difference between the groups in terms of the amounts of analgesics given to the patients within the first 24 hours after the operation ($p>0.05$).

Table 1. *Introductory characteristics of patients (N=90)*

Introductory Characteristics		Experiment (n=30)	Control (n=30)	Placebo (n=30)
		Mean ± SD	Mean ± SD	Mean ± SD
Age		46.93 ± 14.74	47.06 ± 11.50	42.06 ± 11.67
		n (%)	n (%)	n (%)
Sex	Female	16 (53)	20 (67)	23 (76)
	Male	14 (47)	10 (33)	7 (24)
Education Level	Illiterate	0 (0)	0 (0)	1 (3)
	Primary school	4 (13)	6 (20)	2 (7)
	Secondary school	1 (3)	0 (0)	2 (7)
	High school	2 (7)	11 (37)	8 (27)
	Undergraduate and Graduate	23 (77)	13 (43)	17 (57)
Marital Status	Married	26 (87)	26 (87)	21 (70)
	Single	4 (13)	4 (13)	9 (30)
Job	Housewife	9 (30)	13 (43)	9 (30)
	Student	0 (0)	1 (3)	0 (0)
	Civil Servant	7 (23)	5 (17)	8 (27)
	Self-Employed	8 (27)	8 (27)	9 (30)
	Retired	6 (20)	3 (10)	4 (13)
Monthly Income	Income less than expenses	3 (10)	3 (10)	2 (7)
	Income equal to expenses	16 (53)	20 (67)	22 (73)
	Income more than expenses	11 (37)	7 (23)	6 (20)
Social Security	SSI	0 (0)	4 (13)	3 (10)
	Private Insurance	29 (97)	26 (87)	26 (87)
	No Social Security	1 (3)	0 (0)	1 (3)
Place of Residence	City	29 (97)	30 (100)	30 (100)
	District	1 (3)	0 (0)	0 (0)
Smoking	Yes	7 (23)	9 (30)	13 (43)
	No	23 (77)	21 (70)	17 (57)
Alcohol Use	Yes	4 (13)	2 (7)	3 (10)
	No	26 (87)	28 (93)	27 (90)

Table 2. *Comparison of the state and trait anxiety scale scores before and after surgery (N=90)*

State and Trait Anxiety Scale Scores	Experiment (n=30)		Control (n=30)		Placebo (n=30)		χ^2	P
	Mean ± SD		Mean ± SD		Mean ± SD			
Trait Anxiety Before Surgery	37.63 ± 6.30		41.40 ± 7.98		36.26 ± 6.37		3.127	0.20
Preoperative State Anxiety (D1)	36.76 ± 10.75		38.40 ± 8.90		39.83 ± 10.66		2.009	0.36
Postoperative State Anxiety (D2)	29.10 ± 6.67		31.76 ± 8.35		31.16 ± 694		1.930	0.38
Multiple Comparison	**Z**	**P**	**Z**	**P**	**Z**	**P**		
D1 – D2	-4.155	<0.001*	-4.112	<0.001*	-4.417	<0.001*		

**p< 0.05 (Wilcoxon test, Kruskal-Wallis test)*

Table 3. *Comparison of patients' mean pain scores before and after foot massage-1 (N=90)*

Pain Monitoring Times	Experiment (n=30)		Control (n=30)		Placebo (n=30)		χ^2	P
	Mean ± SD		Mean ± SD		Mean ± SD			
1. Before reflexology massage (A1)	7.46 ± 1.87		6.70 ± 2.49		7.46 ± 1.96		1.984	0.371
1. After reflexology massage 10th min (A2)	5.23 ± 2.22		5.70 ± 2.79		6.83 ± 2.26		6.178	0.04*
2. Before reflexology massage (A3)	4.60 ± 2.02		3.80 ± 2.85		5.13 ± 2.45		5.263	0.07
2. After reflexology massage 10th min (A4)	2.70 ± 2.15		3.16 ± 2.19		4.36 ± 2.44		7.184	0.02*
Multiple Comparison	**Z**	**P**	**Z**	**p**	**Z**	**P**		
A1 – A2	-4.575	**<0.001***	-2.956	**<0.001***	-2.724	**<0.001***		
A1 – A3	-4.302	**<0.001***	-4.301	**<0.001***	-4.292	**<0.001***		
A3 – A4	-4.529	**<0.001***	-1.978	**0.04***	-3.630	**<0.001***		

**p<0.05 (Wilcoxon test, Kruskal-Wallis test)*

Table 4. *Comparison of patients' mean pain scores before and after foot massage – 2 (N = 90)*

	Groups	n	Mean Rank	U	Z	P
1. (A2) Pain Score after Reflexology Massage	Experiment Placebo	30 30	24.82 36.18	279.50	-2.547	0.01
	Experiment Control	30 30	28.67 32.33	419.50	-0.455	0.64
	Placebo Control	30 30	34.12 26.88	341.50	-1.620	0.10
2. (A2) Pain Score after Reflexology Massage	Experiment Placebo	30 30	24.73 36.27	277.00	-2.579	0.009*
	Experiment Control	30 30	28.67 32.33	395.00	-0.822	0.41
	Placebo Control	30 30	34.62 26.38	326.50	-1.843	0.06

*(*p<0.01) (Bonferroni test)*

4. DISCUSSION

In the literature, it has been reported that most patients who undergo surgical interventions experience different degrees of anxiety and increased perioperative anxiety levels raise postoperative morbidity and mortality rates, delay wound healing and extend hospital stay, as well as increasing postoperative pain levels and requirement of analgesics (7-11).

In the study by Hudson et al. on the effects of hand reflexology massage performed on patients undergoing minimally invasive varicose vein surgery on their pain and anxiety, it was found that the levels of the anxiety felt by the patients during the procedure in the treatment group were significantly lower than those in the control group (25).

Koraş and Karabulut (2018) examined the effects of foot massage performed on patients undergoing laparoscopic cholecystectomy on their pain and anxiety levels, and the postoperative state anxiety score of the experiment group was found to be lower than that in the control group (12). In a study with 60 patients (36.7% of whom underwent cholecystectomy) who had undergone abdominal surgery, similarly, there was no difference between the basal anxiety levels of the experiment and control groups, and the anxiety level was lower in the foot massage group after the third application (35). In another study conducted with patients who had undergone digestive system surgery, it was stated that there was no difference between the baseline anxiety levels of the experiment and control groups, as well as finding a decrease in the anxiety levels of the experiment group after the intervention (19). Besides, the decrease in the anxiety levels of the experiment group after the implementation was significantly higher (19).

In many studies examining the effect of foot massage on different levels of anxiety after surgery with different protocols in different patient groups such as abdominal surgery, tibial fracture, total abdominal hysterectomy, coronary artery bypass grafting and coronary angiography, it has been reported that the anxiety levels of the patient groups that received foot massage were significantly lower than those in the control groups (17, 22, 25, 36-38).

In this study, the mean postoperative state anxiety scores of the patients in the experiment, control and placebo groups decreased significantly in comparison to their mean preoperative scores ($p<0.05$). In the intragroup comparisons, no significant difference was found between the mean preoperative and postoperative state anxiety scores in any of the groups ($p>0.05$). Although there was a decrease in the levels of postoperative anxiety in all three groups, the finding the difference between the groups in terms of this drop was not significant was interpreted to be dependent on factors such as the duration of the massage, application areas, application technique and application amount.

The effective treatment of postoperative pain in surgical patients contributes to the psychologic state of the patient, increases their quality of life and comfort and prevents the negative effects of pain on body systems (13). In a study examining the effects of ear acupuncture and foot reflexology massage on patients undergoing appendectomy, it was revealed that, although the pain levels in all groups decreased after receiving the acupuncture intervention, the decrease was greater in the patients in the experiment group (39). In the study by Shehata et al. (2016), the effect of foot massage performed on patients undergoing abdominal surgery in two sessions on these patients' pain levels was examined, and after the first and second applications, the expression of the pain by the patients in the experiment group was found to be lower than that of the patients in the control group (26). In the study by Çankaya and Sarıtaş on patients undergoing laparoscopic cholecystectomy, it was revealed that the pain level in the experiment group was lower than that in the control group as a result of conventional foot massage performed for 10 minutes (16). Although there was no significant difference between the pain levels of the experiment and control groups before the massage intervention in the studies by Youssef and Hassan (2017) (N=60) and Chanif (2012) (N=40) among patients who underwent abdominal surgery, it was reported that the degree of pain reduction was significantly greater than that in the control group (35, 36).

Similar results have been achieved in the literature after foot massage in different patient groups such as breast surgery, cesarean surgery, tibia fracture surgery, CABG surgery and total abdominal hysterectomy patients (14, 17-19, 22, 23).

Unlike these studies, Koraş's study with patients who underwent cholecystectomy revealed no statistically significant difference between the mean pain intensity scores at the 5th and 30th minutes after massage in the experiment and control groups. Additionally, when the mean pain intensity scores of the patients at the 60th, 90th and 120th minutes were compared, the mean scores of the experiment group were found to be lower than those of the control group (12). In the study by Hudson et al. (2015), the effect of hand reflexology massage performed on patients

undergoing minimally invasive varicose vein surgery during the procedure on pain was examined, it was determined that the pain intensity scores of the experiment and control groups were not significantly different, and the duration of the pain in the experiment group was shorter (25). In the study conducted by Silverdale and Roodhouse (2019) with 38 patients who underwent radical cystectomy due to bladder cancer, the patients were divided into 3 groups as the whole-body massage group, foot massage group and a group where both massages were performed together, and the protocols were performed in two sessions. It was revealed that pain levels after both treatments were lower in all groups than before the massage, but the degree of reduction did not differ between the groups (40).

In this study, although the mean pain scores of the patients in all groups decreased over time, the pain felt in the experiment group after the second massage was found to be significantly lower than that in the placebo group ($p<0.01$). However, it was not possible to precisely determine between which groups the pain score after the 1st massage differed significantly ($p=0.01$) (Table 4 – 15). In the study, the decrease in the mean pain scores in all patient groups in the postoperative period in comparison to their preoperative scores was consistent with the literature; however, the fact that the decrease in the mean pain scores was not significantly different between the experiment group and the other groups differed from the literature. Koraş and Karabulut (2018) identified no significant difference between the pain levels of the experiment and control groups at the 5th and 30th minutes after foot massage. This difference might have been due to the long-term evaluation of the effects of massage, similar to that of Koraş and Karabulut (12).

Analgesics, which form the basis of pain therapy, are drugs that are used to control pain in acute and chronic pain syndromes (41). In spite of the new knowledge and experience in this field, complementary and alternative treatment methods such as reflexology have become important in the management of post-operative pain and anxiety, considering that pharmacological methods may cause complications such as respiratory depression, nausea, vomiting, itching, convulsions and decreased gastrointestinal motility (12).

In a study in which the effect of foot reflexology massage performed with ear acupuncture on the use of analgesics in patients undergoing appendectomy was analyzed, there was no difference between the analgesic use amounts of the patients in the placebo and control groups, whereas the amount of analgesic use in the experiment group was lower (39). In a study performed with a 3rd session of foot reflexology massage after cancer surgery of the digestive system, it was revealed that the patients in the experiment group used smaller quantities of opioid analgesics than those in the control group (19). In a study conducted using similar methods in patients undergoing cholecystectomy, the quantities of analgesics required in the patients in the experiment and control groups were compared, and it was revealed that the control group used analgesics at higher quantities than the experiment group (12). Similar results were obtained in Asadisker's (2011) CABG group and Öztürk et al.'s (2017) study in patients undergoing abdominal hysterectomy (14, 22).

In this study, when the quantities of the analgesics given to the patients in the experiment, control and placebo groups within the first 24 hours after the operation were examined based on the types of drugs, it was observed that all patients were treated with one or more types of medications such as tenoxicam, paracetamol and tramadol after surgery. It was observed that there was no significant difference in the quantities of the analgesics given to the patients within the first 24 hours after the operation between the groups, and accordingly, these results were not similar to those reported in the literature ($p>0.05$). It is thought that this incompatibility between this study and others in the literature may have been due to the use of pharmacological pain relief methods rather than non-pharmacological pain relief methods in postoperative care for various reasons (e.g., the crowdedness of the hospital, the excess workload of the nurses) in the hospital where the study was conducted.

5. CONCLUSION

As a result of this research, it was found that foot reflexology massage performed in the postoperative period did not have a significant effect on the mean state anxiety scores and physiological parameters of the patients. Additionally, the patients' pain scores after the first and second massage decreased in all groups in comparison to their scores before the massage, and the severity of the pain that the patients in the experiment group reported after the second massage was lower than that reported by the patients in the placebo group. Consequently, it may be stated that using reflexology with pharmacological methods to reduce postoperative pain will increase the effectiveness of pain treatment and the quality of nursing care.

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Clinical and Experimental Health Sciences

Access to Health Services by the Parents of Individuals with Intellectual Disability, According to their Health Literacy Levels

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ABSTRACT

Objective: Health literacy is designated as a "Social Barrier" to accessing health services. The aim of the study was to determine the access status of the parents of mentally disabled individuals to health services according to their health literacy levels.

Methods: The universe of this descriptive study consisted of the parents of individuals with intellectual disability (N:118). The data collection instruments used in the study were the Health Literacy Scale for Turkey-32 and an Access to Health Services Assessment Questionnaire. Ethics Committee permission and approvals were obtained.

Results: A statistically significant difference was found between the caregivers' health literacy levels and their knowledge of how to benefit from health services, their ability to understand the information given to them by health personnel, their ability to correctly communicate their needs, their access to health services and to adequate information, their ability to understand the information gathered and to obtain an appointment from health institutions, their tendency to give up due to the difficulties they faced in procuring an appointment, and their physically being unable to reach health services ($p<0.001$).

Conclusion: The health literacy levels of many of the participants were limited and inadequate. Those with high levels of health literacy were better able to access health information resources, health facilities and services compared to those with low levels of health literacy.

Keywords: intellectual disability, parent, caregiver, health literacy, access to health services, policy

1. INTRODUCTION

A poor level of health literacy is generally regarded as a strong social barrier to accessing health services (1). Health literacy (HL) refers to an individual's capacity to access health services and fundamental health information, and to process and understand this information in order to use it efficiently. This capability allows an individual to not only access health services but to develop an enhanced competence to maintain the ability to deal with different types of data that will help in the management of a medical condition and increase the individual's future navigation of the health system (2,3).

Health literacy levels (HLL) are indirectly proportional to health care utilization and expenditure. Individuals with HLL's below the basic level are seen to have more prescriptions than those with a basic HLL; these prescriptions usually apply to health services and display a greater frequency but are sometimes unnecessary, thus causing a rise in n health services expenditure (4). It has been reported in many studies that low HLL's can affect or worsen the health outcomes of elderly and disabled individuals due to a decreased utilization of preventive healthcare services, increased difficulty with obtaining medicines and the inability to interpret medicine leaflets and other information (3-5). It is estimated that there are about 978 million individuals with disability in the world today. Approximately 1%-3% of the global population (200 million people) have a disability (6). In Turkey, this rate is 6.9% of the national population (4 million people) (7).

Disadvantaged groups such as individuals with disability and their relatives face the adversities of limited access to health services and they are also affected by an inadequate or limited level of health literacy. For this reason, they run the risk of

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obtaining insufficient and/or inappropriate healthcare (8,9). In recent years, researchers have reported that individuals' health literacy levels hold much more significance for the caregivers of these individuals (10,11).

In a study conducted with disabled adults in the United States in 2013, it was found that the frequency at which individuals with disability accessed facilities to obtain Pap smear tests, mammograms and colorectal cancer scans was lower than it was among non-disabled individuals (12). It was reported in a study by Chinn that the failure of interventions designed to protect and improve the health of individuals with intellectual disability was to a large extent the result of the fact that the health literacy levels of the intellectually disabled and their caregivers were not taken into consideration (13).

Health literacy levels are one of today's most important public health issues (14). The responsibility of identifying the current health literacy levels of individuals with disability and their caregivers, especially the parents of these individuals, providing them with needed information, and acting in line with this information falls upon the shoulders of healthcare professionals. In the light of this knowledge, it is noted that both academics and practitioners refer to inadequate health literacy as the "silent epidemic" that encompasses most of the world population (15). Some of the reasons the present study was conducted with the caregivers of individuals with intellectual disability include the fact that this group represents the most severely disadvantaged group in the population. Individuals in this group are generally unable to manage their own health and inevitably need the assistance of a caregiver and, because of their vulnerability, it will most likely be their parents who will be their primary caregivers and the ones who are in a position to make decisions on their behalf (16,17). Because of this, caregivers need to have a high degree of sensitivity. Targeting and raising the level of health literacy of individuals with disability and their caregivers has been formulated as a political goal in national plans of action (18). Caregivers who provide care to individuals with intellectual disability urgently need to be a part of the conversation surrounding health literacy (14). There is only a limited number of studies looking into the health literacy levels of individuals with disability and their caregivers and the access of this group to healthcare services. The aim of this study is to determine the health literacy levels of the parents of individuals with intellectual disability and their access to health services based on their HL levels.

2. METHODS

2.1. Study Design

The study was conducted with descriptive design.

2.2. Sample

This study was conducted over the period January – May 2018. The universe of the study comprises the parents of individuals with intellectual disability attending a training program at three separate private training and rehabilitation centers (N:280). A sample selection was not made; an attempt was made to reach the entirety of the study universe (n:118). Inclusion criteria for the study were being a primary caregiver to the disabled individuals in the study and consent to participate in the research. Parents who themselves had a disability were excluded from the study. The research question was formulated as: Are there differences between the health literacy (HL) levels of the parents of individuals with intellectual disabilities and their access to health services?

2.3. Measurements

Data collection tools were the Sociodemographic Questionnaire, Access to Health Services Assessment Questionnaire and the Health Literacy Scale for Turkey-32.

Sociodemographic Questionnaire: This questionnaire contains 8 questions on gender, age, health insurance, civil status, education level, income level, employment status, relationship to the individual with intellectual disability. This form was drawn up by the authors in line with the literature (2,3,5,13,14).

Access to Health Services Assessment Questionnaire: This questionnaire, consisting of 10 items, was drawn up by the authors on the basis of a scan of the literature. The questions relate to physical access to health services, information provided about health services, training, understanding materials, being able to communicate one's needs, and being able to organize appointments. The form was drawn up by the authors in line with the literature (1,2,5,9,11-13,15).

Turkish Health Literacy Scale-32: This scale, developed by Okyay and Abacıgil, evaluates the health literacy levels of individuals over the age of 15 or with minimum language literacy. There are 32 questions. The scale is based on a conceptual framework that was developed by the European Health Literacy Consortium Each item is scored on a 4-point scale as: 1=Very easy, 2=Easy, 3=Difficult, and 4=Very difficult. The response "I have no idea" was coded 5. The level of health literacy was assessed in four categories according to the scores obtained: (0-25): inadequate; (>25-33): problematic-limited; (>33-42): adequate; and (>42-50): excellent (19). Cronbach's alpha coefficient for the original questionnaire is 0.92. In this study, Cronbach's alpha was 0.80.

2.4. Data Collection

The participants were informed about the study and their consent was obtained. Data were collected from each participant in an average of 30-45 minutes through face-to-face interviews.

2.5. Data Analysis

The data collected were evaluated using descriptive statistics and chi-square analysis. Komolgornov Smirnov test was used for

normal distribution. Statistical analyses were carried out using the statistical software package SPSS Statistics for Windows, version 17. The level of significance was accepted to be 0.005.

2.6. Ethical Considerations

Approvals for the study were obtained from the University Ethics Committee (08.01.2018-3) and the institutions involved. The participants provided their voluntary consent to be a part of the study. Since we would be working with a vulnerable/special needs group, a specialist in Clinical Psychology was asked to review our questions and the necessary adjustments were made. The research conforms to the provisions of the Declaration of Helsinki (20).

3. RESULTS

All of the participants were the caregivers of individuals with intellectual disability; 8.4% were women, 18.6% were men. Their mean age was 42.6±10. Among the participants, 6.9% had no health insurance, 12.8% were divorced/widowed and 67.6% were unemployed. Only 28% were university graduates. A group of 43.1% revealed their income to be less than their expenditure; 27.1% had at least one chronic illness. Of the parents participating in the study, 76.6% were mothers of the disabled individuals, 14.4% were fathers. There were significant differences between the health literacy levels and the variables of age, education, income level, and relationship to the disabled individual ($p<0.005$). Of the participants, 63.3% stated that their access to health services was difficult; 92% said they would like to access health services more easily. The open-ended question, "What are the factors that would made access easier?" was answered by 53.2% of the participants. Of the responders, 31.4% mentioned social services, 9.6% referred to economic factors, 6.9% cited more transportation support, 3.7% pointed to facilitating services for obtaining appointments, and 1.6% stated that eliminating the collection of additional medication costs would make access to health services easier.

In looking at the participants' health literacy levels, it was found that 7.4% had inadequate, 55.9% had limited, 23.9% had adequate and 12.8% had excellent HLLs (Graph 1). The overall mean health literacy score was 32.51 (inadequate; >25-33).

Of the participants, 24.46% said they were unable to meet their needs at the health facilities, 72.34% revealed that they were unable to understand the informative materials that would lead them to health services, and 36.7% said they had physical difficulty in reaching health services. A statistically significant difference was found between health literacy levels and the ability to meet one's needs from health facilities in the city of residence (e.g., hospitals, rehabilitation centers, family health centers, pharmacies) ($x^2=21.51$; $p<0.005$), understanding publications providing information about easily accessible health services (e.g., webpages, brochures, journals) ($x^2=19.10$; $p<0.005$),and having physical difficulty reaching health services ($x^2=26.82$; $p<0.001$) (Table 1).

Table 1. *Access to health services by the parents of individuals with intellectual disability according to their health literacy levels*

Access to Services		Health Literacy Levels							
		Inadequate Health Literacy		Limited Health Literacy		Adequate Health Literacy		Excellent Health Literacy	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Can you meet your needs from the healthcare facilities (hospitals, rehabilitation centers, family health centers, pharmacies and others)in the city you live in?	Yes (n=142;75.5%)	5	2.7	80	42.6	35	18.6	22	11.8
	No (n=46; 24.4%)	9	4.8	25	13.3	10	5.3	2	1
Total	n=188	14	7.4	105	55.9	45	23.9	24	12.8
Statistics:		$x^2 = 21.51$				p= 0.001			
Can you understand materials that give you information about accessing health services (webpages, brochures, journals and others)?	Yes (n=52; 27.7%)	1	0.5	23	12.2	18	9.6	10	5.3
	No (n=136; 72.4%)	13	6.9	82	43.6	27	13.3	14	7.4
Total	n=188	14	7.4	105	55.9	45	23,9	24	12.8
Statistics:		x^2 =19.10				p= 0.004			
Do you find it physically hard to get to health services?	Yes (n=119;63.2%)	14	7.4	64	11.7	11	17.5	8	4.3
	No (n=69; 36.7%)	0	0.0	41	21.8	12	6.4	16	8.5
Total	n=188	14	7.4	105	55.9	45	23,9	24	12.8
Statistics:		x^2 =26.82				p= 0.000			

x2= Pearson Chi-Square

Of the participants, 45.2% said they were unable to understand the information health professionals provided (e.g., diagnoses, tests, prescriptions), 64.36% could not understand the materials distributed after information/ training was provided, 38.29% could not comprehend the training given**,** and 19.14% revealed that they could not express their needs accurately. A statistically significant difference was found between health literacy levels and the difficulty experienced in understanding the information provided by health professionals (e.g., diagnosis, tests, prescriptions) (x^2=35.43; p<0.001), understanding the materials distributed after information/training was given (x^2=30.65; p<0.001), understanding the health training (x^2=33.64; p<0.001), the ability to express a complaint or need accurately (x^2=25.19; p=<0.001). The number of individuals with limited and inadequate HLLs who experienced difficulty in accessing HS was greater than those with adequate or excellent health literacy (Table 2). Caregivers with a low level of HL use health services less productively than those with adequate or excellent HL levels.

Additionally, 70.74% of the participants said they had difficulty in obtaining appointments from health facilities, 50.53% said they gave up on trying to make appointments and 69.14% stated that they ended up not getting an appointment because of the long waiting period. There is a statistically significant difference between health literacy levels and having difficulty obtaining an appointment from a health facility (x^2=19.62; p<0.005), giving up on getting an appointment due to the difficulty involved (x^2=20.08; p<0.005), and not getting an appointment because of the long waiting period (x^2=42.49; p<0.001) (Table 3).

Of the participants, 27.65% stated that they could not reach the financial and social resources provided for access to health services. There is a statistically significant difference between health literacy levels and the ability to reach financial and social resources in order to access health services (x^2=14.6; p<0.005) (Table 4).

Table 2. *Understanding the Information, Training and Materials and Expressing Needs regarding HS, by Level of Health Literacy*

The Information, Training and Materials and Expressing		Health Literacy Levels							
		Inadequate Health Literacy		Limited Health Literacy		Inadequate Health Literacy		Excellent Health Literacy	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Do you have difficulty understanding the information (diagnosis, tests, prescriptions and others) healthcare professionals give you?	Yes (n=85; 45.2%)	13	6.9	51	27.2	16	8.6	5	2.7
	No (n=103; 54.7%)	1	0.5	54	28.7	29	15.4	19	10.1
Total	n=188	14	7.4	105	55.9	45	23.9	24	12.8
Statistics:		x^2 = 3 35.4		p= 0.000					
Can you understand the information sheets or materials (short notes, brochures, etc.) given to you after training?	Yes (n=67; 35.63%)	1	0.5	34	18.1	16	8.5	16	8.5
	No (n=121; 64.36%)	13	6.9	71	37.8	29	15.4	8	3.2
Total	n188	14	7.4	105	55.9	45	23.9	24	12.8
Statistics:		x^2= 30.65		p= 0.000					
Can you understand the health training given to you?	Yes (n=116; 61,70%)	0	0.0	69	36.7	28	14.9	19	10.1
	No (n=72; 38.29%)	14	3.2	36	19.2	17	2.1	5	2.7
Total	n=188	14	7.4	105	55.9	45	23.9	24	12.8
Statistics:		x^2= 33.64		p= 0.000					
Can you accurately explain/ask questions about your condition/needs?	Yes (n=152; 80,85%)	5	2.7	88	46.8	37	19.7	22	11.7
	No (n=36; 19.14%)	9	4.8	17	9.0	8	4.2	2	1.0
Total	n=188	14	7.4	105	55.9	45	23.9	24	12.8
Statistics:		x^2 = 25.19		p=0.000					

x2= Pearson Chi-Square

Table 3. *Access to services of parents of individuals with intellectual disability according to their health literacy levels*

Access to Services		Health Literacy Levels							
		Inadequate Health Literacy		Limited Health Literacy		Inadequate Health Literacy		Excellent Health Literacy	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Do you have difficulty obtaining appointments from healthcare facilities?	Yes (n:133;70.74%)	13	6.9	55	29.3	28	14.9	7	3.8
	No (n=85; 45.21%)	1	0.5	50	26.6	17	9.0	17	9.0
Total	n =188	14	7.4	105	55.9	45	23.9	24	12.8
Statistics:		x^2 = 19.62				p= 0.003			
Do you ever give up on getting an appointment when you have difficulty?	Yes (n=95; 50.53%)	13	6.9	51	27.1	24	12.8	7	3.8
	No (n=93; 49.46%)	1	0.5	54	28.7	21	11.2	17	9.0
Total	n=188	14	7.4	105	55.9	45	23.9	24	12.8
Statistics:		x^2 = 20.08				p= 0.003			
Have you ever been unable to get an appointment because of the long waiting period?	Yes (n=130;69.14%)	14	7.4	69	36.8	36	19.3	11	11.9
	No (n=58; 30.85%)	0	0.0	36	19.1	9	4.8	13	6.9
Total	n= 188	14	7.4	105	55.9	45	23.9	24	12.8
Statistics:		x^2 = 42.49				p =0.000			

x2= Pearson Chi-Square

Table 4. *Access to health and social support services according to the health literacy levels of parents of individuals with intellectual disability*

Access to Health and Social Support Services		Health Literacy Levels							
		Inadequate Health Literacy		Limited Health Literacy		Inadequate Health Literacy		Excellent Health Literacy	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Accessing financial and social resources in order to access health services	Yes (n=136;72.34%)	13	5.9	76	40.4	34	18.0	13	6.9
	No (n=52;27.65%)	1	1.5	29	15.4	11	5.9	11	5.9
Total	n=188	14	7.4	105	55.8	45	23.9	24	12.8
Statistics:		x^2 = 14.68				p = 0.023			

x2= Pearson Chi-Square

4.DISCUSSION

According to the Turkish health literacy survey, the average score of the Turkish population on the general health literacy index is 30.4. A group of 64.6% of the population has an inadequate or problematic level of health literacy (21). Okyay and Abacıgil have found the general health literacy score of the Turkish population to be 29.5. The health literacy levels of 69.4% of their study participants were "inadequate – problematic." This is a very high level, and the researchers reported that most of the population exhibited "inadequate-limited" levels of health literacy. In our study, the mean general health literacy score of the participants was 32.51; 63.3% showed "inadequate – problematic" levels of health literacy. In the United States, only one out of every 10 adults are health-literate. Among adults in the USA, 12% are reported to have adequate health literacy levels (HLL), while 53% and 22% have moderate and basic health literacy, respectively, and 24% have sub-optimal basic health literacy (22). The likelihood of living with suboptimal health conditions, having less access to the healthcare system and inadequately benefiting from health services is greater among individuals with low levels of health literacy (1).It was promising to find that the individuals in this study sample had similar or even better HLLs compared to individuals in the general population. At the same time, the percentage

of individuals with "inadequate-problematic" literacy levels is similar to what is reported in other studies. Although HL scores appear to be a little better than in the general population, the mean still represents an inadequate (>25-33) level of health literacy (8). The reason the scores of the study participants were not lower/less than those of the general population might have stemmed from the fact that these individuals had long years of experience in caring for the individuals they supervised. There is a study that supports this belief. Murphy, et al. assert in their research that the health literacy scores of caregivers living with individuals who are constantly or frequently being treated may increase over time (23). We also think that the rehabilitation reports used in the Children's Special Needs Report have a positive impact on caregivers. The fact that a large majority of the population and the parents participating in this study are at an "inadequate-problematic" HLL despite all that is being done makes it evident that the health literacy of primary caregivers, particularly those that care for individuals with disability, as well as persons with diseases such as cervical cancer or chronic diseases such as HIV, needs to be raised in order to improve the health, social and economic outcomes of the individuals they care for (24,25).

Parents' capacity to serve as advocates must be enhanced so that they can demand the health information and care they seek, ask questions and easily access the healthcare system (23). A statistically significant difference was found between parents' HL levels and the difficulties they experience in easily accessing health services, understanding informative publications and experiencing trouble in physically accessing health services (Table 1). Again, a statistically significant difference was found between HL levels and the ability to understand health training and educational materials, to understand and ask questions about the sickness/or individual needs, comprehend the information provided by healthcare professionals (e.g., diagnoses, tests, prescriptions) (Table 2). Parallel to these findings, Schmidt et al. have reported that parents with low levels of health literacy do not make productive use of health services. Moreover, a statistically significant difference was found between HL levels and easy access to health services and being able to understand informative publications (26). Robinson C, Graham J et al. report that those with limited/poor HL and inadequate skills in informatics or in seeking out, assessing and using information on health are not able to make effective use of such important resources (27). Health training usually seems quite complex and unsettling to those with limited levels of health literacy (1). In the light of what has been reported by Peters E, Hibbard J, Slovic P, Dieckmann N, who said that if individuals cannot understand health information (e.g., diagnoses, treatments, tests) they will have difficulty in making informed decisions and in participating effectively in the process of health services provision, it can be said that our findings are considerably disquieting (28). On the other hand, when clients understand health information, this increases their capacity to access health services (29). In our study, those with inadequate+limited HL levels did have difficulty with understanding information on diagnoses, tests and prescriptions. Not being able to understand how to use prescribed medications increases the risk of inadvertent dosage repetitions and other errors of medication usage, leading to high medical costs (30). More thought-provoking is that it has been observed that even adult caregivers with excellent health literacy can sometimes make mistakes in medication compliance and administering proper dosages (31). Indeed, Fransen et al. and Loke et al. have shown through their most recent scan of the literature that there is a significant correlation between health literacy and drug compliance (32,33). Those with disabilities have more of a need for health services than others. When the high levels in the usage of health services are considered, it can be seen that those with disabilities are disproportionately affected by the barriers to access created by their disability. It is striking to note that in the case of two persons with the same medical condition, where one has a disability and the other is non-disabled, the individual with disability is likely to use 2-3 times more health services than the non-disabled individual and also has more of a need for these services (9,34). These circumstances and the fact that individuals with disability have both physical and mental health issues and have differences in their medical treatment and cognitive processes indicates that the health literacy levels of caregivers and parents must be raised.

We found in our study a statistically significant difference between parents' health literacy levels and the difficulties they face in obtaining an appointment from health facilities and in not being able to get an appointment due to a long waiting period (Table 3). In a study by Schultz et al., the authors evaluated the unmet oral health needs of developmentally disabled children (35). The study indicated that one of the factors that had the most influence on the parents' perception of the need for oral health services was their low levels of health literacy. It was reported that adults with limited health literacy were unable to understand appointment reminders, information sheets, consent forms and lacked the skills to use the healthcare system for access to care. Palumbo has reported that limited health literacy is known to be a social barrier in accessing healthcare services among individuals with HIV. Because of this, limited health literacy has been associated with the poor management of chronic and long-term conditions. Providing the parents of individuals with disability with health education programs is of great importance in improving these circumstances. To manage and avoid adverse circumstances, caregivers and families need to be supported with individual and group programs that should be repeated as necessary.

Our findings suggest that the influence parents with limited levels of health literacy can have on the health of the individuals with disability they care for cannot be ignored. Also, healthcare providers largely neglect the need for health information of parents with low health literacy levels who live with individuals with disability, a circumstance that leads to their inability to obtain effective health services.

Although the variables used in this study are an indication of the access to health services, the fact that the data was collected indirectly constitutes a limitation of the research.

5. CONCLUSIONS

There are significant differences between the participants' HL levels and their ability to access health services. It was concluded that individuals with high levels of health literacy are better able to reach health information resources and access healthcare facilities and services. A large majority of the participants said that their access to health services was difficult and they could not obtain access as much as needed. Our findings on the access to health services of disadvantaged groups have pointed to a strong need for the advocacy of public health nurses and other specialists in this context. Because neither policymakers nor healthcare administrators have adequate awareness about the role HL plays in access to health services, there must be much more of an effort to raise this awareness. In particular, institutional interventions should not be delayed while attempting to achieve effective and prompt access to health services. In this context, we recommend the urgent development of programs that will increase the health literacy of parents of individuals with disability, especially mothers.

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Clinical and Experimental Health Sciences

Effect of Text Messages Intervention on Pregnancy Healthcare Practices in Pregnancy: A Three Group Non-Randomized Controlled Trial

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ABSTRACT

Objective: The purpose of this study was to investigate the effect of text messaging and group training on health care behaviors of pregnant women.

Methods: A three group non-randomized controlled design was used in this study. The study was conducted with pregnant women who applied to four family health centers in different regions of Istanbul between November 2016 and June 2017. The Health Practices in Pregnancy Scale (HPPS) was used to evaluate the health behaviors of pregnant. In total, 150 pregnant women were included in the study, which contained text messages group, education group, control group. The text message group received 105 messages in total, 4 text messages per week for 6 months. The education group attended three weeks of group sessions held for pregnant women. The control group received routine prenatal care in the family health center.

Results: The health practice of the text message and education group were significantly higher than that of the control group. In addition, the text message group health practice scores significantly increased posttest ($p<0.05$), whereas the education and control groups health practice scores did not significantly change ($p>0.05$).

Conclusion: Text messages could be an effective education method for improving the health practices of pregnant women.

Keywords: Education, health practice, health education, pregnancy, text message.

1. INTRODUCTION

Pregnancy, a limited and special period in a woman's life, is an individual experience influenced by sociocultural, behavioral and economic factors (1). During this period, health behaviors such as rest, healthy eating and exercise significantly affect the health of both mother and baby (2).

The main goal of antenatal care services is to eliminate or minimize maternal mortality. However, hundreds of women die due to preventable complications of pregnancy and childbirth each year. Antenatal care includes education, counseling, screening, and treatment to assure the best possible health for mother and fetus (3).

As an important component of antenatal care, particularly for women who are pregnant for the first time, education contributes to better obstetrical outcomes (4). Health education provides an important resource to promote the personalized and integral care of the needs of pregnant women. It allows pregnant women to expose their possible doubts and questions, aiming at reducing uncertainties through an orientation in health, making autonomy possible (5). There have been many studies conducted that found that educated women have better pregnancy outcomes compared with uneducated women and that education during the antenatal period can reduce pregnancy and delivery complications. Considering the potential outcomes of health education for pregnant women, healthcare professionals must strive to execute and incorporate educational activities to prepare pregnant women for childbirth and the postpartum period (6).

In recent years, the use of mobile health (mHealth) applications that provide easier and lower cost access to healthcare has increased (7). mHealth is defined by the World Health Organization as the "medical and public health applications supported by mobile phones, patient follow-up monitors, electronic organizers or other wireless devices" (8). Mobile health has so far been used in many health promotion applications such as smoking cessation, weight management, healthy nutrition, exercise promotion, diabetes management, and health education (9). Text

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messaging is a simple, cost-effective and widely available method that can be used in mobile healthcare applications (10). Due to its characteristic of ubiquity and the possibility of personalization, it is expected to be a powerful tool for patient-centered care.

Mobile health, which is widely used in health improvement applications around the world, has started to be used in antenatal care. Studies have shown that text messages regarding mobile health practices are effective in improving the health knowledge, attitudes and behaviors of pregnant women (11, 12). Mobile health practices provide significant opportunities to support medical and public healthcare practices for pregnant women (9).

There are no studies in Turkey on the education of pregnant women through text messages. Pregnant women receive routine antenatal care in family health centers or benefit from group training in pregnancy education programs in some hospitals. It has been reported that group training is useful for pregnant women, but there are problems involved, such as the limited number of pregnant women who have access to the service, the implementation of applications only in some hospitals, lack of a standard education model, and environmental limitations (13-15). It is feasible then that women can be provided with health information by means of new methods that are easily available and require no limits put on place or time. Text messages constitute one such method.

The purpose of this study was to investigate the effect of text messaging and group training on health care behaviors of pregnant women. Study tested to hypotheses; there will be increase in the health practices of the text message group than education and control group.

2. METHODS

2.1. Design

This study used a pre and posttest quasi-experimental design with two experimental groups and a control group (Figure 1).

2.2. Setting and Participants

This study was conducted at four family health centers in Istanbul, Turkey. Included participants were pregnant who (a) gestational week 1-15, (b) had never received any education on health practice, (c) healthy woman, (d) owned a smart phone. Consent forms were obtained from the pregnant who volunteered to participate. Participants were divided into three groups: text messages group (Experimental 1: TMG), education group (Experimental 2: EG), or Control group: (Con). The sample size was calculated with the Power and Sample Size Calculation Program. A 5% significance level and 90% power (mean difference=13 and standard deviation =12.91) required 66 pregnant women (22 pregnant women in each group) for sampling. However, 150 pregnant women (50 pregnant women in each group) were included in the study considering that there would be losses in the research process.

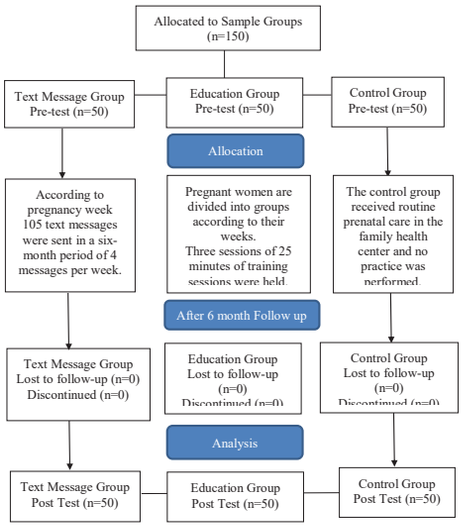

Figure 1. *Flow chart of the study*

2.3. Instruments

Data were collected using a sociodemographic form prepared by the researchers and the Health Practices in Pregnancy Scale (HPPS).

Sociodemographic Form

The sociodemographic form prepared by the researchers was based on existing literature. The form included ten questions determining the socio-demographic/obstetric characteristics of the pregnant women.

Health Practices in Pregnancy Scale

The Health Practices in Pregnancy Scale (HPPS) is a 34-item scale developed by Kelly Lindgreen to evaluate health practices related to pregnancy outcomes during pregnancy; its cronbach alpha coefficient is .81. The scale was adapted into Turkish by Er (2006). In this study, the scale had 33 items and the cronbach alpha coefficient was .74 (16). The scale measures the adequacy of the health practices of pregnant women in six areas; rest and exercise, safety, nutrition, avoidance of harmful substances, health care, and information. There is also an item describing all health practices in pregnancy. The lowest possible score to be obtained from the scale is 33; the highest score is 165. High scores indicate an increase of positive health behaviors.

2.4. Intervention Procedures

2.4.1. Creation of content and obtaining an expert opinion

Education and text messages content were prepared by the researchers using the relevant literature in a specific for each trimester (17-19). For the suitability of the contents of text messages were evaluated by seven obstetric nursing specialists (academics), an education specialist, two public health nursing specialists, and two midwives working in obstetrics clinics. The experts evaluated each text message,

checking for the range of inappropriate to very appropriate expressions (1 to 4 points). A high level of agreement was found between the scores rated by the experts (W= .27, p= .00). The text messages in the content are shown in Table 1.

Table 1. *Text messages and group education topics*

Text Messages Samples			Group Education Topics
Changes in Pregnancy	Drug Use	Smoking/Alcohol	Session 1: First Trimester
During the first 3 months of pregnancy, you may experience psychological ups and downs due to changes in your hormone balance. Do not worry.	Using medication in the first 3 months of pregnancy can harm the baby. Do not use the medication without consulting your doctor, even if it is pain relieving.	When you smoke, your baby is exposed to 7000 chemicals. Do not use alcohol during your pregnancy.	Changes in pregnancy Baby growth and development during pregnancy Oral health Smoking, alcohol, drug use Sexual life Hygiene during pregnancy
Oral Health	Sexual life	Hygiene	
Your gums bleed more often, use a soft brush. Always brush your teeth after each meal and before bedtime.	It is safe for you to continue having sex as long as your doctor allows.	You can make your bathroom as a standing shower or by sitting in the bathtub.	
Baby development	Diet	Exercise	Session 2: Second Trimester
At the end of the 12th week, your baby is approximately 12 cm tall and weighs around 23 grams.	Consume at least 5 servings of fruit and vegetables per day (1 apple = 1 por., 3 tbsp vegetables = 1 por.).	Walking is an easy exercise. Come on, take a 30-minute walk at a light tempo outdoors every day.	Baby growth and development healthy eating habits exercise Living a healthy lifestyle Safe travel during pregnancy Sleep
Safe travel	Sleep	Danger Signs	
If your trip will exceed 2 hours, you can take a break every 2 hours and walk.	Outside of sleeping during the night, take a 1-hour rest during the day	If you have complaints such as bleeding, dizziness, loss of consciousness, multiple vomiting, bad headache, contact your doctor immediately.	
Birth process	Birth Preparation	Breastfeeding	Session 3: Third Trimester
Think of beautiful things in the delivery room, imagine your baby and breastfeeding.	Get your hospital bag ready with a few personal items, such as a toothbrush and toothpaste, lip balm, deodorant, a brush and comb, makeup (if you're planning to use it), and hair ties or a headband	Give your baby your breast every time they get hungry and breastfeed every 2 hours at the latest.	What are the signs of danger in pregnancy Birth process Preparing a birth bag General principles of breastfeeding

2.4.2. Pilot Practice

The clarity of the text messages and training content, set up according to the expert opinions, were tested in ten pregnant women with a pilot application. The final form of the content was created after the pilot implementation.

2.4.3. Inclusion of pregnant women

Those who met the criteria for recruitment from among the pregnant women in the family health centers were identified. The pregnant women were divided into three groups; text messages group (TMG), education group (EG) and control group (CG).

2.5. Educational Intervention

The educational intervention consisted of physiological and psychological changes in pregnancy, what the mother should do, the growth and development of the baby, hygiene and general body care, eating, exercise, sleep, oral health, smoking, alcohol-substance-drug use, travel, sexual life, pregnancy danger signs, birth preparation, and breastfeeding.

The TMG received the education with text messages, a total of 105 text message were sent to the pregnant women at the same time every day (12:00 p.m.), a period of six months, at a rate of 4 messages per week.

The EG received the same education content with text messages gruop in three sessions on different days. Each session lasted about 25 minutes and was in the form of lectures, group discussion, and a question and answer session. The sessions were held at the family health centers taken into the study. The group sessions were attended by 3-4 pregnant women. The trainer was a nurse and PhD student.

The CG received routine prenatal care in the family health center and no intervention was performed.

2.6. Data Collected

Data were collected between November 2016 and June 2017. Participants completed the questionnaires just before study (pre-test) and six months after study (post-test).

2.7. Ethical considerations

Prior to the start of the study, approval was granted by Marmara University Institutional Review Board in Turkey (MIRB-2015-079). Pregnant were informed about all aspects of the study and were assured that there was no disadvantage for non-participation. After pregnant were informed, those who were willing to participate in this study signed a consent form.

2.8. Data Analysis

Data analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive and inferential statistics were used to describe the demographic of participants and their scores on the questionnaire. The chi-square and one-way analysis of variance tests were used to compare the demographic data of the groups in the pre-test. Effect size was calculated using Cohen's d to estimate effect sizes between groups. The dependent sample t-test was used to determine the intra-group differences in each group, and one-way analysis of variance with Duncan post-hoc was used to test for differences in scores among the three groups. All results were evaluated at a 95% confidence interval and a significance level of $p \leq 05$. Normal distribution was evaluated with the Kolmogorov-Smirnov Z test.

3. RESULTS

3.1. Homogeneity test among the three groups' baseline variables

One hundred and fifty pregnant women participated. Participants were between 18 and 42 years (mean: 29.22 ± 4.47). There were no statistically significant differences among the three groups regarding typical characteristics (age, education, income status, before pregnancy, during pregnancy, gestational week number of pregnancy, smoking health education about pregnancy) or the score of health practices (Table 2). Therefore, the three groups were considered homogeneous.

3.2. Mean differences in health practices

There were statistically significant mean differences among the three groups health practices (F=2.88, p=.05) (Table 3). After the intervention, the post-hoc analysis showed that the health practice score was significantly higher for the two experimental groups (TMG and 2) than for the control group. Post hoc analysis of this interaction indicated that the pregnant women in the text messages group had significantly increased health practice scores (t=3.45, p=.01). There was no statistically significant difference between the mean pretest and posttest HPPS scores of the EG (p> .05). There was no statistically significant difference between the mean pretest and posttest HPPS scores of the CG (p> .05) (Table 3).

Table 2. *Comparison of sociodemographic characteristics of groups (n=150)*

Introductory features		TMG (n=50)		EG (n=50)		Con (n=50)		statistics
Variables		mean±sd		mean±sd		mean±sd		F/p
Age	years	28.32±5.14		29.24±4.54		30.12±3.49		2.04/0.13
BMI before pregnancy	kg/m^2	24.22±4.07		23.51±2.84		24.14±3.77		0.59/0.55
BMI during pregnancy	kg/m^2	24.70±3.86		25.18±2.80		25.17±3.66		0.30/0.73
		N	%	n	%	n	%	x^2/p
Gestational week	I. Trimester	20	40	14	28	21	42	18.41
	II. Trimester	30	60	36	72	29	58	/.56
Number of pregnancy	First pregnancy	25	50	29	58	32	64	2.01
	Second and over	25	50	21	42	18	36	/.36
Education	Primary school	15	30	9	18	7	14	6.36
	High school	10	20	12	24	18	36	/.17
	University	25	50	29	58	25	50	
Income status	Good	3	6	5	10	1	2	2.83
	Moderate	47	94	45	90	49	98	/.24
Smoking	Smoker	2	4	5	10	0	0	6.15
	Non-smoker	39	78	36	72	38	76	/.18
	Quit smoking	9	18	9	18	12	24	
Health education about pregnancy	Educated	2	4	6	12	5	10	2.19
	Non-educated	48	96	44	88	45	90	/.33

F=one way anova test, x2= kikare test, TMG=text messages group, EG= education group, Con=control group, sd=standard deviation

Table 3. *Differences in health practices among the three group (n=150)*

	[1]TMG (n=50)	[2]EG (n=50)	[3]Con (n=50)	Inter-group comparison mean difference (%95 CI), p		
	mean(SD)	mean(SD)	mean(SD)	**1 vs 2**	**1 vs 3**	**2 vs 3**
Pre-intervention	107.52 (9.14)	111.34 (9.25)	109.48 (10.79)	-3.82 (-8.44 to 0.80), 0.12¥	-2.06 (-6.68 to 2.56), 0.54¥	1.76 (-2.86 to 6.38), 0.64¥
Post-intervention	110.62 (8.39)	113.44 (8.85)	109.14 (9.98)	-2.82 (-1.49 to 7.13); 0.27¥	1.48 (-2.83 to 5.79); 0.69¥	4.30 (-0.01 to 8.61); *0.05¥
Intra-group comparison mean difference (%95 CI), p	-3.10 (-4.90 to – 1.29), *0.01€	-2.10 (-4.33 to 0.13); 0.06€	0.44 (-0.63 to 1.51); 0.41€	**Effect size TMG (1), EG(2), Con (3)**		
				1 vs 2	**1 vs 3**	**2 vs 3**
				0.32	0.16	0.45

*HPPS=Health Practice in Pregnancy Scale, TMG=text messages group, EG=education group, Con=control group, sd=standard deviation, €; paired sample t test, ¥; one way Anova, Post-hoc Duncan test (2>3), *p≤0.05*

4. DISCUSSION

This study is the first study conducted in Turkey to evaluate the effect of text messages on the health practices of pregnant women. The results of the study showed that text messages are an effective method to improve the health practices of pregnant women.

At the end of the study, it was found that the health practices of the pregnant women in the education group had not effectively improved. Some studies in the literature suggest that education is not effective in reducing pregnant women's fear of childbirth (25), or in terms of reducing smoking, increasing knowledge about infant care, improving psychosocial health (26), enhancing knowledge about breastfeeding (27). In some other studies, however, the education group was found to be effective in reducing the fear of childbirth (28) and increasing nutritional knowledge (29). The differences are thought to stem from the duration and number of training sessions, the materials used, and the diversity of the working groups.

In previous studies, text messages have been shown to increase the health knowledge of pregnant women (19), to encourage smoking cessation or reduction in pregnant women (20), to encourage breastfeeding (21), and to develop a positive attitude toward vaccination (22). Also, in meta-analysis and systematic review studies, it has been stated that text messages increase the number of prenatal care visits of pregnant women and is an effective tool in pregnancy education (23, 24).

In this study, the content of text messages was made short, simple, and understandable and then evaluated by the experts. It has been reported in studies that families want to receive short and simple messages and do not want to read lengthy messages (30).

To ensure reliability, the information in health messages presented with text messages should be provided by health professionals. It has been reported that health professionals are the primary source of health information that women will trust the most (31).

At the end of the study, there was a positive increase in the health practices of the text message group, but the education group recorded no difference in their health practices. This result showed that text messages are seen as more advantageous compared to group education methods (traditional education, brochures, posters, etc.) in reaching women during pregnancy, especially in rural areas where access to health services is less. These advantages include lower costs, time saving, and ease of access (32).

4.1. Limitations

The non-randomized design of this study induced a risk of bias due to the unequal distribution of confounding factors between the groups. Against the presence of this bias, however, are the observations that the groups were similar both demographically and in terms of health practices.

The pregnant women included in the study could not be randomized. There were some reasons for this. Firstly, the study was carried out with pregnant women in their first trimester and since, in the Turkish family medicine system, pregnancies are registered as soon as they are detected, each pregnant woman applying to the family health center were eligible for inclusion in the research. Another factor was that registration records change every day and access to these records can only be achieved individually with the permission of the family physician, even if institutional permission has already been granted by the Ministry of Health. Some family physicians did not want to share the records needed for the scope of the study. For this reason, records could only be obtained from family physicians willing to share the records. Finally, there were time limitations in the conduct of the study since the pregnant women who were in their first trimester had to be contacted during that period of time.

5. CONCLUSION

At the end of the study, it was concluded that text messages are effective in delivering cheap, reliable, and accessible information to pregnant women. It may be suggested that the use of text messages, shown to be effective in this study, should be integrated into the provision of maternal and child health services within the scope of primary health care services, and the effectiveness of text messages should be tested in different samples.

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Clinical and Experimental Health Sciences

The Investigation of Tissue Composition Effects on Dose Distributions Using Monte Carlo Method in Permanent Prostate Brachytherapy

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ABSTRACT

Objective: Radiation dose calculations in the prostate brachytherapy practices have very high importance in terms of the success of treatment. The purpose of the present study is to determine whether there is a significant dose difference between the radiation dose calculations performed in water medium and prostate cancer-diagnosed patients by using the Monte Carlo method.

Methods: The radiation dose calculations were performed on 20 prostate patients by using the BrachyDose Monte Carlo code. Phantom geometry derived from real patients computed tomography (CT) data was created to use in dose calculations. Water material was assigned to all voxels within the prostate volume for dose comparison with CT derived phantom. ^{125}I (Amersham, OncoSeed, 6711), ^{103}Pd (Theragenics Co., TheraSeed, 200) and ^{131}Cs (IsoRay Medical) commercial brachytherapy seed models were used in dose calculations.

Results: It was observed that there are significant dose differences between the water medium and the prostate tissue. The differences between D90 dose values in prostate tissue and water medium were calculated as 7.2-10.5%, 9.1-13.4% and 5.4-8.3% for ^{125}I, ^{103}Pd and ^{131}Cs brachytherapy seed sources, respectively.

Conclusion: It was concluded that material compositions of different organs and tissues in the human body should be considered for more accurate brachytherapy dose calculations.

Keywords: Brachytherapy, prostate cancer, tissue composition, Monte Carlo, brachydose

1. INTRODUCTION

Low dose rate (LDR) permanent seed sources have been used frequently in the treatment of early-stage prostate cancer (1). In the dose results of brachytherapy seed sources used in the treatment of such cancers, the choice of appropriate dosimetry and dose calculation formalism is very important issue in terms of patient dose. Because the dominant interaction type is the photoelectric effect in brachytherapy seed sources such as ^{125}I, ^{103}Pd and ^{131}Cs, tissue heterogeneity-induced dose differences need to be considered in the treatment planning systems (TPS) for sensitive dose calculations (2,3). The ^{125}I, ^{103}Pd and ^{131}Cs brachytherapy seed sources are often preferred in permanent prostate cancer brachytherapy. However, high dose rate (HDR) ^{192}Ir radioactive sources are used especially in cervical and breast cancers.

The dose calculation formalism recommended by the Task Group No. 43 (TG-43) report of the American Association of Physicists in Medicine (AAPM) is used to obtain dose distributions in tissue or organs in current brachytherapy TPSs (4). One of the basic assumptions of this formalism is that the infinite and homogeneous water phantom can be used instead of tissue and organ materials. According to AAPM TG-43 report, the dose distributions are 2-dimensional, and this formalism is still used in determining the dosimetric characteristics of brachytherapy sources. So far, in the literature, there have been various studies carried out on water and tissue mediums related to low-energy brachytherapy seed sources (5,6). The dose values in some of these studies were calculated by using the dose calculation formalism recommended by the TG-43 report of AAPM.

Task Group No. 186 (TG-186) report of AAPM on model-based dose calculation algorithms (MBDCAs) has been recently published to calculate precise dose values in LDR brachytherapy dosimetry, which was developed as

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an alternative to the TG-43 formalism. In this report, photoelectric cross-sectional effect and scattering conditions for different mediums were analyzed with high precision in brachytherapy dose calculations (7). The TG-43 formalism is predominantly a good approximation method in high-energy photon interactions where Compton scattering occurs. It has been shown in studies that the dominant interaction type is the photoelectric effect and whole-body tissues cannot be accepted as water equivalent, when considering the photon energies (< 100keV) emitted from brachytherapy seed sources. It has been known that there are substantial dosimetric differences between the actual dose delivered to the patient and the dose values calculated using TG-43 formalism during treatment planning because the photoelectric cross-section is proportional to the effective Z value (8).

Unlike TG-43 formalism, MBDC algorithms can perform sensitive brachytherapy dose calculations with Monte Carlo-based simulations in a heterogeneous tissue based on real patient data. However, this approach is not yet used in LDR ^{125}I, ^{103}Pd and ^{131}Cs brachytherapy applications in TPS. Therefore, Monte Carlo simulations are needed to evaluate the dose differences between MBDCAs, and TG-43 based dose calculations. The MBDCAs using computed tomography (CT) data allow more precise dose calculations in different mediums such as inhomogeneous tissue and water since the data information about the mass density of each voxel and elemental composition of the mediums are available in the Monte Carlo method and other model-based dose calculation algorithms. Using the CT data in anatomical imaging and brachytherapy treatment planning can provide accurate density information for each voxel using the electron density of the tissue and the Hounsfield Unit (HU) calibration curves (9). Thus, the Monte Carlo simulation technique is proposed as an alternative to accurately transport low-energy photons emitted from seed sources in CT-based real patient geometry (10). Dose calculations in other studies were made on virtual phantoms and were prepared with the help of the information obtained from the CT data of real patients. The obtained results showed that the dose distributions in different tissue phantoms are different from the dose values obtained from the water phantom, and the elemental composition variations had a direct effect on the brachytherapy dosimetry. Therefore, it is still a matter of debate in the literature that the TG-43 formalism is preferable in brachytherapy dose calculations in the TPSs (11,12).

The aim of the present study is to determine whether there are significant dose differences between prostate tissue and water medium in LDR prostate brachytherapy using BrachyDose code Monte Carlo simulation.

2. METHODS

The radiation dose calculations in this study were performed by using the Monte Carlo technique, and the phantom geometry was obtained from CT images of patients diagnosed with 20 prostate cancer. The dose differences were calculated and compared between the prostate tissue and water medium for ^{125}I, ^{103}Pd and ^{131}Cs multiple brachytherapy seed sources. The phantom data were obtained from the CT images which are in the digital imaging and communications in medicine format (DICOM) and resized to be used in dose calculations effectively. Material information and mass density values of each organ and tissue were estimated by the reinterpretation of HU values at each point in the CT images. To do this, a calibration curve was used (13,14). In addition, HU numbers obtained only from patient data depend on the anatomical components of tissues and tissue density for each patient. Information about the medium material and XCOM cross-section values of different interaction types were obtained by the (Elektron Gama Shower national research center) EGSnrc data preparation program (15).

The brachytherapy seed source models used for dose calculations in this study are the LDR brachytherapy seed sources such as ^{125}I (Amersham, OncoSeed, 6711), ^{103}Pd (Theragenics Co., TheraSeed, 200) and ^{131}Cs (IsoRay Medical) (16–18). The Yegin's multi-geometry technique was used in the creation of the complex geometries of the brachytherapy seed source models used in the Monte Carlo particle transport calculations (19).

BrachyDose Monte Carlo code which is a model-based dose calculation algorithm was performed as a dose calculation tool. BrachyDose program uses a track length estimator calculating the kerma value by collecting the particle tracks in a certain volume to estimate the absorbed dose (20). $5x10^{10}$ photon histories were used during each simulation to reduce statistical error below 2.0%. Photon cut-off energy was taken as 1.0 keV in all dose calculations. Rayleigh scattering, photoelectric absorption, bound Compton scattering, and the characteristic X-rays released from K and L shells of related atoms were taken into consideration in Monte Carlo particle transport.

Phantom materials were defined as $D_{w,w}$ (TG-43) for the water phantom and $D_{m,m}$ (TG-186) for the prostate tissue. To perform Monte Carlo particle transport simulations, brachytherapy multi-seed sources were placed into the prostate tissue and homogeneous water phantom in all dose calculations. In addition, different scenarios were produced using a different seed source model each time. In each scenario, radiation dose distributions in the patient's body were calculated separately for ^{125}I, ^{103}Pd and ^{131}Cs brachytherapy multi-seed sources. In this study, dose calculations were performed using CT images of patients diagnosed with prostate cancer. To perform brachytherapy patient dose calculations, CT section images were resized to calculate patient dose more accurately. Therefore, voxel sizes of the phantom were reconstructed as $0.3x0.3x0.1cm^3$ cubic voxels in $91x91x27$ cm^3 cubic volume. Then, brachytherapy multi-seed sources were placed into the prostate volume in a 3-D grid of 1.0 cm spaced combination. During the simulation process, to minimize the dose that the urethra should be exposed to a minimum, particular attention was paid to the fact that the location of the seed sources in prostate tissue could not coincide with the

volume of the urethra. By choosing a convenient point in the prostate volume, where the dose gradient is minimized, the dose value at this point was normalized considering the dose distributions within the prostate volume. This study was conducted with approval from the Ethics Committee of the Necmettin Erbakan University, Faculty of Meram Medicine in Turkey (Approval number: 15/176).

ΔD (%), V_{100} and V_{150} clinical dosimetry parameters for prostate tissue and water medium, and dose homogeneity index (DHI) in prostate tissue volume were calculated through equations 2.1 and 2.2, respectively (21).

$$\Delta D(\%) = \frac{Dwater - Dprostate}{Dwater} \text{x}100 \qquad 2.1$$

$$DHI = \frac{V100 - V150}{V100} \qquad 2.2$$

Considering the TG-43 dose calculation formalism used in current TPS, the CT-based prostate patient phantoms were converted into a water equivalent homogeneous phantom. According to the idealized TG-43 formalism, $D_{w,w}$ (TG-43) dosimetric procedures in homogenous water phantom geometry were performed under the same conditions with CT-based prostate brachytherapy, and dose distributions were obtained from different multi-seed sources.

3. RESULTS

Figures 1-3 show the obtained isodose distributions using CT data of the prostate patient in a plane where multiple brachytherapy seed sources are sequenced. Dose to critical organs such as bone, bladder, and rectum, which were exposed to 125 Gy and higher dose for different seed sources, water and prostate tissue received in the same transverse plane is also illustrated in these figures. As a result of the calculations, it was observed that the differences in the dose distributions calculated at the same points in the prostate tissue and water medium for ^{103}Pd seed source were less when compared to ^{125}I and ^{131}Cs seed sources.

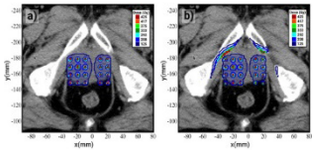

Figure 1. *Isodose curves for ^{125}I source. Monte Carlo dose calculations are carried out (a) in full $D_{w,w}$ water medium (b) in patient body which is made up of $D_{m,m}$ tissue materials.*

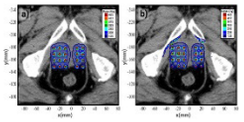

Figure 2. *Isodose curves for ^{103}Pd source. Monte Carlo dose calculations are carried out (a) in full $D_{w,w}$ water medium (b) in patient body which is made up of $D_{m,m}$ tissue materials.*

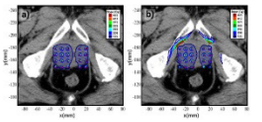

Figure 3. *Isodose curves for ^{131}Cs source. Monte Carlo dose calculations are carried out (a) in full $D_{w,w}$ water medium (b) in patient body which is made up of $D_{m,m}$ tissue materials.*

Dose differences were calculated and compared between the prostate tissue and water medium for ^{125}I, ^{103}Pd and ^{131}Cs multiple brachytherapy seed sources. Dose volume histograms (DVHs) with maximum dose differences between 20 patients are shown in Figures 4, 5 and 6, respectively. According to the results obtained from DVHs, it was observed that there are significant dose differences between the water medium and the prostate tissue. The minimum D_{90} values for prostate tissue and water medium were calculated as 7.2-10.5%, 9.1-13.4% and 5.4-8.3% for ^{125}I, ^{103}Pd, and ^{131}Cs multiple brachytherapy seed sources, respectively. Since the energy emitted from the ^{103}Pd brachytherapy seed source is about 30 keV, the photoelectric cross-section is dominant in this low dose range, and the D_{90} difference due to tissue composition mostly occurs from this seed source.

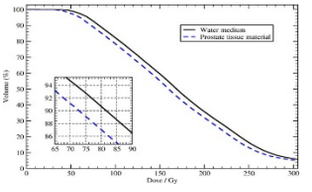

Figure 4. *Dose volume histogram obtained from the $D_{w,w}$ water medium and the $D_{m,m}$ prostate tissue for the Amersham OncoSeed 6711 125I brachytherapy seed source*

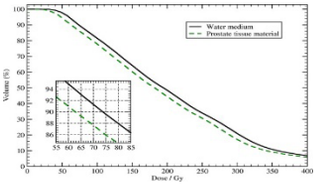

Figure 5. *Dose volume histogram obtained from the $D_{w,w}$ water medium and the $D_{m,m}$ prostate tissue for the Theragenics Co. TheraSeed 200 ^{103}Pd brachytherapy seed source*

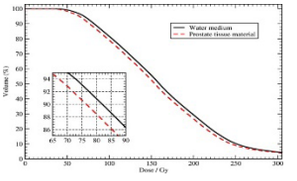

Figure 6. *Dose volume histogram obtained from the $D_{w,w}$ water medium and the $D_{m,m}$ prostate tissue for the IsoRay Medical ^{131}Cs brachytherapy seed source*

V_{100} and V_{150} values for prostate tissue were obtained on DVHs, and dose homogeneity index (DHI) was calculated

for different brachytherapy seed sources. DHI values were calculated as 0.28-0.54, 0.23-0.45 and 0.34-0.62 for ^{125}I, ^{103}Pd, and ^{131}Cs seed sources, respectively (Table 1). Since the DHI parameter is dependent on the V_{100} and V_{150} dosimetric parameters, this value is around 0.5 depending on the V_{150} / V_{100} ratio. However, it is ideally desired to be 1 in the treatment planning system.

Table 1. *Percentage dose differences between values of D_{90} calculated in prostate tissue and water medium for the ^{125}I, ^{103}Pd and ^{131}Cs brachytherapy seed sources*

Brachytherapy seed sources	Percentage (%) dose differences between D_{90} prostate tissue and D_{90} water medium	DHI
^{125}*I*	7.2-10.5%	0.28-0.54
^{103}*Pd*	9.1-13.4%	0.23-0.45
^{131}*Cs*	5.4-8.3%	0.44-0.62

D_{90}: Dose covering 90% of volume; DHI: Dose Homogeneity Index

In the dose calculations performed by sequencing the ^{125}I, ^{103}Pd, and ^{131}Cs multiple brachytherapy seed sources to the same coordinates of the prostate tissue and water phantom, significant dose differences were observed in transverse sections due to the difference of water phantom and prostate composition. Isodose distributions of water medium and prostate tissue in a slice are shown in Figure 7-9.

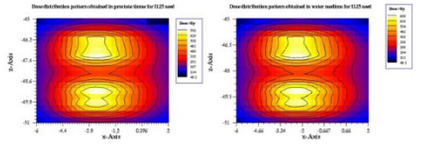

Figure 7. *Dose distribution pattern obtained in the same transverse section of prostate tissue and water medium for ^{125}I brachytherapy seed source*

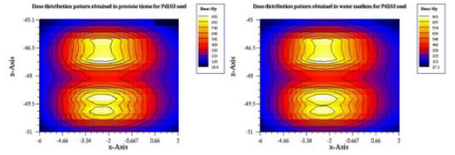

Figure 8. *Dose distribution pattern obtained in the same transverse section of prostate tissue and water medium for ^{103}Pd brachytherapy seed source*

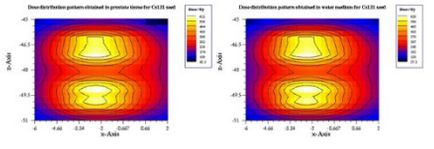

Figure 9. *Dose distribution pattern obtained in the same transverse section of prostate tissue and water medium for ^{131}Cs brachytherapy seed source*

4. DISCUSSION

When considering photon energies (< 100keV) released by brachytherapy seed sources, the dominant interaction type is the photoelectric effect. So, whether whole body tissues will be accepted as equivalent to the water is debated in the literature (22). The LDR seed sources are frequently used in permanent prostate brachytherapy. Especially in prostate brachytherapy due to the tissue composite, the photoelectric cross-section is dominant, so the dose distributions of LDR sources were examined in the study. In this study, the effect of phantom material compositions on the dose values calculated for prostate tissue and water medium was precisely investigated with a model-based dose calculation algorithm by considering TG-43 and TG-186 reports.

In prostate, breast and gynecology brachytherapy treatment planning systems, treatment planning is still carried out according to the AAPM TG-43 dose calculation formalism. However, some shortcomings in this formalism increase the need for using model based TG-186 dose calculation formalism in routine brachytherapy treatment planning systems. $D_{w,w}$ (TG-43) water medium and $D_{m,m}$ (TG-186) CT-based prostate tissue medium showed significant differences in 2-dimensional dose distribution patterns and minimum D_{90} values obtained from DVHs. Dose differences on the minimum D_{90} values for prostate tissue and water medium were calculated as 10.5%, 13.4% and 8.3% for ^{125}I, ^{103}Pd and ^{131}Cs multiple brachytherapy seed sources, respectively. These minimum D_{90} dosimetric values obtained from prostate tissue and water medium for different brachytherapy seed sources were found to be compatible with the literature (23,24). Until now, there have been various studies in the literature that were carried out on both $D_{w,w}$ water medium and $D_{m,m}$ prostate tissue regarding LDR multiple brachytherapy seed sources recommended in the reports of TG-43 and TG-186. In these studies, Chibani and Williamson calculated the difference between D_{100} dose values in prostate and water medium for ^{125}I and ^{103}Pd seed sources as 6% (25). Carrier et al. calculated the differences between water medium and prostate tissue as 4.4-4.8% for the D_{90} (26). Landry et al. calculated that D90 differences are up to 4% for prostate tissue and water medium. They also showed that dose distributions in prostate tissue differ from water and are influenced by density, mean tissue composition, and patient-to-patient composition variations (27). Landry et al. calculated as 8-9% the difference between D_{90} dose values in prostate and water medium for ^{125}I and ^{103}Pd brachytherapy seed sources (28).

In all these studies, when the dose distributions in the prostate tissue phantom were compared to the dose values obtained in virtual water phantoms such as TG-43, dosimetric differences due to composition variations occurred. In addition, the effect of dose differences induced tissue compositions has been demonstrated to be important in permanent implant prostate brachytherapy patient simulations by using multi-seed sources (29). The use of dose calculation algorithms such as Monte Carlo

in brachytherapy TPSs should be supported to obtain the sensitive dose values in tissue and water medium. The use of CT-based simulations, which contain detailed information about anatomical structures instead of water phantoms such as the idealized TG-43, can reduce dosimetric uncertainties in the brachytherapy treatment planning process due to tissue compositions. In addition, Monte Carlo model-based dose calculation algorithms can provide high dose accuracy in brachytherapy dosimetry.

A limitation of our study is that we only investigated the effect of the composition structure related to CT-based prostate tissue and water phantom on dose distributions using different LDR multiple brachytherapy sources. However, in further studies, it is necessary to consider not only the medium composition effect but also dosimetric parameters such as inter-seed effect and source positioning for multiple seed implant applications.

In addition, the dose homogeneity values depending on the V_{100} and V_{150} parameters for the ^{131}Cs brachytherapy source within the prostate target volume were better compared to ^{125}I and ^{103}Pd, and our results were found to be compatible with the literature (30).

We also observed that prostate brachytherapy isodose distributions have high dose values in bone tissue and its vicinity. Considering the dose distributions obtained in Fig. 1-3 for ^{125}I, ^{103}Pd and ^{131}Cs multi-seed sources, it was concluded that the dose distributions obtained by using the TG-43 formalism were inadequate in determining sensitive dose distributions especially in the vicinity of the bone region where heterogeneity was dominant. Based on the TG-43 and TG-186 recommendations, there are not enough studies in the literature regarding high radiation dose values that bone tissue may be exposed to in prostate brachytherapy (31,32), and this deficiency of literature can be investigated in further studies. Our results showed that TG-43 based dose calculations are quite insufficient for accurate dose estimation including tissue compositions. Therefore, it was concluded that MBDCAs in TPS can contribute to precise brachytherapy dose calculations by taking such effects into account. It was concluded that rearrangement of TG-43-based dose calculations to take organ and tissue materials into account or use of Monte Carlo-based dose calculation programs that take these effects into consideration in treatment planning systems is necessary for precise and accurate dose calculations.

Study limitations: In this study, the effects of tissue composition on dose distributions in the prostate medium were investigated. In future studies, it is necessary to examine the dose distribution in tissues such as the breast where tissue composition is dominant.

5. CONCLUSION

The dose simulation results calculated by the BrachyDose Monte Carlo code in this study make a significant contribution to the literature regarding the transition from TG-43 to MBDCA in clinical systems for sensitive dose calculations based on the effect of tissue composition. So, it was concluded that MBDCAs should certainly be considered for more accurate dose calculations in TPSs.

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Clinical and Experimental Health Sciences

The Estimation of Standard Portion Sizes Using Food Photographic Booklet Among University Students in Turkey

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ABSTRACT

Objective: To examine estimation of standard portion size and, the influence of different food plates on the estimation of food portion size, using photograph booklet in students.

Methods: The sample consisted of 300 students, aged 18-35 years old from different academic departments. The student's general characteristics were asked by using a questionnaire, and anthropometric measurements were taken. Students were asked to select the standard portion sizes of each food from a photographic booklet, which was developed by the researchers. Dietary guidelines for Turkey were used for assessing the quantity of standard portion size of foods of a photographic booklet (1).

Results: The findings of the present study indicate that the food group which the most students provided the accurate estimation was protein foods (36.0%), pursued by grains (35.1%), dairy (29.2%), fruits and vegetables group (28.9%). The most and the least accurate estimated foods were found boiled potato (64%) and cheese (3.7%), respectively. The percentages of the accurate estimation of standard portion size for five foods (meatball, meat cubes, shredded chicken, egg, yogurt) in 24 foods were higher in females than men ($p<0.05$). There was no significant relationship between various plate sizes and shape with the perception of standard portion size.

Conclusion: It was found that many students were unable to identify the correct photo that represented a standard portion size. Future studies are needed to validate the food atlas for use in the Turkish population.

Keywords: Portion size estimation, food photographs, standard portion size.

1. INTRODUCTION

In recent years, nutrition scientists have stated that there is a need for research in portion size estimation because the perception of the correct amount of portion sizes of food consumed is an important factor for analyzing nutrient intake (2). Assessment of dietary intake with self-report methods such as dietary diaries, 24-h recall, and food frequency questionnaire, rely on the individual's ability to recall their amount of food intake. For this reason, the major sources of errors occur in the assessment of the portion size of food consumption (3, 4). Methods that have been used to reduce measurement error and help individuals to express amounts of food intake include portion-size models, food models, and photographs (5). Photographs have different advantages in comparison with three-dimensional models like being easily copied, including a wide range of different kinds of foods and it is easy for questionnaires to carry them (6). For this reason, food photographs are usually used in dietary surveys to help estimate portion size (7). As food habits differ between the countries and within, the food photographs used in a dietary study should represent the most important local food items and dishes of the area (6).

The photographs, which show a range of portion sizes, are beneficial for estimating portion sizes, and using such photographs can reduce the misclassification of subjects. Also, it was indicated that age, sex, body mass index were potentially important confounders in the perception of portion size (5). Small errors were seen in using photographs and virtual portion sizes. The findings supported that a food photographic atlas is a valid tool for estimating portion sizes in nutritional epidemiological studies. Different factors may influence these three elements, such as food variety, dishware size, number of various portion sizes and their arrangement in the food photograph booklet, size of photographs, and camera angle (8). The effect of dishware size on the serving behavior of an individual is significant (9). Since 1960, the size of the average dinner plate has increased by 36% (8). Based

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on some studies, a positive correlation was seen between dishware size or plate and the amount of food consumption portion (10-13). Recently, obesity has been rapidly increasing and, has become a major health problem worldwide. Epidemiological data suggest that obesity and lifestyle changes lead to increased incidence of chronic diseases such as cardiovascular diseases, cancer, osteoporosis, high blood pressure, and obesity (14,15). Higher energy consumption due to increasing portion size is associated with higher prevalence of overweight or obesity (16). In this regard, to prevent overweight/obesity, estimating the amount of food intake and measuring the portion size seem necessary. The aim of this study was to assess the estimation of standard portion size and analyze the effect of the dishware on the estimation of food portion size in the students of Hacettepe University.

2. METHODS

2.1. Subjects and Data Collection

This cross-sectional study was conducted on Faculty of Economics and Administrative Sciences and Faculty of Engineering at Hacettepe University, during the fall of 2014 and spring 2015 semesters (October 2014-February 2015) to to assess the perception of standard portion size in students. The sample size was calculated by G-Power analysis program and determined as at least 270 students with β=0.3 and α=0.05 and 80% power. Three hundred students (male: 138, 46.2%; female: 162, 54%), aged 18-35 years, who volunteered to participate in the study. The inclusion criteria for the participants were to be healthy students and not to be following a special diet. Participants with any chronic diseases and eating disorders were excluded. Also, pregnant women were excluded. Data were collected through face-to-face interviews. The questionnaire consisted of two parts, including general information and 35 food photographs of 24 foods (milk, yogurt, cheese, meatball, meat cubes, chicken, shredded chicken, baked beans, egg, French fries, boiled potato, boiled green pea, boiled spinach, apple, orange, banana, strawberry, watermelon, bread, rice, pasta, cornflakes, tomato soup, pizza) for estimating the standard portion size of typical Turkish foods. To determine the standard amounts of foods, Dietary guidelines for Turkey were used (1). Students were asked to estimate standard portion sizes of each food using a photographic booklet which was developed by the researcher. Some anthropometric measurements were taken. The body weights were measured to the nearest 0.5 kg with a portable scale. Height, waist, and hip circumferences were measured to the nearest 0.1 cm with a fiber-glass tape. The students wore minimal clothing without shoes during measurements (16). Body mass index (BMI; kg/ m^2) was calculated for each subject. The cut-off points for BMI were defined as 18.5–24.9 kg / m^2 for normal weight and 25–29.9 kg m^2 for overweight according to the World Health Organization (WHO) standards (17). According to WHO recommendation optimal cut-off points for waist circumference were 94 for men and 80 for women; 94-102 cm in men and 80-88 cm in women is associated with an increased risk of metabolic complications and the risk is significantly increase with ≥102 cm in men and ≥88 for women. Also, the waist-hip ratio cut-off points recommended by WHO expert consultation to detect obesity were ≥ 0.9 and ≥ 0.85 for men and women, respectively (18). The cut-off points for waist to height ratio according to Ashwell et al, were defined as; <0.4 take care, 0.4 – 0.5 no increased risk, ≥0.5 and <0.6 'increased risk' and ≥0.6 'very high risk' (19).

Ethical approval was endorsed by Hacettepe University Ethics Committee with B.30.2.HAC.016969557-238 number on 20.10.2015. The participants of this study have confirmed the Informed Consent Form statements.

2.2. Design of the Photographic Booklet

2.2.1. Food selection

The food items were selected among some popular foods which daily consumed in Turkish population (milk, yogurt, cheese, meatball, meat cubes, chicken, shredded chicken, baked beans, egg, French fries, boiled potato, boiled green pea, boiled spinach, apple, orange, banana, strawberry, watermelon, bread, rice, pasta, cornflakes, tomato soup, pizza). It has been noted that foods were selected from different food groups. The participants were asked to select the photo reflecting one portion of a food from different photos shown for each food

2.2.2. Food digital photographs

To reflect the various amount of food intake, photos from three or four different portion sizes were taken. To determine portion size amounts; a coefficient equal to 1 was assessed as medium portion size, the small portion was calculated by multiplying the medium portion size by 0.5. The large portion size was calculated by multiplying the medium one by 1.5, while the extra-large was calculated by multiplying the large portion by 1.5. All foods and beverages were prepared in the kitchen and subdivided into three or four different weighed portions in accordance with previous computed. In the following, they placed on white dining plates, bowls, and glasses against a white background, and photographed from the same angle using a digital camera (Canon SX200IS Power Shot). As the photos were taken with the same angle camera and distance the photographs were demonstrated the unique frame. In the first form, photos were arranged side by side with the paint program, coded by numbers and each portion identified by alphabetical capital letters (A, B, C, and D), and then printed in color. To avoid response bias, actual weights of portions were not shown in photographs. The weights of portion size were indicated at the end of the photograph booklet. The photographs, which were presented to the selection of students, were evaluated with two forms: 1. In 24 food photographs, foods were weighted in different

amounts and were presented in the same size of dishware to examine the knowledge of standard portion size (Figure 1.A) ; 2. In 11 food photographs, foods were weighed in the same amount but presented in different sizes of plates (small (19 cm), medium (23 cm), large (28 cm) diameter) and different dishware (soup plate, bowl, glass) to examine the influence of dishware size in estimating standard portion size (Figure 1.B). For assessing the quantity of standard portion size of foods, Dietary guidelines for Turkey were used (1).

2.3. Data Analysis

Statistical evaluation of results was performed using IBM SPSS 22 package program in the Windows operating system to calculate the frequency, percentage, average, and standard deviation according to gender. The data normality was assessed by Kolmogorov-Smirnov test. The number and percentage of participants with correct estimation for standard portion size were calculated for each portion size foods. The percentages of correct and incorrect estimation of standard portion size of foods were compared for gender using the χ2-test, with 95% confidence.

3. RESULTS

The general characteristics of the subjects are demonstrated in Table 1. The average age of students was 21.5 years old that was found similar in males and females. The percentages of normal BMI (18.5-24.99 kg/m2) were 69.6% and 71.6% in men and women, respectively. 17.7 % of the total samples were overweight, and a further 1.7 % of them were obese. We found that 13.7% of men had a waist circumference ≥94 cm, and 20.4% of women had a waist circumference ≥80 cm. Also, 13.8 % of men (WHR ≥ 0.9) and 7.4% of women (WHR ≥ 0.85) demonstrated central adipose tissue distribution. The percentages of males and females with central obesity according to the waist-to-height ratio (WHtr >0.5) were 31.2 % and 13.0%, respectively.

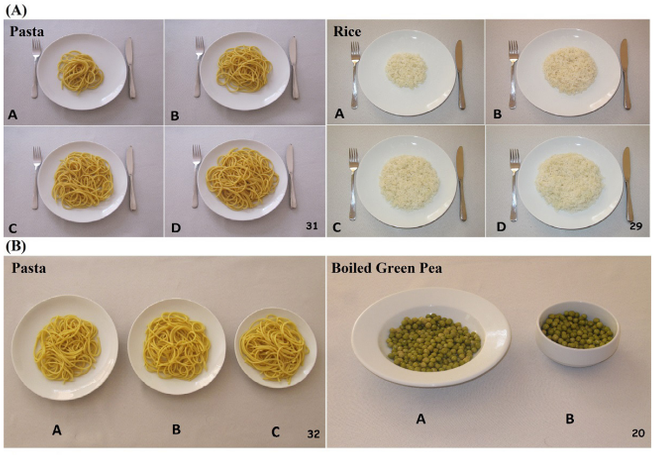

Figure 1. *(A) Different amount of food in same plate (B) Same ammount of food in different dishwares*

Table 1. *General characteristics and anthropometric measurements of students*

	Male (n:138) Mean ±SD	Female (n:162) Mean ±SD
Age (year)	21.9 ± 3.0	21.0 ±2.2
Height (cm)	177.2 ± 6.0	163.7 ± 5.9
Weight (kg)	74.5 ±10.6	57.3 ± 9.2
BMI(kg/m²)	23.9 ± 3.0	21.3 ± 2.8
Waist circumference (cm)	84.8 ± 9.4	74.2 ±9.0
Waist/hip ratio	0.86 ± 0.05	0.77 ± 0.05
Waist/height ratio	0.48 ± 0.05	0.45 ± 0.05
BMI classifications*		
<18.5	1 (0.7)	29 (17.9)
18.5-24.99	96 (69.6)	116 (71.6)
25.0-29.9	37 (26.8)	16 (9.9)
≥ 30	4 (2.9)	1 (0.6)
Waist classifications*		
<94	119 (86.2)	-
94-102	13 (9.4)	-
≥102	6 (4.3)	-
<80	-	129 (79.6)
80-88	-	21 (13.0)
≥88	-	12 (7.4)
Waist/hip ratio classifications*		
<0.9	119 (86.2)	-
≥ 0.9	19 (13.8)	-
<0.85	-	150 (92.6)
≥0.85	-	12 (7.4)
Waist/height ratio classifications*		
<0.4	4 (2.9)	15 (9.3)
0.4-0.5	91 (65.9)	126 (77.8)
0.5-0.6	40 (29.0)	18 (11.1)
>0.6	3 (2.2)	3 (1.9)

Correct estimation of the standard portion size of 24 typical Turkish foods shown in Table 2. The food group that the most students provided the accurate estimation, was for protein group (36.0%), followed by grains/ starches (35.1%), dairy (29.2%), and fruits and vegetables (28.9%) (Figure 2). The food item for which most of the participants provided the accurate estimation was boiled potatoes (64.0%), followed by meat

cubes (55.3%). The most and the least food item which students estimated the accurate portion size was yogurt (47.3%) and cheese (3.7%) from the dairy group, cube meat (55.3%), and chicken (13.7%) from the protein group, boiled potato (64.0%), and orange (5.0%) from fruit and vegetable group, cooked rice (40.0%) and cornflakes (19.3%), respectively.

Among 24 foods, there were significant gender differences in the estimation of portion size of 5 foods ($p<0.05$). The female students came done to an accurate estimation compare with the male students, to appraisal of the standard portion size for meatball ($p < 0.001$), meat cubes ($p <0.001$), shredded chicken ($p < 0.01$), egg ($p < 0.001$), yogurt ($p<0.05$). Only for cornflakes, males provided the correct estimation of standard portion size comparison to females ($p<0.05$). The proportion of female students in estimating of standard portion size of foods, were higher than male students.

The standard portion size estimation of the same amount of some foods in different plate size and dishware are shown in Table 3 and Table 4. In estimating the same amount of foods, which were offered in three sizes of plates (meatball, chicken, shredded chicken, French fries, rice, and pasta), the majority of students were selected medium-sized plates. For the same amount of yogurt in glass and bowl, 74.0% of the students were chosen the yogurt showed in the bowl as standard portion size. In tomato soup, 66.7% of students had preferred the bowl as standard portion size. The differences in selecting dishes were statistically significant ($p< 0.01$).

Table 2. *Correct estimation of standard portion size of typical Turkish food*

	Male (n=138)	Female (n=162)	Total (n=300)	
Variables	N (%)	N (%)	N (%)	p
Dairy				
Milk (225g)	39 (28.3)	35 (21.6)	74 (24.7)	0.183
Yogurt (225g)	55 (39.9)	68 (42.0)	123 (41.0)	0.031*
Cheese (60g)	5 (3.6)	6 (3.7)	11 (3.7)	0.710
Protein Foods				
Meat ball (100g)	37 (26.8)	77 (47.5)	114 (38.0)	0.000*
Meat cubes (100g)	49 (35.5)	117 (72.2)	166 (55.3)	0.000*
Chicken (100g)	22 (15.9)	19 (11.7)	41 (13.7)	0.290
Shredded chicken (100g)	44 (31.9)	77 (47.5)	121 (40.3)	0.006*
Baked beans (130g)	42 (30.4)	54 (34.6)	98 (32.7)	0.447
Egg (50g)	34 (24.6)	73 (45.1)	107 (35.7)	0.000*
Vegetables				
French fries (90g)	47 (34.1)	58 (35.8)	105 (35.0)	0.752
Boiled potato (150g)	86 (62.3)	106 (65.4)	192 (64.0)	0.576
Boiled green pea (150g)	50 (36.2)	77 (47.5)	127 (42.3)	0.061
Boiled spinach (200g)	49 (35.5)	68 (42.0)	117 (39.0)	0.252
Fruit				
Apple (150g)	12 (8.7)	6 (3.7)	18 (6.0)	0.070
Orange (150g)	9(6.5)	6 (3.7)	15 (5.0)	0.264
Banana (150g)	24 (17.4)	32 (19.8)	56 (18.7)	0.601
Strawberry (150g)	31 (22.5)	37 (22.8)	68 (22.7)	0.938
Watermelon (150g)	34 (24.6)	49 (30.2)	83 (27.7)	0.302
Grains and Starches				
Bread (50g)	43 (31.2)	55 (34.0)	98 (32.7)	0.607
Rice (90g)	52 (37.7)	68 (42.0)	120 (40.0)	0.479
Pasta (90g)	45 (32.6)	68 (42.0)	113 (37.7)	0.095
Corn flakes (30g)	35 (25.4)	33 (14.2)	58 (19.3)	0.015*
Tomato soup (200g)	45 (32.6)	62 (38.3)	107 (35.7)	0.307
Pizza (100g)	41 (29.7)	42 (25.9)	83 (27.7)	0.465

**Significant differences between categories defined at P < 0.05. Using chi-square test*

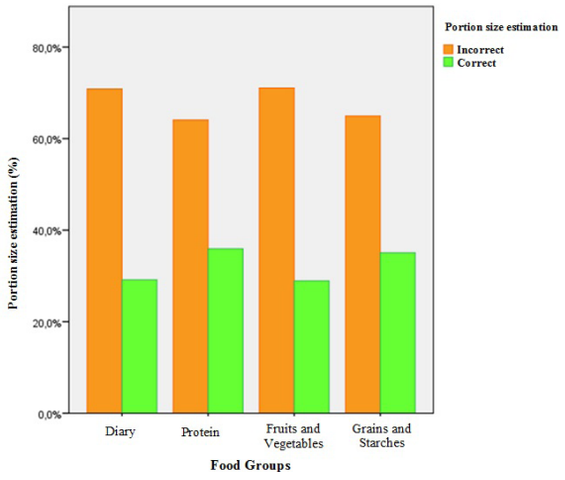

Figure 2. *Estimation of standard portion size of food groups*

Table 3. *Estimation of standard portion size in order to the same amount of foods in different plate size*

Foods (standard portion size)	Plate size n (%)			
	Small	Medium	Large	p
Meat ball (100g)	62 (20.7)	155 (51.7)	83 (27.7)	0.000*
Chicken (100g)	43 (14.3)	158 (52.7)	99 (33.0)	0.000*
Shredded chicken (100g)	61 (20.3)	163 (54.3)	76 (25.3)	0.000*
French fries (100g)	88 (29.2)	129 (43.0)	83 (27.7)	0.002*
Rice (90g)	82 (27.3)	131 (43.7)	87 (29.0)	0.001*
Pasta (90g)	88 (29.3)	134 (42.7)	78 (26.0)	0.000*

**Significant differences between categories defined at P < 0.05. Using chi-square test*

Table 4. *Estimation of standard portion size in order to the same amount of foods in different dishware*

Foods (standard portion size)	Dishware	n (%)	p
Yogurt (225g)	Bowl	222 (74.0)	0.000*
	Cup	78 (26.0)	
			0.000*
Tomato soup§ (200g)	Bowl	200 (66.7)	
	Plate	100 (33.3)	

**Significant differences between categories defined at P < 0.05. Using chi-square test*

4. DISCUSSION

Portion-size estimation is the main factor for assessing dietary intake for managing weight and subsequently managing chronic diseases. In this study, we found that the knowledge of students in estimation of standard portion sizes of foods from food photographs was poor. We found gender differences in the estimation of portion sizes of five foods (meatball, meat cubes, shredded chicken, egg, yogurt). We did not find any statistically differences between different plate sizes and dishware with the estimation of food portion size.

Studies, which examine the accuracy of portion size estimation have reported contradictory results. While in the study of Naska (20), about 53% of participants and in the study of Nikolić (21) about 60.3% (44.3–82.9%) of participants selected the correct portion size from food photographs. In this study, we found that many students were unable to identify the photo that represented a standard portion size. According to the results of this study and previous studies (16, 22,23), the low percentages of accurate estimation, approved that estimating portion size is a challenging task for participants.

The findings of the present study indicate that the food group which the most students provided the accurate estimation was protein foods (36.0%), pursued by grains (35.1%), dairy (29.2%), fruits and vegetables group (28.9%). In this study, the food photographs which most students gave the correct answer was for the standard portion size of boiled potato (64.0%), followed by meat cubes (55.3%). In an earlier study, Choi et al. (24) were evaluated the estimation of the energy content of standard portion size among students and reported that the estimate of the calorie content was correct for grains/starches (25.6 %), followed by dairy products (22.5%) and protein foods (15.6%). Another study (25) showed that the most accurate estimated food groups were dairy products (48%) grains/starches (37.5%), and fruits (22.6%), respectively. Venter et al. (26) stated that more than 80.0% of correct answers were for estimating of standard size of foods, which were presented as solid pieces (sausage, fried fish, apple, and dumplings). On the other hand, the least correct responses (<60.0%) were provided for fairly amorphous foods like soft porridge, lamb and beans, cooked cabbage, and margarine spread on bread. In one study, portion sizes of rice, collard green, and cookies were largely estimated correctly (14). Lillegaard et al. (27) showed that the highest correct responses were for mashed potatoes, pizza, meat, sauce, salad, and cornflakes, while the lowest percentages of correct answers for both portion size of fat-spread bread and French fries. Also, we found that while the largest error in rates of portion size estimation occurred for cheese, orange, and apple, the smallest error occurred for boiled potato and meat cubes. However, Nelson et al. (28) reported that the most and the least error in estimating portion size were for mashed potato and cornflakes, respectively.

The study showed that gender is a major factor in portion size estimation and compared with women, men usually have inaccurate portion size estimation (29). In addition, previous studies indicated that males prefer larger portion sizes of foods than females (30,31). However, Pfrimer et al. (32) showed that there were no significant differences in the

accuracy of perception of portion sizes for different genders. In this study, compared with men, a high rate of women perceived the correct standard portion size for meatball, meat cubes, chicken, egg, bread, cooked rice, pasta, tomato soup, and pizza. Only for cornflakes, men provided the correct estimation of standard portion size compared with women. Gender differences in portion size estimation can be related to the truth that men had higher nutrient requirements and tend to consume more foods in comparison with women (33-35). On the other hand, women are more interested in nutrition-related issues and have more concern about their body weight which can be the result of estimating correct portion size (35,36). The shape and color of foods and the size of the plate on which foods are presented are important factors that affect the perception of the portion size of food. We observed that the majority of students were selected medium-sized plates (Figure 1.B) in the estimation of the same amount of foods (meatball, chicken, shredded chicken, French fries, rice, and pasta) which were offered in 3 sizes of plates. In this study, more selection of medium-sized plates can be contributed to being the middle plate in the photograph. For the same amount of yogurt in glass and bowl, 74.0% of the students chose yogurt in the bowl as standard portion size. In the present study, the usual presentation form of yogurt in the bowl may be the reason for choosing the yogurt in the bowl as the standard portion size. More than half of the students selected a bowl as a standard portion size instead of the plate for tomato soup, also its presentation form in restaurants can be effected to select bowl as a standard one. While some studies (15, 37,38) reported that plate size did not have any effect on the perception of food portion size. On the other hand, other studies (9-13, 39) showed that increasing plate size leads people to eat more food. One study has presented that half of the study participants notified that they tended to eat until they 'clean their plates'. Thus, the tendency means consuming the entire portion available on the plate as a result of visual signaling of a 'clean plate' and not just being satisfied with a smaller quantity (12). Van et al. (9) demonstrated that increasing the size of dishware can encourage an individual to eat at least 50 more calories a day that causes a five-pound increase in weight each year. One study indicated that participants who served themselves cereal in a larger bowl ate 30% more cereal and underestimated their portion size by 14% compared with those given smaller bowls (40). Wansink et al. (11) reported a similar relationship between glass size and amounts of drinking beverages. Another study indicated that in comparison with wide glasses, tall and slender glasses cause to consume more quantity of beverages (41). In this study, we showed that many students were unable to correctly identify the photo that represented a standard portion size. Also, we did not find a significant relationship between different dishware size and shape with the perception of standard portion size. So, training programs and policies should be planned and implemented for university students to improve knowledge of standard portion size as an important factor in preventing the most important health problem, obesity.

5. CONCLUSION

In conclusion, our finding in this study showed that the students have poor portion size estimation skills from food photographs. We found gender differences in the estimation of some foods portion sizes. We could not determine any statistically differences between various dishware and perception of food portion size. The food photographic booklet can be a useful tool to assess the quantification of foods during dietary assessment. So, university students should have education with food-portion tools which is an effective way to enhance estimation skills to improve the accuracy of dietary assessment. Also, Future studies are needed to validate the food atlas for use in the Turkish population.

Author contributions

N.A collected the data, analyzed and interpreted the results, wrote the manuscript. N.R designed the research and had primary responsibility for final content. All authors reviewed the manuscript rigorously and approved the final version submitted for publication.

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Clinical and Experimental Health Sciences

The Determination of *IL-6* rs1800795 Polymorphism Distribution in Turkish National Cross-Country Skiing Athletes Sub-groups Created Referring to the 1km CCSTAs

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ABSTRACT

Objective: Interleukin-6 (IL-6) is an effective and functional protein with a cytokine structure. IL-6 produces a pro/anti-inflammatory response in the immune system and muscle tissue hypertrophy and repair. We aimed to investigate the *IL-6* rs1800795 polymorphism in athletes who were members of the Turkish national cross-country skiing team in order to determine the advantageous genotype for endurance performance.

Methods: A total of 34 athletes within three groups–general (group-1), female (group-2), and male (group-3)–were enrolled in the study. Each group was also divided into two sub-groups as faster and slower referring to their 1-kilometer cross-country skiing time averages (1km CCSTAs). Blood samples were used for DNA isolation, and genotyping was completed by real-time PCR. Chi-square Fisher's and descriptive tests were used for statistical analysis.

Results: The groups' 1km CCSTAs were 188.7 ± 22.4, 212.3 ± 17.8, and 177.4 ± 14.1 seconds, respectively. We detected the genotypes as follows: 17 GG (50%) and 17 GC (50%) in Group 1, 5 GG (45%) and 6 GC (55%) in Group 2, and 12 GG (52%) and 11 GC (48%) in Group 3. We detected no CC genotype in the groups.

Conclusion: Although the GC genotype ratio was higher in the faster athletes of each group compared to the slower sub-groups, we detected a statistically significant difference only in Group 3 ($p<0.0001$). Additionally, the C-allele frequency was higher in the faster sub-groups. However, the significant allele gene distribution was obtained only in Group 3 ($p=0.0002$). According to our results, we can speculate that the GC genotype is more advantageous than the GG genotype in cross-country skiing athletes.

Keywords: *IL-6* Genotypes, Cross-country skiing, Endurance, Athletic performance, Sports genetics

1. INTRODUCTION

The first goal of sports genetics research is to identify the genes that affect athletic performance which is a concept with multifactorial components determined by both polygenetic and epigenetic mechanisms. Today, we know that there are quite a few genes associated with athletic performance (1). We can categorize these genes into groups such as those that affect endurance, muscle strength, energy metabolism, muscle repair, ligament-tendon strength, and heart-lung capacity. The second goal of sports genetics research is to analyze the polymorphism distribution of the determined genes in successful athletes. The third goal of sports genetics research is to use the data obtained to direct individuals in childhood to sports branches in which they may be predisposed according to their genotype.

Cross-country skiing is a winter sport based on completing a heat in the shortest time possible on snow with very heavy protective clothing using skis and poles. It is an endurance sport that requires long-term muscle activity depending on the type of competition (2). There are many national and international competitions for this sport branch. The experimental group in our research included athletes who are specialists in this field.

The Interleukin-6 (*IL-6*) gene is located on chromosome 7 (7p15.3). IL6 is a cytokine protein consisting of 184 amino acids. IL-6 effects the immune system, muscle tissue repair, and muscle hypertrophy. It is accepted that it creates a pro-inflammatory effect as a cytokine as well as an anti-inflammatory effect as a myokine (3).

IL-6 rs1800795 polymorphism attracts a great deal of attention in sports genetics studies (4, 5, 6, 7, 8, 9). According to *IL-6* rs1800795 polymorphism, the G allele is associated

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with more IL-6 plasma levels and is considered to be more advantageous in strength-oriented sports while the C allele, which causes a low IL-6 plasma level, is considered to be more advantageous in endurance sports.

It is the purpose of our study to investigate the relationship between the 1km CCSTA of the athletes of the Turkish cross-country skiing team and *IL-6* rs1800795 polymorphism. We aim to determine the advantageous allele and genotype for cross country skiing, an endurance-focused sport. We can hypothesize that the GC genotype is more advantageous than the GG genotype in cross-country skiing athletes. We believe that our study will be a meaningful part of big data to better understand the paradoxical identity of *IL-6* and contribute scientifically to the spread of cross-country skiing sport in our country and the world.

2. METHODS

2.1. Sample Selection

A total of 34 athletes participated in our study. We formed three experiment groups among the athletes: general (Group 1), female (Group 2), and male (Group 3). There were 34, 23, and 11 athletes in Group 1, Group 2, and Group 3 respectively. There was no sedentary group in our study. We divided each group into faster and slower sub-groups referring to their 1km CCSTAs. There were 19 athletes in the faster sub-group and 15 athletes in the slower sub-group of Group 1, and 7 athletes in the faster sub-group and 4 athletes in the slower sub-group of Group 2, and 12 athletes in the faster sub-group and 11 athletes in the slower sub-group of Group 3. Our study was carried out with a protocol under the Declaration of Helsinki. Ethics committee approval was given by the Uskudar University ethics committee (Approval Number: B.08.6.YOK.2.US.0.05.0.06/2018/553).

2.2. Genotyping

DNA isolation for the blood samples taken from all athletes was carried out with a PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, USA) following the user protocol. Generation of gene amplicons from isolated DNA and genotyping of *IL-6* rs1800795 was realized by the real time-PCR method. For this purpose, the PCR device Roch Light Cycler Nano, Taqman's genotyping kit (Applied Biosystems, Foster City, CA, USA), and VIC/FAM probes were used.

2.3. Statistical Analysis

The data obtained were analyzed using the statistics program for the social sciences (SPSS, version 25.0, IBM, USA). While descriptive statistics of demographic data was performed with a descriptive test, predictive statistics of the data related to genotypes and alleles gene distribution between the sub-groups was performed with the chi-square Fisher's test. Significance was accepted as less than 0.05 ($p<0.05$).

3. RESULTS

A total of 34 national cross country skiing athletes, 23 (68%) male and 11 (32%) female, participated in our study. According to the *IL-6* rs1800795 polymorphism, we formed three experimental groups.

Table 1 shows the phenotypic features of the groups and the number of athletes in the sub-groups. We summarized the age, height, and weight averages, 1km CCSTAs, and the sub-groups athlete numbers for each group. The respective average ages were 18.0 ± 1.4, 17.2 ± 0.8, and 18.4 ± 1.5, for Group 1, Group 2, and Group 3. The height (cm) averages were 168.7 ± 6.5, 163.5 ± 6, and 171.1 ± 5.4, respectively; and the weight (kg) averages were 59.6 ± 6.2, 71.1 ± 5.4, and 62 ± 5.4, respectively. The 1km CCSTAs were 188.7 ± 22.4 s, 212.3 ± 17.8 s, and 177.4 ± 14.1 s, respectively for the groups. There were 19 athletes in the faster sub-group and 15 athletes in the slower sub-group of Group 1, there were 7 athletes in the faster sub-group and 4 athletes in the slower sub-group of Group 2, and there were 12 athletes in the faster sub-group and 11 athletes in the slower sub-group of Group 3. The faster and slower sub-groups were formed referring to the 1km CCSTA of each group.

Table 1. *Groups' phenotypic features and sub-groups athletes*

Phenotypic features	Group 1 General Athletes (n=34)	Group 2 Female Athletes (n=11)	Group 3 Male Athletes (n=23)
Average age	18 ± 1.4	17.2 ± 0.8	18.4 ± 1.5
Average height (cm)	168.7 ± 6.5	163.5 ± 6	171.1 ± 5.4
Average weight (kg)	59.6 ± 6.2	54.5 ± 4.5	62 ± 5.4
1km CCSTA* (s)**	188.7 ± 22.4	212.3 ± 17.8	177.4 ± 14.1
Faster sub-group	19 athletes	7 athletes	12 athletes
Slower sub-group	15 athletes	4 athletes	11 athletes

**1km CCSTA: 1-kilometer cross-country skiing time average, **s: second*

The *IL-6* rs1800795 genotype polymorphism distribution of the three groups is summarized in Table 2. There were 17 GG (50%) and 17 GC (50%) genotypes in Group 1, there were 5 GG (45%) and 6 GC (55%) in Group 2, and there were 12 GG (52%) and 11 GC (48%) genotypes in Group 3.

Table 2. *IL-6 rs1800795 genotype polymorphism distribution of groups*

Genotype	Group 1 (n=34)	Group 2 (n=11)	Group 3 (n=23)
GG	17 (50%)	5 (45%)	12 (52%)
GC	17 (50%)	6 (55%)	11 (48%)

Table 3 shows the *IL-6* rs1800795 allele gene polymorphism distribution of the three groups we formed. The distribution

of the G and C alleles of the *IL-6* rs1800795 polymorphism in the groups were counted as 51 G (75%) and 17 C (25%) in Group 1; 16 G (73%) and 6 C (27%) in Group 2, and 35 G (76%) and 11 C (24%) in Group 3. The distributions were not normally distributed. The common allele was found to be G allele, as expected.

Table 3. *IL-6 rs1800795 allele polymorphism distribution of groups*

Allele	Group 1 (n=34)	Group 2 (n=11)	Group 3 (n=23)
G	51 (75%)	16 (73%)	35 (76%)
C	17 (25%)	6 (27%)	11 (24%)

Table 4 shows the genotypes and their average 1km CCSTAs time. Individuals with the GC genotype completed the 1km cross-country skiing heat 7.16 ± 22.3 s earlier in Group 1, 3.6 ± 10.3 s earlier in Group 2, and 10 ± 7.3 s earlier in Group 3 than the group averages. In all groups, it was observed that the individuals with the GC genotype completed the 1km cross-country skiing heat in less time.

Tablo 4. *The genotypes differences of 1km CCSTAs*

GROUPS	IL-6 rs 1800795 polymorphism	The differences of genotypes' 1km CCSTAs* from groups' 1km CCSTAs* (s)**
Group 1 (n=34)	GG (n= 17)	7.16 ± 20.9 (slower than average)
	GC (n= 17)	-7.16 ± 22.3 (faster than average)
Group 2 (n=11)	GG (n=5)	4.2 ± 24.9 (slower than average)
	GC (n=6)	-3.6 ± 10.3 (faster than average)
Group 3 (n=23)	GG (n=12)	9.8 ± 11.5 (slower than average)
	GC (n=11)	-10 ± 7.3 (faster than average)

**1km CCSTA: 1-kilometer cross-country skiing time average, **s: second*

Table 5. *Significance value of IL-6 rs1800795 polymorphism genotype distribution between the sub-groups*

GROUPS	SUB-GROUPS	GG	GC	CC	Significance value
Group 1 (n=34)	Faster (n=19)	8 (42%)	11 (58%)	0 (0%)	p= 0.490
	Slower (n=15)	9 (60%)	6 (40%)	0 (0%)	
Group 2 (n=11)	Faster (n=7)	2 (29%)	5 (71%)	0 (0%)	p= 0.240
	Slower (n=4)	3 (75%)	1 (25%)	0 (0%)	
Group 3 (n=23)	Faster (n=12)	1 (8%)	11 (92%)	0 (0%)	p < 0.0001
	Slower (n=11)	11 (100%)	0 (0%)	0 (0%)	

The genotype distribution values between the faster and slower sub-groups formed referring to their 1km CCSTAs are listed in Table-5. In Group 1, the faster sub-group had 8 GG (42%) and 11 GC (58%) genotypes, and the slower sub-group had 9 GG (60%) and 6 GC (40%) genotypes (p=0.49). In Group 2, the faster sub-group had 2 GG (29%) and 5 GC (71%) genotypes, and the slower sub-group had 3 GG (75%) and 1 GC (25%) genotypes (p=0.24). In Group 3, the faster sub-group had 1 GG (8%) and 11 GC (92%) genotypes, and the slower sub-group had only 11 GG (100%) genotypes. Only the allele genes distribution of Group 3 was statistically significant (p<0.0001).

The ratio of GC genotypes in all the faster sub-groups was higher than in the slower sub-groups. However, only the genotype distribution of Group 3 was statistically significant (p<0.0001). In Group 3, it was observed that the ratio of GC genotypes was higher in the faster sub-group than the slower sub-group. The ratios of GC genotypes of Group 3 were 0% in the slower sub-group and 92% in the faster sub-group. These ratios had a significant distribution (p<0.0001).

Table 6 shows the significance values of the allele gene distribution between the faster and slower sub-groups formed referring to their 1km CCSTAs. In Group 1, the faster sub-group had 27 G (71%) and 11 C (29%) allele genes, and the slower sub-group had 24 G (80%) and 6 C (20%) allele genes (p = 0.57). In Group 2, the faster sub-group had 9 G (64%) and 5 C (36%) alleles genes, and the slower sub-group had 7 G (88%) and 1C (12%) alleles genes (p=0.35). In Group 3, the faster sub-group had 13 G (54%) and 11C (46%) alleles genes, and the slower sub-group had 22 G (100%) and 0 C (0%) alleles genes (p=0.0002).

Table 6. *Significance value of IL-6 rs1800795 polymorphism allele distribution between sub-groups*

GROUPS	SUB-GROUPS	G	C	Significance value
Group 1 (n=34)	Faster (n=19)	27 (71%)	11 (29%)	p= 0.57
	Slower (n=15)	24 (80%)	6 (20%)	
Group 2 (n=11)	Faster (n=7)	9 (64%)	5 (36%)	p= 0.35
	Slower (n=4)	7 (88%)	1 (12%)	
Group 3 (n=23)	Faster (n=12)	13 (54%)	11(46%)	p= 0.0002
	Slower (n=11)	22 (100%)	0 (0%)	

The G allele gene's rate was over 50% in all groups, and the frequency of C alleles in all the faster sub-groups was higher than in the slower sub-groups. However, only the allele gene distribution of Group 3 was statistically significant (p=0.0002). The ratios of C alleles in Group 3 were 0% in the slower sub-group and 46% in the faster sub-group. These ratios had a significant distribution (p=0.0002). In our cohort, there was only the GC as genotypes with C, but there was no CC genotype.

4. DISCUSSION

IL-6 is an effective cytokine in the immune system, in muscle tissue repair, and in muscle hypertrophy. In recent studies, it has been reported that the IL-6 molecule produces a pro-inflammatory response in monocytes or macrophages and an anti-inflammatory response in the muscle (10). The IL-6 molecules can rise to 100 times the normal level in blood and muscle tissue during exercise, depending on the amount of physical activity (11).

Factors that increase *IL-6* gene expression in skeletal muscles include the increase in muscle contraction activity, the

increase in the number of calcium ions in the cytoplasm, the increase in the amount of calcineurin, some metabolic changes as a result of sports activity (decrease in glucose and glycogen content, increase in oxidative stress, increase in temperature, increase in catecholamines); and it can be counted as an increase in some hormones (12).

Some studies have reported that vigorous and intense exercise reduces the number of pro-inflammatory receptors on the surface of monocytes and some other cells (13). Based on information in the literature, we thought that the reason for the decrease in the number of these receptors is to reduce the destructive effect of the excessively increased number of cytokines. Therefore, we hypothesized that the GC genotype may be more advantageous than GG for endurance-type athletes.

Our study is one of the first to investigate the relationship among the 1km CCSTA of the Turkish cross-country skiing athletes with *IL-6* rs1800795 polymorphism distribution. In our study, we formed three experimental groups among the athletes on the Turkish national cross-country skiing team as Group 1, Group 2, and Group 3. We also divided each group into two sub-groups that were relatively faster and slower referring to their 1km cross-country skiing time averages (1km CCSTAs). In addition, the genotype distribution between these sub-groups according to the *IL-6* rs1800795 polymorphism was investigated.

There are some studies that attempt to associate the rs1800795 SNP polymorphism of the *IL-6* with athletic performance in terms of strength or endurance. In the literature, significant relationships have been established between the G allele of the *IL-6* rs1800795 polymorphism and increased power performance (4, 6). In a meta-analysis of this topic, nine polymorphisms were defined for strong athlete status, and, according to the *IL-6* rs1800795 polymorphism, the G allele and thus the GG genotype was found to be associated with the power phenotype. (14).

However, there are also studies in the literature reporting that there is a significant relationship between the G allele of the *IL-6* rs1800795 polymorphism and the endurance phenotype (8). However, there are also studies reporting a significant relationship between the C allele and the endurance phenotype (9).

When we look at the difference in genotypes and 1km CCSTAs, it was observed that the athletes with GC genotype completed the 1km cross-country skiing faster than the group averages in all three groups.

The GC genotype ratio was higher in the faster sub-groups of each group compared to the slower sub-groups. However, a significant genotype distribution was detected only in Group 3 ($p<0.0001$). The ratios of the GC genotypes of Group 3 were 0% in the slower sub-group and 92% in the faster sub-group. We can say that the GC genotype is more advantageous than GG for endurance performance, and there was no CC genotype in our cohort.

The C allele frequency was higher in the faster sub-groups of each group compared to the slower sub-groups. However, a significant allele gene distribution was detected only in Group 3 ($p=0.0002$). The ratios of C alleles of Group 3 were 0% in the slower sub-group and 46% in the faster sub-group. In our cohort, there was only the GC as genotypes with C, and there was no CC genotype.

According to our results, we can speculate that the GC genotype is more advantageous than GG in sports branches that require endurance. We believe that the GC genotype, which may be less affected by the destructive effect of excessively increased cytokine amount, may be more advantageous in sports branches that require endurance compared to the GG genotype. However, there was no CC genotype in our cohort, which makes it difficult to make a hypothesis about CC. The main limitation of our study was the low number of subjects. The reason for this was to eliminate the environmental differences of the athletes. The athletes who enrolled in our study had the best average times and represented the national team. They have similar training programs and very similar nutritional habits; that was the reason we just wanted to have a single team to analyze the effect of the *IL-6* rs1800795 polymorphism. The second limitation of our study, which we are planning to complete in further studies, was the lack of serum IL-6 amounts. In spite of these limitations, we believe that this study will guide researchers to further studies.

5. CONCLUSION

High performance occurs in athletes as a result of the interaction of appropriate genetic structure and environmental factors such as proper nutrition and training. Therefore, genetic and environmental factors that can affect athletic performance should be analyzed separately. We can suggest that the GC genotype is more advantageous than GG for endurance performance; however, since a limited number of athletes were included in our study, we think that this gene polymorphism should be re-studied in experimental groups with larger numbers of participants, and its results should be evaluated with meta-analyzes. Our results should also be confirmed by further studies.

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Clinical and Experimental Health Sciences

Related Factors of Sexual Abstinence Behavior of College Women: A Transtheoretical Model Based Examination

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ABSTRACT

Objective: This research was carried out to determine the relationship between college women's transtheoretical model stages of change and their sexual health knowledge, decisional balance, and self-efficacy towards sexual abstinence behavior.

Methods: The sample of this descriptive study consisted of 559 college women. Data were collected using the Socio-Demographic Form, Stages of Change for Sexual Abstinence, Sexual Health Knowledge Test, Decisional Balance Scale, and Self-Efficacy Scale. The data were analyzed using frequency (n), percentage distributions (%), chi-square test, Mann-Whitney U Test, and Kruskal-Wallis Test.

Results: The mean age of the sample is 19.88±1.42 (Min-Max: 17-24). It was determined that the students who had no sexual experience had higher the internal and external pros perception and self-efficacy scores and lower cons perception scores ($p<0.05$). The internal and external pros perceptions and self-efficacy of the students in the precontemplation stage were found lower than those in the contemplation stage ($p<0.05$). At the same time, it was determined that the external pros perceptions of the students in the contemplation stage were lower than those in the action stage ($p<0.05$).

Conclusion: It was found that the perception of internal and external pros and self-efficacy increased with the progress towards the action stage among the sexual abstinence stages of change. Decisional balance and self-efficacy were found to be important components for the effectiveness of planned sexual health education.

Keywords: Sexuality, sexual health, sex education, transtheoretical model, college women

1. INTRODUCTION

The youth period is considered as the transition stage from adolescence to adulthood (1). Young individuals who did not receive proper and sufficient sexual health education until the university period may engage in risky sexual behaviors such as sexual intercourse without feeling physiologically and psychologically ready, unprotected intercourse, unwanted pregnancies and miscarriages, early marriage, having more than one sexual partner or frequent partner change, having sexual experiences with drug effect due to reasons such as separation from their families, changes in their lifestyles and peer pressure (2,3). When the literature is examined, these risky behaviors are mostly seen in first-year college students (4–6).

In countries where sexuality is seen as taboo and sexual health education is insufficient, it is seen that the method of sexual abstinence is effective in protecting young individuals from sexually transmitted diseases and unwanted pregnancies (7). Most of the studies abroad carry out programs supporting sexual abstinence (8). For the effectiveness of the programs related to planned sexual health education, factors related to sexual abstinence should be determined.

The Transtheoretical Model (TTM) can provide an opportunity to identify factors associated with sexual abstinence behavior. TTM argues that behavioral change is a process, it will facilitate behavioral change by making appropriate interventions to the change stage of the individual and it is a model with proven effect (9,10). The stages of change, which are the main components of this model, include the stages of pre-contemplation, contemplation, preparation, action, and maintenance, which evaluate the individual's readiness to change behavior. Also, the model includes cognitive and behavioral processes, self-efficacy, and decisional balance regarding the behavior change. Decisional balance includes the perception of pros and cons that affects behavior change (9). Self-efficacy is the confidence of an individual to perform the desired behavior in any situation (11). When the studies

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on TTM in the literature were analyzed, it is seen to be used to investigate different health behaviors such as smoking, alcohol and substance addiction, nutrition, physical activity, HIV/AIDS prevention, and cancer screening (10,12,13). There are very few studies evaluating the relationship between sexual abstinence behavior and TTM structures (14,15). In Turkey, there are no studies on this subject. In Turkey, the problems related to sexuality in adolescents and young individuals are not defined sufficiently, and sexual health education is insufficient, which causes limited programs to be made on this subject. To increase the effectiveness of programs aimed at preventing risky sexual behaviors, related factors should be examined and interventions should be made against them. This study was conducted to (a) examine the sexual health behaviors of college women, (b) determine the factors related to the stages of change regarding sexual abstinence, and (c) evaluate the relationship of the stages of change with decisional balance, self-efficacy, and sexual health knowledge levels.

2. METHODS

2.1. Study Design and Setting

The universe of this descriptive research consisted of the first-year female students (N=709) studying at a university in Istanbul in the 2019-2020 academic year at a health services vocational school. Ethics committee approval (20.06.2019-143) and institutional permission were obtained. The sample of the study consisted of 559 students who agreed to participate in the study and filled in the data collection tools.

2.2. Data Collection Tools

Research data were collected online between October 4-11, 2020. Data collection tools were The Socio-Demographic Form, Stages of Change for Sexual Abstinence, the Sexual Health Knowledge Test, Decisional Balance Scale for Sexual Abstinence, and Self-Efficacy Scale for Sexual Abstinence.

2.2.1. Socio-Demographic Form

This form consists of 9 questions including age, marital status, sexual health education status, sexual activity status, and opinion on premarital sexual experience.

2.2.2. Stages of Change for Sexual Abstinence (SCSA)

The stages of change developed by Hulton (2001) in line with the transtheoretical model were described with questions (14). It consists of a 5-choice question that evaluates the stage at which people are including the stages of pre-contemplation, contemplation, preparation, action, and maintenance.

2.2.3. Decisional Balance Scale for Sexual Abstinence (DBSSA)

The scale, which was developed by Hulton (2001) (14) in line with TTM and whose Turkish validity and reliability study was conducted by Karatana et al. (16), consists of three sub-dimensions of sexual abstinence: internal pros, external pros, and cons. It consists of a total of 16 questions including 5 items for the internal pros sub-dimension, 4 items for the external pros sub-dimension, and 7 items for the cons sub-dimension. The scale is a 5-point Likert type that determines the importance of the participants' decisions about sexual abstinence between strongly agree and strongly disagree. High scores for pros sub-dimension and low scores for cons sub-dimension are considered as factors that positively affect sexual abstinence. The Cronbach's alpha coefficients of the Turkish form were 0.61 for internal pros, 0.77 for external pros, and 0.76 for cons sub-dimension (16). For this study, the Cronbach's alpha coefficients were 0.62 for internal pros, 0.76 for external pros, and 0.76 for cons sub-dimension.

2.2.4. Self-Efficacy Scale for Sexual Abstinence (SESSA)

The scale, which was developed by Hulton (2001) (14) in line with TTM and whose Turkish validity and reliability study was conducted by Karatana et al. (16), consists of 6 questions with a 5-point Likert type that evaluate the self-efficacy of individuals in sexual abstinence. High scores indicate high self-efficacy for sexual abstinence. The Cronbach's alpha coefficient was found to be 0.88 for the Turkish form (16), and 0.83 in this study.

2.2.5. Sexual Health Knowledge Test (SHKT)

It is a 40-question multiple-choice test that was developed and whose validity and reliability studies have been conducted by Evcili and Golbasi (2017) to measure the knowledge level of the participants (17). The score to be taken from the test varies between 0-40. It is accepted that the higher the score, the higher the level of sexual health knowledge. The Cronbach's alpha reliability coefficient of the test was reported as 0.88 (17). In this study, it was found to be 0.80.

2.3. Data Analysis

Socio-demographic characteristics were evaluated with mean, standard deviation, frequency (n), percentage distributions (%). Mann-Whitney U, Kruskal-Wallis and chi-square tests were used to compare socio-demographic characteristics and TTM stages of change and perceptions of pros/ cons from sexual abstinence, self-efficacy, and sexual health knowledge scores. The statistical significance level was 0.05.

3. RESULTS

The mean age of the students is 19.88±1.42. It was observed that 98.9% of the students were single and 55.8% did not receive sexual health education. 20.6% of the students had sexual experience starting at the age of 17.98±1.89 (min: 14 – max: 23). It was determined that 65.2% of the students were protected during sexual intercourse, 69.7% preferred condoms as a protection method, 26.1% had an sexually transmitted disease test and 36.5% had sexual intercourse while using alcohol (Table 1).

Table 1. *Socio-demographic Information of Students*

Variables	Min. – Max.	M±Sd
Age (n=559)	17-24	19.88±1.42
Age of First Sexual Experience (n=115)	14-23	17.98±1.89
	n	**%**
Marital status(n=559)		
Single	553	98.9
Married	6	1.1
Sexual Health Education (n=559)		
Yes	247	44.2
No	312	55.8
Sexual Activity Status (n=559)		
Yes	115	20.6
No	444	79.4
Protection During Sexual Intercourse (n=115)		
Yes	75	65.2
No	13	11.3
Most of the time	17	14.8
Rarely	10	8.7
Choice of Prevention Method (n=102)		
Contraceptive pill	2	1.9
Condom	71	69.7
Morning-after pill	8	7.8
Withdrawal	21	20.6
Sexually Transmitted Disease Testing Status (n=115)		
Yes	30	26.1
No	85	73.9
Sexual Experience While Using Alcohol (n=115)		
Yes	42	36.5
No	73	63.5

Sd: Standard deviation, Min: Minimum, Max: Maximum, M: Mean

In our study, it was determined that 66.7% of sexually active students who received sexual health education and 63.8% of those who did not receive health education were protected during sexual intercourse and there was no significant difference between the groups (X^2= 4.70, p>0.05).

3.1. Factors Related to TTM Sexual Abstinence Decisional Balance, Self-Efficacy and Sexual Health Knowledge Score

When the students' age and their perceptions of pros and cons in sexual abstinence, self-efficacy, and sexual health knowledge scores were compared, it was found that the internal pros, external pros perceptions and self-efficacy of students aged between 17 and 19 were higher than those of those aged 20 and over (p<0.05). It was found that married students had higher self-efficacy scores than single students (p<0.05). It was found that the self-efficacy and sexual health knowledge of the students who had previously received sexual health education was higher than those who did not (p<0.05). It was determined that the students who had no sexual experience had higher the internal and external pros perception and self-efficacy scores and lower cons perception scores (p<0.05), and there was no significant difference in sexual health knowledge scores (p>0.05) (Table 2).

3.2. Relationships Between TTM Stages of Change for Sexual Abstinence and Decisional Balance, Self-Efficacy and Sexual Health Knowledge

It was determined that 14.7% of the students were at the stages of pre-contemplation, 28.8% contemplation, 44.2% preparation, and 12.3% action.

When sexual abstinence decisional balance, self-efficacy, and sexual health knowledge mean scores were compared according to the stages of change, while a statistically significant difference was found between internal pros, external pros, and self-efficacy scores (p<0.05), there was no difference in perception of cons and sexual health knowledge levels (Table 3) (Figure 1).

The internal and external pros perceptions and self-efficacy of the students in the precontemplation stage were found lower than those in the action stage (p<0.05). At the same time, it was determined that the external pros perceptions of the students in the contemplation stage were lower than those in the action stage (p<0.05) (Table 3).

Figure 1. *Comparison of Students' Stages of Change for Sexual Abstinence with Decisional Balance, Self-Efficacy and Sexual Health Knowledge Mean Scores*

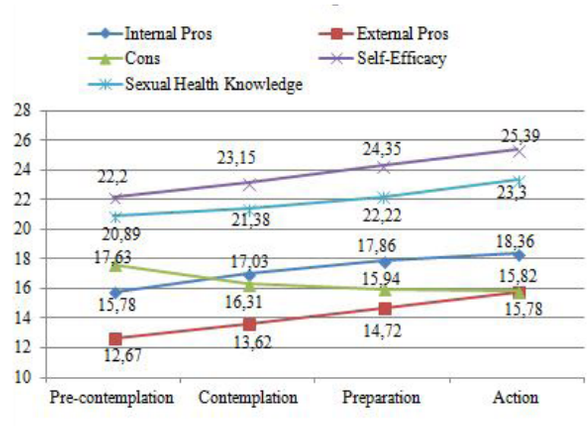

Table 2. *Comparison of Students' Sexual Abstinence Decisional Balance, Self-Efficacy and Sexual Health Knowledge Mean Scores and Descriptive Characteristics (N=559)*

Characteristics	DBSSA			SESSA	SHKT
	Internal Pros Mean±SD	External Pros Mean±SD	Cons Mean±SD	Mean±SD	Mean±SD
Age					
17-19 (n=394)	17.80±3.62	14.77±3.99	15.98±5.36	24.26±4.99	22.09±5.78
20-24 (n=165)	16.36±4.76	13.00±4.65	16.74±5.77	22.75±6.07	21.50±6.30
U/p	**-3.80/.000***	**-4.19/.000***	-1.42/.155	**-2.437/.015***	-.671/.502
Marital Status					
Single (n=553)	17.38± 4.06	14.26± 4.28	16.23± 5.49	23.77± 5.37	21.87± 5.94
Married (n=6)	17.00± 2.00	12.50± 2.58	13.83± 5.03	27.83± 3.12	26.00± 4.73
U/p	-.58/.558	-1.37/.168	-1.08/.279	**-2.08/.037***	-1.92/.055
Sexual Health Education					
Yes (n=247)	17.48± 4.02	14.27± 4.20	15.97± 5.11	24.61± 4.84	22.92± 5.95
No (n=312)	17.29± 4.06	14.22± 4.33	16.39± 5.77	23.18± 5.68	21.12± 5.82
U/p	-.724/.469	-.015/.988	-.767/.443	**-2.843/.004***	**-3.86/.000***
Sexual Activity					
Yes (n=115)	15.01± 4.09	13.16± 4.14	19.51± 5.63	21.36± 6.21	21.94± 5.58
No (n=444)	17.99± 3.80	14.52± 4.26	15.35± 5.12	24.45± 4.94	21.91± 6.03
U/p	**-6.86/ .000***	**-3.42/ .001***	**-6.75/ .000***	**-4.93/ .000***	-075/.940

*DBSSA: Decisional Balance Scale for Sexual Abstinence, SESSA: Self-Efficacy Scale for Sexual Abstinence, SHKT: Sexual Health Knowledge Test, U: Mann-Whitney U Test, * p<0.05*

Table 3. *Comparison of Students' Stages of Change for Sexual Abstinence with Decisional Balance, Self-Efficacy and Sexual Health Knowledge Mean Scores (N=559)*

Variables	*PC (n = 82)*	*C (n = 161)*	*P (n = 247)*	*A (n =69)*	*Test and p value*		*Paired Comparison*
	Mean ±Sd	Mean ±Sd	Mean ±Sd	Mean ±Sd	X^2	*p*	*Mann-Whitney U Test*
Internal Pros	15.78±5.13	17.03±4.26	17.86±3.67	18.36±2.43	**18.78**	**.000**	PC<P, A
External Pros	12.67±4.67	13.62±4.41	14.77±3.99	15.69±3.63	**23.76**	**.000**	PC<P, A; C<A
Cons	17.10±7.14	16.31±5.51	15.94±5.27	15.82±3.63	1.57	.664	
Self-Efficacy	22.20±6.18	23.15±6.11	24.35±4.80	25.39±3.49	**10.69**	**.014**	PC<A
Sexual Health Knowledge	20.89±6.83	21.38±6.09	22.22±5.44	23.30±5.93	6.69	.082	

Pc: Pre-contemplation, C: Contemplation, P: Preparation, A: Action, X^2: Kruskal-Wallis test

4. DISCUSSION

This is the first study on sexual abstinence to examine the relationship between the stages of change of the transtheoretical model and decision-making, self-efficacy, and sexual health knowledge. In our study, it was determined that the perception of internal and external pros and self-efficacy scores of the students who were at the pre-contemplation stage of TTM sexual abstinence stages of change were the lowest, and the scores of the students in the action stage were the highest. This result was consistent with the theoretical structure of TTM and the results of TTM studies conducted on different subjects in the literature (9,10). According to the studies in Turkey, the age of first sexual experience was found to be between 16-20 (18–20). Similar to the literature, it was found that 20.6% of the students had sexual experiences and the mean age of first sexual experience was 17.98±1.89 (min: 14 – max: 23).

In this study, it was determined that 55.8% of the students did not receive sexual health education. When studies in Turkey are examined, most of the college women did not receive sexual health education and have insufficient knowledge levels (19–21). This can result from the limited sexual health education in the Turkish education system, the perspective of the community on sexuality, socio-cultural differences, the influence of religion and culture. The fact that a large proportion of the young population does not know about sexual health education emphasizes the need for education on this subject. Considering that the first sexual experience reduced to the age of 14, it can be suggested to do sexual

health education in the pre-university period to prevent early and risky behaviors.

4.1. Decisional Balance

Decisional balance reveals the perceptions of pros and cons of changing behavior towards sexual abstinence behavior. Pros are defined as the benefits of changing a behavior, while cons are defined as barriers or disadvantages of change. Increasing perceived pros and decreasing perceived cons show that a person is more likely to change behavior (9).

Prat et al. (2012) (22) study on the use of condoms with university students, and Lipschitz et al. (2013) (23) study with female university students on the HPV vaccine, it was stated that the pros perception scores of the students in the action stage were higher than the other stages. In our study, parallel to the literature, it was determined that the pros perception score of sexual abstinence increased from the pre-contemplation stage to the action stage. In line with these results, it can be suggested to include content that will increase the perception of pros in sexual health education programs in terms of the effectiveness of the programs. Increasing the perception of the pros of young people who are at the stage of pre-contemplation and contemplation will make it easier for them to move to the action stage.

In studies examining the perception of cons according to the stages of change, it was found that the cons perception scores of students in the action stage were lower than the other stages (22–25). Similarly, in this study, it was observed that the perception of cons score was the lowest during the action stage. Since the low scores of the cons sub-dimension support sexual abstinence, it can be suggested to prepare content to reduce the perception of cons in sexual health education. In our study, the pros perception was found to be higher in those aged between 17 and 19 compared to those aged 20 and over. In addition, those with no sexual experience had higher pros perception and lower cons perception than those with no sexual experience. These results show that young people who are older and have sexual experiences are at risk in terms of sexual behavior. It is recommended to give priority to these risky groups in sexual health education programs.

4.2. Self-efficacy

Self-efficacy is a key concept in healthy sexual behavior and focuses on the ability to control one's sexual health-related behaviors, to engage in safe sexual behavior, and to choose the right sexual partner (26). Studies show that high self-efficacy prevents risky sexual behaviors (27–29). In studies comparing stages of changes and self-efficacy scores, higher self-efficacy scores were found in the action and maintenance stage (23,25,30). Similarly, in our study, it was determined that the self-efficacy scores increased from the pre-contemplation stage to the action stage. In line with these results, interventions to increase the level of self-efficacy in sexual health education can be suggested to support sexual abstinence behavior.

In our study, sexual abstinence self-efficacy scores of those who did not receive sexual health education, had sexual experience, and those who were 20 years and older were found to be lower than others. For these reasons, it is important to increase self-efficacy to avoid sexuality before sexual experience and at an early age.

4.3. Sexual Health Knowledge Levels

It is important for young people to acquire correct information, attitude, and behavior about their sexual health. It enables young people to develop their sexual identity, develop healthy and safe sexual behavior, and prevent sexual dysfunctions (31). It is stated in the literature that sexual health knowledge is influenced by family, society, and cultural factors (32,33). In our study, it was found that the sexual health knowledge level of the students was at a medium level (21.92±5.94). 66.7% of the sexually active students who stated that they received sexual health education and 63.8% of those who did not receive sexual health education stated that they were protected during sexual intercourse. The lack of significant difference between the groups may be related to the content and quality of health education. Comprehensive sexual health education programs (34) which is a proven effective program in this area, may be recommended in future studies.

There is no study investigating the relationship between sexual health knowledge levels and stages of change. Although there was no significant difference between the stages of change and sexual health knowledge levels in our study, it was observed that the scores increased from the pre-contemplation stage to the action stage. Sexual health knowledge has an important effect on the healthy sexual attitudes and behaviors of young people (35) and increasing their knowledge level is necessary to protect them from risky sexual behaviors.

5. CONCLUSION

It was determined that the perception of internal and external pros and self-efficacy scores of the students who were at the pre-contemplation stage of TTM sexual abstinence stages of change increased towards the action stage, while the perception of cons decreased. Our study results will form the basis for health education programs. To support sexual abstinence behavior effective sexual health education can be planned to increase pros perceptions and self-efficacy, and to reduce the perception of cons.

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Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Committee Approval

The study protocol was approved was obtained from the Ethics Committee of Marmara University (20.06.2019-143).

Restrictions of the Study

The main limitation of the study is that it is limited to university students studying at a foundation and a state university.

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Clinical and Experimental Health Sciences

Does Double-layer Application of a Universal Adhesive Affect Its Bonding to Different Tooth Substrates?

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of a single or double layer application of a Universal adhesive on bond strength to different tooth substrates.

Methods: Seventy-two extracted sound human teeth were used for the study. The teeth were divided into three groups according to tooth substrates; enamel, coronal and radicular dentin (n=24). The roots of the teeth were separated from the crowns and then abraded to obtain flat surfaces. The specimens were randomly allocated into 2 groups in accordance with application layers of universal adhesive (n=12). The universal adhesive, Adhese Universal was applied in self-etch mode as single – or double-layer to different substrates. Following the adhesive application, enamel, coronal and radicular dentin substrates were restored with a resin composite (Tetric N-Ceram). The specimens were subjected to shear bond strength (SBS) test after storage in distilled water (37°C, 24 h). Representative fractured and interface samples from each group were examined by a scanning electron microscope. The data were analyzed using two-way ANOVA($\alpha = 0.05$).

Results: There was no statistically difference in SBS values between single – and double-layer applications of the tested universal adhesive on different dental substrates ($p>0.05$). Whilst no difference was occurred between the different dental substrates in the single-layer application ($p>0.05$), double-layer application on coronal dentin presented significantly higher bond strength than enamel ($p<0.05$).

Conclusion: Double-layer application of the tested universal adhesive did not improve the bond strength regardless of different dental substrates.

Keywords: Universal adhesive, Shear bond strength, Double-layer application

1. INTRODUCTION

Contemporary dental adhesive systems can be categorized based on the adhesion strategies in etch-and-rinse and self-etch or the number of application steps (1). Three fundamental steps that can be either separate or combined are required to adhesion resin-based materials to the dental substrates: priming, etching and bonding. In order to shorten the treatment time, all-in-one self-etch adhesive systems have been introduced owing to decreasing the three procedures into a single process (2). Recently, the novel family of dental adhesives known as “multi-mode” or “universal”, similar to the all-in-one concept but also provides a variety of uses, have been introduced to the market.

Although the etch-and-rinse strategy is still the gold standard in dentistry practice, the current trend is to improve simplified adhesive systems (3,4). In spite of the fact that a significant decrease was achieved in the total treatment time and less sensitivity to dissimilarity in the clinicians’ technique compared to multi-step etch-and-rinse adhesives, there are some concerns about the etching capability and vulnerable adhesive layer due to the hydrophilic nature (5,4,6). Various approaches have been proposed for clinical conditions such as selective etching, hydrophobic coating, double adhesive layer applications or extended application time to overcome the deficiencies of simplified self-etch adhesives (3,7-10).

Universal adhesives, which can be considered as a new category of self-etching adhesives, are thought to have as vulnerable adhesive layers as other self-etch adhesives (11). The current trends in adhesive applications seem to be tending towards one step applications; however, it is speculated that the double-layer application might be an effective technique to improve the bonding to dentin (12).

The main difficulty for prevailing adhesive systems is to ensure a homogeneously effective bond to different tooth

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substrates such as enamel, coronal dentin and radicular dentin (4,5).

Thus the aim of the present study was to assess the influence of the double-layer application of a universal adhesive on the bonding efficacy to enamel, coronal and radicular dentin. The null hypothesis purposed in this in-vitro study were that (i) the double-layer application technique of the tested universal adhesive would not influence bond strength; (ii) there would be no difference between the tooth substrates' bond strength regardless of single and double application.

2. METHODS

2.1. Specimen Preparation

Seventy-two sound, caries-free human maxillary anterior teeth extracted for periodontal reasons were included in the study. The study under a protocol (GO 20/677) was confirmed by the Ethics Committee of the local university after obtaining the informed consent of the patients. Following the disinfection in 0.5% aqueous chloramine-T solution, the teeth were stored in distilled water and used within six months after extractions. To separate the roots, the crown of the teeth were sectioned along the cementoenamel junction using a slow-speed diamond saw. The teeth were then embedded in moulds with a self-curing acrylic resin and allocated into three groups according to tooth substrates; enamel, coronal and radicular dentin (n=24). The buccal surfaces of the crowns were wet ground to obtain enamel and coronal dentin specimens. To prepare radicular dentin specimens, the radicular part of the teeth was abraded from the buccal surface. Afterwards, enamel and dentin were verified under the light microscope. All specimens were prepared using 180 – and 600 grit SiC papers for 60 seconds at 300 rpm using the grinding machine (Presi Mecapol 220, Eybens, France) under running water to have a standardized smear layer.

2.2. Adhesive Procedure

Each substrate group was then randomly divided into two according to the adhesive's application layer (n=12).

Single application: The universal adhesive, Adhese Universal (Ivoclar Vivadent, Schaan, Lichtenstein) was applied with scrubbing to each tooth substrate for 20 seconds in self-etch mode according to the manufacturers' recommendations. Then the adhesive was dispersed with oil/moisture-free compressed air until a glossy film layer. The adhesive was light-cured for 10 seconds using an LED light-curing unit (Radii Plus, SDI, Victoria, Australia) with a light intensity of 1500 mW/cm².

Double application: The adhesive was scrubbed into the tooth surfaces for 20 seconds, as in the single application, then the second layer of adhesive was exerted for another 20 seconds and light-cured for 10 seconds with the same curing unit.

The flow chart of the study is shown in Figure 1.

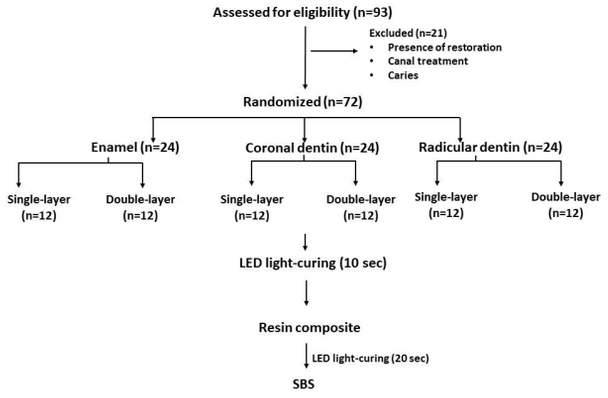

Figure 1: *Flow chart of the study*

2.3. Restorative Procedure

Following the adhesive procedure, a nano-hybrid resin composite, Tetric N-Ceram (shade A2) was placed on prepared tooth substrates through cylindrical-shaped plastic tubes (Tygon Medical Tubing Formulations 54-HL, Saint Gobain Performance Plastics, Akron, OH, USA) with a height of 2 mm and a diameter of 2 mm. Examined under the light microscope, the coronal dentin area was determined apical to the enamel-dentin junction, and the radicular dentin area was determined to be apical to the enamel-cementum junction (CEJ). After the resin composite (Tetric-N Ceram) was inserted into each tube, a clear Mylar matrix strip was laid over and gently pressed and then cured with a LED for 20 seconds as instructed by the manufacturer. Table 1 lists the materials, compositions and manufacturers used in the study.

Table 1. *Materials used in the study*

Product	Product name(lot #)	Manufacturer	Composition
Adhesive	Adhese Universal #V13743	Ivoclar Vivadent Schaan, Lichtenstein	10-MDP, bis-GMA, 2-HEMA, MCAP, D3MA, ethanol, water, initiator, stabilizer, silicon dioxide
Resin Composite	Tetric N-Ceram #T38568	Ivoclar-Vivadent, Schaan, Liechtenstein	Bis-GMA, UDMA, TEGDMA, Bis-EMA, 57% of filler: Barium glass, ytterbium trifluoride, mixed oxides and silica dioxide particles

Abbreviations: MDP, Methacryloyloxydecyl dihydrogen phosphate; HEMA, Hydroxyethyl methacrylate; Bis-GMA, Bisphenol A glycidyl methacrylate; D3MA, Decandiol dimethacrylate; MCAP,Methacrylated carboxylic acid polymer; TEGDMA, triethyleneglycol dimethacrylate.

2.4. Shear Bond Strength Analysis

The specimens were stored at 37°C for 24 hours in distilled water, then the tubes were removed with a bistoury. After examining each sample at 10X magnification, specimens with any porosities or voids were excluded from the study. The specimens were attached to the fixture then tested in Instron universal testing machine (Lloyd Instruments, Leicester, UK) with a crosshead speed of 1 mm/min. The SBS data in MPa was calculated from the peak load at failure divided by the bonded surface area. Following the testing, the fractured specimens were examined under a light microscope (Olympus SZX7, Hamburg, Germany) at 40X magnification to determine the location and the type of the bond failure. The classification was assessed according to the types of failure observed in tooth substrate / composite bonding areas: a) adhesive failure, b) cohesive failure, c) mixed failure.

2.5. Scanning Electron Microscopy (SEM) Evaluation

One extra sample from each group was prepared to observe the resin-tooth interface using SEM. Teeth substrates were exposed, and then adhesives and resin build-ups were performed according to the same procedure previously described for each group. Samples with resin bonded were placed in self-curing acrylic resin and then separated longitudinally with a diamond saw. The exposed cross-sectional interfaces were polished using high gloss abrasive discs (Kerrhawe Hiluster Plus, Kerr, CA, USA) and diamond pastes (Universal polishing paste, Ivoclar Vivadent, Schaan, Liechtenstein) were used respectively. The samples were treated with 10% phosphoric acid (10 sec) subsequently, subjected to 5% sodium hypochlorite (5 min) followed by rinsing with distilled water.

In addition, a fractured specimen from each group was processed directly for SEM observation. Each treated specimen for SEM was dehydrated in raising degrees of tert-butyl alcohol and later conveyed from the latest 100% bath to a critical point dryer. Afterwards, each SEM specimen was coated in a vacuum evaporator with a thin gold film layer. Resin/dental substrate interfaces and fracture fields were examined by field emission SEM (FIB-SEM, GAIA3, Tescan, Oxford XMax 150 EDS).

2.6. Statistical Analysis

The data analysis was performed in SPSS software (21.0, SPSS, Chicago, IL). All data sets were analyzed for normality by the Kolmogorov-Smirnov test. The mean bond strength values of the independent groups were statistically analyzed by repeated-measures analysis of variance, while the two-way ANOVA test was used in dependent groups. All tests were used at the 5% significance level.

3. RESULTS

Mean shear bond strength (SBS) values (MPa) and standard deviations are shown in Table 2. Regardless of dental substrates, no statistically significant difference was noticed in bond strengths between the single – and double-layer applications of the universal adhesive (p> 0.05).

No significant differences were found in bond strength between enamel, coronal dentin and radicular dentin in single-layer application (p> 0.05). For the double-layer application, the bond strength of coronal dentin was statistically higher than enamel's (p<0.05), whereas the bond strength of radicular dentin was not statistically different from neither enamel nor coronal dentin (p>0.05).

The failure modes that occured after the SBS test performed after different adhesive layer applications were examined under a light microscope, and are shown in Table 3. Examination of double and single application interfaces under a light microscope is shown in Fig 2.

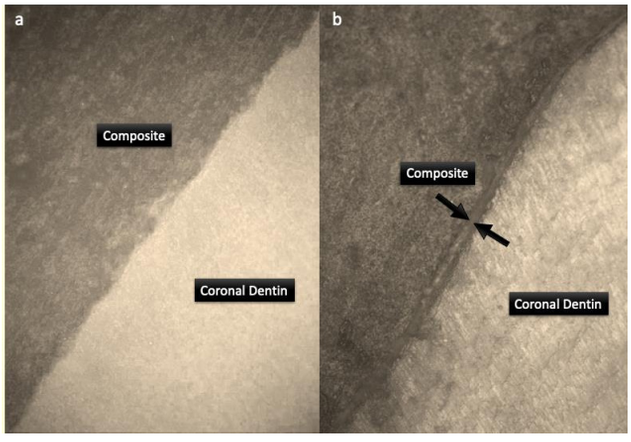

Figure 2. *Light microscopy images showing restoration/coronal dentin interfaces (a) Single adhesive layer. (b) Double adhesive layer.*

Representative SEM images of tooth substrate surfaces are shown in Fig. 3 (a-f). It is noteworthy that the fracture surfaces seen in the double application examples show a more homogeneous appearance than a single application. The complex distribution of enamel rods across the layer could not be viewed clearly in both double and single applications. Representative SEM images of the restorative-tooth substrate interface are shown in Fig 4 (a-f).

Tablo 2. *Means and Standard Deviations of the Shear Bond Strengths (MPa)*

Tooth Subsrates	Application Number	
	Single-layer Application	Double-layer Application
	Mean (MPa ± SD)	Mean (MPa ± SD)
Enamel	24.00 ± 3.54	20.58 ± 2.14*
Coronal Dentin	36.33 ± 7.15	39.33 ± 10.16*
Radicular Dentin	38.58 ± 8.38	35.00 ± 6.23

** Indicates significant differences in vertical columns; p<0.05*

Table 3. *Failure modes after SBS tests for all groups*

Aplication technique	Tooth substrates	Failure Types		
		Adhesive Failure (%)	Cohesive Failure (%)	Mixed Failure (%)
Single application	Enamel	83.3	0	16.7
	Coronal dentin	83.3	8.3	8.4
	Radicular dentin	75	0	25
Double application	Enamel	91.7	0	8.3
	Coronal dentin	75	16.7	8.3
	Radicular dentin	83.3	8.3	8.4

Percentage of specimens' fracture modes

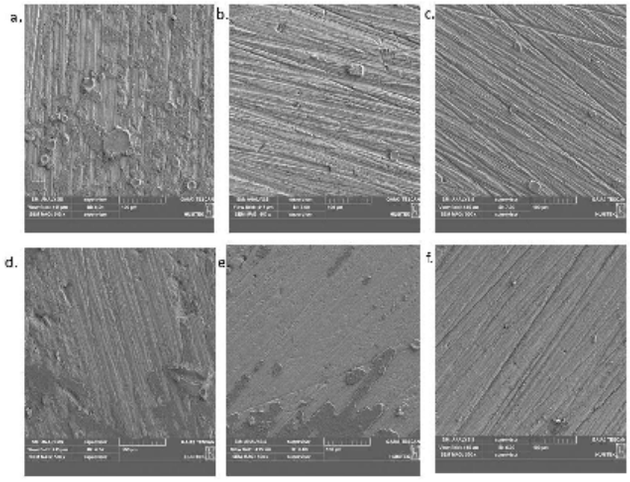

Figure 3. *(a) Single application on enamel (×500). (b) Single application on coronal dentin (×500). (c) Single application on radicular dentin (×500). (d) Double-layer application on enamel (×500). (e) Double-layer application on coronal dentin (×500). (f) Double-layer application on radicular dentin (×500).*

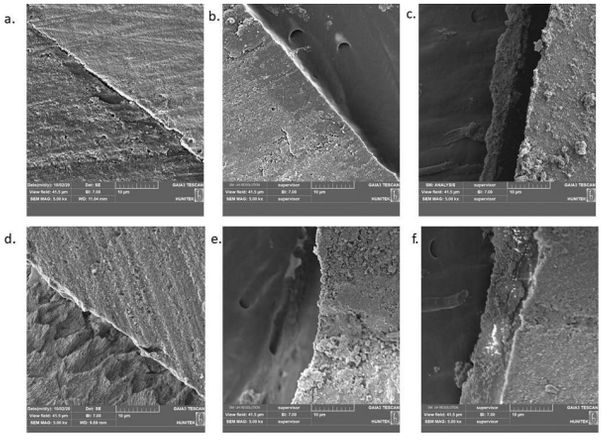

Figure 4. *(a) Single adhesive layer applied restoration/enamel interface (×5000). (b) Single adhesive layer applied restoration/ coronal dentin interface (×5000). (c) Single adhesive layer applied restoration/radicular dentin interface (×5000). (d) Double adhesive layer applied restoration/enamel interface (×5000). (e) Double adhesive layer applied restoration/coronal dentin interface (×5000). (f) Double adhesive layer applied restoration/ radicular dentin interface (×5000).*

4. DISCUSSION

Adhesive restorations are negatively affected by many physical and chemical deteriorations in the oral environment. Therefore, adhesive technology is an important factor for durable and long-lasting restorations. (7,13).

In the present study as no statistically difference was found between the single – and double-layer application modes of the tested universal adhesive, the null hypothesis which a double-layer application technique of the tested universal adhesive would not influence bond strength was accepted. However, our findings are not in line with the results of previous studies that found double application of universal adhesives enhance bond strength between resin composites and tooth structure (6,14,15). In an in-vitro study of Fujiwara et al. (6), it has been reported that double-application technique increased universal adhesives' bond strength to the hard structures of the tooth. This result may depend on the different compositions or layer thickness of the universal adhesives tested. In another study that examined the influence of multiple coating of adhesives on dentin bond strength, the bond strength increased until the fourth coating, yet no increase was observed when more than four coatings were applied (9). This result was found to be related to the optimum thickness of each adhesive layer however, it is not clear what this thickness was. Therefore, the possibility of the adhesive used in this study might have provided sufficient thickness of the adhesive layer in a single application, which could be considered as a reason why the extra layer did not improve the bonding (7,16-20). Moreover, the adhesive tested may have been recommended by its manufacturer for single-layer application due to achieved qualified thickness. On the other hand, some manufacturers recommend that unfilled adhesives should be applied to at least two coats (18,21). Adhese Universal used in the present study is a filled adhesive containing silicon dioxide filler particles. It was indicated that filled adhesives form a thicker adhesive layer after thinning with air (22). In a controlled in-vitro study evaluating the bonding properties of universal adhesives to dentin, these adhesives have been reported to exhibit different bonding performances even if they shared the same multi-mode application properties (23).

Regarding the enamel substrate, there are conflicting results in terms of application numbers of adhesives (14,18). While some studies reported a significant increase in enamel bond (6,14), some authors reported that the double application effect differs according to the adhesive system used (18,24). In a recent *in vitro* study, it was reported that double application increased bonding strength in enamel, unlike the current study, universal adhesives were applied with curing of the first applied layer (14). Our results are in line with previous studies that confirm double application effect on enamel bonding differs according to the specifications of the used adhesive system.

Although double application is expected to improve the quality of the adhesive layer (9), it has been reported to be less important for resin-enamel bond strength (18). Yaguchi et

al. reported that MDP-based self-etch adhesive applications created more calcium salts in dentin than enamel (25). Yoshihara et al. (26) reported that nanolayering on dentin was remarkably improved compared to nanolayering on enamel, especially with actively scrubbing. As a result, the technique of application mode could affect dentin more than enamel (26). Although no statistical difference was noticed between the single – and double-layer application, this finding may explain the tendency to increase dentin bond strength, while no increase was observed in enamel. However, the double-layer application tended to improve the bond strength only for coronal dentin. In most of the studies, double application of adhesives increased the dentin bond strength (6,18). The amplified adhesive layer might enhance the mechanical properties and as a result of this, the larger plastic zone can spread stress accumulation (10). Besides, the double-layer application may produce a more homogeneous adhesive layer and compensate for potential application deficits that occurred in the first layer (3,21,27). On the other hand, Erhardt et al. (28) reported that the potential improvement in bonding strength due to changes in the application techniques of adhesives depends on the adhesive system itself. Furthermore, MDP, which is included in many universal adhesives, is different purity and quality, which affects the performance and bonding strength of the adhesive (14,29).

The discrepancy of our enamel bond strength results with previous studies might be related to the pH of the used adhesive system. The universal adhesive systems evaluated in the former study had variable pH values around 2-2.5. The tested universal adhesive, Adhese Universal has a pH of approximately 2.5 – 3.0 which is classified as an ultra-mild universal adhesive (4). Within this pH range, adhesives in self-etch mode applied do not etch enamel as effectively as other universal adhesives (1). In a recent review, it has been declared that while prior acid etching is recommended for using universal adhesive on enamel, it is not required for dentin (4). The results of this study also support this finding. While it is said that sufficient bonding strength can be provided for dentin, prior acid-etching may be a better choice for enamel (4,5,30,31).

The discrepancy of the present dentin bond strength results with previous studies might be related to the actively scrubbing. In many studies, it has been reported that when self-etch adhesives are actively applied on dentin, they can accelerate solvent evaporation hence, a higher rate of monomer impregnation into the smear layer (3,32). The active application of the adhesive can lead to the transport of fresh acidic monomers into the deeper enamel and dentin, thereby enabling more aggressive demineralization and ultimately better diffusion of monomers that improve the quality of the hybrid layer (3,18,31). In this study the adhesive system was applied actively, therefore a quality adhesive layer could have been achieved just with the single-application and caused no improvement with the double application.

The double-layer application without light curing at the first layer may be associated with the prolonged application of adhesive systems. In an in-vitro study comparing the prolonged – and immediate – applications of universal adhesives on enamel, a significant increase in bond strength was reported when using Adhese Universal with prolonged self-etching mode (33). On the other hand, in the present study, there was no statistical increase in enamel. However, the prolonged application mentioned in the study was 20 seconds as in the manufacturer's instructions (corresponding to the single-layer in this study) for Adhese Universal. In this case, applying 20 seconds as the manufacturer's instructions should not be considered as a prolonged application.

Some studies have reported that the effect of double-layer or long-term application on bonding performance is specific to the adhesive system used, especially when the adhesive system is water/ethanol-based (28,34) However, the Adhese Universal system tested in the study is ethanol-based. It has been indicated that when water is added as a solvent to comonomer-ethanol mixtures, air drying or prolonged application time cannot provide better solvent evaporation since hydrogen bonding to the monomers will increase (28). It can be said that the bonding of water/ethanol-based universal adhesive, Adhese Universal, is not technically sensitive.

When considering the results of the study in terms of adhesive's application numbers, single application of the adhesive shown similar bonding performance for enamel, coronal and radicular dentin substrates, while double application mode of the adhesive showed statistically higher results for coronal dentin than enamel. Therefore, the null hypothesis which there would be no difference between the tooth substrates' (enamel, coronal and radicular dentin) bond strength regardless of the application technique (single/double application) was partially accepted. Dentin shear bond strength of the universal adhesives might be more susceptible to application technique than enamel (5,18). The adhesive layer of universal adhesives is a hydrophilic selectively permeable membrane and, as is known, dentin is formed by much more water and less hydroxyapatite than enamel. Therefore, the scrubbing action and double-application of the universal adhesive could have benefits such as infiltration of the functional monomer, evaporation of the solvent/water, providing a uniform adhesive layer in the dentin (1,8,31,35).

Differences such as closeness to the pulp tissue, mineral density, mineral content and direction of the dentinal tubules may cause the dentin substrate to differ in terms of regional mechanic properties (36-37). For this reason, both radicular and coronal dentin were examined in this study. However, no statistical difference was detected between these two substrates' bond strength.

According to the current literature, there is no consensus on whether double application produces higher bond strength values on adhesive systems or not (1,3,6,7,18). Some methodological differences, such as whether the

adhesive is applied passively or actively; application time or manufacturer recommendations, or application with light-curing at the first layer should be considered (1,3,33).

Additionally, as a limitation of this study, the present study was performed under *in vitro* conditions and only one commercial universal adhesive was tested. More studies are needed to elaborate on this subject using different adhesive systems. Moreover, the teeth used in this study are relatively older teeth with periodontal extraction indications. Different results could be obtained in young teeth. Further clinical studies should also be performed to confirm these results.

5. CONCLUSION

Within the limitations of the present *in vitro* study, for enamel, coronal and radicular dentin substrates, the double application of the universal adhesive in the self-etch mode was not effective in enhancing the shear bond strength of the tested universal adhesive. Coronal dentin bond strength of the universal adhesives might be more susceptible to application technique than enamel. Despite the longer application time, the double-layer application did not have a noticeable impact on bond strength.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all participants included in the study who had an indication for extraction and had teeth suitable for this study.

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Clinical and Experimental Health Sciences

Pharmacopoeia Researches and Antimicrobial Activity Studies on *Matricaria chamomilla* L.

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ABSTRACT

Objective: The aim of this study is to compare *Matricaria chamomilla* samples collected from nature and supplied from pharmacies and herbalists by performing quality control analyses and investigate the antimicrobial activities of them.

Methods: These samples were examined according to Matricariae flos monograph analysis in European Pharmacopoeia. Macroscopic and microscopic examination, loss on drying and total ash experiment were performed. Essential oil was obtained from the drugs and analyzed by thin layer chromatography. Additionally, aqueous and ethanolic extracts of all samples were compared by thin layer chromatography. Antimicrobial activity was determined by microdilution method in aqueous and ethanolic extracts.

Results: According to the pharmacopoeia analysis; in macroscopic and microscopic examinations, all samples except 2 herbalist samples were found to suitable for the pharmacopoeia standards. In loss on drying, none of the samples were found to suitable for the standards except 2 herbalist samples. In total ash, all samples were suitable for the standards. While the essential oil of *M. chamomilla* samples collected from nature was yellow-orange colored, we obtained the blue essential oil containing chamazulene from pharmacy samples and 3 of herbalist samples. The highest activity in antimicrobial activity assay was observed in ethanol extracts against S*taphylococcus aureus, Staphylococcus epidermidis and Candida tropicalis* strains.

Conclusion: Examples of true *M. chamomilla* were found among herbalist samples, contrary to the previous studies. Pharmacy samples were qualified but limited in terms of variety. Essential oil analyses pointed out the importance of geographical location among natural samples. Antimicrobial activity tests did not provide significant results.

Keywords: *Matricaria chamomilla*, Chamomile, Pharmacopoeia analysis, Antimicrobial activity

1. INTRODUCTION

Matricaria chamomilla L. is a member of the Asteraceae family and known by names such as “Mayıs papatyası, Tıbbi papatya, Adi papatya, Babunç, Akbubaç, Papatya and Papaçya” in Turkey (1,2). The name of the plant is a combination of the words "chamos" meaning place in Greek and "malos" meaning apple, as it grows on the ground and the scent of its fresh flowers reminds of the smell of apples (3). *M. chamomilla* grows naturally in Southern and Eastern Europe, Western Asia, North America and Australia. Chamomile is abundant in roadsides and in empty fields in Turkey (1,4).

Matricariae flos are shade-dried capitulums of *M. chamomilla*. According to studies on the chemical composition of *M. chamomilla*, the plant contains mainly flavonoids (apigenin, luteolin, chrysoeriol, apigenin-7-*O*-glucoside, apigenin glucoside acetate, luteolin-7-*O*-glucoside, rutin, hyperoside, isorhamnetin, patuletin, jaceidinem), phenolic compounds, essential oil (chamazulene, (-)-α-bisabolol, bisabolol oxide A, bisabolol oxide B, bisabolone oxide A, (*E*)-β-farnesene, (*E*) – and (*Z*)-spiroethers, spathulenol), hydroxy coumarins (umbelliferone, herniarin) and sesquiterpene lactones (matricin, matricarin and desacetylmetricarin). Plant also contains mucilage, polysaccharides, amino acids, fatty acids, triterpene hydrocarbons, tannin and choline (3-6). Chamazulene, which is formed by the hydrolysis of matricin during distillation, gives the essential oil a blue color (5,6). It has been observed that some Anatolian-origin chamomile don’t contain chamazulene in their essential oils. The essential oils of Turkey-origin drugs are rich in bisabolon oxide and bisabolol oxide (1).

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The plant is commonly used internally as a diuretic, an appetite-stimulant, a carminative and a cholagogue in public. Externally, it is used for the treatment of throat inflammation, hemorrhoids, abscess and acne (1). *M. chamomilla* has anti-inflammatory, antispasmodic, antimicrobial, antiviral and mild sedative effects. In the German Commission E monographs, it is approved for use in fevers and colds, cough, bronchitis, tendency to infection, inflammation of the skin, inflammation of the mouth and pharynx, wounds and burns (5,6).

There are many biological activity studies on *M. chamomilla*. Among these studies, antimicrobial, antioxidant and anti-inflammatory activity studies are the most common (7-10). In addition, studies on wound healing, anxiolytic, antispasmodic, anticancer, analgesic, antiallergic, antihyperglycemic and antiparasitic effects are also included in the literature. Further, the effects on stomach, mucosa and memory are studied (11-19). Recent antimicrobial activity studies on *M. chamomilla* mainly performed with its' extracts which obtained by using various solvents and extraction techniques or essential oil of the plant. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus* and *Salmonella typhi* bacterial strains and *Candida albicans, Aspergillus flavus* and *Aspergillus niger* fungal strains were the most common strains in these studies (7,8,20-31).

Today, there are many plants are considered as chamomile by mistake, because of their similarity to chamomile. These plants include *Anthemis, Bellis, Tanacetum, Tripleurospermum* and *Chrysanthemum* species (32). Accidental use of some of these species, instead of *Matricaria*, may cause serious problems for human health.

Medicinal plants are generally obtained from herbalists, markets, online stores and chain stores, rarely collected from nature and supplied from pharmacies. Usually, complete form of the plants can be collected from nature or supplied from pharmacies and herbalists. To understand the differences among these three resources, in this study, quality control analyses were performed on chamomile samples obtained from 5 herbalists in Istanbul, 2 Matricariae flos obtained from pharmacies and *M. chamomilla* capitulum samples collected from 3 different regions in Istanbul, based on the monograph of Matricariae flos in the European Pharmacopoeia (EP) (33). Since it is a widely used drug in phytotherapy, the investigation of the antimicrobial activities of ethanolic and aqueous extracts of all samples was also included in this study.

2. METHODS

2.1. Materials

Diethyl ether, ethanol, chloroform, methanol, n-hexane, toluene, sulfuric acid were obtained from Merck (Darmstadt, Germany). Benzene and ethyl acetate were from Riedel-de-Haën (Seelze, Germany). Vanillin, acetaldehyde and Roswell Park Memorial Institute (RPMI) medium were from Sigma-Aldrich (Steinheim, Germany). Mueller-Hinton broth were obtained from Difco (Detroid, Michigan, USA).

2.2. Plant Materials

Samples sold as "medicinal chamomile" were purchased from 5 different herbalists randomly selected in Istanbul. *M. chamomilla* drugs were obtained from 2 different companies whose products are only sold in pharmacies. Also, *M. chamomilla* samples were collected from Çatalca and 2 different regions in Çekmeköy in Istanbul (Turkey) in May 2018. The collected plant samples were determined by Emine Akalın Uruşak (Istanbul University Faculty of Pharmacy Department of Pharmaceutical Botany). Voucher specimens were deposited in the Herbarium of the Istanbul University Faculty of Pharmacy (ISTE: 115521, 115522, 115654). The flowering parts of the collected plants were dried in the shade. The drug samples used in this study were coded as MD1, MD2, MD3 for the samples collected from nature, ME1 and ME2 for pharmacy samples and MA1, MA2, MA3, MA4 and MA5 for herbalist samples.

2.3. Macroscopic Analysis

Morphological characteristics of flower specimens were examined using a Leica S8AP0 stereomicroscope (Leica, Wetzlar, Germany). Capitulum, involucre, receptacle, ray and disc florets parts were analyzed according to pharmacopoeia standards. The capitulum sizes of the samples were measured with a ruler.

2.4. Microscopic Analysis

The preparations prepared using chloral hydrate reagent from powdered drug samples were determined under microscope (Olympus CX21FS1, Olympus Corporation, Tokyo, Japan) using 10x and 40x lenses. Characteristic elements for Matricariae flos such as pollen grains, stigma, glandular trichomes were examined (33,34).

2.5. Loss on Drying

Glass weighing bottles were kept in the drying oven and then cooled in a desiccator to constant weight. 1 g of each sample was weighed in tared bottles and dried in the drying oven at 105 °C for 2 hours. Then bottles were cooled in a desiccator, reweighed and the percentage of weight loss was calculated.

2.6. Total Ash

The crucibles were heated for 30 minutes and then cooled in a desiccator to constant weight. 1 g drug from each sample was weighed in the tared crucibles. After drying at 105 °C for one hour, it was burned in an ashing furnace (Protherm PC442T, Protherm Furnaces, Ankara, Turkey) at 600 °C. Then the crucibles were cooled in a desiccator, weighed and the percentage of total ash was calculated.

2.7. Isolation of Essential Oil

Essential oil was isolated using clevenger apparatus according to the distillation method in the EP. 50 grams of chamomile drug was put into a 1000 mL balloon. 500 mL of distilled water was added on it. After 3 hours of distillation, the volume of

essential oil was measured. The isolated essential oil was stored at +4 °C until it was used.

2.8. Preparation of the Extracts

5% ethanolic and aqueous extracts of all *M. chamomilla* samples were prepared by 3 different methods. For the 1st method (P); 20 g drug was extracted using ethanol (400 mL) in percolator for 24 hours. In the 2nd one (E5); 5 g drug was macerated using ethanol (100 mL) for 24 hours and shaked in a magnetic stirrer (Dragon Lab MS-H-S, DLAB, Beijing, China) for 4 hours. After extraction, both samples were filtered. Then the ethanol was evaporated under reduced pressure at 45 °C using rotary evaporator (Buchi R-210, Flawil, Switzerland). For the 3rd method (S); 5 g drug was macerated using distilled water (100 mL) for 24 hours and shaked in a magnetic stirrer for 4 hours. The extract was filtered and lyophilized (Labconco Free Zone 4.5, Labconco, Kansas City, MO, USA). The crude extracts were used in TLC analysis and antimicrobial activity assays.

2.9. Thin Layer Chromatography (TLC) of Extracts and Essential Oils

TLC analyses of essential oils and extracts were performed according to the "Matricariae flos" monograph in the EP. In addition, 2 different mobile phase systems which used for the TLC analysis of *M. chamomilla* in the literature were also included in this study (35). Essential oils were dissolved in hexane. 20 mg of each extracts were dissolved in methanol (2 mL). Silica gel TLC plate (Silicagel 60F254, Merck, Darmstadt, Germany) was used as the stationary phase. Ethyl acetate-toluene (5:95), chloroform-toluene (75:25) and chloroform-toluene-ethyl acetate (65:30:5) solvent mixtures were used as mobile phase. Anisaldehyde-sulfuric acid reagent was used for ethyl acetate-toluene (5:95) mobile phase system, and vanillin-sulfuric acid reagent was used for chloroform-toluene (75:25) and chloroform-toluene-ethyl acetate (65:30:5) mobile phase systems. After the reagents were sprayed, the plates were heated at 105 °C for 10 minutes. They were examined under daylight and UV light (254-366 nm).

2.10. Antimicrobial Activity

The antimicrobial activities of all of the aqueous and ethanol extracts of Matricariae flos samples were examined by the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul University. The microorganisms used in this study were; *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Candida tropicalis* ATCC 750, *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 1023. Minimum Inhibitory Concentration (MIC) values were determined by microdilution method according to the Clinical Laboratory Standards Institute (CLSI) guidelines (36,37).

A $5x10^5$ colony forming unit (cfu)/mL bacterial inoculum was sampled from 4-6 hours culture in Mueller-Hinton broth medium. A $2.5x10^3$ cfu/mL inoculum of *Candida* species was sampled from 24 hours culture in RPMI-1640 medium which buffered with 3-(N-morpholino) propanesulfonic acid (MOPS).

The microplates were incubated for 18-24 hours at 35 °C for bacteria and for 46-50 hours at 35 °C for *Candida* species. The lowest concentration of substance that prevents visible growth was determined as the MIC value. Cefuroxime, Ceftazidime, Amikacin, Clotrimazole and Amphotericin B were used as reference antimicrobial agents.

3. RESULTS

3.1. Macroscobic Analysis

Disc florets of all plants were yellow, ray florets were white. Expect MA4 and MA5, all of the involucral bracts had brownish-grey scarious margins. For MA4 and MA5 bracts had white and none-scarious margins. Expect MA4 and MA5, all of the receptacles were elongated-conical or hemispherical (for young capitula). MA4 and MA5 had generally flat or slightly curved receptacles. The receptacle of 2 herbalist samples (MA4 and MA5) was hollow, with paleae. Capitulum size, number of ray and disc florets, other specifications of involucre and receptacle are demonstrated in the Table 1.

Table 1. *Morphological properties of samples.*

Samples	Capitulum sizes	Incolucral bracts	Involucral bracts	Receptacle	Ray florets	Disc florets
MD1	0,4-0,5 cm	Ovate	1-2 rows	Without paleae, hollow	15-18	Several dozen
MD2	0,4-0,6 cm	Ovate-lanceolate	2-3 rows	Without paleae, hollow	12-15	Several dozen
MD3	0,4-,0,5 cm	Ovate-lanceolate	2-3 rows	Without paleae, hollow	12-15	Several dozen
ME1	0,4-0,6 cm	Ovate	2-3 rows	Without paleae, hollow	18-21	Several dozen
ME2	0,4-0,75 cm	Ovate-lanceolate	1-2 rows	Without paleae, hollow	18-21	Several dozen
MA1	0,5-0,7 cm	Ovate	1-2 rows	Without paleae, hollow	18-20	Several dozen
MA2	0,5-0,8 cm	Ovate-lanceolate	1-2 rows	Without paleae, hollow	16-18	Several dozen
MA3	0,45-0,7 cm	Ovate-lanceolate	1-2 rows	Without paleae, hollow	Were not intact	Several dozen
MA4	0,7-1,2 cm	Ovate	1-2 rows	With paleae, full	14-17	Several dozen
MA5	0,7-0,9 cm	Ovate	1-2 rows	With paleae, full	16-18	Several dozen

MD1, MD2, MD3: the samples collected from nature; ME1, ME2: pharmacy samples; MA1, MA2, MA3, MA4, MA5: herbalist samples.

3.2. Microscobic Analysis

Druse crystals in the inner tissues of the ovaries and the anther lobes, glandular trichomes, stigma, pollen grains with 3 pores, inner and outer epidermis of the corolla of the ray florets and corolla of the disc florets were seen in all samples. Covering trichomes and papillae were detected in MA4 and MA5 samples on the contrary of the other samples (Figure 1).

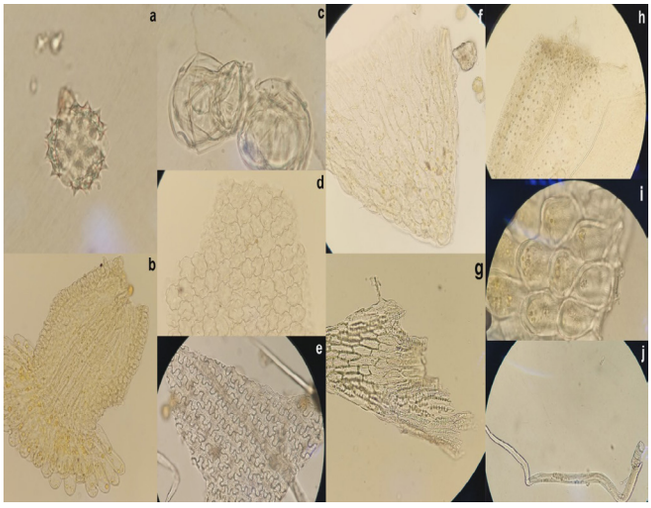

Figure 1. *Microscope images of the samples (a) pollen in MA5; (b) stigma in ME1; (c) glandular trichomes in MA3; (d) inner epidermis of the corolla of the ray florets in ME2; (e) outer epidermis of the corolla of the ray florets MA4; (f) corolla of the disc florets MD3; (g) anther in MD2; (h) druse in ME2; (i) papillae in MA4; (j) covering trichome in MA4.*

3.3. Loss on Drying and Total Ash

In the "Matricariae flos" monograph, weight loss after drying is specified as max 12% and the total amount of ash is specified as max 13%. It was determined that the total ash amount of all samples remained below standard value. However, it was observed that the moisture content of all samples except for MA4 and MA5 were above 12% (Table 2).

Table 2. *Results of analyses of loss on drying and total ash.*

Samples	Loss on Drying (%)	Total Ash (%)
MD1	13,0112	8,7123
MD2	15,5977	9,0199
MD3	14,4521	10,642
ME1	14,2476	10,1841
ME2	15,3156	9,6448
MA1	14,4579	9,5455
MA2	14,9551	9,1168
MA3	14,2757	12,0265
MA4	11,4349	10,9044
MA5	11,0601	12,1064

MD1, MD2, MD3: the samples collected from nature; ME1, ME2: pharmacy samples; MA1, MA2, MA3, MA4, MA5: herbalist samples.

3.4. Essential Oil Isolation

Essential oil was obtained from the samples which identified as *M. chamomilla* after our analyses. While the distillation of pharmacy and herbalist samples of *M. chamomilla* resulted with blue essential oil, the distillation of samples collected from nature of *M. chamomilla* resulted with yellow-orange essential oil. According to the pharmacopoeia, a dry drug should contain at least 4 mL/kg of blue essential oil. The volume of essential oil was determined as 0.6-0.8% mL in the samples collected from nature, 0.4-0.5% mL in pharmacy samples and 0.4-0.6% mL in herbalist samples.

3.5. Thin Layer Chromatography (TLC)

As a result of the TLC analysis of essential oils according to the pharmacopoeia method, it was observed that the essential oils of samples collected from nature did not contain chamazulene. Bornyl acetate and (-)-α-bisabolol zones specified in the pharmacopoeia were detected in all essential oil samples (Figure 2).

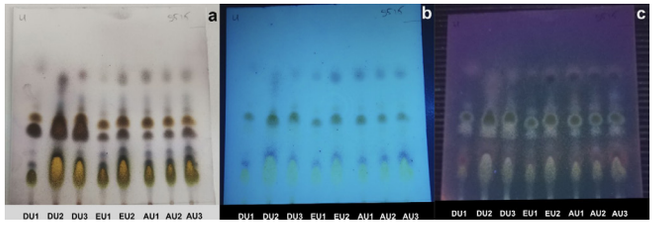

Figure 2. *TLC chromatogram-1 visualized (a) under white light; (b) at 254 nm; (c) at 366 nm. Mobile phase: ethyl acetate-toluene (5:95); derivatization: Anisaldehyde-sulfuric acid reagent. DU1, DU2, DU3: essential oils of the samples collected from nature; EU1, EU2: essential oils of pharmacy samples; AU1, AU2, AU3: essential oils of herbalist samples.*

As a result of TLC analysis using chloroform-toluene (75:25) and chloroform-toluene-ethyl acetate (65:30:5) mobile phase systems, the retention factor (Rf) values of cis-en-in-dicycloether and chamazulene were accordant with the literature (35). Rf values are given in the Table 3. Chamazulene zone was not detected in the chromatograms of samples collected from nature. Selected TLC chromatograms are presented in Figures 3-7.

Table 3. *Rf values of essential oil samples.*

	Chloroform-toluene (75:25) mobile phase system		Chloroform-toluene-ethyl acetate (65:30:5) mobile phase system	
	Rf1	**Rf2**	**Rf1**	**Rf2**
DU1	-	0,58125	-	0,6125
DU2	-	0,5875	-	0,6
DU3	-	0,6	-	0,60625
EU1	0,74375	0,59375	0,725	0,5875
EU2	0,74375	0,6	0,7375	0,6
AU1	0,75	0,6	0,7375	0,60625
AU2	0,74375	0,6	0,7375	0,6
AU3	0,75	0,6	0,7375	0,60625

DU1, DU2, DU3: essential oils of the samples collected from nature; EU1, EU2: essential oils of pharmacy samples; AU1, AU2, AU3: essential oils of herbalist samples.

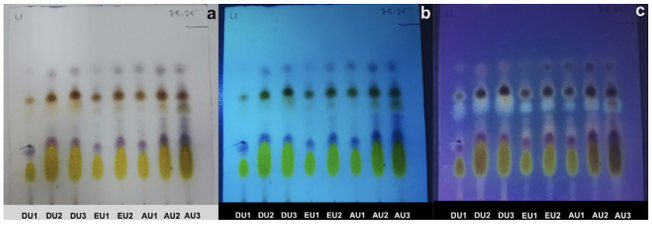

Figure 3. *TLC chromatogram-2 visualized (a) under white light; (b) at 254 nm; (c) at 366 nm. Mobile phase: chloroform-toluene (75:25); derivatization: Vanillin-sulfuric acid reagent. DU1, DU2, DU3: essential oils of the samples collected from nature; EU1, EU2: essential oils of pharmacy samples; AU1, AU2, AU3: essential oils of herbalist samples.*

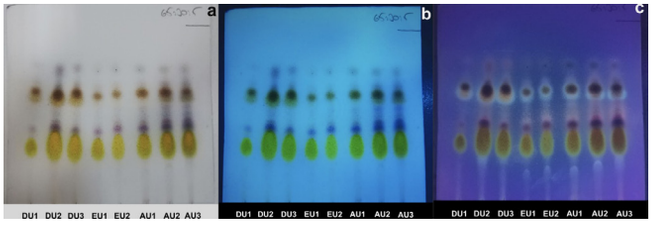

Figure 4. *TLC chromatogram-3 visualized (a) under white light; (b) at 254 nm; (c) at 366 nm. Mobile phase: chloroform-toluene-ethyl acetate (65:30:5); derivatization: Vanillin-sulfuric acid reagent. DU1, DU2, DU3: essential oils of the samples collected from nature; EU1, EU2: essential oils of pharmacy samples; AU1, AU2, AU3: essential oils of herbalist samples.*

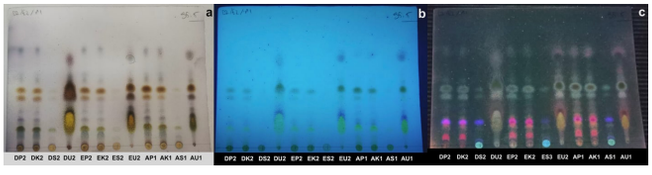

Figure 5. *TLC chromatogram-4 visualized (a) under white light; (b) at 254 nm; (c) at 366 nm. Mobile phase: ethyl acetate-toluene (5:95); derivatization: Anisaldehyde-sulfuric acid reagent.*

From MD2 sample (collected from nature): (DP2) Ethanol extract prepared by the 1st extraction method; (DK2) Ethanol extract prepared by the 2nd extraction method; (DS2) aqueous extract; (DU2) essential oil. From ME2 pharmacy sample: (EP2) Ethanol extract prepared by the 1st extraction method; (EK2) Ethanol extract prepared by the 2nd extraction method; (ES2) aqueous extract; (EU2) essential oil. From MA1 herbalist sample: (AP1) Ethanol extract prepared by the 1st extraction method; (AK1) Ethanol extract prepared by the 2nd extraction method; (AS1) aqueous extract; (AU1) essential oil.

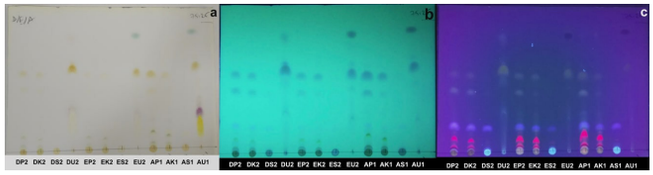

Figure 6. *TLC chromatogram-5 visualized (a) under white light; (b) at 254 nm; (c) at 366 nm. Mobile phase: chloroform-toluene (75:25); derivatization: Vanillin-sulfuric acid reagent.*

From MD2 sample (collected from nature): (DP2) Ethanol extract prepared by the 1st extraction method; (DK2) Ethanol extract prepared by the 2nd extraction method; (DS2) aqueous extract; (DU2) essential oil. From ME2 pharmacy sample: (EP2) Ethanol extract prepared by the 1st extraction method; (EK2) Ethanol extract prepared by the 2nd extraction method; (ES2) aqueous extract; (EU2) essential oil. From MA1 herbalist sample: (AP1) Ethanol extract prepared by the 1st extraction method; (AK1) Ethanol extract prepared by the 2nd extraction method; (AS1) aqueous extract; (AU1) essential oil.

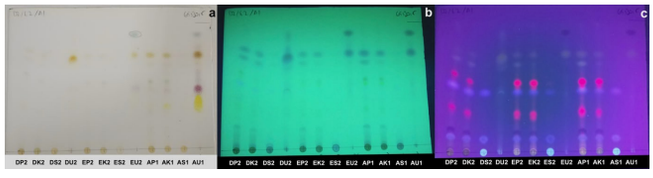

Figure 7. *TLC chromatogram-6 visualized (a) under white light; (b) at 254 nm; (c) at 366 nm. Mobile phase: chloroform-toluene-ethyl acetate (65:30:5); derivatization: Vanillin-sulfuric acid reagent.*

From MD2 sample (collected from nature): (DP2) Ethanol extract prepared by the 1st extraction method; (DK2) Ethanol extract prepared by the 2nd extraction method; (DS2) aqueous extract; (DU2) essential oil. From ME2 pharmacy sample: (EP2) Ethanol extract prepared by the 1st extraction method; (EK2) Ethanol extract prepared by the 2nd extraction method; (ES2) aqueous extract; (EU2) essential oil. From MA1 herbalist sample: (AP1) Ethanol extract prepared by the 1st extraction method; (AK1) Ethanol extract prepared by the 2nd extraction method; (AS1) aqueous extract; (AU1) essential oil.

3.6. Antimicrobial Activity

According to the results of the antimicrobial activity tests, all extracts showed antibacterial activity against *S. aureus* strain and the highest activity was detected in $MA1_{P}$ extract with a MIC value of 312.5 µg/mL. The highest activity against *S. epidermidis* strain was detected in $MD2_{ES}$, $ME1_{P}$ and $ME1_{ES}$ extracts with a MIC value of 625 µg/mL. Against *E. faecalis* strain, it was detected in $ME1_{ES}$ and $MA3_{ES}$ extracts with a MIC value of 625 µg/mL. Only the aqueous extracts of the MD1 and MD3 samples showed antibacterial activity against *E. coli* strain with a MIC value of 1250 µg/mL. In our study, it was determined that none of the extracts showed antibacterial activity against *P. aeruginosa*, *K. pneumoniae* and *P. mirabilis* strains. Antifungal activity against fungal strains was observed only in extracts prepared from herbalist samples. As a result of the antimicrobial activity assay, it was found that the highest antimicrobial activity was in the ethanol extracts prepared with a magnetic stirrer after maceration with ethanol (Table 4).

Table 4. *Antimicrobial activity results of samples.*

	S. aureus ATCC 29213	*S. epidermidis ATCC 12228*	*E. coli ATCC 25922*	*E. faecalis ATCC 29212*	*C. albicans ATCC 10231*	*C. parapsilosis ATCC 22019*	*C. tropicalis ATCC 750*
$MD1_P$	1250	1250	-	-	-	-	-
$MD1_{ES}$	625	1250	-	-	-	-	-
$MD1_S$	1250	-	1250	-	-	-	-
$MD2_P$	1250	1250	-	-	-	-	-
$MD2_{ES}$	1250	625	-	-	-	-	-
$MD2_S$	1250	-	-	-	-	-	-
$MD3_P$	1250	1250	-	1250	-	-	-
$MD3_{ES}$	625	1250	-	1250	-	-	-
$MD3_S$	1250	-	1250	-	-	-	-
$ME1_P$	1250	625	-	1250	-	-	-
$ME1_{ES}$	1250	625	-	625	-	-	-
$ME1_S$	1250	-	-	-	-	-	-
$ME2_P$	1250	1250	-	1250	-	-	-
$ME2_{ES}$	1250	1250	-	1250	-	-	-
$ME2_S$	1250	-	-	-	-	-	-
$MA1_P$	312,5	1250	-	-	-	-	-
$MA1_{ES}$	625	1250	-	-	-	312,5	312,5
$MA1_S$	1250	-	-	-	-	-	312,5
$MA2_P$	1250	1250	-	-	-	-	-
$MA2_{ES}$	1250	1250	-	-	-	-	312,5
$MA2_S$	1250	-	-	-	-	-	-
$MA3_P$	1250	1250	-	1250	-	-	-
$MA3_{ES}$	1250	1250	-	625	-	-	312,5
$MA3_S$	1250	-	-	-	625	-	-
Cefuroxime	1,2	9,8	4,9	-	-	-	-
Amikacin	-	-	-	128	-	-	-
Clotrimazole	-	-	-	-	4,9	-	-
Amphotericin B	-	-	-	-	-	0,5	1

MD1, MD2, MD3: the samples collected from nature; ME1, ME2: pharmacy samples; MA1, MA2, MA3, MA4, MA5: herbalist samples. P: ethanolic extract prepared with the 1st extraction method, E5: ethanolic extract prepared with the 2nd extraction method, S: aqueous extract
(Since antimicrobial activity was not detected against P. aeruginosa, K. pneumoniae and P. mirabilis strains, these strains were not included in the table.)

4. DISCUSSION

In this study, quality control analyses were performed on a total of 10 samples, including 3 *M. chamomilla* samples collected from nature, 2 Matriciariae flos drugs obtained from the pharmacy and 5 chamomile samples taken from the herbalist, according to the EP.

As a result of macroscopic and microscopic examinations, all of the samples collected from nature and supplied from pharmacies were determined as *Matricaria chamomilla*. Among the 5 herbal samples, 3 were determined as *M. chamomilla*, but 2 samples did not meet the pharmacopoeia standards. As a result of the macroscopic and microscopic examinations, the samples were thought to be *Anthemis*, *Chamaemelum, Tanacetum or Bellis*, but could not be identified because they had only flowering parts. Thus, these two samples (MA4 and MA5) were not included in the essential oil, TLC and antimicrobial activity analyses. On the other hand, the loss of drying value of all samples except two herbalist samples (MA4 and MA5) was found to be higher than pharmacopoeia standards. The total ash amount of all samples conformed to the standards.

M. chamomillla is considered as an aromatic plant because its' flowers contain essential oil. It was observed that the volume of essential oil obtained from the samples collected from nature was higher than the volume of the essential oil of pharmacy and herbalist samples. The volume of essential oil of freshly collected samples was higher than that of the other samples. It is confirmed that the time of collection of medicinal and aromatic plants containing essential oil is important for essential oil yield. Yellow-orange essential oil, which was isolated from the samples collected from nature by distillation, indicates that these samples do not contain chamazulene. The fact that no blue essential oil was detected in the analyzed samples collected from nature is compatible with the literature (1,38). Blue essential oil containing chamazulene was obtained from pharmacy and herbalist samples. TLC analyses also confirmed these findings.

Our study is the first study in which *M. chamomilla* samples collected from nature, obtained from pharmacy and herbalist were analyzed together and compared with each other according to the EP. Three of 5 different herbalist samples were identified as *M. chamomilla*. Identification of these 3 samples, which also contain blue essential oil, is a positive result. However, our study has limited number of herbalist samples from a certain region, thus, it is hard to claim that most of the herbalists in Turkey provide true *M. chamomilla* plants.

During the time of this study, there were only 2 medicinal drugs for *M. chamomilla* which sold in pharmacies, thus, to create a comparable group of samples, we preferred to limit the number of herbalist samples to 5.

In our study, the antimicrobial activity test were also performed on all samples determined as *M. chamomilla*. The highest activity was observed in ethanolic extracts against *S. aureus*, *S. epidermidis* and *C. tropicalis* strains. These results are consistent with the literature data (7,20,28). However, the activity levels in our study were not as high as the activity levels in the similar studies on *M. chamomilla*. The difference in collection time and geographical location or extraction methods may have caused this inconsistency.

5.CONCLUSION

This study examined ten different *M. chamomilla* samples from three different resources to compare their qualifications based on EP. Contrary to the literature and conventinal knowledge, this study showed that *M. chamomilla* also can be found in some of the herbal stores and can meet the EP standardards. Pharmacy samples also meet the EP standards in our study, however there were only two of them. This limited availability and variety might divert people from pharmacies to other resources. In terms of natural resources, this study demonstrated once again geographical location is important for the medicinal plants' chemical composition such as presence of blue essential oil. Lastly, antimicrobial activity tests did not provide significant results in our study.

To achieve more significant and generalisable results, studies with more pharmacy samples can be conducted, natural samples can be collected from wider regions, number of herbalist samples can be increased. Also, different extraction methods could be useful for further studies.

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Clinical and Experimental Health Sciences

Premolar Agenesis Prevalence and Patterns in a Sample of Turkish Children

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ABSTRACT

Objective: The aim of this study was to evaluate the characteristics of premolar tooth agenesis patterns in Turkish children and confirm a valid cutoff age.

Methods: Included in this study were panoramic radiographs of 3637 Turkish pediatric patients aged 7–12 years (1819 males and 1818 females), whose panoramic radiographs were taken. The prevalence of premolar hypodontia, sex distribution, distribution of premolar hypodontia in the maxilla and mandible, unilateral/bilateral incidences and cutoff ages were examined in radiographs.

Results: The prevalence rate of agenesis of one or more premolars was 3.1% (n = 111) for both sexes combined, and 1.4% for boys and 1.6% for girls, with no significant difference between the sexes (p = 0.498). No significant difference was observed between younger and older groups at the cutoff ages examined.

Conclusion: An early diagnosis of premolar agenesis enables ideal and conservative treatment planning, which may involve less invasive treatment options for patients diagnosed in the early period. For these reasons, the awareness of dentists regarding these treatments should be enhanced. Therefore, there is a need for more studies regarding the prevalence of this condition, which will provide important clinical value in the timely diagnosis of hypodontia.

Keywords: Hypodontia, premolar, prevalence, children

1. INTRODUCTION

Tooth agenesis is the most common dental anomaly in permanent dentition that occurs due to genetic or environmental factors (1, 2). It is categorized according to the number of missing teeth: hypodontia if < 6 teeth are missing, oligodontia if ≥ 6 teeth are missing, and anodontia is the agenesis of all teeth (3, 4). The prevalence of congenitally missing teeth in the permanent dentition, excluding the third molars, varies from 0.15% to 16.2% (5). In previous studies, the lateral incisors and premolar agenesis were the most common congenitally missing teeth. Over the last few decades, research suggests the prevalence of tooth deficiencies has increased (6).

Previous studies have reported that a deep bite and diastema are seen in individuals with hypodontia. Individuals with premolar or molar tooth agenesis tend to have a deeper bite, but this condition leads to non-working interferences, poor gingival contours, and an over eruption of the opposing teeth. In addition, patients with premolar hypodontia have more difficulty in chewing due to having a smaller occlusal table (6, 7).

Tooth agenesis (except the third molar) involves a multidisciplinary approach (orthodontics, prosthodontics, pediatric dentistry, and surgery), which can be a costly and complex process for health insurance providers, patients and families. If a tooth deficiency is diagnosed early, treatment costs and its psychosocial effects can be reduced (8, 9).

Calcification of premolars starts between the ages of 2 and 2.5 years, and calcification of enamel is usually completed at 6–7 years of age (10). Sometimes calcification of premolars is delayed. For instance, the second premolars can develop long after what would ordinarily be expected. There is no consensus on the age criteria for detecting dental agenesis. Some authors have reported that tooth agenesis cannot be detected before the age of 9 or 10 years (11–13). Nevertheless, other authors have refused this idea, arguing that any time after 7 years of age is sufficient to detect a tooth deficiency (14). The minimum cutoff age in previous epidemiologic studies has varied.

Results of studies conducted over last few decades have reported in increased prevalence of hypodontia (15). The

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aim of this study was to evaluate the characteristics of premolar tooth agenesis patterns in Turkish children and confirm a valid cutoff age. The null hypothesis states that there will be no difference between ages for detecting premolar agenesis.

2. METHODS

This retrospective cross-sectional study was conducted with archived panoramic radiographs taken at the Istanbul Gelisim University School of Dental Medicine Affiliated Hospital. Ethical approval was obtained from Istanbul Gelisim University Ethics Committee (22/04/2021:2021-15).

Included in this study were Turkish pediatric patients aged 7–12 years, whose panoramic radiographs were taken between June 2016 and December 2020. Poor image quality radiographs and patients with developmental anomalies, such as ectodermal dysplasia or a cleft lip or palate, were excluded from this study. Tooth extraction was not performed on patients. If the patient had more than one radiographic image, the most recent image was included in the study. Patient names and information were anonymized prior to the analysis.

Teeth with no mineralization as observed on panoramic radiographs were considered agenesis. All radiographs were evaluated by an observer with 11 years of experience (E.E.). To test the reliability of agenesis, 30 randomly selected radiographs were reassessed after 2 weeks, and the Cohen's Kappa was calculated to measure the strength of agreement for intra-observer reliability. The prevalence of premolar hypodontia, sex distribution, distribution of premolar hypodontia in the maxilla and mandible, and unilateral/bilateral incidences was examined in radiographs.

Descriptive statistics for age (mean, standard deviation [SD], minimum, maximum) were calculated for the sample. The rates and percentages of the variables were determined through a frequency analysis. The differences between groups were tested with chi-square tests or Fisher's exact tests. The odds ratio (OR) was calculated at a 95% confidence interval (CI) to measure the strength of associations between the sexes and the groups.

The number of missing teeth was identified for each patient. For each cutoff age (8 to 11 years), patients were grouped as a cutoff age below (younger group) and above (older group). The mean, SD, median, and interquartile range (IQR) were calculated for each group. The distribution of normality was evaluated with Shapiro-Wilk tests. Since the groups did not show a normal distribution, Mann-Whitney U tests were used for comparisons and these were repeated for each cutoff age.

The result was considered statistically significant for a p value <0.05. All statistical analyses were performed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

Intra-examiner reproducibility was 100% in the diagnosis of tooth agenesis.

We examined 3637 radiographs, consisting of 1819 boys and 1818 girls with a mean age of 9.82 years (SD: 1.48, minimum: 7, maximum: 12). The prevalence rate of agenesis of one or more premolars was 3.1% (n = 111) for both sexes combined, and 1.4% for boys and 1.6% for girls, with no significant difference between the sexes (p = 0.498).

In this study, the most common missing premolar was the mandibular right second premolar (2.3%), followed by the mandibular left second premolar (2.1%) (Table 1). As shown in Table 2, both males and females had significantly increased prevalence rates of unilateral or bilateral second premolar agenesis. There were significantly more missing second mandibular premolars than maxillary premolars. In comparing the prevalence of a bilateral absence of the second premolars with sex, the prevalence was higher for females compared to males in the mandible (p = 0.028). In the maxilla, unilateral absence of second premolars, the prevalence was higher for males than females (p = 0.046) (Table 2).

Table 1. *Numbers of Subjects with Agenesis of Second Premolars Between Sexes*

	Females n=1818 (%)	Males n=1819 (%)	Both sexes n=3627 (%)	p value*	Odds ratio	95% Confidence interval
15	5 (0.3)	4 (0.2)	9 (0.2)	0.754[a]	1.25	0.33-0.66
25	7 (0.4)	1 (0.1)	8 (0.2)	0.039[a*]	6.97	0.85-56.73
35	46 (2.5)	32 (1.8)	78 (2.1)	0.109[b]	1.45	0.91-2.28
45	47 (2.6)	38 (2.1)	85 (2.3)	0.322[b]	1.24	0.80-1.00

*[a]Fisher exact test, [b]Chi-square test, *p<0.05*

As a result of this study, 11 different agenesis patterns were observed (Figure 1). A bilateral second premolar agenesis pattern was observed for the mandibular arch as 42.3%. Mandibular right second premolar agenesis pattern was observed as 26.1%. The tooth agenesis pattern with the highest prevalence was second premolar agenesis (75.6%). Five patients had a premolar agenesis pattern in all four quadrants. No significant difference was observed between younger and older groups at the cutoff ages examined (Table 3).

Table 2. *Second Premolar Agenesis in the Maxilla and Mandible*

	Number (%) of subjects with unilateral agenesis of second premolars		
	Females	Males	Both sexes
Maxilla	2 (3.4)	4 (7.7)	6 (5.4)
Mandible	24 (40.7)	31 (59.6)	55 (49.5)
p value	<0.001 [b*]	<0.001 [b*]	<0.001 [a*]
Odds ratio	0.051	0.056	0.058
95 % Confidence interval	0.011-0.230	0.018-0.180	0.024-0.144
	Number (%) of subjects with bilateral agenesis of second premolars		
	Females	Males	Both sexes
Maxilla	5 (8.5)	2 (3.8)	7 (6.3)
Mandible	35 (59.3)	20 (3805)	55 (49.5)
p value	<0.001 [a*]	<0.001 [b*]	<0.001 [a*]
Odds ratio	0.063	0.064	0.069
95 % Confidence interval	0.22-0.182	0.014-0.293	0.029-0.160
	Number (%) of congenitally missing second premolars		
	Females (n=59)	Males (n=52)	Both sexes (n=111)
Maxilla	12 (10.1)	5 (4.8)	17 (7.6)
Mandible	93 (78.8)	70 (67.3)	163 (73.4)
p value	<0.001[a*]	<0.001[a*]	<0.001[a*]
Odds ratio	0.030	0.025	0.030
95 % Confidence interval	0.014-0.064	0.009-0.066	0.017-0.166

*[a]Chi square, [b]Fisher exact test, *p<0.05*

Table 3. *Comparison of Means, SDs, Medians, and IQRs of Numbers of Missing Teeth Per Patient According to Different Cutoff Ages*

	<Cutoff age (younger group)					≥ Cutoff age (older group)					
	Missing teeth per patient					Missing teeth per patient					
Cut of age	n	Mean	SD	Median	IQR	n	Mean	SD	Median	IQR	*p** value
8	12	1.416	0.514	1	1	99	1.656	0.771	2	1	0.369
9	26	1.615	0.136	2	1	85	1.635	0.834	2	1	0.938
10	51	1.509	0.944	1	1	60	1.733	0.103	2	1	0.114
11	79	1.557	0.780	1	1	32	1.812	0.859	2	1	0.130

**Mann-Whitney U test, IQR:interquartile range*

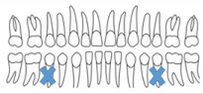
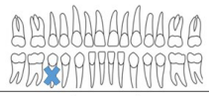
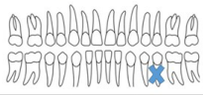
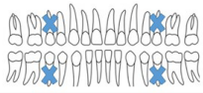
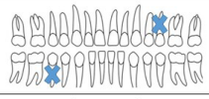
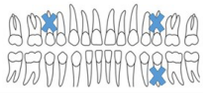
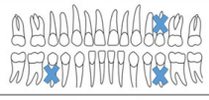
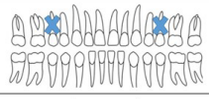
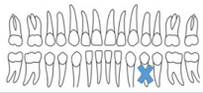
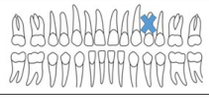
Rank	Pattern	Number of patients	Prevalence of pattern(%)
1		47	42.3
2		29	26.1
3		27	19.8
4		5	4.5
5		2	1.8
6		1	0.9
7		1	0.9
8		1	0.9
9		1	0.9
10		1	0.9
11		1	0.9

Figure 1. *Premolar Agenesis Patterns*

4. DISCUSSION

Tooth agenesis is the most commonly seen dental anomaly in the permanent dentition.

Tooth agenesis appears most frequently in the second premolars (excluding third molars), with variations in the frequency and sequence of the agenesis, which may be affected by ethnic differences (12, 16). The aim of this study was to establish the prevalence and agenesis patterns of premolars in a group of nonsyndromic Turkish children.

The prevalence of tooth agenesis has varied between 0.15%–16.2% in previous studies (17). Although there are many studies on the prevalence of hypodontia, the number of publications reporting premolar agenesis is limited. The prevalence of agenesis has previously been reported at 3.4%–6.6% for the second premolars (18). The prevalence of premolar agenesis also varies from country to country: 5.8% in the Italian population (19), 1.9% in Slovenia, and 5% in the Turkish population (20). Our results showed that 3.1% of the patients had one or more instances of premolar agenesis; thus, our results are in agreement with previous studies. On the contrary, Koc et al reported second premolar agenesis of 6.7% (21). Gelgor et al. (22) reported a mandibular second premolar agenesis of 3%, while Sumer et al. (23) reported the prevalence at 2.59%. Dzemidzic et al. reported the teeth most affected by agenesis were the lower second premolars in orthodontic patients (24). In addition, although there are many studies regarding the prevalence of hypodontia in our country, the prevalence values of premolar agenesis have not been specified in these studies. Therefore, the number of studies in which the prevalence of premolar hypodontia can be compared was insufficient. Considering this, more studies are needed on this condition.

Bilateral premolar agenesis is more prevalent in females, while unilateral agenesis is found more frequently in males; however, these differences are not statistically significant. Previous studies have suggested that bilateral premolar agenesis is more common than unilateral premolar agenesis (25–27). In our study, there were no significant differences between unilateral and bilateral premolar agenesis. The numbers for both unilateral and bilateral premolar agenesis were significantly higher in mandibular premolars than in maxillary premolars.

The effect of a cutoff age was evaluated in patients with premolar agenesis in the present study. There was no significant difference between the groups at the determined cutoff ages. More studies on premolar agenesis are required to validate the results of this study.

This study has some limitations. The sample group consisted of patients who applied to the dental clinic. Socioeconomic differences may have affected the patients' dental visits and their ability to receive treatments. Therefore, the results of this study may not reflect the actual biological variation within the population.

An early diagnosis of premolar agenesis enables ideal and conservative treatment planning, which may involve less invasive treatment options for patients diagnosed in the early period. Restoring the primary tooth with a minimally invasive approach and retaining it in the mouth increases the survival rate of the tooth and decreases the need for complicated treatments, such as endodontic treatments. With a late diagnosis, extraction of the primary tooth may be the only treatment option. In such cases, a space maintainer should be placed to protect the cavity from closure due to mesial/rotational movements of the first molar or overturning of the first molar into the cavity. For these reasons, the awareness of dentists regarding these treatments should be enhanced. Therefore, there is a need for more studies regarding the prevalence of this condition, which will provide important clinical value in the timely diagnosis of hypodontia.

Pediatric dentists are likely to be the first specialist to diagnose congenital tooth agenesis. Therefore, pediatric

dentists are best able to educate the child and family members about dental care and help guide the child to cope with the situation by preventing possible malocclusions. In a multidisciplinary approach, the aim is to preserve the presence of the existing primary tooth, improve aesthetics and speech, provide appropriate chewing, and improve the psychological and emotional health of the child. The role of the pediatric dentist within this dental team is to guide the child's behavior, maintain good oral hygiene, manage malocclusions, and when necessary, provide restoration of the tooth.

5. CONCLUSION

The prevalence of agenesis of one or more premolars was 3.1%. There was no significant difference between the groups with cutoff ages ranging from 7 to 12 years. Therefore, further longitudinal studies of premolar agenesis are required to confirm the results of the present study.

Ethics Committee Approval: Ethical approval was obtained from Ethics Committee of Istanbul Gelisim University, Turkey (22/04/2021:2021-15).

Author contributions: EE designed the study, generated the data for the study, analyzed of the data, wrote the original draft of the paper, and approved the final version of this paper.

Conflict of Interest: Author declared no conflict of interest.

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Clinical and Experimental Health Sciences

Thoracic Oncologic Surgery in Covid-19 Pandemic: Short Term Outcomes

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ABSTRACT

Objective: After sustained community spread of Coronavirus Disease – 19 (COVID-19), guidelines regarding safe practice are still evolving. In this article, we evaluated our clinic's oncologic thoracic surgery practice in COVID-19 pandemic via an observational retrospective study.

Methods: All cases (including local anesthesia only), were recorded in the hospital electronic medical record system. Patients undergoing procedures for cancer related causes (resection, diagnosis, palliation, treatment facilitation) were retrospectively identified. Patient demographic data, type of operation/procedure, 30 day SARS-COV-2 PCR test data, hospital readmission due to COVID-19, 30 and 90 day mortality were gathered. Group 1 and 2 were operated between 1 June 2019 – 11 March 2020 and 12 March 2020 – 31 December 2020, respectively.

Results: 344 (Group 1= 178, Group 2= 166) patients underwent thoracic surgery due to cancer related causes during the study period. Surgical or COVID-19 related mortality were not encountered in either group. After initiation of mandatory testing, 3 asymptomatic patients were found to be PCR positive and had surgery rescheduled (2 lung resections, 1 mediastinoscopy). Number of lung resection cases increased post-COVID-19 (25 vs 45, $p<0.001$). None of the postoperative patients experienced PCR conversion.

Conclusion: Safe elective thoracic surgery practice is possible via following basic safety precautions and following evolving national guidelines. Even though routine preoperative SARS-COV-2 PCR testing catches asymptomatic cases that can cause mortality/morbidity for patients and staff, social distancing and other infection control measures have to be in place to prevent in hospital and post-operative SARS-COV-2 PCR conversion.

Keywords: COVID-19 pandemic, Elective surgical procedure, Lung cancer, Thoracic surgery

1. INTRODUCTION

Since early 2020, COVID-19 pandemic has forced substantive change in surgical practices all over the world. Elective surgical procedures are restricted in many parts of the world due to the conservation of limited resources (manpower, personal protective equipment, ventilator, intensive care bed) and resource distribution for acute needs, maintaining patient and employee safety (1). In places where the epidemic can be controlled, elective procedures can continue with various security measures (2). However, the benefit/risk analysis of delaying surgery due to COVID-19 is still controversial for some particular surgical practices (cardiovascular surgery, oncologic surgery) or safe amount of wait time for certain patient groups which are more problematic in terms of urgency, when for example, compared to elective knee or hip replacement is not well defined (3).

The risk of clinically serious events was found to be higher in patients who were operated in the month before contracting SARS-CoV-2 compared to those who did not have surgery (4). Currently, there are no published international guidelines for decisions regarding specific considerations for surgical intervention in cancer patients during the pandemic (5). However, lung cancer was observed with a rate of 28% in patients with a diagnosis of malignancy among COVID-19 patients (4), so lung cancer patients stand out as a particularly vulnerable group in the pandemic population.

Although there are flow charts and suggestion booklets created by many experts on this subject, limited field knowledge on specific surgical branches delays creation of an evidence based guideline (1,2,5).

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The objective of this study is to summarize and characterize our clinic's experience of thoracic oncologic surgery during COVID-19 pandemic.

2. METHODS

Patients who underwent thoracic surgery due to cancer related causes (resection, diagnosis, palliation, treatment facilitation) between June 1 2019 and December 31 2020 were identified from the hospital electronic medical record system and included in the study. 11th March (identification of first COVID-19 case in Turkey) was chosen for separation of the pre (Group 1) and post (Group2) COVID-19 groups. Patient admissions, demographic parameters (age, sex, in or out of province), type of surgery, presence of SARS-COV-2 screening, length of hospital stay, postoperative complications, 30 and 90 day mortality, COVID related postoperative morbidity-mortality during follow up were recorded.

SARS-COV-2 PCR was mandatory after 15th July, so was performed for every case with planned general anesthesia. Otherwise it was left to surgeon discretion. Cases without planned general anesthesia were only directed to testing in case of clinical suspicion (contact history, symptoms, radiologic findings).

All patients underwent standard preoperative testing and clinical staging work-up, including pulmonary function tests. Informed consent was introduced for pulmonary function tests, per health ministry quality guidelines. Post pandemic, staging and preoperative testing were done in as few days possible to prevent exposure to public transport etc.

Data was analyzed using SPSS 22.0. $p<0.05$ was used for statistically significance. Mean, standard deviation, median values were calculated accordingly. Kolmogorov Simirnov test was used to determine distribution appropriate choice for comparison of independent quantitative variables.

Institutional ethical board (decision number 2020/0754) and Ministry of Health approval were obtained before conduction of the study.

3. RESULTS

344 patients underwent cancer related thoracic surgery during the study period. 178 patients (101 male, 77 female) were assigned to Group 1 (pre – COVID), 166 patients (107 male, 59 female) were assigned to Group 2 (post-COVID). Mean age was 55.1±14 and 56.1 ± 15.3 for groups 1 and 2 respectively. Sex and age distribution were comparable among groups ($p>0.05$ for both, Chi-Square and Mann-Whitney-U respectively). 41 patients in Group 1, 37 patients in Group 2 traveled to Istanbul from out of province to get treatment ($p>0.05$, Chi-Square).

Preoperative evaluation via American Society of Anesthesiologists (ASA) Physical Status Classification System revealed that both groups were equivalent. Significantly more patients underwent lung resection (anatomic and non-anatomic) in Group 2 (25 vs 45, p=0.002, Chi-Square), while chest wall resections and mediastinal procedures were found to be decreased (13 vs 1, p=0.001 and 22 vs 9, p=0.02, Chi-Square). Number of chemotherapy port insertions, pleural diagnostic/palliative procedures, lymph node biopsies were comparable among groups. Operation type breakdown is provided in Table 1.

Table 1: *Patient characteristics and operation type breakdown and mortality*

	Group 1	Group 2	p Value
Age	55.1 ± 14.0	56.1 ± 15.3	0.30
Sex (Male/Female)	101/77	107/59	0.17
ASA class (1/2/3/4)	31/125/21/1	8/130/28/0	N/A
Operation type	Chemotherapy port = 96 Lung Resection = 25* *Lung resection with mediastinal lymph node dissection =15* *Wedge = 10* Mediastinal = 22 *Mediastinoscopy =12* *Resection = 10* Lymph node biopsy = 16 Pleural = 6 Chest wall resection = 13*	Chemotherapy port = 93 Lung Resection = 45* *Lung resection with mediastinal lymph node dissection = 25* *Wedge = 10* Mediastinal = 9 *Mediastinoscopy=6* *Resection= 3* Lymph node biopsy = 10 Pleural = 8 Chest wall resection = 1*	* p<0.05
Mortality (n)	19 30 day mortality=5 90 day mortality=4	4 30 day mortality=0 90 day mortality=1	P=0.002

In both groups, chemotherapy port cases and lymph node biopsies were conducted as day case operations, 2 and 5 chemotherapy port cases in Group 1 and 2 respectively and 6 lymph node biopsies in both groups needed inpatient stay for symptom control/palliation. Lung resection cases had a comparable length of stay (8.3±5.6 vs 7.7±5.9 days, p>0.05, Mann-Whitney-U).

43 cases (20 under general anesthesia, 10 lung resections) were operated after the identification of the first COVID-19 patient in the country but before the health ministry guideline change regarding mandatory SARS-COV-2 PCR screening. 1 patient (anatomic lung resection) underwent SARS-COV-2 PCR testing due to post-operative clinical and radiologic deterioration at that period and found to be negative, recuperated well afterwards. No post-operative COVID-19 related mortality and morbidity was observed.

Rest of the Group 2 was operated under current policy, with a negative SARS-COV-2 PCR test within 48 hours of the operation, 3 asymptomatic and radiology negative patients were found to be positive and had their surgery postponed for 2-3 weeks (2 lung resections, 1 mediastinoscopy) and re-admitted after a negative PCR for the rescheduled procedure, none of those patients experienced further adverse events.

Significantly more deaths were recorded in Group 1 (19 vs 4, p=0.002, Chi-Square), 14 of 19 deaths in Group 1 occurred in chemotherapy port cases and were not attributable to the procedure itself. There was no mortality observed in either group's lung resection cases.

4. DISCUSSION

After declaration of COVID-19 as a global health emergency in January 2020, first case was identified in Turkey at 11th March 2020. Since the beginning of the pandemic, COVID-19 has put a considerable strain on health services as a whole, affecting staffing levels, inpatient bed and ICU capacity, need for personal protective equipment etc. Many countries, including Turkey, introduced unprecedented measures when compared to the last 7 decades, to limit the contagion so the health systems are not overwhelmed and the resources are appropriately prioritized.

Careful evaluation and determination of the severity of population level spread is very important for setting appropriate guidelines for preoperative screening and case selection. In a recent case series from Aminian et al, illustrates the point with 3 cases of subclinical COVID-19 patients undergoing elective surgeries for cholecystectomy, hernia repair and hysterectomy. Two operations were conducted right before the identification of the index case in the country, one right after. 1 patient died post-operatively, also another fatality happened for a scheduled gastric bypass surgery on the planned day, as the patient arrived to emergency with respiratory distress (6).

Since surgical needs of oncology patients are time sensitive and has less tolerance for deferral, also considering the waxing/waning nature of COVID-19 together with precautionary measures, many expert groups tried to come up with prioritization and screening protocols as exampled by Center for Disease Control (CDC), ASA and Anesthesia Patient Safety foundation (APSF) (7). These institutions also advise for universal symptom and contact checking for all cases and standard preoperative PCR testing if the are prevalence is high.

In terms of classification of surgery types for appropriate prioritization, Stahel reported 5 main groups as with deferral times as urgent (<24 hours), urgent elective (<2 weeks), Essential elective (1-3 months), Discretionary elective (indefinite). Cardiothoracic operations and Cancer surgery/ biopsies were classified as urgent elective and essential elective respectively (9, 10).

3 asymptomatic, PCR positive cases were identified after introduction of mandatory testing and were operated on uneventfully afterwards. While the decision to postpone surgery and re-test for clearance of infection seems straightforward for both patient and staff safety, exact timelines were not apparent at the time of the decision and we moved forward per infectious disease clinic consultations. Further literature upon the subject do advice up to 7 weeks of interval after SARS-COV-2 infection in the absence of risk of disease progression (11). To consider an oncology predominant cohort, Baiocchi et al did a case control study where 49 cases with a positive PCR test for SARS-COV-2 (9 symptomatic) underwent surgery with a median of 25 days from the positive PCR test, with a comparable complication profile with the control cases, but their cohort is mostly formed by general surgery cases (12).

In-hospital precautions are also as important as pre-admission testing of SARS-COV-2. In a report summary encompassing over 70000 patients, Wu et al (13) emphasize that even though it is not responsible for most of the cases, one of the leading settings for secondary infections is hospitals and health workers are the ones mostly at risk in this scenario.

Cases with either cancer or emergency related causes are predominantly represented in normal thoracic surgery practice as there are very few other type operations (thoracic symphatectomy, chest wall deformity correction etc) that are within the scope of thoracic surgery. So it may be considered as expected to have near normal surgical volume during this pandemic. We did experience decreased 30 and 90 day mortality due to decrease in mortality after chemotherapy port insertions as well, since particularly morbid/advanged stage patients might have postponed this comfort measure due to COVID-19.

Due to dynamic and flexible organization and resource prioritization with multidisciplinary and administrative collaboration, thoracic oncologic procedures of radical intent, diagnosis, palliation and treatment facilitation were able to be performed without changing the rhythm of the normal workflow, as evidenced by largely comparable case volume and composition. Health ministry preoperative screening

guidelines were adequate in preventing COVID-19 related postoperative morbidity/mortality without needing extra precautions. Also mobility restrictions enforced throughout the country did not significantly affect the number of out of province patients accessing our services. This experience might not be universal as Wang et al describe decreased oncologic case volume decreases and a backlog of at least 6 weeks worth of cases after a peak time of COVID-19 (January-April 2020), and underlines the need of adaptation of precautionary measures as pandemic evolves (eg: keeping the preoperative PCR test but dropping thorax computerized tomography and blood count requirement after the infection curve is flattened) for more straightforward access and cost-effectiveness (14).

This study is conducted in a single center setting, therefore is limited in case numbers and events captured, including an assessment of overall surgical mortality and morbidity. Further preferably prospective studies are needed to delineate the effects of COVID-19 on thoracic surgery practice and patient population to ensure timely diagnosis and treatment.

5. CONCLUSION

Thoracic oncologic practice can be safely continued during COVID-19 pandemic while abiding by simple precautions. Prospective studies are needed to answer important questions like how the health service accessibility can be improved. While complete preoperative testing is possible during COVID-19 pandemic, facilitation can be achieved with protected time slots for those vulnerable oncology patients. Centralized systems should offer guidance regarding COVID-19 prevalence in particular healthcare system's population to determine optimal pre-operative PCR testing strategy.

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Data Sharing: The author will make anonymized data available per request.

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Clinical and Experimental Health Sciences

Covid-19 Pandemic and Its Effects on Dentistry: A Retrospective Study

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ABSTRACT

Objective: The purpose of this study was to compare patient admissions, treatment needs, and treatments in our hospital before and after the Covid-19 pandemic.

Methods: The patients who presented to Karabük Oral Dental Health Training and Research Hospital between March 2019 and March 2021 were retrospectively evaluated. The data obtained were used to compare the 1-year process after the Covid-19 pandemic and the 1-year process before the Covid-19 pandemic. In this 2-year period, the number of hospital registrations, distribution of patients by gender, age data, type, and the number of procedures performed were obtained from the software system used in the hospital.

Results: The average number of patients admitted to the hospital per month in 1 year before the pandemic is approximately 3.5 times in the next 1 year. When the number of patients applying was evaluated in terms of gender, it is seen that the number of male patients decreased more than women during the pandemic period. It has been observed that there is a dramatic decrease in the pandemic period in all treatments.

Conclusion: With the Covid-19 pandemic, there has been a serious decrease in the number of patients. However, with the ongoing treatment needs and normalization processes of the patients, necessary precautions were taken and the treatment of the patients continued.

Keywords: Covid-19, Dental procedures, Patient admission

1. INTRODUCTION

In 2019, a coronavirus that has not previously been detected in humans was identified in the Wuhan region of China. The World Health Organization (WHO) named this virus, which causes atypical pneumonia, as a novel coronavirus (2019-nCoV). It was later named SARS-CoV-2 because of its taxonomic similarity to the causative virus (SARS-CoV) of severe acute respiratory syndrome (SARS) (1, 2). WHO first announced this unique pneumonia as an epidemic threatening international public health on 30 January 2020, and then declared it a pandemic on 11 March 2020, as cases of infection occurred in many countries (3). The rapidly spreading coronavirus infection (Covid-19) has become a major health crisis worldwide. The first Covid-19 case in Turkey was officially announced by the Ministry of Health of the Republic of Turkey on 10 March 2020 (4).

Current data show that human-to-human transition of the new coronavirus type is easier and faster than for other coronavirus types (5). It has been reported that the route of transmission is directly from person to person or through infected saliva, respiratory tract, and droplets (6). Similar to SARS-CoV, SARS-CoV-2 enters the cell via the angiotensin converting enzyme-2 (ACE-2) cell receptor (7). ACE-2 cells are morphologically similar to salivary duct epithelium and are abundant along the airways. It has been found that ACE-2 cells are more abundant in the oral mucosa than in buccal and periodontal tissues, particularly on the dorsum of the tongue, and therefore dental practices are at high risk for Covid-19 (8).

Although it is not possible to identify people who are carriers of the virus without testing in the early period, it has also been reported that the infection can be transmitted through contact by persons who are asymptomatic carriers (9). In the pandemic period we are in, it is extremely important in terms of infection control that any patient coming into the clinic can transmit this virus without showing any signs and symptoms, and that the dentists and the entire team of assistants

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take appropriate precautions. Dental clinics are considered contamination areas that increase the spread of the disease in terms of cross-infection (10). Therefore, the measures and protocols required to control the risk of cross-infection during the Covid-19 epidemic have been established, and patient management has been implemented.

The Ministry of Health of Republic of Turkey published Covid-19 disease guide for health professionals and specified the measures to be taken. Then, with the recommendation of Coronavirus Scientific Advisory Board of General Directorate of Ministry of Health Services, a circular was issued that dental treatments should be postponed except for emergency and compulsory services (11). It was recommended that air-water sprayers and round hand tools be used at a minimal level, and ultrasonic hand tools should not be used in applications during this period. It was reported that procedures should be performed with hand tools and minimally invasive/atraumatic as much as possible (12). Some measures that can be taken are as follows: the clinic waiting area and examination room should be adequately ventilated, no companions should be brought into the treatment area except for patients who require assistance, aerosol-generating surgeries should be performed in isolated areas and scheduled as the last appointment of the day if possible. In addition to standard precautions (hand hygiene, use of gloves and surgical masks), personal protective equipment including FFP2/FFP3 (N95/N99) filtered mask, face/eye protection and gown is required (13).

On 21 April 2020, the definition of Emergency and Compulsory Services in Dentistry Practices in the Covid-19 process was also made, and it was reported that dentists should continue to recruit patients in accordance with these guidelines (14). In the later stages of the pandemic, with the decline in the number of cases, the transition to the normalization period was made and the "Working Guide in Health Institutions in Normalization Period in Covid-19 Pandemic" was published by the Ministry of Health on 1 June 2020. Deferred elective treatments are now again performed according to the working principles of this guideline, especially in emergency and compulsory care (15).

The purpose of this study was to compare patient admissions, treatment needs, and treatments in our hospital before and after the Covid-19 pandemic. The study questions were as follows: 1) How have hospital admissions changed during the pandemic period compared to previous periods? 2) Is there a relationship between socio-demographic characteristics such as gender and age in the number of hospital admissions during the pandemic period? 3) How did the treatments provided in the hospital change during the pandemic period compared to the previous periods? The hypothesis of the study was that there is a significant decrease in the number of patient admissions in the early period of the epidemic compared to previous periods, and the majority of procedures performed on patients treated are in the context of emergency treatments.

2. METHODS

The study protocol was approved by the Karabük University Ethics Committee for Non-Interventional Clinical Research (Decision No: 2021/467). In this study, patients who presented to Karabük Oral Dental Health Training and Research Hospital between March 2019 and March 2021 were retrospectively evaluated. The data obtained were used to compare the 1-year process after the Covid-19 pandemic and the 1-year process before the Covid-19 pandemic. In this 2-year period, the number of hospital registrations, distribution of patients by gender, age data, type, and the number of procedures performed were obtained from the software system used in the hospital. For the 1-year periods before and after the Covid-19 pandemic, these data were collected separately for each month. Since the first Covid-19 case in our country was reported in March 2020, the distinction between the pre-and post-pandemic periods was made in March. Data were recorded monthly in order to evaluate the effects of the Covid-19 pandemic on the number of patients and the procedures performed. When evaluating patient admission, the classification by age was divided into 3 groups: 0-14, 14-65, and over 65 years old. The procedures performed are prosthodontic (crown recementation, removable denture applications, removable denture repair, and reline procedures, veneer crown removal), endodontic (root canal treatment, extirpation), pedodontic (fluoride application, fissure sealing, and space retainers), and surgical (extraction) were classified as procedures. All dental treatments were not included in the study. Only dental treatments were selected for the study, which are thought to be able to obtain more detailed data and can be considered as emergency or non-essential treatments that can be associated with the Covid-19 pandemic.

The number of patient admissions and the number of dental procedures performed were compared by calculating monthly averages for the 1-year periods before and after the Covid-19 pandemic. The distribution of patients according to the determined by age groups and gender was evaluated. The alterations in the numbers and types of dental procedures performed were determined.

3. RESULTS

In the retrospective screening, the average number of patients admitted to the hospital per month in the 12 months before March 2020 when the epidemic was first seen in Turkey and the 12 months after the epidemic, the change in the number of patients by age groups and by gender as 0-14, 15-64 and 65+ are shown (Table 1, Figure 1 and Figure 2). In addition, the numbers of patients who have had Covid-19 and patients admitted the dental hospital every month since March 2020 shown in Table 2. While there were a total of 183.275 patient applications in the one-year period before the Covid-19 pandemic, a total of 64.145 patient applications were made in the one-year period after the Covid-19 pandemic. The correlation of the number of patients with Covid-19 and the number of monthly patients admitted to our oral and dental health center is shown in Figure 3. The

average number of patients admitted to the hospital per month in 1 year before the pandemic is approximately 3.5 times in the next 1 year. When the number of patients applying is evaluated in terms of gender, it is seen that the number of male patients decreased more than women during the pandemic period. In addition, the average numbers of procedures performed (repair, cementation, prosthesis, veneer crown, crown removal, denture reline, fluoride application, fissure sealant, fixed retainer, extraction, extirpation, root canal treatment) per months are shown in Table 3 and Figure 4.

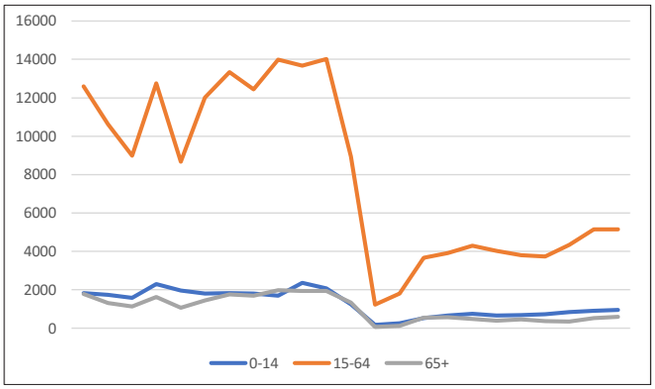

Figure 1. *Distribution of the average numbers of patients admitted to the hospital per month in the 12 months before March 2020 and in the 12 months after the Covid-19 by age groups*

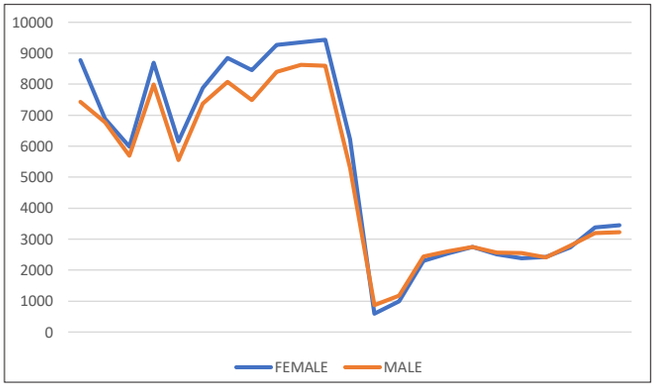

Figure 2. *Distribution of the average numbers of patients admitted to the hospital per month in the 12 months before March 2020 and in the 12 months after the Covid-19 by gender*

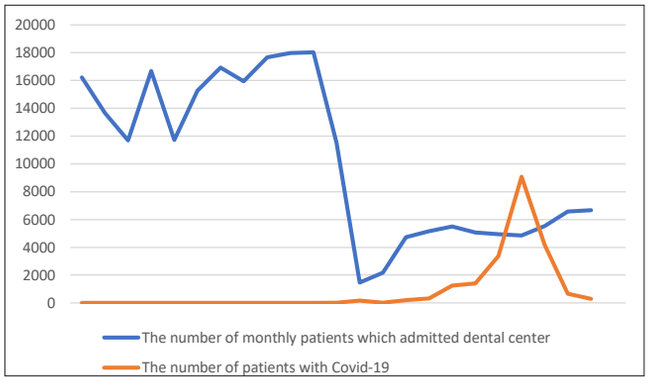

Figure 3. *The correlation of the numbers of patients with Covid-19 and the average numbers of patients per month which admitted dental center*

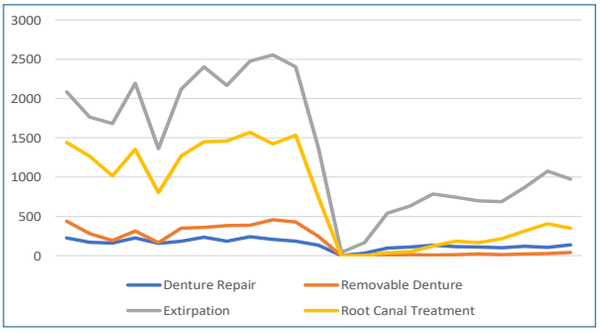

Figure 4. *Average numbers of mainly dental procedures performed per month in the 12 months before March 2020 and in the 12 months after the Covid-19.*

Table 1. *Distribution of the average number of patients admitted to the hospital per month in the 12 months before March 2020 and in the 12 months after the Covid-19 by age groups and gender*

	Before Covid-19	After Covid-19
0-14 age	1853.33	627.33
15-64 age	11838	3708.75
65+ age	1581.58	389.41
Female patient	7999.83	2273.16
Male patient	14549.83	2333.41

Table 2. *Distribution of the number of patients with Covid-19 by months since the beginning of the pandemic in March 2020 in the city and numbers of patients who admitted to the dental hospital*

Date	Numbers of Covid (+) patients	Numbers of admitted patient
March 2020	11	11520
April 2020	154	1462
May 2020	20	2168
June 2020	208	4735
July 2020	325	5148
August 2020	1250	5500
September 2020	1405	5075
October 2020	3364	4927
November 2020	9078	4837
December 2020	4163	5515
January 2021	657	6582
February 2021	278	6676
March 2021	1059	9366
April 2021	3628	7796

Table 3. *Average number of dental procedures performed in the hospital per month in the 12 months before March 2020 and in the 12 months after the Covid-19*

Dental procedures	Before Covid-19	After Covid-19
Removable Denture Repair	192.58	88.66
Cementation	292.66	194.41
Removable Denture	333.83	14.5
Veneer Crown	604.16	0.83
Crown Removal	188.33	14.75
Denture Reline	22.91	3.66
Fluoride Application	72.41	11.83
Fissur Sealing	114.41	8.5
Fixed Retainer	21.16	9.6
Extraction	2048	621.25
Extirpation	145.25	60.33
Root Canal Treatment	1276.75	164.08

4. DISCUSSION

In our study, primary treatment needs were evaluated due to the changes in the number of patient applications to Karabük Oral and Dental Health Training and Research Hospital and the number of treatments during the 12 months before the Covid-19 pandemic and the following 12 months. As a result of the study, it was found that there was a dramatic decrease in the number of patients admitted to the hospital after the pandemic and in all treatment types. In the literature, it has been reported that SARS-CoV-2 virus was isolated in saliva samples obtained from Covid-19 cases (16). In addition, it has been reported that the SARS-CoV virus, which is a member of the same family but has similar features to this virus, can form the main source of the virus by infecting the salivary gland epithelial cells (17). Therefore, saliva contamination has an important place in the transmission of the disease (18). When asked to evaluate basic dentistry branches according to the risk they pose for saliva contamination in Covid-19, most of the participants in all three groups answered prosthetic dental treatment as the highest risk branch. Prosthetic dental treatment is a clinical branch that is very prone to the spread of intraoral fluids, as it includes operations such as tooth preparation and impression taking (19). In Benli's study, the most risky dental applications are; tooth preparation, tartar scaling, pulp extirpation and filling process (20). In line with this result, the highest rate of decline was observed in prosthetic applications (denture repair, crown cementation, removable denture, veneer crown, crown removal, denture reline) in our study.

Since our study was conducted on patients who applied to Karabük Oral and Dental Health Training and Research Hospital, when we examined the relationship between the distribution of the number of patients and the number of patients who had Covid-19 since March 2020, the first date of the pandemic, the increase in the number of cases showed a significant correlation in the number of patients admitted and the number of procedures performed. found to be absent.

Since all treatments applied by dentists are carried out very close to the mouth and nose, it increases the risk of transmission for both physicians and auxiliary staff, patients and relatives of all these individuals (21, 22). According to the current study by Meng et al. (23), it is not possible to definitively determine whether there is a Covid-19 infection in patients undergoing dental treatments, because there are individuals who are in the incubation period, have the disease without symptoms, or hide their disease. For this reason, Covid-19 has caused significant anxiety problems in dentists in terms of dental treatments. In addition, another reason for the decrease in the number of patients can be shown as the anxiety and reluctance of patients in need of treatment to go to hospitals and other health institutions due to the risk of contamination.

In a survey conducted by Ovalioğlu et al. (24), 11.2% of the patients reported that they would come to the endodontic clinic in non-emergency situations, and 88.7% of them reported that they would only apply to the endodontic clinic in an emergency. The women participants surveyed stated that they were more anxious and fearful than man participants who were more willing to go to the dentist appointment. The fact that the rate of wanting to apply even to a department such as endodontics, which requires the most urgent treatment and where the majority of patients apply with severe pain, can provide us with an idea for other dental procedures. In addition, when we look at the types of dental procedures performed, it is seen that there is a greater decrease in the procedures other than the applications considered as emergency dentistry applications after Covid-19, compared to the emergency ones. According to a study conducted by Aras et al. (25) to evaluate the knowledge level of the Turkish Community about emergency dentistry practices after the Covid-19 Epidemic. Although some of the participants are still not aware of the seriousness of Covid-19, it has been shown that the majority of them are aware of the contamination risk in dental clinics and have sufficient knowledge about emergency dental treatments. In addition, the dramatic decrease in the number of treatments and patients, especially in the first months after the declaration of the pandemic, may be due to the fact that physicians and other clinical staff do not have enough information about the Covid-19 outbreak. In a study by Dikilitaş et al. (26), it was stated that the level of knowledge of dentistry health workers on the prevention and control of Covid-19 in general is low and they should be trained on this subject and regularly renew this knowledge and experience in order to protect against new infections that may occur in the future.

Ağmaz et al. (27) evaluated the applications made to the pediatric dentistry clinic retrospectively as a period of 3 months and 5 years before the Covid-19 pandemic. It has been shown that there is a significant decrease in the applications made to the hospital and the procedures performed during the Covid-19 pandemic period. They stated that the treatment aims and methods in dentistry, which is an area with a very high risk of transmission, will be reviewed after Covid-19. The reason for the decrease in patient admissions due to the treatments given may be the curfews applied especially for children and the elderly. One study found that restrictions imposed to prevent the spread of Covid-19 caused a significant decline (81%) in oral health care intake for older adults (28). In addition, in pediatric patients, parents may have made their own palliative treatments by looking at their old prescriptions, and therefore, applications and treatments may have decreased. In the study of Tunç et al. (29), in which they aimed to evaluate the knowledge, attitudes and practices of parents about self-medication for their children's dental problems during the Covid-19 epidemic, it was stated that the majority of parents (n=273; 70.2%) used self-medication for their children's dental problems. It was concluded that drug treatment with previously prescribed drugs was generally preferred by parents (n=179; 62.2%) and analgesics (98%) were the most self-administered drugs for their children's dental problems.

Due to the unpredictability of the pandemic, the measures that can be taken and additional protective procedures before treatment are practically insufficient. For this reason, interventional procedures have been reduced and even stopped since the declaration of the pandemic. In our study, we preferred a public institution because it is easier to obtain patient records and covers a wider population. However, we think that this dramatic decrease in treatments and number of patients is similar in private clinics. It is inevitable that patient flows are not as high as they used to have economic effects. Among these effects, the decrease in working hours with the transition to flexible working hours can be counted as the dismissal of salaried dentists and other clinical staff (30, 31). These situations can lead to unpredictable long-term results such as unemployment. While the lockdowns and reductions in dental activities have a profound impact on the economy of this sector, all dental procedures during the Covid-19 outbreak should be continued with all precautions taken (32). An important consequence of this dramatic decrease in the number of treatments is that patients cannot have the necessary dental treatments, so there may be serious deterioration in oral and dental health, and advanced treatments require more costs.

The fact that patients prefer private clinics instead of public institutions may also be effective in the dramatic decrease in the number of patients in our hospital after the epidemic. Şahin et al. (33), in a study they conducted, aimed to evaluate the patients' thoughts about dental practices and preventive measures and to compare their thoughts on the risk of transmission according to the preferences of the institution. As a result of the study, it was determined that 69.30% of the participants preferred private clinics for dental treatments, 18.80% preferred dental faculties and 11.90% preferred oral and dental health centers and hospitals. It has been determined that there is a statistical relationship between the state of the patients thinking that they are adequately protected against the risk of Covid-19, the state of thinking that the Covid-19 epidemic is under control, and the anxiety caused by the crowd in the waiting area and institution preferences.

The limitations of this study are that the study is limited to only one public hospital and covers a limited time. Future studies can be planned to be evaluated over more clinics providing dental services and for longer time intervals.

5. CONCLUSION

With the increase in Covid-19 cases in the early stages of the pandemic, there has been a significant decrease in the number of patients admitted to dental hospitals and the procedures performed. However, first of all, since the treatment needs of the patients continued, necessary precautions were taken and emergency treatments were started. Then, with the normalization period, routine dental procedures of the patients began to be performed. Dentistry is a profession that has a high risk of cross infection due to its nature, but infection control training is very strict. For this reason, the dental treatment of the patients should be continued by taking the necessary precautions.

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Clinical and Experimental Health Sciences

Evaluation of the Effect of the Covid-19 Pandemic on the Education Process of Dentistry Faculties in Turkey: A Survey Study

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ABSTRACT

Objective: The aim of this study is to determine the existing changes in the education process in dentistry faculties in Turkey from the spring term of 2020 to the present day and to provide information exchange between universities.

Methods: In total 79 dentistry faculties in Turkey were invited to this survey study. Data were collected between April 15, 2021 and May 1, 2021. In the 32-item questionnaire, questions were asked about the characteristics of the faculties (state or private institution, foundation year) and how they carried out their educational activities (online, face-to-face, etc.) since the beginning of the pandemic. Chi-square test and Fisher's Exact test were used to determine the differences between the groups ($p<0.05$).

Results: The questionnaire was answered by 67 dentistry faculties (84.8%). Theoretical education in dentistry faculties continued online at a rate of 71.6% in all three terms. Preclinical and clinical education, which continued through distance education in the spring of 2020, was carried out mostly face-to-face (51.6% preclinical, 88.2% clinical) in the spring of 2021. The rate of continuing the theoretical education online (72.5%) of public universities in the spring term of 2020 was statistically higher than that of private universities (70.4%) ($p<0.05$). While most of the faculties performed their theoretical (83.6%) and preclinical (51.5%) exams online in the spring of 2020, they preferred to conduct the practical exams mostly (47.8%) face-to-face in the 2021 spring term.

Conclusion: During the pandemic process, a hybrid education model was preferred in dentistry faculties in Turkey, in which theoretical courses are offered by distance education and practical courses are provided face-to-face.

Keywords: Covid-19, Dentistry, Distance education

1. INTRODUCTION

The World Health Organization declared the Covid-19 disease caused by the SARS-CoV-2 (corona) virus as a pandemic on March 11, 2020 (1). Until today, no clear treatment has been found for Covid-19, and vaccine production studies have begun to be carried out in different countries of the world in order to prevent the pandemic. There are countries in the world that already have intensive vaccination, and currently, individuals over the age of 18 are vaccinated in our country. Since the beginning of the pandemic, various preventive measures, including quarantine processes, have been implemented in most countries in order to break the chain of transmission of the infection. In addition, social life has changed considerably on a global scale due to the pandemic (2).

Along with the quarantine processes, face-to-face education activities at undergraduate and postgraduate level have been greatly interrupted in most countries. In order to manage this process in our country, the Council of Higher Education (CoHE) has decided to transition to distance education in the spring term of 2020 (3). Since the quarantine and social isolation times are unpredictable, most universities have turned to alternative education tools in order to continue their education and training activities and have engaged in various researches on this subject (4). In this process, educators around the world had to switch to the distance education model (such as online courses, exams) (5,6). Many dentistry faculties have also faced various difficulties due to the pandemic (7). In a recent study investigating how dentistry faculties in European countries continue their educational activities during the pandemic, 72% of them are online live videos, 48% are links to additional online materials, 65% are online meetings and fewer small-scale

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study groups, social media groups or magazine clubs have been reported (8). However, there is little evidence and study about the real impact of these education models on dental education (9). On the other hand, considering the preclinical and clinical education processes in dentistry faculties, the available alternative options are very few (5).

In the near future, it is foreseen that distance education will be the main ground of education instead of being an alternative or a support tool in face-to-face learning (10). In the literature review, no studies were found on how the dentistry faculties in Turkey carried out their educational activities during the pandemic process. Therefore, the aim of this study is to determine the existing changes in the educational processes in dentistry faculties in Turkey from the spring term of 2020 to the present, and to investigate existing techniques and new perspectives in this field. With this study, information exchange was ensured with dentistry faculties across the country, and it will be possible to be prepared for possible demands for dental education in similar pandemic processes that may be encountered in the future. The hypothesis of this study is that dentistry faculties would prefer a hybrid education model where online education will be provided in theoretical courses and face-to-face education will be provided in preclinical/clinical education.

2. METHODS

In order to carry out the study, permission was obtained from the scientific research studies department on COVID-19 in the Scientific Research Platform of the Ministry of Health. Subsequently, this study was accepted by Biruni University Ethics Committee (Date: 09.04.2021 No: 2021/50-21). This research, which was planned as a cross-sectional survey study, was carried out between April 15, 2021 and May 1, 2021. Participation in the study was completely voluntary, and answering the survey questions was interpreted as consent to participate in the study. All dentistry faculties whose educational process started in Turkey were included in the study (n=79). The sample size required for the research consists of 79 dentistry faculties still continuing their education activities in Turkey. For this study, the minimum sample size was 66 according to the 95 % power calculation. The questionnaire, which was prepared using the "Google Forms" application and consisted of 32 questions in total, was sent to all dentistry faculty deans via e-mail. The survey questions are multiple choice and more than one option can be marked separately. The questionnaire form consists of two parts. In the first part, there are questions about the characteristics of the faculties (name, public or private institution, year of establishment). In the second part, detailed questions were asked about how they carried out their educational activities and exams (online, face-to-face, hybrid, video, homework, etc.) since the beginning of the pandemic. In this section, how the theoretical and practical courses as well as clinical applications are carried out were questioned separately. In addition, in this part of the questionnaire, the opinions of the faculty deans were taken about precautions taken in clinics for infection control and extra materials provided, compensation for practical and clinical applications that could not be made during the pandemic period, virtual models related to distance education in practical courses, reliability of online exam results and precautions taken for the reliability of these exams.

2.1. Statistical Analysis

IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for statistical analysis of the findings obtained in the study. The conformity of the study parameters to the normal distribution was evaluated with the Shapiro Wilks test. When comparing quantitative data, Mann Whitney U test was used to compare parameters that did not show normal distribution. Chi-square test, Fisher's Exact test, Fisher Freeman Halton test and Continuity (Yates) Correction were used to compare qualitative data. Significance was evaluated at the $p<0.05$ level.

3. RESULTS

A total of 67 (84.8%) faculty deans answered the survey questions completely in the 2-week period given for answering the survey. The education period of the faculties participating in the research varies between 1 and 113 years, 59.7% of them are state universities and 40.3% are private universities. Approximately 42% of the universities participating in this study provide education between 0-5 years, 20.9% between 6-10 years and 37.3% 11 years or more. The distribution of parameters regarding how the faculties continue their theoretical, practical and clinical applications in the 2020 spring, 2020 autumn and 2021 spring terms of the pandemic was demonstrated in Table 1.

While the level of students' participation in online courses was very low in 4.5% of the faculties, it was found to be low in 6%, moderate in 47.8%, high in 31.3% and very high in 10.4%. In response to the question "What level do you think the learning level of students is in online courses compared to face-to-face courses", 65.7% of the faculties reported that they were at medium level and 23.9% at low level. The distribution of the extra materials provided to clinical students for infection control and the precautions taken for clinical practice was demonstrated in Table 2. Accordingly, the majority of faculties (72.5%) provided all personal protective equipment for infection control. In addition, most of the faculties reduced the number of patients treated in clinics (80.4%) and the number of students in the internship group (86.3%).

The distribution of the theoretical and practical course exams in the 2020 spring-autumn and 2021 spring terms was showed in

Table 1. *Distribution of parameters related to the method of continuing theoretical, preclinical and clinical education during the pandemic*

		n	%
The method of continuing theoretical education in the 2020 spring semester of the pandemic	Online	48	71.6
	With offline videos	7	10.4
	With hybrid education	5	7.5
	With lecture notes	7	10.4
The method of continuing theoretical education in the 2021 spring semester of the pandemic	Online	48	71.6
	With offline videos	2	3
	With hybrid education	15	22.4
	With lecture notes	2	3
Presence of preclinical students	Yes	62	92.5
	No	5	7.5
The method of continuing preclinical education in the 2020 spring semester of the pandemic (n=62)	Face to face	9	14.5
	Online	25	40.3
	With videos	11	17.7
	By giving homework	8	12.9
	Other	9	14.5
The method of continuing preclinical education in the 2020 autumn semester of the pandemic (n=62)	Face to face	15	24.2
	Online	28	45.2
	With videos	8	12.9
	By giving homework	7	11.3
	Other	4	6.5
The method of continuing preclinical education in the 2021 spring semester of the pandemic (n=62)	Face to face	32	51.6
	Online	19	30.6
	With videos	7	11.3
	By giving homework	1	1.6
	Other	3	4.8
Presence of clinical students	Yes	51	76.1
	No	16	23.9
Continuation of clinical internships in the 2020 spring pandemic period (n=51)	Face to face	8	15.7
	Postponed to autumn semester	12	23.5
	Not done – online exam only	18	35.3
	With videos	3	5.9
	By giving homework	1	2
	With case discussions	4	7.8
	Other	5	9.8
Continuation of clinical internships in the 2020 autumn pandemic period (n=51)	Face to face	25	49
	Postponed to spring semester	11	21.6
	Not done – online exam only	9	17.6
	With videos	1	2
	By giving homework	2	3.9
	With case discussions	3	5.9
Continuation of clinical internships in the 2021 spring pandemic period (n=51)	Face to face	45	88.2
	Won't – only online exam will be done	4	7.8
	With case discussions	1	2
	Other	1	2

Table 2. *Distribution of the extra materials provided to clinical students for infection control and the precautions taken for the clinical practice*

		n	%
Extra materials provided to clinical students for infection control (n=51)	All personal protective equipment provided	37	72.5
	N95 mask	9	17.6
	Surgical gown or overalls	8	15.7
	Visor	2	3.9
	Bonnet	13	25.5
	Surgical mask	14	27.5
	Other materials	2	3.9
Measures taken for the clinical practice (n=51)	Teledentistry support	6	11.8
	Creating a triage area	24	47.1
	Areas with multiple units were dividing one by one.	30	58.8
	Patients are seated in the units at intervals	35	68.6
	Surgical suctions, double suction or special suction apparatus are used	15	29.4
	Ventilation systems rearranged (hepafilter etc.)	25	49.0
	The number of patients has been reduced	41	80.4
	Appointment duration extended	33	64.7
	The number of students in the internship group has been reduced	44	86.3
	Internship time reduced	26	51.0

table 3. According to this, most of the faculties (83.6%, 95.5%, 77.6%; respectively) conducted the theoretical exams online. On the other hand, half of the faculties (51.5%) performed online the practical exams in the spring term of 2020, while 43.3% performed face-to-face in the autumn term of 2020. In the spring term of 2021, 47.8% of the faculties reported that they performed practical exams face-to-face. While 4.5% of faculties believe that online exams measure students' knowledge correctly, 43.3% do not believe and 52.2% partially believe. In addition, 80.6% of the faculties reported that they took an extra precaution to prevent cheating in online exams, while 19.4% did not take extra precautions. The most frequently taken precautions include shortening the exam time (70.4%), conducting multiple-choice exams (66.7%), and setting time limits per question (53.7%). While 47.8% of the faculties created a separate program for compensation of the missing education in the 2020 spring term, 52.2% did not create any program. In the trainings given during the pandemic period, attendance was mostly ignored (67.2%) and the practical thresholds were lowered (61.2%).

While 29.9% of the faculties consider using virtual reality devices for clinical and preclinical education, 23.9% do not

Table 3. *Distribution of the methods of conducting the theoretical and practical course exams for 2020 and 2021*

		University			
		State	Private	Total	p
		n (%)	n (%)	n(%)	
The method of conducting the theoretical course exams in the spring term of 2020	Face to face	2 (5%)	1 (3.7%)	3 (4.5%)	[1]**0.010***
	Online	37 (92.5%)	19 (70.4%)	56 (83. 6%)	
	Other	1 (2.5%)	7 (25.9%)	8 (11.9%)	
The method of conducting the theoretical course exams in the autumn term of 2020	Face to face	0 (0%)	3 (11.1%)	3 (4.5%)	[2]**0.061**
	Online	40 (100%)	24 (88.9%)	64 (95.5%)	
The method of conducting the theoretical course exams in the spring term of 2021	Face to face	6 (15%)	3 (11.1%)	9 (13.4%)	[1]**0.108**
	Online	33 (82.5%)	19 (70.4%)	52 (77.6%)	
	Other	1 (2.5%)	5 (18.5%)	6 (9%)	
The method of conducting the practical course exams in the autumn term of 2020 (n=66)	Face to face	4 (10%)	2 (7.7%)	6 (9.1%)	[1]**0.373**
	Online	22 (55%)	12 (46.2%)	34 (51.5%)	
	Homework	12 (30%)	7 (26.9%)	19 (28.8%)	
	Other	2 (5%)	5 (19.2%)	7 (10.6%)	
The method of conducting the practical course exams in the spring term of 2020 (n=66)	Face to face	5 (12.5%)	7 (25.9%)	12 (17.9%)	[1]**0.210**
	Online	20 (50%)	9 (33.3%)	29 (43.4%)	
	Homework	10 (25%)	10 (37%)	20 (29.9%)	
	Other	5 (12.5%)	1 (3.7%)	6 (9%)	
The method of conducting the practical course exams in the spring term of 2021 (n=66)	Face to face	19 (47.5%)	13 (48.1%)	32 (47.8%)	[1]**0.376**
	Online	10 (25%)	4 (14.8%)	14 (20.9%)	
	Homework	7 (17.5%)	9 (33.3%)	16 (23.9%)	
	Other	4 (10%)	1 (3.7%)	5 (7.5%)	

[1]*Fisher Freeman Halton Test* [2]*Fisher's Exact Test* **p<0.05*

consider about it. The reason why are mostly due to high cost and lack of appropriate infrastructure.

While 50% of state universities continue education for 11 years or more, 51.9% of private universities provide education between 0-5 years. The rate of continuing the theoretical education online (72.5%) in the 2020 spring term of the pandemic of state universities was statistically higher than that of private universities (70.4%) (p:0.033; p<0.05). In the autumn of 2020 and spring of 2021, there was no significant difference between state and private universities (p>0.05) (Table 4). There was no statistically significant difference between the ways in which state and private universities continued practical courses and clinical internships during the pandemic period (p>0.05).

Table 4. *Evaluation of the parameters related to the method of continuing the theoretical education of the faculties during the pandemic according to the university*

		University		p
		State	Private	
		n (%)	n (%)	
The method of continuing theoretical education in the 2020 spring semester of the pandemic	Online	29 (72.5%)	19 (70.4%)	**0.033***
	With offline videos	6 (15%)	1 (3.7%)	
	With hybrid education	4 (10%)	1 (3.7%)	
	With lecture notes	1 (2.5%)	6 (22.2%)	
The method of continuing theoretical education in the 2020 autumn semester of the pandemic	Online	32 (80%)	16 (59.3%)	**0.099**
	With offline videos	2 (5%)	1 (3.7%)	
	With hybrid education	6 (15%)	8 (29.6%)	
	With lecture notes	0 (0%)	2 (7.4%)	
The method of continuing theoretical education in the 2021 spring semester of the pandemic	Online	31 (77.5%)	17 (63%)	**0.573**
	With offline videos	1 (2.5%)	1 (3.7%)	
	With hybrid education	7 (17.5%)	8 (29.6%)	
	With lecture notes	1 (2.5%)	1 (3.7%)	

Fisher Freeman Halton Test **p<0.05*

A comparison of the measures taken for clinical practice of the extra materials provided to clinical students for infection control by universities was showed in table 5. Accordingly, the rate of providing all personal protective equipment to interns (84.8%) by state universities was statistically significantly higher than private universities (50%) (p:0.019; p<0.05). In addition, the rate of providing students with bonnet (12.1%) and surgical masks (15.2%) was statistically lower in state universities (p<0.05). While the areas with multiple units in state universities were mostly closed one by one (63.3%), private universities preferred to use the units intermittently (88.9%) and to renew their ventilation systems (72.2%) (Table 5).

Table 5. *Evaluation of the extra materials provided to clinical students for infection control and the precautions taken for the clinical practice according to the university*

		University		p
		State	Private	
		n (%)	n (%)	
Extra materials provided to clinical students for infection control	All personal protective equipment provided	29 (84.8%)	9 (50%)	[1]**0.019***
	N95 mask	5 (15.2%)	4 (22.2%)	[1]**0.703**
	Surgical gown or overalls	4 (12.1%)	4 (22.2%)	[1]**0.430**
	Visor	1 (3%)	1 (5.6%)	[1]**1.000**
	Bonnet	4 (12. 1%)	9 (50%)	[1]**0.006***
	Surgical mask	5 (15.2%)	9 (50%)	[1]**0.019***
	Other materials	1 (3%)	1 (5.6%)	[1]**1.000**
Measures taken for the clinical practice	Teledentistry support	6 (18.2%)	0 (0%)	[1]**0.078**
	Creating a triage area	16 (48.5%)	8 (44.4%)	[2]**1.000**
	Areas with multiple units were dividing one by one.	21 (63.6%)	9 (50.0%)	[2]**0.517**
	Patients are seated in the units at intervals	19 (57.6%)	16 (88.9%)	[2]**0.047***
	Surgical suctions. double suction or special suction apparatus are used	9 (27.3%)	6 (33.3%)	[2]**0.895**
	Ventilation systems rearranged (hepafilter etc.)	12 (36.4%)	13 (72.2%)	[2]**0.031***
	The number of patients has been reduced	27 (81.8%)	14 (77.8%)	[1]**0.727**
	Appointment duration extended	19 (57.6%)	14 (77.8%)	[2]**0.256**
	The number of students in the internship group has been reduced	28 (84.8%)	16 (88.9%)	[1]**1.000**
	Internship time reduced	20 (60.6%)	6 (33.3%)	[2]**0.117**

[1]*Fisher's Exact Test* [2]*Continuity (Yates) Correction* **p<0.05*

The rate of online theoretical course exams (92.5%) in state universities in the spring term of 2020 was found to

Table 6. *Evaluation of the parameters related to the method of continuing the theoretical education of the faculties during the pandemic according to the education period of the faculties*

		Faculty education period			p
		0-5 years	6-10 years	11 years and above	
		n (%)	n (%)	n (%)	
The method of continuing theoretical education in the 2020 spring semester of the pandemic	Online	19 (67.9%)	10 (71.4%)	19 (76%)	[2]**0.001***
	With offline videos	2 (7.1%)	4 (28.6%)	1 (4%)	
	With hybrid education	0 (0%)	0 (0%)	5 (20%)	
	With lecture notes	7 (25%)	0 (0%)	0 (0%)	
The method of continuing theoretical education in the 2020 autumn semester of the pandemic	Online	19 (67.9%)	9 (64.3%)	20 (80%)	**0.618**
	With offline videos	2 (7.1%)	1 (7.1%)	0 (0%)	
	With hybrid education	7 (25%)	3 (21.4%)	4 (16%)	
	With lecture notes	0 (0%)	1 (7.1%)	1 (4%)	
The method of continuing theoretical education in the 2021 spring semester of the pandemic	Online	21 (75%)	9 (64.3%)	18 (72%)	**0.492**
	With offline videos	1 (3.6%)	1 (7.1%)	0 (0%)	
	With hybrid education	6 (21.4%)	4 (28.6%)	5 (20%)	
	With lecture notes	0 (0%)	0 (0%)	2 (8%)	
Presence of clinical students	Yes	25 (89.3%)	12 (85.7%)	25 (100%)	[1]**0.174**
	No	3 (10.7%)	2 (14.3%)	0 (0%)	
Continuation of clinical internships in the 2020 spring pandemic period (n=51)	Face to face	5 (20%)	1 (8.3%)	3 (12%)	[2]**0.045***
	Postponed to autumn semester	8 (32%)	6 (50%)	11 (44%)	
	Not done – online exam only	1 (4%)	3 (25%)	7 (28%)	
	With videos	3 (12%)	1 (8.3%)	4 (16%)	
	By giving homework	8 (32%)	1 (8.3%)	0 (0%)	
Continuation of clinical internships in the 2020 autumn pandemic period (n=51)	With case discussions	1 (4%)	7 (58.3%)	7 (28%)	[2]**0.040***
	Other	14 (56%)	2 (16.7%)	12 (48%)	
	Face to face	4 (16%)	1 (8.3%)	3 (12%)	
	Postponed to spring semester	3 (12%)	2 (16.7%)	2 (8%)	
	Not done – online exam only	3 (12%)	0 (0%)	1 (4%)	
Continuation of clinical internships in the 2021 spring pandemic period (n=51)	With videos	8 (32%)	9 (75%)	15 (60%)	[2]**0.132**
	By giving homework	10 (40%)	3 (25%)	6 (24%)	
	With case discussions	3 (12%)	0 (0%)	4 (16%)	
	Face to face	1 (4%)	0 (0%)	0 (0%)	
	Won't – only online exam will be done	3 (12%)	0 (0%)	0 (0%)	

*[1]Fisher Freeman Halton Test [2]Chi-square Test *p<0.05*

be statistically significantly higher than private universities (70.4%) (p:0.010; p<0.05). Alternatively, private universities gave homework instead of exams. On the other hand, the rate of believing that online exams accurately measure students' knowledge in state universities (0%) was statistically lower than that of private universities (11.1%) (p:0.032; p<0.05). However, there is no difference between universities in terms of precautions taken for exam security.

The evaluation of the parameters related to the theoretical and practical education continuation during the pandemic according to the education duration of the faculties was given in Table 6. The rate of continuing the theoretical education online (67.9%) in the 2020 spring term of the pandemic of the faculties with education duration between 0-5 years was found to be statistically significantly lower than the other groups (p<0.05).

4. DISCUSSION

Covid-19 pandemic has adversely affected every field of life all over the world; but the largest impact was seen in the field of education (11). With the beginning of the pandemic, almost all faculties have been start to distance education instead of face-to-face training. Especially the faculties, which has practical training, were more affected by this situation (12,13). Dental education is a training, in which theoretical information has been supported by practical training. According to the results of this study, dentistry faculties in our country continued online education in theoretical courses during the three terms of the pandemic. But they have preferred to return to face-to-face education for preclinical and clinical trainings together with the initiation of vaccination and providing protective equipment.

Dental education consists of 3 parts. The first part is theoretical lectures and are very easy to adapt to the distance education

model. The latter is preclinical education (simulation laboratory courses). In this training, students work on the models and the instructor controls each stage. The distance education alternative to this part can only be with videos or virtual reality devices. When the time and human power required for this training is considered, it is very difficult to teach preclinical training online (6). In addition, according to the results of our study, many faculties do not have the grant and infrastructure for virtual reality devices. The third part is the clinical practice. In the clinical practice, students treat real patients and no alternative methods, including haptic devices, cannot fully meet this training. For this reason, the last part is the most difficult part of dental education to deal with pandemic environment (2).

With the onset of the Covid-19 pandemic, dentistry has been reported to be the most risky profession, and practical dental education has been suspended almost all over the world (14). Educational alternatives have been started to be considered in order to continue the education process, to enable students to gain the necessary competence, but also to protect them from the virus. As an alternative to direct learning strategies, most dental schools have adopted distance education strategies such as online courses and lectures, case discussions and webinars (13). According to the results of this study, depending on the decision of CoHE, all lectures were given online in the spring 2020 in our country, and it was thought as an alternative that the clinical trainings could be postponed until after the pandemic. Some universities, on the other hand, have made online case discussions instead of clinical practice. This result is in agreement with studies conducted in Europe, America, Pakistan, Saudi Arabia and Asia-pacific countries (2,6,8,13,15). In China, where the pandemic emerged, 97% of dentistry faculties switched to online education during the pandemic, and less online education was preferred for practical training (16). Similarly, 71.6% of the universities in our country preferred online education for theoretical courses in the spring term of 2020. Preclinical education was conducted online at a lower rate (40.3%), while clinical education was either not done (35%) or postponed (23%). In many studies in the literature, the authors agree that providing preclinical and clinical education by distance education method or virtual reality systems cannot replace face-to-face education (13,16).

In the autumn 2020, CoHE gave the initiative regarding education model during the pandemic to universities (17). Considering that the pandemic will last longer, it has emerged that education in dentistry faculties should continue and it will not be possible to graduate for students who do not clinical practice (8). For this reason, the universities aimed to create an environment that provides safety for students, faculty members and staff and to continue their preclinical and clinical training. The results of the present study demonstrated, clinical practice, which was mostly concluded with online exams in the spring 2020 (35.3%), continued by face-to-face training in the autumn of 2020 (49%). Similar results are obtained for preclinical training. In line with our country, a concept that returns to “face-to-face” training has started in Germany in July 2020, despite the COVID-19 pandemic (18). According to the results of the present study, more than half of the faculties have provided all the protective equipment to the students in order to return face-to-face training. In addition, the vast majority of them restored the multi-units open clinics in a way that divided the units, and made adjustments to the appointment durations, daily number of patients and ventilation systems. The state's inclusion of all practicing students in the vaccination program has also been very effective in returning to face-to-face education.

The academicians participating in this study stated that the learning level of the students with online education was moderate (65.7%) and less (23.9%) compared to face-to-face education. Similarly, in many studies in the literature, students reported that the efficiency they received from online lectures was less than face-to-face education (12,15). In addition, students stated that they wanted to continue face-to-face education especially for practical courses after the pandemic and that distance education can only be considered as a complement to traditional education methods (11,15,18). In studies conducted in Germany and Turkey, the majority of both, students and educators, reported that they preferred the hybrid education model in which distance education continues to a certain extent in the future. In our country, many universities still continue their education with a hybrid education model. Again, in these studies, students wanted clinical practice to be supported by detecting their deficiencies during the pandemic period (12,18). This deficiency has been tried to be eliminated with case discussions in our country, and 7.8% of universities continued their clinical education with this education method during the pandemic. Although case discussions are helpful in adaptation theoretical knowledge to practice, it is not a substitute for clinical experience. Additional training such as courses for new graduates should be given in order to eliminate the clinical deficiencies. As a matter of fact, nearly half of the faculties (47.8%) have created an additional program to make up for the missing education in the 2020 spring term.

Updates had to be made not only in education methods but also in assessment and evaluation systems. In the study of Hardy et al. (13), academicians stated that the most appropriate preclinical exam technique is to conduct virtual exams in simulation laboratories (39.3%) and postpone exams until after the pandemic (24.6%). For clinical practice, they suggested postpone the exams (22.1%). Contrary to this result, online exams (51.1%) and homework (28.8%) were preferred for preclinical education in our country. It is thought that the practical deficiencies of the students will be covered with the compensation programs (47.8%) to be made later. However, the faculties preferred to postpone (23.5%) the education for clinical practice. Universities in Europe have planned to extend internship periods instead of reducing the clinical requirements in order to graduate (8). In the present study, reducing the clinical requirements (61.2%) was preferred rather than extending the internship

period (4.5%). Additionally, nearly half of the faculties in our study did not believe that online exams measure knowledge correctly, and 80.6% of them had to take extra precautions such as time restrictions and log records to avoid cheating. Because audit mechanisms are weak in online exams. A special online exam platform may also need to be developed for the reliability of online exams in dental education in the future.

In this study, the education methods of state and private universities during the pandemic period were compared. Only in the spring term of 2020 (in the first period of the pandemic), the rate of continuing theoretical education online in state universities was found to be higher than in private universities. However, private universities quickly completed their deficiencies (distance education platform, online course materials, etc.) and there was no significant difference between universities in terms of coursework in other terms. Contrary to this study, it was reported that state schools in Pakistan were less successful than private schools in distance education (15). In addition, this study demonstrated that the rate of continuing theoretical education online in the spring 2020 of the faculties with a education period between 0-5 years was found to be lower than other groups. They continued their education mainly with lecture notes. The reason for this can be interpreted as the fact that the new faculties do not have the distance education infrastructure and sufficient faculty members to cope with this extraordinary situation. When examined in terms of clinical practice, in this study, the rate of providing all personal protective equipment to clinical internship students of state universities was found to be higher than private universities. It has been observed that state universities can obtain protective equipment faster with the support of the state, while private universities demand that some of the protective equipment be covered by the students. In a study in which many countries from Europe, America, Asia and Africa participated, only 44% of academicians said that they could access N95 masks (19). According to the results of this study, we can assume that the rate of access to personal protective equipment is much higher in our country (72.5%).

Despite all these findings, it is possible to say that the pandemic has pros as well as cons. Although the pre-pandemic distance education model started slowly in some countries, it was rarely used especially in the field of dentistry (13). However, with the pandemic, the hybrid education model, in which the theoretical courses are given with distance education and the practical courses are theach face-to-face in dental education, has been adopted by both educators and students. Therefore, we can state that our hypothesis was confirmed. In addition, work on modern virtual simulation devices, haptic technologies, and the use of artificial intelligence in dental education has accelerated, but needs further development.

There are limitions of the present study. First, due to the cross-sectional design of this study, the educational methods of universities were recorded over a period of time, and changes may have occurred in the methods of some institutions after data collection. Second, all dentistry faculties in Turkey could not be reached. Long-term studies including several dentistry faculties from different countries are needed in the literature.

5. CONCLUSION

Distance education model was preferred for theoretical courses in our country during the Covid-19 pandemic. However, it was more preferred to return to traditional methods in practical training. The hybrid education model, which has just entered our lives in the field of dentistry, is used in almost all universities. All faculties that include practical courses, such as dentistry faculties, should accept this pandemic as an opportunity, share their experiences in this process, identify their deficiencies, and develop appropriate education policies that include distance education model permanently against possible future pandemics.

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Conflicts of interest

The authors have no conflict of interest to disclose.

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Clinical and Experimental Health Sciences

Pediatricians' Knowledges, Attitudes, and Practices on Parafunctional Oral Habits and Orthodontic Problems in Children

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ABSTRACT

Objective: Treatment of dentofacial deformities via orthodontics can improve the health of the teeth and the gums and also numerous malpositioned teeth and jaws, and increase the quality of life in children affected by a malocclusion. The purpose of this research was to examine if pediatricians referred their patients to pediatric dentists, if they had sufficient knowledge and awareness in parafunctional habits and basic orthodontic principles, and if they examined their patients for parafunctional habits and malocclusions.

Methods: A descriptive, cross-sectional survey was sent to a sample of pediatricians in Turkey. The questionnaire consisted of 42 questions in five domains. A total of 166 pediatricians participated in the survey.

Results: In terms of the examination of the oral cavity, a low frequency of examination for the malocclusion (28.9%) and oral functional habits (43.4%) was detected. The reasons pediatricians referred patients to dentists varied from over-bite (24%) to crowding (87%). In the Chi-Square Test for the effect of the location of practice, the duration of practice, training about parafunctional oral habits, and orthodontic problems, patients examined per day on knowledge, attitude, and practices of pediatricians regarding orthodontic problems and parafunctional oral habits in children, significant relations were detected ($p<0.05$).

Conclusion: Although the majority of the participants were aware that pediatricians have an important place in the prevention of parafunctional oral habits and orthodontic anomalies, they did not have the pertinent knowledge and practice to apply a complete and systematic examination for parafunctional oral habits and malocclusion.

Keywords: children, malocclusion, pediatricians, perception, referral.

1. INTRODUCTION

In dentistry, malocclusion is a healthcare issue considered deeply. It is also the third most prevalent oral disease and is described as a condition associated with abnormal relations between the teeth or dentition in any of the dimensions. Malocclusion, which may cause dental caries, periodontal disease, and increased possible temporomandibular disease, is considered a public health problem (1). Dentofacial appearance has a significant impact on individuals, especially children (2,3). Shaw et al. reported that children were teased about their dentition more than any other factors (4). Malocclusion may therefore affect the individual's quality of life and self-esteem (3). Thus, a referral may significantly and positively change an individual's life. Early intervention performs the same function as interceptive orthodontics and prevents or reduces progression to fully developed malocclusion later in life and also excludes factors that inhibit the regular development of dental arches (5). Furthermore, early orthodontic treatment has been found to improve both psychosocial development and masticatory function in children. Posterior crossbites that prevent function, anterior crossbites resulting in traumatic occlusion and damage to the lower anterior teeth, anterior open bites that cause masticatory dysfunction as well as esthetic distress are some of the many indications for early treatment. In addition, prevention of trauma to the anterior teeth of individuals with severe Class II malocclusions with increased overjet is an indication for early orthodontic treatment (6,7). Otherwise, people who receive orthodontic treatment can clean teeth more effectively. This might end up in decreased dental caries and periodontal diseases at significant levels (8).

Oral harmful habits include thumb or lip-sucking, bottle-feeding, tongue-thrusting, nail-biting, lip-biting, and mouth-breathing patterns, which have direct effects on the quality of life as they affect the stomatognathic systems (9). Previous authors argued that oral habits played significant roles in the development of dental anomalies and malocclusion

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if persisted beyond preschool age because they caused a disequilibrium between intra-and extra-oral muscular acts (10-12). These habits might cause poor dental health, be socially abasing, and avoid the development of speech clearance. For this reason, habits need a multidisciplinary approach for providing integral care for child patients. These habits need to be eliminated early using the appropriate protocols, including habit breakers to reduce the risk factors of developing malocclusions (13). There is a need for educating and increasing awareness of the pediatricians as well as teachers, children, parents about the harmful effects caused by such habits on the development of normal occlusion and the importance of timely intervention (14). The early treatment of orthodontic anomalies in the deciduous and early mixed dentition aims to prevent the development of significant anomalies in late mixed and permanent dentition to reduce or even eliminate the need for subsequent orthodontic treatment (15).

Pediatricians are pivotal in coordinating, collaborating, and communicating with interdisciplinary professionals to provide comprehensive care (16). Pediatricians are often the first healthcare professionals examining children in this respect. They are more likely to examine children earlier than pediatric dentists. Oral healthcare has an integral part of overall health. Therefore, they have a unique position in detecting and guiding oral and dental health diseases. They could serve as a good point of referring patients with malocclusion and parafunctional habits. Pediatricians may help in the early diagnosis of orthodontic issues, which might advance the treatment effect and its constancy over the years (17,18).

Cooperation between pediatricians and pediatric dentists is essential for the continuity of general health and oral health (19). For this reason, it is important that pediatricians perform the first orthodontic examinations to diagnose abnormalities early and refer the patients consequently. As fas as we are concerned, there is only one study published worldwide conducted by Koufatzidou et al. (20) focusing on the assessment of awareness, knowledge, attitude, and practices of pediatricians on orthodontic problems and parafunctional habits in children.

The purpose of this research is to investigate the knowledge, attitudes, and practices of pediatricians who work in Turkey, concerning the prevention of parafunctional oral habits and malocclusion, and to raise awareness regarding the importance of pediatricians in preventing orthodontic problems in children.

2. METHODS

This report is based on a questionnaire conducted among Turkish pediatricians including those with post-graduation levels, working in government, private, and other healthcare sectors. Ethical permission required for the study to be carried out was obtained from Gaziosmanpaşa Training and Research Hospital, Medical Research Local Ethics Board (Ethical board date/number: 26.05.2021/256).

The questionnaire used in our study consists of five chapters containing questions about the sociodemographic information of the participants, the risk factors of malocclusion, the evaluation of the attitudes of pediatricians towards the prevention of malocclusions and parafunctional habits, the evaluation of the behaviors of pediatricians on the examination and treatment of malocclusion and parafunctional habits, and the evaluation of knowledge levels and sources of information on malocclusion and parafunctional habits of pediatricians. The first section of questionnaire included sociodemographic data such as age, gender, title, institution where the specialty training was obtained, years of clinical experience, hours of patient care per week, and the number of patients seen per day. The second part included questions measuring the level of knowledge of pediatricians about risk factors related to malocclusion. In the third and fourth sections, physicians were asked about the attitudes and practices regarding the prevention of malocclusion and orthodontic examination practices (knowledge about orthodontic problems, whether they have performed orthodontic examinations, ways of referring patients to pediatric dentists); and in the fifth part, it was questioned if pediatricians received training on parafunctional oral habits and prevention of malocclusion and the source of their knowledge.

Google forms were used to create the questionnaire. Before starting the study, to test the comprehensibility and consistency of the questionnaire within the scope of the study, the questions were sent to 5 experts, 2 pediatric dentists, 2 pediatricians, and 1 biostatistician. Therefore, biased and confusing questions were omitted. A pilot study was performed before uploading the questionnaires online, to assess the appropriateness and clarity of the questions. The pilot study sample included participants representing ten pediatricians, and each received a hard copy of the questionnaire. The links to questionnaires were sent to pediatricians in Turkey by email, WhatsApp, and private social network platforms starting May 28, 2021, and the questionnaires were closed on June 18, 2021. A brief introduction was presented at the beginning of the survey to inform the respondents of the purpose and content of this study, and electronic informed consent was obtained if they agreed to complete the questionnaire. The minimum required respondents were predicted to be 160, with an estimated margin of error of 5% and 80% sample power in the OpenEpi power analysis program. A total of 166 Turkish pediatricians, all volunteers, answered the questionnaire.

2.1. Statistical Analyses

The statistical evaluations were made by using the SPSS (ver. 22.0. Chicago. II. USA) program. The relationship between pediatricians' age, gender, work sector, years of experience, patients per day, subspecialty, orthodontic treatment in children and relatives, training about parafunctional oral habits

and orthodontic problems, and the knowledge, attitude, and practices of pediatricians regarding orthodontic problems and parafunctional oral habits in children were determined by the Chi-Square Test. Percentage frequencies were used in descriptive statistics. The significance level was accepted as $p<0.05$.

3. RESULTS

The number of participants agreeing to take part and completed the forms was 166 (43.4% female, and 56.6% males; 30.1% subspecialists in pediatrics, and 69.9% non – subspecialists in pediatrics). The fields of participants in the pediatrics subspecialty are as follows; endocrine, pediatric neurology, pediatric cardiology, hematology, neonatology, allergy, gastroenterology, emergency, newborn, pediatric genetics, pediatric infection, pediatric pulmonology, social pediatrics. According to the duration of practice, 64.5% reported experience of more than 10 years, and 48.8% said they were working in a state hospital. Many reported working duration up to 40 hours a week (86.1%). Various sociodemographic details of the study participants are available in Table 1.

Table 1. *Socio-demographic characteristics of respondents*

Gender	
Male	72 (43.4)
Female	94 (56.6)
Age	
20-30	10 (6)
31-40	107 (64.5)
41-50	37 (22.3)
>50	12 (7.2)
Subspecialty	
No	116 (69.9)
Yes	50 (30.1)
Location of Practice	
Government Hosp.	81 (48.8)
Private Hosp.	28 (16.9)
University	54 (32.5)
Years at work	
0-9	59 (35.5)
10-19	79 (47.6)
>19	28 (16.9)
Hours per week	
<40	72 (43.4)
>=40	143 (87.2)
Patients per day	
<=10	18 (10.9)
11-20	24 (14.5)
21-30	30 (18.2)
>30	93 (56.4)

Of the total sampling, 71.7% knew the risk factors of malocclusion. However, a deficiency of knowledge was found on the argument that the severity of ankyloglossia increases and the likelihood of malocclusion increases (45.2%). However, more than half of the sample, 86.1% had sufficient knowledge regarding that long-term feeding of breast milk or formula with a bottle increases the likelihood of parafunctional oral habit and malocclusion. Similarly, 81.5% of the participants had sufficient knowledge regarding that if the duration of pacifier use is prolonged, the possibility of open-bite increases. For the knowledge regarding the probability of occurrence of malocclusion in children, 72.3% of the participants were aware of the effect of parafunctional oral habits. Moreover, 54.4% of the participants knew that premature and low birth weight increases the susceptibility to malocclusion by affecting tongue functions in children. Similarly, 54.4% were knowledgeable regarding that breastfeeding for longer than 6 months reduces the possibility of malocclusion; and 71.7% of the participants answered the question that supporting healthy chewing function in early childhood can help to prevent malocclusion correctly. For the possibility of malocclusion in children with snoring and respiratory problems (mouth breathing), 71.7% of the participants answered this question correctly. Regarding pediatricians' knowledge levels about various orthodontic problems, knowledge of diastema was the most (80.1%), while knowledge of overbite was the least (24.4%) (Table 2).

Table 2. *Pediatricians' knowledge levels about orthodontic problems and risk factors of malocclusion*

Question Response		n (%)
Children with parafunctional oral habits are more likely to develop malocclusion. Do you have any information about this?	No Yes	46 (27.7) 120 (72.3)
Breastfeeding for longer than 6 months reduces the likelihood of developing parafunctional oral habits and malocclusion. Do you have any information about this?	No Yes	74 (44.6) 92 (55.4)
Long-term feeding of breast milk or formula with a bottle increases the possibility of developing parafunctional oral habits and malocclusion. Do you have any information about this?	No Yes	22 (13.3) 143 (86.1)
Preterm birth and low birth weight increase susceptibility to malocclusion by affecting tongue functions. Do you have any information about this?	No Yes	74 (44.6) 92 (55.4)
If the pacifier use time is prolonged, the possibility of open-bite increases. Do you have any information about this?	No Yes	30 (18.1) 136 (81.9)
Children with snoring and breathing problems (mouth breathing) are more prone to malocclusion. Do you have any information about this?	No Yes	46 (27.7) 119 (71.7)
The higher the severity of the tongue ligament (short, thick frenulum), the more likely it is to develop malocclusion. Do you have any information about this?	No Yes	75 (45.2) 91 (54.8)
Supporting healthy chewing function in early childhood can help prevent malocclusion. Do you have any information about this?	No Yes	47 (28.3) 119 (71.7)

A total of 91.6% believed that they played important roles in preventing malocclusion and parafunctional oral habits in

children. Also, 97.0% considered dental visits for preventing malocclusion and parafunctional oral habits. About the fact that pediatricians have to examine children's teeth and oral cavities, 92.8% of the participants responded positively. Moreover, when they were asked if the malocclusion can be prevented 91.0% of the participants replied positively (Table 3).

Table 3. *Questions related to attitude domain among the pediatricians*

Question	Response	n (%)
The pediatricians have to examine the oral cavity.	No Yes	12 (7.2) 154 (92.8)
Dental examination is important in the prevention of malocclusion and parafunctional oral habits.	No Yes	5 (3) 161 (97)
Malocclusion can be prevented.	No Yes	22 (13.3) 143 (86.1)
Pediatricians play an important role in the prevention of malocclusion and parafunctional oral habits in children.	No Yes	14 (9) 151 (91)

They were also asked if they evaluated the oral functional habits of their patients. While 41.6% of the sample indicated that they evaluated when the patient had a complaint about this subject, 43.4% of the sample indicated that they evaluated every time. Only 28.9% of the participants reported performing routine oral examinations for malocclusion. Participants were asked regarding the time for the child for the first oral health examination. A total of 30.1% of the sample indicated that they counseled the first oral health examination when the child was 0-2 years old. Moreover, when they were asked regarding how often an oral health examination was recommended, only 49.4% of the participants indicated it as six months. Moreover, participants were asked if they would refer a child to pediatric dentists when they identified a child with malocclusion or any parafunctional habits. Most of the sample (97.6%) indicated that they would refer. The type of referral was mostly advising parents/guardians to go to a pediatric dentist (80.1%). The answer to the question when your patient, whom you referred to a pediatric dentist, comes to you for a check, do you check whether a pediatric dentist examination has been done was mostly "Yes" (75.9%) (Table 4). Their answers for the reasons for referral due to orthodontic problems to pediatric dentists differed for each condition from 24% for over-bite to 87% for crowding. On the other hand, their answers for the reasons for referral due to parafunctional oral habits and developmental anomalies to pediatric dentists differed for each condition from 5.3% for nail-biting to 38.4% for prolonged use of pacifiers. On the other hand, prolonged use of pacifiers was the most common reason for referral due to parafunctional oral habits and high labial frenulum attachment was the most common reason for referral to (38.5%) due to developmental anomalies.

The overall good scores of the knowledge, attitudes and practices with a combined overall score in which a discrepancy was detected between the knowledge, attitudes, and practices of the participants are given in Table 5. Many participants had a high percentage of good attitudes (94.0%) when compared with good knowledge (71.2%) and good practice (61.2%) (having good practice was the least). No significant differences were detected between females and males in terms of knowledge, attitudes, and practices ($p > 0.05$). In the distribution of good practice, it was seen that the highest rate was among those working in private hospitals, and the lowest rate was among those working in state hospitals. When the distribution of good combination scores was compared according to years of experience, it was found that dental professionals who had >19 duration of practice had the highest level of good combination scores. Good knowledge, good practice, and good combination scores respectively 87.8%, 78.0%, 100% were found to be statistically significantly higher in those who received training about parafunctional oral habits and orthodontic problems than those who did not. On the other hand, good practice scores of dental professionals (76.2%) who saw less than 20 patients a day were significantly higher than those who saw more than 20 (56.6%).

Table 4. *Questions related to practice domain among the pediatricians*

Question	Response	n (%)
Do you evaluate the oral functional habits of your patients?	No If any problem Yes	25 (15.1) 69 (41.6) 72 (43.4)
Do you perform oral health examinations for malocclusion for your patients?	No If any problem Yes	39 (23.5) 79 (47.6) 48 (28.9)
When do you perform the first oral health examination for your patients?	I don't Immediately after birth 0-2 >=2	13 (7.8) 90 (54.2) 50 (30.1) 12 (7.2)
When would you recommend the first oral health examination to your patients?	Immediately after birth 0-2 >=2	68 (41.0) 80 (48.2) 17 (10.2)
How often are you recommended oral health examination?	If any problem per 6 mo. per one year	24 (14.5) 82 (49.4) 59 (35.5)
Do you refer your patient to a pediatric dentist when you diagnose malocclusion or any parafunctional oral habit?	No Yes	3 (1.8) 162 (97.6)
If your answer to the previous question is yes, how do you do the referral?	I advise families to go to a pediatric dentist. I refer the pediatric dentist I work with by giving information myself. I consult the pediatric dentist through the system.	133 (80.1) 12 (7.2) 18 (10.8)
When your patient, whom you referred to the pediatric dentist, comes to you for a check-up, do you check whether the pediatric dentist examination is performed?	No Yes	38 (22.9) 126 (75.9)
Do you have any patients you consult with pediatric dentistry?	No Yes	67 (40.4) 96 (57.8)

The participants preferred several methods to receive dental education and training on parafunctional oral habits and orthodontic anomalies. Workshops and colleagues were the most preferred method, (99.4%). Furthermore, previous dental training about orthodontic problems in children was reported by only 12.6% of participants. On the other hand, their responses for receiving dental education regarding orthodontic problems and protective measures in children during their medical or specialty training were mostly "I did not receive any dental education and training" (80.7%). They were asked if there were any children among their children or relatives who received orthodontic treatment, and 59.6% of the sample indicated that there were. Most of the participants (96.4%), indicated that they needed more knowledge about parafunctional oral habits and prevention of malocclusion (Table 6).

Table 5. *Relationship of overall scores of each domain–knowledge, attitude, and practice, and combined to various factors*

	good knowledge		good attitude		good practice		good combination	
	n (%)	p	n (%)	p	n (%)	p	n (%)	p
Gender								
Male	52(72,2)	0,893	68(98,4)	0,824	46(63,9)	0,535	65(90,3)	0,321
Female	67(71,3)		88(93,6)		55(59,1)		80(85,1)	
Work sector								
State hospital	60(74,1)	0,031*	74(91,4)	0,415	38(47,5)	0,001*	68(84,0)	0,08
Private hospital	24(85,7)		27(96,4)		24(85,7)		28(100,0)	
University	32(59,3)		52(96,3)		36(66,7)		46(85,2)	
Duration of practice								
0-9	42(71,2)	0,912	57(96,6)	0,342	37(62,7)	0,173	54(91,5	0,01*
10-19	56(70,9)		72(91,1)		43(55,1)		63(79,7)	
>19	21(75,0)		27(96,4)		21(75,0)		28(100,0)	
Subspecialty								
Yes	33(66,0)	0,286	112(96,6)	0,068	25(51,0)	0,081	42(84,0)	0,394
No	86(74,1)		44(88,0)		76(65,5)		103(88,8)	
Training about parafunctional oral habits and orthodontic problems								
Yes	36(87,8)	0,008*	40(97,6)	0,454	32(78,0)	0,011*	41(100,0)	0,005*
No	83(66,9)		116(92,8)		69(55,6)		104(83,2)	
Orthodontic treatment in pediatricians' children and relatives								
Yes	70(70,7)	0,825	95(96,6)	0,174	62(62,6)	0,539	90(90,9)	0,079
No	47(72,3)		59(90,8)		37(57,8)		53(81,5)	
Patients per day								
<=20	30(71,4)	1,000	40(95,2)	0,739	32(76,2)	0,024*	39(92,9)	0,252
>20	89(72,4)		115(93,5)		69(56,6)		106(86,2)	

Chi-square test **p<0.05*

Table 6. *Evaluation of training and education among the pediatrician*

	Variable Response	n (%)
I need more education and training about parafunctional oral habits and malocclusion prevention.	No Yes	6 (3.6) 160 (96.4)
Have you received any training on orthodontic problems in children and the approaches to be applied?	No Yes, I got it in my medical school education. Yes, I got it my specialist training.	134 (80.7) 18 (10.8) 3 (1.8)
Have you received any training on parafunctional habits (bad oral habits) and approaches to be applied in children?	No Yes, I got it in my medical school education. Yes, I got it my specialist training.	125 (75.3) 21 (12.7) 20 (12)
Do any of your children or relatives receive orthodontic treatment?	No Yes	65 (39.2) 99 (59.6)

4. DISCUSSION

This research was conducted to emphasize the need to improve the relationship between the pediatrician and the pediatric dentists, and also to work on the areas that physicians need to improve their knowledge about orthodontic problems and to recognize the importance of pediatric dentistry. Orthodontic anomalies cause important global oral health issues, but parents do not take their children to the dentist for regular examination of orthodontic problems as early as they do with pediatricians; and therefore, pediatricians are considered credible and excellent sources to promote oral health and prevent malocclusion (21). However, our study showed a lack of knowledge and practice among pediatricians regarding parafunctional oral habits and orthodontic problems in children. The results of our study indicated a discrepancy between the practice, knowledge, and attitudes of participants whose practice score was lower (61.2%) compared with attitude scores (94.0%) and knowledge scores (71.2%). The majority of the sample had admissible attitude levels on malocclusion and risk factors, but few of them reported oral health-related activities, which comply with the data of an American national survey of Lewis et al. (22). We could only identify one survey regarding pediatricians' awareness of orthodontic problems and related conditions in the literature (20). Since only one related previous study was found on this subject, we could only use this pre-existing survey/questionnaire as a valid source (20). According to the results of this previous survey with 96 pediatricians in Greece, the majority were aware of the importance of examination of the oral cavity, but they did not have appropriate knowledge for performing a full and systematic screening in terms oforthodontic problems. In this previous study, a low frequency was detected in the examination of the position of teeth (54%) and jaws (51%), which is in line with this study. Similarly, the results of this study show that physicians examine the oral cavity for malocclusion when the patient has a complaint about this subject (47.6%). The reasons pediatricians referred patients to specialists varied from mouth breathing-snoring 24% (23/96) to face or teeth asymmetry 87% (84/96) (20). In this study, the reasons pediatricians referred patients to specialists varied from over-bite 24% to crowding 87%.

However, we could also compare our results with studies that assessed pediatricians' practice, knowledge, and attitudes for oral health and dental caries (22-24). Some studies reported poor knowledge on oral health among pediatricians (22,25,26) and studies from Turkey (27), as well as in Canada (28), and some others reported contrary results (22,29).

The knowledge and understanding of healthcare providers showed that approximately 55.4% of pediatricians could relate the importance of breastfeeding and oral health. Infants' oral muscles are exercised strenuously in suckling in breastfeeding, which is an important effect on the thrust and growth of the mandible (30).

Moreover, many participants (%92.8) believed that pediatricians must examine the teeth of children as part of their daily practice. The study of Di Giuseppe et al. (31) reported that pediatricians (%96.6) considered their roles in the examination of children's teeth. Most participants (%91) considered their roles in children's teeth examination, but few of them reported evaluating children's oral functional habits (%43.4) and the oral health examination for malocclusion (%28.9). Similarly, Alshunaiber et al. (21) and Di Giuseppe et al. (31) reported a low percentage of pediatricians who performed oral health examinations for children. However, the study of Indira et al. (32) found better practice levels, and many pediatricians (%98.9) reported that they included children's teeth examination in routine practice. Considering that pediatricians encounter pediatric patients earlier and more frequently and can play an important role in early diagnosis, it becomes clear that pediatricians in our country should be informed about parafunctional oral habits and orthodontic problems in children and encouraged.

While easily diagnosed conditions such as early loss of primary teeth and crowding in dentition resulted in high referral frequency, other anomalies were not common reasons for referral. Therefore, it might be considered that pediatricians have limited basic dental training, and this causes low confidence in oral cavity screening, recommendation, or consultation (21). A previous study found that many orthodontists recommended that the first assessment of occlusion must be done before the age of 7, and crossbites must be preferably applied during primary and early-mixed dentition stages (33). Proper and timely evaluation of malocclusions and the associated factors with malocclusions in primary dentition might help to prevent and manage occlusion-related problems better in life.

In the present study, as in the study of Sezer et al. (27), low-level knowledge on some aspects of children's parafunctional oral habits and orthodontic problems together with the associated practice among pediatricians might be related to the lack of required dental training and education of the majority of participants. Also, decreased frequency in the practice of children's oral health-related activities among pediatricians who saw more than 20 patients a day and worked in state hospitals may be associated with a lack of clinical time for detailed examination.

In the present study, a significant portion of the participants (91%) reported that pediatricians have important roles in preventing malocclusion and parafunctional oral habits. However, 96.4% of them stated that their knowledge level on the prevention of malocclusion and parafunctional oral habits is not sufficient and they need more information. This result reveals that pediatricians should be informed more about the subject both during and after medical education.

Limitations

The study has certain limitations. First of all, online self-assessment questionnaires may be affected by the difficulty of completing them. This could affect the validity of the data provided. In the questionnaire, it was asked whether

the participants refer the patients to a pedodontist when orthodontic problems are the issues. However, some pediatricians might refer the patient to an orthodontist or even a dentist. But it doesn't mean that they don't guide the patient when they refer the patient to an orthodontist or dentist. This also might affect the results.

5. CONCLUSION

Malocclusion and parafunctional habits can negatively affect the quality of life, self-esteem, and health of the periodontium. Pediatricians have the potential to make a significant impact on their clients' oral health by identifying parafunctional habits and malocclusion early and making referrals. Establishing an oral and occlusal assessment during general health appointments reinforces comprehensive client care and provides greater opportunities for interprofessional collaboration. Understanding the etiology, risk factors, and impact of malocclusions will support pediatricians in making informed decisions and in achieving comprehensive person-centered care plans.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Clinical and Experimental Health Sciences

The Evaluation of Knowledge and Behavior of Medical Doctors about Toothbrush Disinfection

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ABSTRACT

Objective: The behavior and knowledge of medical doctors about toothbrush disinfection was evaluated in this study.

Methods: In this study 170 medical doctors (63 females, 107 males) were included. Participants filled out the standard questionnaires which contained 18 questions into three parts: demographic data, personal oral hygiene practices-patient recommendations about toothbrush disinfection, and toothbrush disinfection. chi-squared and Fisher's exact tests were used for data analyses, where appropriate.

Results: Regarding personal oral hygiene practices; most medical doctors reported toothbrushing at least twice a day and changed their toothbrushes once every three months. The answers of males and females for these questions were significantly different ($p < 0.05$). Most participants used the same toothpaste and stored their toothbrushes in an open-topped toothbrush holder shared with other individuals. The doctors mostly did not any advice their patients about toothbrush usage.

Conclusion: Regarding toothbrush disinfection; most medical doctors did not disinfect their own toothbrushes and reported a lack of knowledge about toothbrush disinfection. On the other hand, the participants mostly thought that disinfection of toothbrush was necessary for every individual. The results of this study showed that majority of the participants were unaware about the subject.

Keywords: Toothbrushing, Disinfection, Medical Doctors

1. INTRODUCTION

Toothbrushes are the most important oral hygiene tool for good oral health and are manufactured as free of microorganisms. After a single use, toothbrushes may become heavily contaminated with several microorganisms in the oral cavity, aerosol contamination, hands, environment and storage conditions (1). Many microorganisms which can remain their viability on toothbrush bristles for periods ranging from a day to a week are found after toothbrushing (2,3). In many systemic and oral diseases (e.g septicemia and gastrointestinal, renal problems, respiratory, cardiovascular), contaminated toothbrushes may play important roles (1).

Although Cobb reported that toothbrush is a cause of repeated infections in the oral cavity as early as 1920, toothbrush disinfection is become attract attention by various authors in last decades (4). Previous studies reported that toothbrush disinfection is required for high-risk patients including children, elders, susceptible populations (*e.g.*, critically ill adults) in a hospital setting and chemotherapy or undergoing organ transplantation or immunosuppressed (1,5). Toothbrushes can serve as reservoir for healthy individuals with poor oral health as well as high-risked patients due to toothbrushes frequently stored in the bathroom or close to the toilet and sink and may be exposed to enteric bacteria dispersed by aerosols (5,6).

Toothbrush contamination much more increases depending on careless usage like sharing toothbrush and improper storage circumstances, and causes the reintroduction of potential pathogens (7). Therefore, proper care of toothbrush is one of the most important issue for good oral health, besides of oral hygiene procedures. Recently, modern dentistry strongly emphasizes storing conditions and disinfection of toothbrushes and changing of toothbrushes at regular intervals, as preventive precautions Various authors agree that toothbrush disinfection is a fundamental component of oral hygiene now. Many studies have suggested different methods for toothbrush disinfection including mouth rinses or immersion/spray in different disinfectant solutions, and bristles impregnated with antimicrobial agents, ultraviolet sanitizer, dishwasher, microwave irradiation etc. (1,7,9-14).

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Patient motivation, education of oral hygiene and preventive procedures are substantially dentist's task (15). In the same way, good oral health behavior and adequate knowledge of the other professionals play an important role for oral health motivation and education of their patients, families, friends and the community (16,17). Because regular dental visit of the individuals and the ratio of dental professional to population were very low in developing countries, oral health motivation and education may be needed to give by medical doctors (18). Additionally, physicians refer to dentists their patients when required, due to oral health and oral care practices may be a predisposing factor for several systemic diseases. In this context, it is essential that physicians are sufficiently informed about oral and dental care such as toothbrush disinfection.

To the best of our knowledge, there is no study investigating the knowledge and behavior of medical doctors about toothbrush disinfection besides of our previous study conducted by dentists. The aim of this study was to evaluate these aspects among medical doctors working at a medical faculty in Turkey. We hypothesized that medical doctors have insufficient knowledge about toothbrush disinfection and they did not regularly carry out any disinfection for their own toothbrush. Our null hypothesis was that knowledge and behavior of medical doctors regarding toothbrush disinfection would be well enough.

2. METHODS

Before starting the study, Approval of Gazi University Ethics Board of the Institutional Ethics Committee was obtained (Date: 25.02.2016 No: E.27143) This study was carried out between June 2016-2017 among medical doctors of Gazi University Faculty of Medicine in Ankara, Turkey.

Written informed consent was given to all participants before enrollment in the study. The standard questionnaires used in our previous study (16) were modified and distributed to medical doctors (Table 1). Table 1 contains three parts in 18 questions 1) 6 questions for demographic data, 2) 7 questions for toothbrush/toothpaste use and recommendations, 3) 5 questions for toothbrush disinfection. The participants (male/female) filled out the questionnaires. The sample size was calculated using G*Power 3.0 software. The calculations were based on effect size =0.3, a 2-tailed test, an α level of .05, and a desired power (β) of 95%. The estimated desired sample size was calculated to be at least 172 participants. However, six forms from 176 questionnaires were excluded from the study due to deficiently filled out in conclusion and 170 questionnaires were evaluated. Therefore, actual power was 0.948.

The questionnaire was given to participants and they filled the forms in person. Six forms from 176 questionnaires were excluded from the study due to deficiently filled out in conclusion and 170 questionnaires were evaluated.

Descriptive statistics, Fisher's exact tests and chi-squared tests (SPSS-15.0, SPSS Inc., Chicago, USA) was used for statistical evaluation, as appropriate. Cramer v correlation coefficients were used to show the power of the correlation. The level of significance was 0.05.

3. RESULTS

Totally 170 medical doctors (63 females, 37.1% and 107 males, 62.9%) age ranged between 30 and 67 years (mean age ± standard deviation: 48±9.4 years) were participated in this study. The results of statistical analyses were shown in Table 2.

Regarding demographics; differences between females and males for age were not significant ($p>0.05$) and most doctors were aged between 41 and 50 years. Most participants were professor, had graduated from medical school over than 11 years ago, with the largest frequency of them graduating from Turkish universities and differences between females and males were not significant ($p>0.05$). Specialists of internal medicine sciences were more common in females than in males, with significantly differed ($p<0.05$).

Regarding personal toothbrush/toothpaste habits and patient recommendations; most doctors brushed their teeth at least twice a day and changed their toothbrushes once every three months. Differences between females and males for these questions ($p<0.05$) were significant. Most participants stored their toothbrushes in open contact with the environment, an open-topped toothbrush holder shared with other family members, thought that contact between toothbrushes was important, and shared their toothpaste with others. The doctors mostly did not any advice their patients about toothbrush usage. There were no statistically significant differences ($p>0.05$) between females and males for the questions (questions no:9-13).

Regarding toothbrush disinfection; most medical doctors reported a lack of knowledge about toothbrush disinfection and did not disinfect their own toothbrushes and no advice gave their patients in this issue. On the other hand, the participants mostly thought that toothbrush disinfection was necessary for everyone. No statistically significant differences ($p>0.05$) were found between females and males for these questions. Details of these results are given in Table 2.

Regarding specialties of the participants and their knowledge and behavior about toothbrush habits and patient recommendations; statistically significant difference ($p<0.05$) was found between specialties of the participants and opinion about the importance of toothbrushes contact'. The more specialists of internal medicine sciences thought that the contact between toothbrushes was important than the specialists of surgical medicine sciences. Also, knowledge of toothbrush disinfection and advice to their patients were more common in the specialists of internal medicine sciences than the others, with statistically significant differed ($p<0.05$). Although the majority of both specialists in two groups indicated that they did not disinfect their own toothbrushes, they were in agreement about the requirement of toothbrush disinfection for everyone, with no statistically significant differed ($p>0.05$). Details of these results are given in Table 3.

Table 1. *Questionnaire used in the study*

Demographics
1. Age
2. Biological sex
3. When did you graduate from the medical school? a) 0–10 years ago b) 11+ years ago
4. What medical school did you graduate from? a) University in Turkey b) University in another country
5. What is your specialty? a) Surgical medicine sciences b) Internal medicine sciences
6. What is your title? a) Specialist and/or Assistant Professor b) Associate Professor c) Professor
Personal toothbrush/toothpaste habits and patient recommendations
7. How often do you brush your teeth? a) Sometimes b) Once a day c) Twice or more a day
8. How often do you change your toothbrush? a) Once in a month b) Bimonthly c) Once every 3 months d) After 3 months or more
9. Where do you store your toothbrush? a) In the bathroom, in open contact with the environment b) In the bathroom, in a closed cabinet
10. How do you store your toothbrush? a) In an open-topped toothbrush holder shared with other family members b) In an closed-topped toothbrush holder shared with other family members c) Separately from the toothbrushes of other family members
11. In your opinion, is the contact between toothbrushes an important issue? a) No b) Yes
12. Do you share your toothpaste with other individuals? a) Yes b) No
13. Do you advise your patients regarding how often they should change their toothbrushes and/or where and how their toothbrushes should be stored? a) I only make suggestions about the frequency of changing their toothbrushes. b) Yes c) No
Toothbrush disinfection
14. Do you have any knowledge about toothbrush cleaning and disinfection? a) No b) Yes
15. Do you disinfect your own toothbrush? a) No b) Yes
16. Do you advise to your patients regarding toothbrush disinfection? a) No b)Yes
17. In your opinion, is toothbrush disinfection necessary? a) No b)Yes
18. If your answer to question 17 is yes, then for whom is toothbrush disinfection necessary? a) Everybody b) Special patient groups, such as immunosuppressed individuals, hospitalized patients, and children c) I have no idea

Table 2. *Descriptive statistical analysis of the questionnaire, per biological sex.*

Questions		Female N (%)	Male N (%)	p value	r
Demographics					
1. Age	a) 30-40 years b) 41-50 years c) 51 + years	15(23.8) 25 (39.7) 23 (36.5)	19 (17.8) 48 (44.9) 40 (37.3)	0.612	0.055
3. When did you graduate from the medical school?	a) 0–10 years ago b) 11+ years ago	11 (17.5) 52 (82.5)	9 (8.4) 98 (91.6)	0.077	0.120
4. What medical school did you graduate from?	a) University in Turkey b) University in another country	62 (98.4) 1 (1.6)	104 (97.2) 3 (2.8)	0.526[a]	0.043
5. What is your specialty?	a) Surgical medicine sciences b) Internal medicine sciences	22 (34.9) 41 (65.1)	59 (55.1) 48 (44.9)	0.011*	0.257*
6. What is your title?	a) Specialist or assistant professor b) Associate Professor c) Professor	11 (17.5) 19 (30.1) 3 (52.4)	15 (14.0) 26 (24.3) 66 (61.7)	0.494	0.077
Personal toothbrush/toothpaste habits and patient recommendations					
7. How often do you brush your teeth?	a) Sometimes b) Once a day c) Twice or more a day	1 (1.6) 13 (20.6) 49 (77.8)	7 (6.5) 37 (34.6) 63 (58.9)	0.033*	0.216*
8. How often do you change your toothbrush?	a) Once in a month b) Bimonthly c) Once every 3 months d) After 3 months or more	8 (12.7) 21 (33.3) 26 (41.3) 8 (12.7)	11 (10.3) 19 (17.8) 38 (35.5) 39 (36.4)	0.005*	0.293*
9. Where do you store your toothbrush?	a) In the bathroom, in open contact with the environment b) In the bathroom, in a closed cabinet	52 (82.5) 11 (17.5)	86 (80.4) 21 (19.6)	0.727	0.041
10. How do you store your toothbrush?	a) In an open-topped toothbrush holder shared with other family members b) In a closed-topped toothbrush holder shared with other family members c) Separately from the toothbrushes of other family members	31 (49.2) 4 (6.3) 28 (44.5)	60 (56.1) 8 (7.5) 39 (36.4)	0.587	0.082
11. In your opinion, is the contact between toothbrushes an important issue?	a) No b) Yes	6 (9.5) 57 (90.5)	16 (15.0) 91 (85.0)	0.308	0.088
12. Do you share your toothpaste with other individuals?	a) Yes b) No	35 (55.6) 28 (44.4)	72 (67.3) 35 (32.7)	0.126	0.147
13. Do you advise your patients regarding how often they should change their toothbrushes and/or where and how their toothbrushes should be stored?	a) I only make suggestions about the frequency of changing their toothbrushes. b) Yes c) No	9 (14.3) 4 (6.3) 50 (79.4)	15 (14.0) 7 (6.5) 85 (79.5)	0.998	0.016
Toothbrush disinfection					
14. Do you have any knowledge about toothbrush cleaning and disinfection?	a) No b) Yes	49 (77.8) 14 (22.2)	79 (73.8) 28 (26.2)	0.565	0.004
15. Do you disinfect your own toothbrush?	a) No b) Yes	49 (77.8) 14 (22.2)	85 (79.4) 22 (20.6)	0.798	0.020
16. Do you advise to your patients regarding toothbrush disinfection?	a) No b) Yes	52 (82.5) 11 (17.5)	90 (84.1) 17 (15.9)	0.790	0.024
17. In your opinion, is toothbrush disinfection necessary?	a) No b) Yes	9 (14.3) 54 (85.7)	25 (23.4) 82 (76.6)	0.153	0.025
18. If your answer to question 17 is yes, then for whom is toothbrush disinfection necessary?	a) Everybody b) Special patient groups, such as immunosuppressed individuals, hospitalized patients and children c) I have no idea	48 (76.2) 7 (11.1) 8 (12.7)	73 (68.2) 8 (7.5) 26 (24.3)	0.164	0.160

*Statistical analysis result according to Fisher exact test. r: Cramer V correlation coefficient, * p<0.05*

Table 3. *The comparison between specialties of the participants and their knowledge and behavior about toothbrush habits and patient recommendations*

Questions		Specialists of surgical medicine sciences	Specialists of internal medicine sciences	p value	r
		N (%)	N (%)		
11. In your opinion, is the contact between toothbrushes an important issue?	a) No b) Yes	15 (18.5) 66 (81.5)	7 (7.9) 82 (92.1)	0.039*	0.159*
14. Do you have any knowledge about toothbrush cleaning and disinfection?	a) No b) Yes	67 (82.7) 14 (17.3)	61 (68.5) 28 (31.5)	0.032*	0.164*
15. Do you disinfect your own toothbrush?	a) No b) Yes	67 (82.7) 14 (17.3)	67 (75.3) 22 (24.7)	0.236	0.091
16. Do you advise to your patients regarding toothbrush disinfection?	a) No b)Yes	73 (90.1) 8 (9.9)	69 (77.5) 20 (22.5)	0.027*	0.170*
17. In your opinion, is toothbrush disinfection necessary?	a) No b)Yes	20 (24.7) 61 (75.3)	14 (15.7) 75 (84.3)	0.145	0.112
18. If your answer to question 17 is yes, then for whom is toothbrush disinfection necessary?	a) Everybody b) Special patient groups, such as immunosuppressed individuals, hospitalized patients, and children c) I have no idea	55 (67.9) 5 (6.2) 21 (25.9)	66 (74.2) 10 (11.2) 13 (14.6)	0.124	0.157

** p<0.05 r: Cramer V correlation coefficient*

4. DISCUSSION

Knowledge and behavior of medical doctors were evaluated regarding oral care and toothbrush disinfection in the present study. Most participants (approximately 75%) were unaware about toothbrush disinfection and did not have disinfect their own toothbrush (approximately 79%). This result confirmed our hypothesis. However, 71% of the participants thought that toothbrush disinfection was necessary for all individuals. These results were in accordance with our previous published study conducted with dentists (16). Toothbrush disinfection has become an issue that needs to be emphasized more especially today, when the covid-19 pandemic has spread all over the world. It is important that all healthcare professionals have knowledge about the subject.

Toothbrushing at least twice a day has been suggested by American Dental Association (ADA) for good oral health (19). In this study, most participants (approximately 66%) brushed their teeth twice or more a day. The frequency of toothbrushing habit and changing the toothbrush were higher in females than in males, with statistically significant difference. Various studies have investigated oral health behavior and attitudes for different study samples such as health care professionals, university students, dentists and dental students, etc. Baseer et al reported that only 3.9% of the health professionals including doctors, medical students, technicians, pharmacists and nursing staff brushed their teeth twice a day (17). Sharda and Sheety reported that 47.8% of the participants including non-medical, para-medical and medical students brushed their teeth twice a day (20). The rate of toothbrushing twice a day has been reported as relatively higher in dentists and dental students (55% to 87%) (15,16,21-23). Females generally pays more attention to their own personal care and appearance in comparison with males. Many studies have been confirmed that oral health behavior of females was better than males (15-17,23). The results of the present study were in accordance with previous reports.

Storage conditions play an important role for toothbrush contamination. Because moist environment allows to increase bacterial survival, it was suggested that toothbrushes should be stored as open-topped instead of closed containers (7,24). Also, toothbrushes should be stored in an upright position and provided to air-dry until reused. The contact among toothbrushes may occurred when stored with others individuals' or in the same toothbrush box, should be kept separately (19). Additionally, ADA suggested that toothbrushes should be replaced every 3 months due to the loss of mechanical effect, not bacterial contamination (19). However, the current study compared to difference of bacterial contamination between toothbrushes used for 3 months and 1-month, also compared to storage conditions they kept with family members and separately (25). The results showed that the toothbrushes used for 3 months and kept with family members had become heavily contaminated than the others. Thus, the authors suggested that toothbrush should be changed after every 3-4 weeks and stored in upright position and separately to avoid cross-contamination (25). The risk of cross-contamination

increases when toothpastes shared with other individuals (7,26). Sharing toothpaste among family members may be high-risk factor related with transmission of hepatitis C virus infection (27). It was reported that dentists generally stored their own toothbrushes in a toothbrush box, and shared the toothpaste with other individuals, and changed theirs every 3 to 4 months (16). In this study, the storage conditions and replacement period of toothbrushes were found to be very similar with our previous study (16).

ADA currently published some recommendations for toothbrush care. Although bacterial contamination of toothbrushes is discussed, there is no tangible evidence related with toothbrush cleaning methods are effective for oral and general health. However, common-sense approaches were suggested for immunosuppressed individuals and high-risk patients with transmissible systemic diseases by blood or saliva. These procedures include replacing toothbrushes more often than 3-4 months, rinsing with the antibacterial mouth rinses before brushing, disposable toothbrushes, but it may be high cost and use of toothbrush sanitizers cleared by Food and Drug Administration (19).

Knowledge about toothbrush disinfection was found more common in specialists of internal medicine sciences than specialists of surgical medicine sciences and the difference was statistically significant. Similarly, the specialists of internal medicine sciences mostly thought that contact between toothbrushes was an important issue and advised to their patients for toothbrush disinfection, statistically significant different from the surgeons. Dentists generally receive various lectures during their undergraduate training about the relationship between oral health and systemic diseases (22,28). However, previous studies showed that medical students receive a few hours' lecture regarding oral health during all training years (29,30). It was reported that knowledge and awareness of medical students and physicians about periodontal diseases, caries, the relationship between oral diseases and systemic conditions were deficient (17,18,30,31). The investigators agree that medical education should be included oral health and dental topics in the curriculum of medical education (17,18,31,32). It was also concluded that severity of the COVID-19 could be potentially impacted by the invidual's oral health status. In addition, an increase in CRP values was correlated with poor oral health. This also portrays a serious condition of the disease. Recovery periods were also observed to be longer for poor oral health patients. For predicting the severity of COVID-19, the cut-off value coming from the oral health scoring used in this questionnaire could be used on a larger scale. For dental practitioners it is also possible to use this type of questionnaire before the clinical examination in order to reduce the exposure time (33).

A limitation of this study was that the specialties of the medical doctors included in the study were not selected in the branches directly related to the risk of infection and inflammation. In further studies, the knowledge and behaviors of healthcare professionals working in intensive care units, infectious diseases clinics, transplantation units and oncology clinics etc. where the risk of infection is high, about toothbrush disinfection can be investigated.

5. CONCLUSIONS

In conclusion, behavior and knowledge of medical doctors about toothbrush disinfection first time investigated to date in this study. The results showed that majority of the participants were unaware regarding the subject and did not have disinfect their own toothbrush and thought that toothbrush disinfection was necessary for all individuals. Even though motivation and education of the patients about oral health practices are dentist's task, medical doctors should be aware oral care such as toothbrush disinfection. In these challenging times of Covid-19 pandemic, the training of medical doctors on the disinfection and storage conditions of toothbrushes has become very important.

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Clinical and Experimental Health Sciences

Assessment of Oral Health in Elders with and without Alzheimer's Disease: A Cross-Sectional Study

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ABSTRACT

Objective: The aim of the study was to assess and compare the oral health status in elders with and without Alzheimer's disease (AD).

Methods: This age and gender-matched cross-sectional study included thirty-six elders with AD and 37 elders without AD from an Alzheimer's daycare center and a nursing home. Mental state, oral health and periodontal parameters, Geriatric Oral Health Assessment Index (GOHAI), unstimulated salivary flow rate (USFR), and other dry mouth conditions were examined. Group comparisons and correlation analysis were performed with T-test, Mann-Whitney U test, chi-square test, Fisher's Exact test, and Spearman's rank correlation.

Results: Probing depth (PD) and clinical attachment loss (CAL) were lower and the percentage of the subjects with non-periodontitis was higher in elders with AD than without AD ($p=0.017$ and $p=0.028$, respectively). Both groups had similar GOHAI scores and USFR levels ($p>0.05$). However, the elders with AD had higher use of mouth-drying medication and presence of halitosis complaint than without AD ($p<0.001$). In both groups number of missing teeth was positively correlated with the DMF-T score ($p<0.001$). In AD group, age was positively related to the gingival index and bleeding on probing ($p=0.005$ and $p=0.001$, respectively). USFR level was positively correlated with GOHAI score ($p=0.027$) in AD group, but it was negatively correlated with the DMF-T score ($p=0.031$) in without AD group.

Conclusion: Personalized care and supporting oral care education of the caregivers could maintain the oral health of elders with AD.

Keywords: Alzheimer disease, oral health, quality of life, dementia, periodontitis

1. INTRODUCTION

Alzheimer's disease (AD) is one of the main causes of dementia in the geriatric population which represents more than half of the dementia cases. The disease is a progressive neurodegenerative disease of the central nervous system (1,2). Impaired memory and failure to learn new information are the most prominent clinical features of the disease. Despite the memory loss in the early stage of the disease, the patient can continue his daily life activities independently. In the middle stage of the disease, the independence in daily living activities is gradually lost, and psychiatric and behavioral symptoms begin to appear. In the last stage of the disease, the patient becomes completely dependent on his caregiver in daily living activities, and serious psychiatric disorders may occur (1).

Various risk factors are associated with AD. The incidence of AD increases with older age, lower education, and female gender. Also hereditary, and environmental factors play a role in the etiology of the disease. History of previous head injury has also been linked with the increased risk of developing AD (3). It has been reported that the incidence of the disease increases in case of blood vessel damages due to hypertension, heart disease, stroke, and diabetes. (4).

Oral health problems are frequently seen because of the behavioral effects of dementia, which could make it difficult for a patient to perform oral care (5). The deterioration of oral, and dental care lead to plaque accumulation, and dental caries (6). Also, dementia patients suffer from gingival bleeding, periodontal pockets, mucosal lesions, and reduced salivary flow. Elders with dementia experience poor oral health, which can be improved with an oral care education for caregivers, and regular professional dental care of the patients (7). However dental treatment of AD patients becomes challenging due to their reduced ability to cooperate (8).

Periodontal disease is an inflammatory status that could lead to tooth loss when it is left untreated (9). The disease is known to be linked with many systematic diseases. Recent

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findings showed that the periodontal pathogens' effect on neural inflammation could lead to cognitive decline, and sporadic late-onset AD (10).

Dry mouth is another factor affecting the oral health of individuals, especially in AD. Sreebny and Schwartz reported that 80% of the commonly prescribed drugs cause dry mouth, and more than 400 drugs cause salivary gland dysfunction as a side effect (11). Thus, the dysfunction in the antibacterial effect of saliva leads to disorders such as lack of lubricant, and buffering function, caries and periodontal disease, difficulty in chewing and swallowing (12,13).

The aim of this study is to compare the oral health of elders with and without AD, through oral examination, oral health quality of life assessment, and evaluation of dry mouth condition.

2. METHODS

This cross-sectional study was conducted in January 2018 – March 2019 in Istanbul, Turkey. A group of 73 elders participated in the study, 36 elders with AD, and 37 elders without AD. Firstly elders with AD were recruited, and the elders without AD were matched for age and gender. Nineteen of the elders with AD were living in the home with their family or caregivers and visiting the daycare center of the Turkish Alzheimer Association few days a week, where caregivers were educated on caring for the elders. The remaining 17 elders with AD and elders without AD were living in a public nursing home. Socio-demographic characteristics and use of medications were obtained via a questionnaire. The medications that cause dry mouth were determined from Sreebny and Schwartz's work (11). The oral health quality of life was examined with the Turkish version of the Geriatric Oral Health Assessment Index (GOHAI) (14). Elders with AD responded to the questionnaire and GOHAI with their caregivers, while elders without AD responded by themselves.

This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethical Committee of Marmara University School of Medicine (protocol number 09.2018.056/05.01.2018). Written informed consent has been obtained from the caregivers of the elders with AD, and the elders without AD involved in the study.

2.1. Mental State Evaluation

Participants' mental state was evaluated with the Mini-Mental State Examination test (MMSE). The test score was categorized as normal (between 24 and 30), mild or moderate dementia (between 23 and 18), and severe dementia (17 or lower) (15).

2.2. Oral and Periodontal Examination

The periodontal assessments were carried out for those who were dentate and gave consent for the examination (23 elders with AD patients and 24 elders without AD). The oral and prosthesis care habits and halitosis complaints were recorded. The oral and periodontal examination was performed by a single dentist (DO) using the University of North Carolina 15 periodontal probe (Hu-Friedy, Chicago, IL), and a dental mirror. The decayed, missing, filled teeth (DMF-T) index (16), prosthetic situation, modified mucosal rating scale (MMRS) (17) were assessed. Plaque index (PI) (18), gingival index (GI) (19), probing depth (PD), bleeding on probing (BOP), and clinical attachment loss (CAL) (16) were recorded. Periodontal status was determined according to the Centers for Disease Control and Prevention-American Academy of Periodontology criteria set in 2012 (20).

2.3. Geriatric Oral Health Assessment Index

Geriatric Oral Health Assessment Index (GOHAI) is a 12-items scale, which evaluates the elders' oral health quality of life (21). Participants respond how often they experience a specific oral health issue stated in the item based on a 5-point Likert scale (1-always and 5-never). The total score was computed as the sum of item scores and ranges from 12 to 60. A higher GOHAI score indicates a better oral health quality of life (14). The scale has four dimensions which are functional restriction (eating, speaking, and swallowing), pain or discomfort (use of medication to relieve pain, eating without discomfort, and sensitivity to hot, cold, or sweets), psychological factors (worry or concern about oral health, dissatisfaction with appearance, self-consciousness about oral health, and feel uncomfortable eating in front of people), and behavioral effects (limiting the amount and kind of food, and avoidance of social contacts because of oral problems) (21).

2.4. Unstimulated Saliva Collection

The unstimulated saliva collection was performed at least one hour after the participant's food and beverage intake, except for water. The participants were requested to sit upright, not to swallow, and lean their head forward over a measuring cup to let the saliva drain in it. A one-minute pretest was performed, and the saliva collection trial lasted for five minutes. The unstimulated salivary flow rate (USFR) was computed by dividing the saliva level into collection period (ml per min). A value lower than 0.1 ml per min was considered as salivary gland hypofunction (22).

2.5. Statistical Analysis

The descriptive statistics were reported as frequency (n, %) for the discrete random variables. The continuous random variables were presented as mean and standard deviation (SD) when the data follow a normal distribution, otherwise reported as the median and interquartile range (IQR). The group comparisons between the elders with and without AD were evaluated with independent samples *T*-test, Mann-Whitney U test, Chi-square test, and Fisher's Exact test. Spearman's rank correlation analysis was performed for

each group. The statistical significance level was considered as p<0.05, and the analysis was carried out with IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA).

3. RESULTS

The participant characteristics of the study groups are presented in Table 1. The mean age was 77.1 (±9.7) for elders with AD, and 77.4 (±7.8) for elders without AD (p=0.876). The mean age onset for AD was 72.6 (±10.6) years. Almost half of the elders with AD (17, 47.2%), and without AD (18, 48.7%) were female (p=0.903). There was no statistically significant difference between elders with and without AD in terms of education level (p=0.667), and smoking habit (p=0.226). More than half of the elders with AD were cared in-home and daycare center by their family or/and a personal caregiver (19, 52.8%). The remaining elders with AD (17, 47.2%), and all elders without AD cared in the nursing home (p<0.001) (Table 1).

The elders with AD group had a significantly lower median MMSE score than the elders without AD group (p<0.001). Severe dementia (20, 55.6%) was observed in more than half of the elders with AD, while the remaining (16, 44.4%) had mild or moderate dementia. The median number of medications that cause dry mouth condition was higher in the elders with AD than the elders without AD (p<0.001). The use of neurotransmitter (p=0.358), antiepileptic (p=0.615), cardiovascular disease treatment (p=0.879), antidiabetic (p=0.113) medications were similar between the elders with and without AD groups. However, the elders with AD had more frequent use of nonsteroidal anti-inflammatory (p=0.046), psychiatric (p<0.001), and stomach (p=0.028) medications than the elders without AD (Table 1).

Table 1. *Inter-group comparisons of the participant characteristics*

Socio-demographic Parameters	**Elders with AD (n=36)**	**Elders without AD (n=37)**	**p-value**
Age years; mean±SD	77.1 ± 9.7	77.4 ± 7.8	0.876[a]
Age onset of the AD years; mean±SD	72.6 ± 10.6	-	
Female n (%)	17 (47.2%)	18 (48.7%)	0.903[b]
Education level n (%)			
Illiterate	7 (19.4%)	11 (29.7%)	
Primary school	12 (33.3%)	12 (32.4%)	
Secondary school	4 (11.1%)	6 (16.2%)	
High school	6 (16.7%)	4 (10.8%)	
University	7 (19.4%)	4 (10.8%)	0.667[b]
Smoking n (%)	4 (11.1%)	8 (21.6%)	0.226[b]
Place of Care n (%)			
Home and day care center	19 (52.8%)	-	
Spouse	7 (19.4%)	-	
Adult children	9 (25.0%)	-	
Family and personal caregiver	3 (8.3%)	-	
Nursing home	17 (47.2%)	37 (100.0%)	
MMSE (points; median (IQR)	16.5 (11.5–20.0)	28.0 (28.0–29.0)	**<0.001[d]**
Mental Status n (%)			
Normal mental status	-	37 (100%)	
Mild or Moderate dementia	16 (44.4%)	-	
Severe dementia	20 (55.6%)	-	
Number of medications that cause dry mouth median (IQR)	4.0 (2.0–5.0)	1.0 (1.0–2.0)	**<0.001[d]**
Use of Medication n (%)			
NSAI drugs	8 (22.2%)	2 (5.4%)	**0.046[c]**
Neurotransmitter drugs	3 (8.3%)	1 (2.7%)	0.358[c]
Psychiatric drugs	29 (80.6%)	3 (8.1%)	**<0.001[b]**
Antiepileptic drugs	2 (5.6%)	1 (2.7%)	0.6150[c]
Cardiovascular disease treatment drugs	23 (63.9%)	23 (62.2%)	0.8790[b]
Antidiabetic drugs	9 (25.0%)	4 (10.8%)	0.113[b]
Stomach drugs	10 (27.8%)	3 (8.1%)	**0.028[b]**

[a] Independent samples T-test; [b] Chi-square test; [c] Fisher's exact test; [d] Mann-Whitney U test. Abbreviations; AD: Alzheimer's disease, SD: Standard deviation, MMSE: Mini-mental state examination test score, IQR: Interquartile range, NSAI: Nonsteroidal *anti-inflammatory.*

The oral and periodontal findings of the study groups are presented in Table 2. More than one-third of elders with AD (12, 33.3%) and elders without AD (13, 35.1%) were edentulous (p=0.871). The prosthesis type used in the upper jaw and lower jaws were similar between the study groups (p>0.05). Oral and prosthetic care of the groups were also similar, almost 60% of the elders practice oral care at least once a day in both groups (p=0.732) (Table 2).

Table 2. *Inter-group comparisons of the oral and periodontal findings*

Oral Health Finding	Elders with AD (n=36)	Elders without AD (n=37)	p-value
Edentulousness n (%)	12 (33.3%)	13 (35.1%)	0.871 [c]
Upper jaw prosthesis type[a] n (%)			
Complete denture	21/31	22/28	0.350 [c]
Removable partial denture	6/31	5/28	0.883 [c]
Fixed prosthesis	4/31	1/28	0.199 [c]
Lower jaw prosthesis type[a] n (%)			
Complete denture	15/29	17/28	0.494 [c]
Removable partial denture	9/29	11/28	0.173 [c]
Fixed prosthesis	5/29	0/28	0.052 [d]
Oral and prosthesis care n (%)			
Less than twice a week	11 (30.6%)	12 (32.4%)	
At least twice a week	5 (13.9%)	3 (8.1%)	
At least once a day	20 (55.6%)	22 (59.5%)	0.732 [c]
Modified Mucosal Rating Scale n (%)			
Normal	32 (88.9%)	34 (91.9%)	
Mild	4 (11.1%)	3 (8.1%)	0.663 [c]
DMF-T median (IQR)	26.5 (18.3–28.0)	28.0 (22.5–28.0)	0.384 [e]
Decayed teeth	0.0 (0.0–0.0)	0.0 (0.0–0.5)	0.352 [e]
Missing teeth	24.5 (8.5–28.0)	25.0 (20.0–28.0)	0.467 [e]
Filled teeth	0.0 (0.0–2.8)	0.0 (0.0–0.0)	0.198 [e]
Number of Teeth	3.5 (0.0–19.5)	3.0 (0.0–8.0)	0.467 [e]
PI[b] median (IQR)	2.0 (1.5–2.0)	2.0 (1.6–2.0)	0.964 [e]
GI[b] median (IQR)	1.4 (1.3–1.8)	1.4 (1.3–1.7)	0.476 [e]
BOP[b] %; median (IQR)	33.3 (14.6–50.0)	33.3 (21.8–48.6)	0.558 [e]
PD[b] mm; mean±SD	2.8 ± 0.8	3.3 ± 0.6	**0.017** [f]
CAL[b] mm; median (IQR)	3.1 (2.5–5.3)	4.3 (3.3–5.7)	**0.028** [e]
Periodontal status[b] n (%)			
Non-periodontitis	10/23	2/24	**0.008** [d]
Moderate Periodontitis	8/23	11/24	0.440 [c]
Severe Periodontitis	5/23	11/24	0.081 [c]
GOHAI			
Total Score mean±SD	50.3 ± 4.9	49.7 ± 4.5	0.551 [f]
Functional Restriction Score median (IQR)	14.0 (11.0 – 15.0)	13.0 (11.0 – 15.0)	0.463 [e]
Pain and Discomfort Score median (IQR)	14.5 (13.0 – 15.0)	15.0 (13.0 – 15.0)	0.656 [e]
Psychological Factors Score median (IQR)	15.0 (14.0 – 17.0)	14.0 (13.0 – 17.5)	0.249 [e]
Behavioral Effects Score median (IQR)	8.0 (7.0 – 10.0)	8.0 (7.0 – 9.0)	0.614 [e]
USFR ml/min; median (IQR)	0.3 (0.1–0.4)	0.3 (0.1–0.5)	0.607 [e]
USFR ≥0.1 ml/min n (%)	27 (75.0%)	31 (83.8%)	-
USFR <0.1 ml/min n (%)	9 (25.0%)	6 (16.2%)	-
Halitosis Complaint n (%)	24 (66.7%)	8 (21.6%)	**<0.001** [c]

[a] Analysis performed for those who wear prosthesis in the relevant jaw; [b] Assessment carried out for 23 AD patients and 24 elders without AD.[c] Chi-square test;[d] Fisher's exact test; [e] Mann-Whitney U test; [f]Independent samples T-test. Abbreviations; AD: Alzheimer's disease, DMF-T: Decayed missing filled teeth index score, IQR: Interquartile range, PI: Plaque index, GI: Gingival index, BOP: Bleeding on probing, PD: Probing depth, CAL: Clinical attachment loss, GOHAI: Geriatric Oral Health Assessment index, SD: Standard deviation, USFR: Unstimulated salivary flow rate.

Most of the elders with and without AD had normal MMRS (p=0.663). The Median DMF-T index score was relatively high in both elders with AD (26.5, IQR:18.3 – 28.0) and without AD (28.0, IQR:22.5 – 28.0) groups (p=0.384). Both study groups had similar number of teeth (p=0.467), PI (p=0.964), GI (p=0.476), and BOP (p=0.5580). The mean PD and median CAL were significantly lower in elders with AD than elders without AD (p=0.017 and p=0.028, respectively). Patients having non-periodontitis were higher in AD group than the elders without AD (p=0.008). All participants had a relatively high mean GOHAI score of 50 points (out of 60), which was similar between the groups (p=0.551). The median USFR level was 0.3 ml/min for elders with and without AD (p=0.607). About 25% of elders with AD and 16% of elders without AD had a median USFR level below 0.1ml/min. Elders with AD had more frequently complained about halitosis than those without AD (p<0.001) (Table 2).

Correlation analysis between age, GOHAI scores, and selected oral health and periodontal findings of study groups was presented in Table 3. In both study groups, the higher number of missing teeth was positively correlated with DMF-T score (p<0.001).

In elders with AD the higher age was associated with the higher number of missing teeth (*r*=0.44, p=0.007), GI (*r*=0.57, p=0.005), and BOP (*r*=0.66, p=0.001). The higher GOHAI score was correlated with the higher level of USFR (*r*=0.37, p=0.027). Also, the higher GOHAI pain and discomfort score was associated with the lower GI (*r*=-0.45, p=0.031). The higher GOHAI psychological factors score was correlated with the lower PD (*r*=-0.49, p=0.018) and CAL (*r*=-0.48, p=0.020). The lower GOHAI behavioral effects score was associated with the higher BOP (%) (*r*=-0.46, p=0.029), PD (*r*=-0.42, p=0.044) and CAL (*r*=-0.53, p=0.009) in elders with AD. The number of missing teeth and DMF-T score were positively correlated with BOP, PD, and CAL (p<0.01) in AD group. (Table 3).

In elders without AD higher DMF-T score was negatively correlated with GOHAI total score (*r*=-0.41, p=0.011), GOHAI behavioral effects score (*r*=-0.36, p=0.028), and USFR level (*r*=-0.36, p=0.031). The higher CAL was positively correlated with GOHAI functional restriction score (*r*=0.41, p=0.050), and USFR (*r*=0.42, p=0.042) in elders without AD. (Table 3).

Table 3. *Correlation between age, GOHAI scores, and selected oral health and periodontal parameter findings of study groups*

Elders with AD	Age	GOHAI					USFR	DMFT	Number of Missing Teeth
		Total Score	Functional Restriction Score	Pain and Discomfort Score	Psychological Factors Score	Behavioral Effects Score			
PI [a]	0.24	0.03	0.12	0.02	0.05	0.10	-0.08	0.02	0.11
GI [a]	**0.57****	0.02	0.40	**-0.45***	0.01	-0.15	0.02	0.03	0.32
BOP [a]	**0.66****	-0.12	0.38	-0.29	-0.29	**-0.46***	-0.11	**0.56****	**0.54****
CAL [a]	0.34	-0.18	0.19	0.07	**-0.48***	**-0.53****	-0.02	**0.63****	**0.58****
PD [a]	0.29	-0.12	0.29	-0.14	**-0.49***	**-0.42***	0.16	**0.61****	**0.43***
USFR	0.04	**0.37***	0.31	0.17	0.13	0.29	-	-	-0.17
DMFT	0.33	-0.03	0.16	-0.14	-0.19	-0.14	-0.11	-	-
Number of Missing Teeth	**0.44****	-0.09	0.10	-0.14	-0.25	-0.11	-0.17	**0.87****	-

Elders without AD	Age	GOHAI					USFR	DMFT	Number of Missing Teeth
		Total Score	Functional Restriction Score	Pain and Discomfort Score	Psychological Factors Score	Behavioral Effects Score			
PI [b]	-0.03	-0.03	0.07	-0.02	0.05	-0.07	0.15	0.04	0.18
GI [b]	0.05	0.04	0.17	0.09	0.04	0.12	0.03	-0.03	0.22
BOP [b]	-0.02	-0.13	-0.10	-0.02	-0.12	0.06	0.13	-0.06	0.02
CAL [b]	0.17	0.04	**0.41***	-0.15	-0.11	-0.07	**0.42***	-0.09	0.04
PD [b]	-0.06	-0.07	0.17	0.14	-0.08	-0.22	0.14	0.05	0.23
USFR	0.18	0.27	0.23	0.13	0.14	-0.11	-	-	-0.19
DMFT	-0.04	**-0.41***	-0.19	-0.07	-0.19	**-0.36***	**-0.36***	-	-
Number of Missing Teeth	-0.04	-0.15	0.06	0.13	-0.08	-0.24	-0.19	**0.83****	-

*Spearman's rank correlation test significant at *p<0.05 and **p<0.01 level. Analysis performed for [a] n=23 and [b] n=24. Abbreviations; AD: Alzheimer's Disease, GOHAI: Geriatric Oral Health Assessment index, PI: Plaque index, GI: Gingival index, BOP: Bleeding on Probing, PD: Probing depth, CAL: Clinical attachment loss, USFR: Unstimulated salivary flow rate, DMF-T: Decayed missing filled teeth index score.*

4. DISCUSSION

In this cross-sectional study, oral and periodontal health, the oral health quality of life, and the dry mouth condition of the elders with and without AD were investigated. The participants were recruited on a voluntary basis from two different institutions by matching for age and gender. The edentulousness was not significantly different between elders with and without AD. Both group participants mostly use complete prosthetics, and the type of prosthesis use was similar between the study groups. These findings agree with the findings of previous studies (23,24).

The self-rated GOHAI scores were found relatively high, and there was no significant difference between the comparison groups, this finding is consistent with the study of Ribeiro et al. (23), and a recent systematic review (25). Although most of the elders with AD in our study were suffering from poor oral health conditions, GOHAI scores were reported relatively high. This finding could be due to the tendency of elders with AD to evaluate their perceived oral health better despite showing poor health indicators (26). Also, it has been reported that providing a personalized oral care routine and treatments to elders with AD would prevent the decline in the quality of life of the patient (27). This could be another reason for the high GOHAI scores of the participants with AD in our study since more than half of the cases were having personalized care in their homes.

Decayed, missing, filled teeth index score was not significantly different between the participants with and without AD, this finding is in concordance with some studies, where participants had similar age and gender characteristics (24,28). However few studies found higher DMF-T scores in AD patients (29,30,31). The number of teeth was not significantly different between the study groups, this finding is in line with the literature, where the study groups had similar age and gender features (24,26,32).

The periodontal health indicator GI was found similar between elders with and without AD in age, gender, and dentate matched study (26), our finding agrees with this literature finding. In this study, we found that the elders with AD had relatively lower PD and CAL, and a higher presence of non-periodontitis than the elders without AD, these findings are not consistent with the previous studies (7,32). It has been reported that nursing homes need improved oral care services (30), also a study in Turkey reported that the elders in the nursing home had poor oral health (33). Since all participants without AD, were residents of the nursing home, they may need more periodontal treatments. Also, the additional analysis showed that elders with AD who had institutional care had higher periodontal indicators of BOP, PD, and CAL than those AD patients who had personal care at home. In our study although elders with AD were using higher number of medications that cause dry mouth condition, the study groups had similar USFR levels. This finding is not in agreement with the literature findings of the lower level of salivary flow in elders with AD (4,7,29). However, the effect of dry mouth in our study could be seen in the higher halitosis complaint (34) in elderly people with AD than those elders without AD.

Although it was difficult to perform oral examinations in elders with AD, we investigated their oral quality of life with important clinical parameters such as PD, CAL, and dry mouth condition by comparing them with the elders without AD, therefore this can be stated as the strengths of our study.

The sample size may be considered relatively small as a limitation of this study, because of impaired memory and capabilities of elders with AD, we achieved a sample size greater than 30 in each group, which is the least acceptable sample size for clinical studies.

5. CONCLUSION

In conclusion, most of the oral health indicators were found similar between the elders with and without AD. The unfavorable effect of dementia on oral health could be seen in the higher presence of halitosis complaint among the elders with AD. However**,** periodontal health status of the elders with AD was relatively better than the elders without AD. This might arise from the difference in the type of care the elders received. Also, the education and information provided by Alzheimer's daycare center might help the caregivers to maintain the oral health condition of the patients. This emphasizes the importance of personalized oral care for elders with AD according to their capabilities, needs, and conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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Clinical and Experimental Health Sciences

Prenatal Psychosocial Profile: Validity and Reliability Study to Its Use in Turkey

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ABSTRACT

Objective: This study was planned to test the statistical properties of the prenatal psychosocial profile assessment tool in Turkish sample and to examine its validity and reliability on healthy pregnant women.

Methods: This reliability and validity study was conducted in the gynecology and obstetrics outpatient clinic of a training and research hospital with 440 healthy pregnant women between March and June 2017.

Results: In the analysis performed for internal consistency in the Prenatal Psychosocial Profile (PPP) reliability study, Cronbach's alpha reliability coefficient was found to be μ=.75 for the stress subscale, μ = .94 for the social support-partner subscale, μ = .96 for the social support-other people subscale, and μ= .80 for the self-esteem subscale. In the construct validity of the PPP-stress subscale, loads of all items except one item were found to be sufficient, and in the social support-partner, social support-other people, and self-esteem subscales, loads of all items were found to be sufficient.

Conclusion: The Prenatal Psychosocial Profile-Turkish Version is a valid and reliable assessment tool that can be used to determine the psychosocial profile of women during pregnancy.

Keywords: Prenatal psychosocial profile, stress, social support, self-esteem

1. INTRODUCTION

Pregnancy is an adaptation process, in which bio-psychosocial changes occur in a woman, a parenting relationship is established between the mother and fetus, and consequently, the birth of a new individual happens (1,2). Changes experienced in this adaptation process may adversely affect the women both physiologically and psychologically and may prevent the healthy progression of pregnancy (3). Therefore, pregnant women, their partners, and other family members need to ensure adaptation to changes that occur during pregnancy (4).

The process of adaptation to pregnancy differs for every woman (1). Some factors that are effective in these differences are unwanted pregnancy, inadequacy in receiving health care, unhealthy housing conditions, communication barriers with the family and environment, malnutrition, tobacco/substance use, the lack of security, violence, and inadequate social support. Each of these factors can be a source of stress in pregnant women, can lead to depression and disrupt the process of adaptation to pregnancy by affecting self-esteem negatively (5,6).

Psychosocial stress in pregnancy is defined as the situation in which the woman feels unworthy and expresses this both behaviorally and psychologically when she is unable to cope with the difficulty she experiences in meeting her requirements. In the study in which Woods et al. (2010) evaluated stress during pregnancy, it was determined that domestic violence, substaence use, and two or more health problems increased the incidence of psychosocial stress during pregnancy by three or four times (7). Studies reported that stress experienced during pregnancy affects both the physiology of pregnancy and the postpartum period negatively (7,8). Accordingly, the incidence rates of physiological problems such as preterm birth, hypertensive disorders, placental anomalies, antenatal bleeding, difficulty in delivery, interventional delivery, spontaneous abortus, intrauterine growth retardation, a low birth weight and APGAR score of a newborn, and perinatal mortality are also known to increase in women with the poor mental state during pregnancy (8-11).

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Social support is another variable of adaptation to pregnancy. Social support is one of the important factors affecting the woman's adaptation to pregnancy and the postpartum period and her coping with stress (12). The studies have revealed that pregnant women at risk need more social support (8), social support plays a preventive role in depression during pregnancy (13,14), and the incidence of anxiety and depression in pregnant women who receive inadequate partner support increases (15,16). The pregnancy process and postpartum period are among the periods when social support is important for the mother and the infant (12,17,18) because the process of the mother's adaptation to the maternal role starts before pregnancy and continues postnatally. One of the most significant factors that help the woman adapt to this period, which is also called the process of learning motherhood, is social support (17,19,20). Similarly, it enhances attachment to her infant and facilitates communication with her family/immediate environment (12,17).

Self-esteem, which forms the basis of an individual's personality structure (21), is known to have a feature that is systematically affected by developmental changes throughout life. According to various theoretical perspectives, life events, and especially the transition to parenting, may be related to changes in self-esteem (22). One of these theoretical perspectives emphasizes the role of biological effects on self-esteem. It focuses on the physiological and neurological changes associated with the transition to motherhood (16,22). Another theoretical perspective asserts that having the sense of motherhood is an important opportunity for the development of a person's self-esteem (22). Self-esteem in pregnancy is associated with neonatal outcomes in the postpartum period such as height, weight, Apgar score, mother-infant attachment and mother's ability to care for her infant, and low self-esteem leads to mental disorders such as postpartum depression (16,23,24).

There is a correlation between the stress experienced during pregnancy, social support received from the husband and relatives, self-esteem and pregnancy outcomes (9-11). Thus, the negative psychosocial profile during pregnancy is an issue to which attention should be paid due to its effect on the mother and infant health postpartum (9-11,16,23,24). The American College of Obstetricians and Gynecologists (ACOG) (2006) advocates the assessment of risk factors by psychosocial screening regardless of the social status, educational level, race or ethnic origin of all women who want to receive prenatal care (5). Moreover, it is recommended to perform psychosocial screening in every trimester regarding the possibility that problems that are not found in the first prenatal follow-up may occur later in pregnancy (5,25,26).

In Turkey, risk assessment is performed by taking the present status and past obstetric history and general medical history of pregnant women. However, the psychosocial profile, which is composed of stress, social support, and self-esteem, is not evaluated. In this context, there is the PPP assessment tool developed by Curry et al. (1994) in the United States. The relationship between psychosocial factors and pregnancy outcomes constitutes its theoretical framework (6). The PPP is a 44-item Likert-type assessment tool consisting of four subscales (stress, social support-partner, social support-other people, and self-esteem) that can be applied to pregnant women. The PPP was designed to measure the stress perceived by women during pregnancy, the social support they receive from their partners/relatives, and their self-esteem. The assessment tool has been used in different cultures such as American, Caucasian, Spanish, African-American, and Brazilian, and it is implemented successfully for a psychosocial assessment (27).

In Turkey, there are tools assessing stress, social support, and self-esteem during pregnancy and studies conducted on this subject. However, there is no assessment tool containing all the components of the psychosocial profile. This study was planned to adapt the prenatal psychosocial profile assessment tool to Turkish sample and examine its validity and reliability on healthy pregnant women.

2. METHODS

2.1. Procedures performed before the data collection

At the beginning of the study, it was attempted to reach Professor Dr. Mary Ann Curry, who developed the PPP assessment tool, via email. However, since she is retired and transferred her rights to Dr. Linda Bullock, permission to use the PPP assessment tool was received from Dr. Bullock. Furthermore, the original version of the assessment tool and the calculation sheet were requested from Dr. Bullock.

2.2. Analysis of linguistic equivalence, content and scope validity

To analyze the linguistic equivalence, the content validity of the prenatal psychosocial profile assessment tool, the Prenatal Psychosocial Profile-Turkish Version was created using six methodological steps suggested by Beaton et al. (2000) (Figure 1) (28). According to this, at the first stage, two separate translations of the current tool (T1 & T2) were done by the researchers and a professional translator not related to the subject. Afterward, the translations (T1 & T2) were brought together, and the inconsistency between the translations was eliminated. After the corrections were completed, with the combination of the translations (T1 & T2), T1-2 was created. T1-2 was retranslated by a professional translator, whose native language was English, by working with the original version of the scale (RT1). RT1 was sent to Dr. Bullock, the owner of the PPP assessment tool, by requesting to assess its linguistic validity. By considering Dr. Bullock's recommendations and working with all reports of T1, T2, T1-2, RT1, whether there was any change in meaning according to the original scale was evaluated, and the Turkish version of the scale (TV1) was created. For the evaluation of cultural appropriateness, linguistic equivalence, and content validity, TV1 was sent to experts in the field, and necessary corrections were made following their recommendations.

Turkish version 2 of the assessment tool (TV2) was created. The Content Validity Index (CVI) was used to evaluate expert opinions. While calculating the CVI score, for each item, what percentage of the ten experts gave three or four points to the item was calculated (Item CVI score). In the evaluation, it was calculated that all items (100%) received 3-4 points, and all items were found to be suitable.

The comprehensibility of the final version of the PPP assessment tool was evaluated by conducting a pilot study in a group consisting of 15 people. With the necessary corrections, the final form of the Turkish version of the PPP was created (PPP-TV).

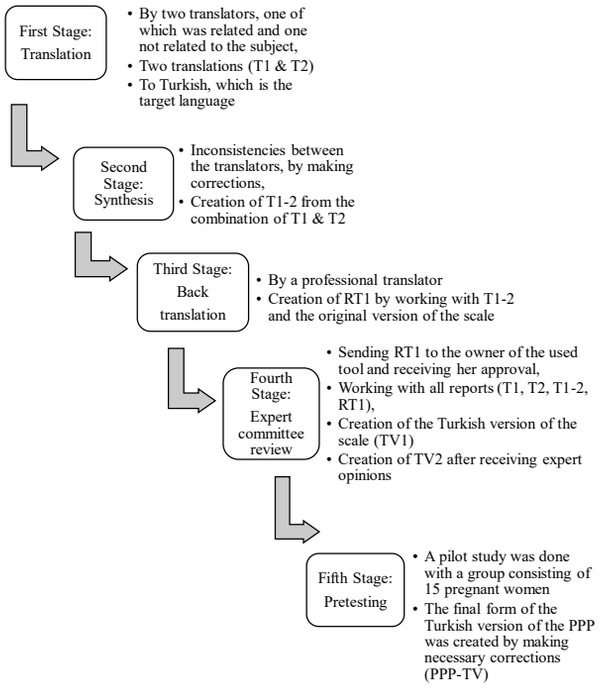


Figure 1. *Steps applied for linguistic equivalence and cultural adaptation*

2.3.Data collection

The study was conducted in the Gynecology and Obstetrics Outpatient Clinic of a training and research hospital between March and June 2017. The study sample consisted of all pregnant women who applied to the Gynecology and Obstetrics Outpatient Clinic. Pregnant women who had no chronic or pregnancy-related disease diagnosed by a physician, who knew Turkish at a level that they could understand, and answer the questions, and who agreed to participate in the research were included in the study sample.

In the literature, it is recommended to reach five to ten times more participants than the number of the items in the scale while the sample size is determined in validity and reliability studies (29,30). Validity and reliability studies were carried out with 440 healthy pregnant women. The questionnaire containing the socio-demographic and obstetric characteristics of pregnant women, which was prepared as a result of the literature review, and the Prenatal Psychosocial Profile were filled out by the researcher face-to-face with the pregnant women, who agreed to participate in the study, and each interview lasted for 10 minutes on average.

2.4. Data Collection Tools

The Questionnaire Containing Socio-Demographic and Obstetric Characteristics of Pregnant Women

The form, which was developed by the researcher as a result of the literature review, consists of 25 questions in total, 12 questions related to socio-demographic characteristics of pregnant women and 13 questions related to obstetric characteristics. This questionnaire includes questions about socio-demographic characteristics such as age, marital status, educational status, perception of economic level, employment status, etc. and questions about obstetrics characteristics such as the number of pregnancies and live births, the status of planning to continue the pregnancy controls, smoking/alcohol use during pregnancy, herself and her partner's status of wanting the pregnancy.

Prenatal Psychosocial Profile

The PPP is a Likert-type assessment tool consisting of four subscales and a total of 44 items. Stress, which is the first subscale, consists of 11 items, including factors such as financial concerns, family-related problems, being pregnant, being exposed to violence, and problems related to working life. All items are answered in four-point Likert type graded between 1 and 4. The four-point Likert-type scale was classified as follows: no stress = 1, little stress = 2, medium-level stress = 3, severe stress = 4. The lowest score to be obtained from this subscale is 11, and the highest score is 44. As the score received from the subscale increases, the stress level also increases.

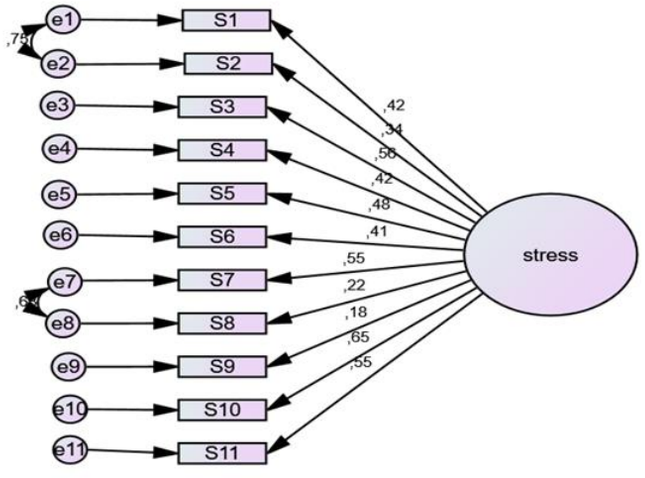
The social support subscale consists of 11 items questioning how satisfied the woman is with the support received during pregnancy. This subscale is the short version of Brown's Support Behaviors Inventory. The social support subscale was repeated twice as social support-partner and social support-other people. In the first repetition, the social support of the spouse/partner is questioned, while in the second repetition, the social support of other people is questioned. If the woman does not have a spouse/partner, this subscale is filled out only once by considering the support of the surrounding people. All items are answered in a six-point Likert type graded between 1 and 6. The six-point Likert-type scale was classified as follows: I am not satisfied at all = 1, I am very satisfied = 6. The lowest score to be obtained from this subscale is 11, and the highest score is 66. As the score received from the subscale increases, the social support level also increases (2).

In the self-esteem subscale, in addition to Rosenberg's self-esteem scale, the item "I feel that I can control my life" was added. Therefore, a new subscale of 11 items was created (2).

All items are answered in four-point Likert type graded between 1 and 4. The four-point Likert-type scale was classified as follows: completely agree = 1, agree = 2, disagree = 3, strongly disagree = 4. While half of the items included expressions related to feeling valued, feeling satisfied, and positive attitude, the other half included negative expressions related to feeling useless and unsuccessful. When calculating the total score to be obtained from the subscale, negative expressions should be included in the calculation by reversing them (5 items will be scored in reverse). As the score obtained from the subscale increases, self-esteem is considered to be high.

2.5. Data analysis

The data were analyzed using the SPSS version 23.0 and AMOS 26 packaged software. We used AMOS 26 in order to perform the confirmatory factor analysis and used SPSS 23.0 in order to perform the descriptive statistics, exploratory factor analysis, and correlation analysis. The descriptive statistics of the continuous variables in the study were presented as mean, standard deviation, minimum and maximum values, whereas the descriptive statistics of the categorical variables were presented as frequency and percentage. The correlation reliability coefficients were used for the PPP reliability study, and Cronbach's alpha coefficients were used for internal consistency. Pearson's product-moment coefficients and the t-test were used for the test-retest measurements of the PPP assessment tool. For validity analysis, the confirmatory factor analysis (Figures 2-3-4-5) and the exploratory factor analysis were used.

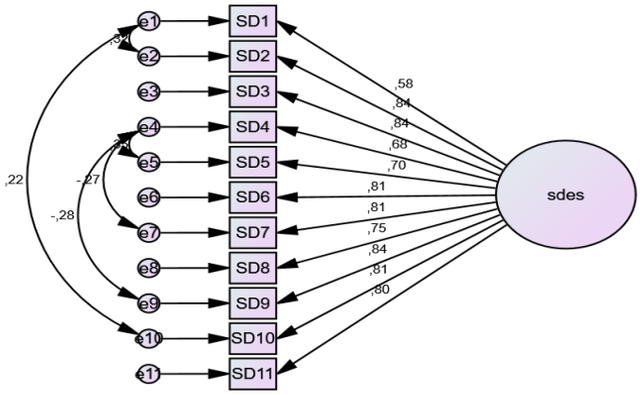


Chi-Square=136,1, Sd=42, RMSEA=0,071

Figure 2. *Confirmatory factor analysis of stress subscale of PPP*

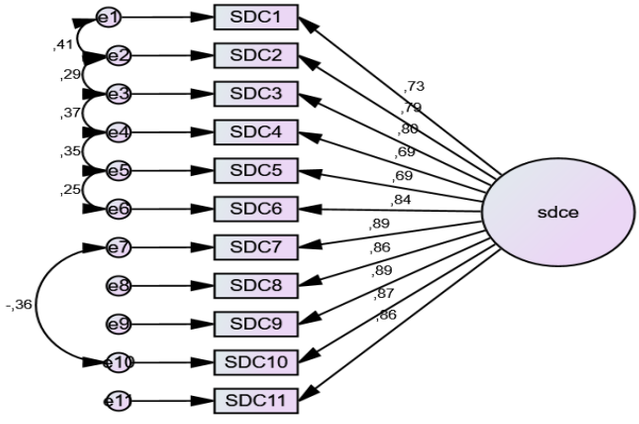
2.6.Ethical issues

In order to conduct the study, permission was obtained from the administrator of the hospital where data would be collected, and written permission was obtained from the ethics committee (Istanbul University Cerrahpaşa Clinical Research Ethics Committee Approval Date: 13.12.2016, Approval Number: A-01). In accordance with the Declaration of Helsinki, written and verbal information about the study and the nature of the study was provided to the participants, and their written consent was obtained.



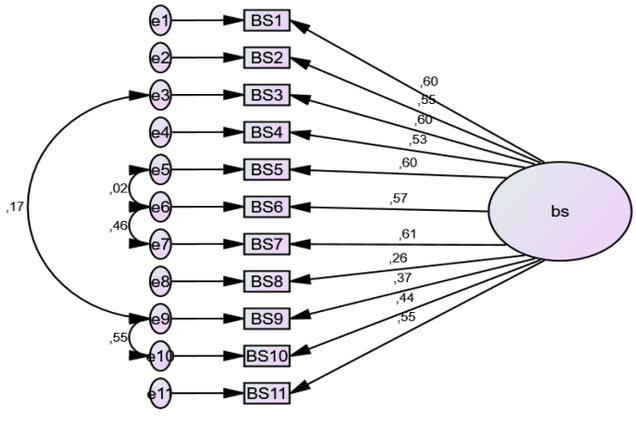
Chi-Square=215,11, Sd=45, RMSEA=0,084

Figure 3. *Confirmatory factor analysis of partner support subscale of PPP*



Chi-Square=224,7, Sd=46, RMSEA=0,088

Figure 4. *Confirmatory factor analysis of other support subscale of PPP*



Chi-Square=143,803, Sd=40, RMSEA=0,077

Figure 5. *Confirmatory factor analysis of self-esteem subscale of PPP*

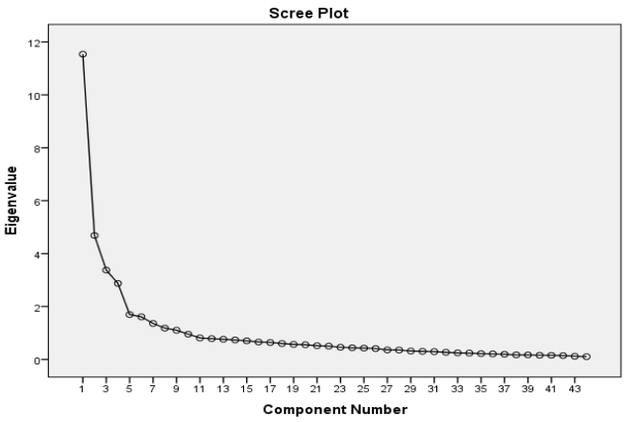


Figure 6. *Scree plot*

3. RESULTS

It was found that the mean age of the pregnant women included in the study was 28.42 ± 5.59 years (min: 18, max: 44), more than half of them (57.7%) received education under 8 years, the majority (72.0%) of them did not work and had an income equal to expenses (70.9%). It was observed that the mean marriage duration of the pregnant women was 6.00 ± 5.05 (min: 1, max: 28) years, a great majority (90.2%) considered their marital relationship as good, and most of them had a nuclear family (86.1%) (Table 1).

Table 1. *Distribution of socio-demographic characteristics of pregnant women (n = 440).*

Characteristics	n	%
Age		
28 years and under	237	53.9
Above 28 years old	203	46.1
Education		
Under 8 years	254	57.7
8 years or more	186	42.3
Employment status		
Employed	121	27.5
Unemployed	319	72.0
Economical situation		
Income less than expenses	111	25.2
Income equivalent to expenses	312	70.9
Income is more than expenses	17	3.9
Family type		
Nuclear family	379	86.1
Extended family	61	13.9
Marriage duration		
Under 5 years	223	50.7
5 years or more	217	49.3
Evaluation of the marital relationship		
Good	397	90.2
Middle	38	8.6
Bad	5	1.2

When the obstetric characteristics of the pregnant women included in the study were examined, it was found that nearly half of them experienced three and more pregnancies (40.5%) and were in the third trimester of pregnancy (48.9%), and most of them had planned pregnancy (73.6%). The mean gestational week of the participants was found to be 26.63±10.30, and the number of living children to be 1.66±0.91 (Table 2).

Table 2. *Distribution of obstetric and pregnancy-related characteristics of pregnant women (n = 440).*

Characteristics	n	%
Number of pregnancies		
One pregnancy	141	32.0
Two pregnancies	121	27.5
Three and more pregnancies	178	40.5
Pregnancy period		
I trimester	66	15.0
II trimester	159	36.1
III trimester	215	48.9
Pregnancy planning status		
Planned	324	73.6
Not planned	116	26.4
	Range	**Mean (SD)**
Gestational age	5-41	26.63 (10.30)
Live birth (n: 261)	1-6	1.66 (0.48)
Living child (n: 258)	1-6	1.66 (0.91)
Stillbirth (n: 13)	1-2	1.23 (0.43)
Abortion (n: 89)	1-3	1.26 (0.53)
Curretage (n: 43)	1-8	1.51 (1.18)

3.1.Reliability

Findings Related to Item Analysis

When the subscale total scores and their correlations were examined for the reliability study of the PPP, it was found that the correlation reliability coefficients were distributed between r = .270 – .646 in the stress subscale, r =.401 – .865 in the social support-partner subscale, r = .763 – .868 in the social support-other people subscale, and r =.464 – .660 in the self-esteem subscale, and that there was a positive correlation.

When the correlations between the PPP subscales were evaluated, a significant negative correlation was found between the stress subscale and social support-partner, social support-other people and self-esteem ($p<0.01$). Accordingly, stress increases as the partner support, the support of other people, and self-esteem decrease. A significant positive correlation was found between the self-esteem subscale and social support-partner and social support-other people ($p<0.01$). In line with this, self-esteem increases as the partner support and the support of other people increase. Moreover, a significant positive correlation was found between the social support-other people subscale and social support-partner ($p<0.05$). As the support of other people increases, partner support also increases (Table 3).

Table 3. *Correlations between the PPP subscales (n = 440).*

Subscales	Stress	Partner Support	Other Support	Self-esteem
Stress	1.00			
Partner Support	-0.247*	1.00		
Other Support	-0.228*	0.434**	1.00	
Self-esteem	-0.262*	0.357*	0.261*	1.00

*$p<0.01$; **$p<0.05$

Findings Related to the Internal Consistency Reliability Coefficient

In the analysis performed for internal consistency in the reliability study of the PPP, Cronbach's alpha reliability coefficient was determined as μ = .75 for the stress subscale, μ = .94 for the social support-partner subscale, μ= .96 for the social support-other people subscale, and μ= .80 for the self-esteem subscale. Cronbach's alpha values of the original and Portuguese versions of the scale are presented in Table 4.

Table 4. *Cronbach's alpha values of the original, Portuguese, and Turkish versions of the PPP assessment tool.*

Subscales	PPP-TV	PPP-PV (2015)	Curry et al. (1994)
Stress	**.75**	.71	.78
Partner Support	**.94**	.95	.93
Other Support	**.96**	.95	.95
Self-esteem	**.80**	.79	.89

PPP-PV: Prenatal Psychosoyal Profile – Portuguese Version
PPP-TV: Prenatal Psychosoyal Profile – Turkish Version

Comparison of the Test and Retest Mean Scores and Findings Related to Their Correlations

In the present study, the test-retest measurements of the PPP assessment tool, conducted with 30 subjects, who met the inclusion criteria, with a four-week break, were evaluated by Pearson's product-moment correlation and the t-test. When the average of the scores obtained by the pregnant women from the test and retest was compared with the t-test in the dependent groups, no significant difference was found between the mean scores ($p>0.05$, Table 5). When there is no significant difference between the measurement averages, it is understood that the scale measures similar results in periodic measurements, and there is consistency between the measurements. Furthermore, in reliability analysis, when the relationship between the scores obtained from the first and second applications was examined with Pearson's correlation analysis, a significant positive correlation was found between the two measurement scores of the four subscales obtained with a four-week break, and the reliability coefficients varied between .79 and .90 (Table 5).

Table 5. *Comparison of the test and retest mean scores of the PPP assessment tool and their correlations (n = 30).*

Subscales	Test Mean (SD)	Retest Mean (SD)	t	p	r	p
Stress	40.23 (3.55)	39.80 (3.76)	1.019	.317	.80	.000
Partner Support	56.53 (9.51)	55.90 (9.50)	.843	.406	.90	.000
Other Support	51.03 (11.96)	50.96 (12.03)	.060	.953	.87	.000
Self-esteem	36.90 (4.14)	37.10 (5.12)	-.351	.728	.79	.000

The t-test in the dependent groups: the degree of freedom = 29, t: t-test in the dependent groups (Paired Samples t-test), r: Pearson's correlation test.

Exploratory Factor Analysis

The skewness and kurtosis values were calculated for the distribution testing of the scale. The skewness value was found as .24, and the kurtosis value as .74. These values show that the distribution is within the range of – 1 and +1, which is a condition for accepting the distribution normal (31).

It was examined with the exploratory factor analysis whether the PPP assessment tool consisted of 4 subscales, as in the original version. The Kaiser-Meyer-Olkin (KMO) and Bartlett's test scores of the factor analysis are stated in Table 6.

Table 6. *KMO and Bartlett's Test*

KMO and Bartlett's Test		
Kaiser-Meyer Olkin		**.900**
Bartlett's Test	Chi-Square	12206.56
	Degree of Freedom	946
	p	.000

The KMO ranges are as follows: .90-1.00 marvelous, .80–.89 meritorious, .70–.79 middling, .60–.69 mediocre, .50–.59 miserable, and .50 and below unacceptable (31). Since the result obtained in our study is .900, the suitability of the sample size appears to be marvelous; on the other hand, Barlett's Sphericity test is valid with p value .000. Accordingly, the results of the KMO test indicate that the data are suitable for the factor analysis. Furthermore, based on the significant outcome of Barlett's Sphericity test, a sufficient correlation existed between the items to conduct the factor analysis. The level of the total variance explained was calculated as 51%. These findings show that the factor analysis is structurally valid. Also the scree plot is shown in Figure 6.

As known, factor analysis is carried out to reveal whether the items in a scale are separated into fewer factors excluding each other (31). The common varimax rotation was used in factor analysis, and the factor number was limited to 4, as in the original version of the assessment tool. With the factor analysis, a 4-factor structure emerged. The results of the factor analysis are shown together with the factor loads. (Table 7).

Tablo 7. *Rotated Component Matrix*

	Component			
	Stress	Partner Support	Other Support	Self-esteem
Being exposed to violence (sexual, emotional, physical)	.717			
Problems about friends	.667			
Family problems (e.g., spouse/ partner, children, etc.)	.556			
Having lost someone you love recently (e.g., death, divorce, being away from each other)	.548			
Generally feeling extreme burnout	.548			
Having recently moved or being obliged to move in the future	.,519			
Problems about consuming alcohol or drugs	.501			
Financial concerns (e.g., foods, shelter, healthcare, transportation)	.496			
Other monetary concerns (e.g., bills, etc.)	.417			
Being pregnant	.353			
Problems about work life (e.g., being dismissed, etc.)	.316			
He helps me keep my morale high		.825		
He clarifies my condition so that I can understand more easily		.816		
He helps me when I need or when I am in trouble		.812		
He spares time to talk to me about personal and private issues		.809		
He takes me serious when I am concerned about something		.786		
He appreciates the things I do for him		.766		
I know he/she will be with me when I need help		.758		
He tries to make something special or thoughtful for me		.751		
He tolerates my ups and downs and unusual behaviors		.720		
He is interested in my daily routine and problems		.700		
He shares similar experiences with me		.640		
I know he/she will be with me when I need help			.847	
He/she takes me serious when I am concerned about something			.844	
He/she helps me when I need or when I am in trouble			.843	
He/she appreciates the things I do for him/her			.839	
He/she clarifies my condition so that I can understand more easily			.830	
He/she tolerates my ups and downs and unusual behaviors			.830	
He/she helps me keep my morale high			.828	
He/she spares time to talk to me about personal and private issues			.822	
He/she shares similar experiences with me			.788	
He/she is interested in my daily routine and problems			.751	
He/she tries to make something special or thoughtful for me			.714	
Generally, I am pleased with myself				-.663
I feel that I do not have many things to be proud of				.654
I feel unsuccessful				.648
I have a positive attitude towards myself				-.634
I feel at least as valuable as other people				-.603
I feel that I can control my life				-.593
I feel that I have some good characteristics				-.574
I feel that I can do things as other people do				-.539
Sometimes I think I am worthless				.528
Sometimes I feel useless				.497
I would like to have more self-respect				.346

All the factor structures were examined according to their factor loads, and the factor loads in the four-factor structure are given in sequence in the table presented above. While interpreting the items with a loading on each factor, the level of .30 was generally considered the minimum factor load in the literature (31). Therefore, it was decided in our study that the items with a factor load above .30 in the six-factor structure explaining 51% of the total variance would be included in the same dimension. In line with the previous studies, the factors were named “Stress,” “Partner Support,” “Other Support,” and “Self-esteem.”

3.2. Comfirmatory Factor Analsis

Findings Related to Construct Validity Analysis

In the construct validity of the PPP-stress subscale, the loads of all items except one (S9) were found to be sufficient (.18-.65). In the construct validity of the social support-partner (.58-.84), social support-other people (.69-.89), and self-esteem (.26-.61) subscales, the loads of all items were found to be sufficient (Figures 2-3-4-5).

4. DISCUSSION

4.1.Reliability

Discussion of the Findings Related to Item Analysis

If the items in a scale have equal weights and are in the form of independent units, the correlation coefficient between each item and total values is expected to be high. With the increase in the correlation coefficient, the relationship of that item with the quality that is desired to be measured increases to the same extent. Although there is no specific standard related to which value of the item-total score correlation coefficient will be considered insufficient, the correlations are recommended to be not negative, and even to be above .25 or .30 and less than .70 (29,32,33). The results obtained in this study are consistent with the literature.

In this study, a negative correlation was found between stress and social support-partner, social support-other people and self-esteem, which are the PPP subscales, and a positive correlation was found between self-esteem and social support-partner and social support-other people. The study results display similarity with the results of the PPP-PV study conducted by Weissheimer and Mamede (2).

Discussion of the Findings Related to the Internal Consistency Reliability Coefficient

To evaluate the internal consistency of the Prenatal Psychosocial Profile, Cronbach's alpha coefficient, which is a method suitable for Likert-type measurement tools, was used (29). Cronbach's alpha values of the original (27), Portuguese (2), and Turkish versions of the PPP assessment tool were similar.

Comparison of the Test and Retest Mean Scores and Discussion of the Findings Related to Their Correlations

Test-retest reliability is the ability of a measurement tool to give consistent results from application to application and to be invariable over time. To find the test-retest reliability, the correlation between the scores obtained from the two applications is calculated. The high correlation coefficients indicate the power of the consistency between the first and second application results (34). In this study, high correlation coefficients indicate that the consistency between test-retest results is high. In the original PPP and the Brazilian version, the test-retest reliability was studied, and similar results were obtained (2,27).

4.2. Validity

Discussion of the Findings Related to Construct Validity Analysis

For construct validity in the adaptation of the Prenatal Psychosocial Profile to Turkish, confirmatory factor analysis was performed to verify the compliance of the factors. In this study, the compliance values were found to be at the desired level and compliance to be good in all subscales. However, in the construct validity of the PPP-stress subscale, the load of an item (S9) was determined to be below .20 (.18). Since the study was an intercultural adaptation, it was decided to keep the item.

5. CONCLUSION

Stress, inadequate social support, and low self-esteem are important determinants of the psychosocial profile affecting pregnancy. Therefore, the negative psychosocial profile during pregnancy is an issue that needs to be considered because of its negative effect on the health of the mother and infant during pregnancy, birth, and the postpartum period.

This tool, which evaluates the psychosocial profiles of women during pregnancy, was adapted to Turkish society, and its reliability and validity were examined on healthy pregnant women. According to the study results, the PPP-TV is a valid and reliable measurement tool in terms of Turkish culture.

In line with the study results, using the Prenatal Psychosocial Profile assessment tool as a valid and reliable assessment tool to determine the psychosocial status of women during pregnancy, trying it in different socio-demographic groups, and using it also in groups with risky pregnancy are recommended. Furthermore, starting the follow-up at the beginning of pregnancy and reapplying the PPP assessment tool in each trimester may provide the follow-up of psychosocial situations that change during pregnancy. Each subscale that makes up the assessment tool can be used independently.

The facts that the gestational weeks of the pregnant women included in the sample were variable (between 5-41 weeks) and the number of pregnant women in the third trimester was high were among the study's limitations. Moreover, since stress, social support, and self-esteem may be affected by acute events, the obtained results may vary during pregnancy. Therefore, they should not be generalized to the whole pregnancy period.

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Clinical and Experimental Health Sciences

An Examination of Nurses' Acceptance of Mobile Health Applications

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ABSTRACT

Objectives: This study aims to examine nurses' acceptance of Mobile Health Applications.

Methods: This cross-sectional study was conducted with 321 nurses in a health practices and research center between March-July of 2020. Data collection instruments were Personal Data Form and Mobile Applications Acceptance Model.

Results: In this study, 97.2% of the nurses believed that mobile health applications should be used more in hospitals. The total mean score of the nurses on the Mobile Applications Acceptance Model (MAAM) was 124.69±15.98. Nurses under the age of 30, those who worked in intensive care units, and those who owned a mobile device exhibited higher total mean scores on the MAAM ($p<0.05$). The MAAM total mean scores were higher among those who thought Mobile Health Applications should be made more widespread ($p<0.04$), who felt that Mobile Health Applications were effective in accessing individual health data ($p<0.001$), who felt that using Mobile Health Applications were easy ($p=0.02$), who believed that using Mobile Health Applications helped patients access health services without having to go to the hospital ($p<0.001$), and who wanted to help patients obtain health services without going to the hospital ($p<0.001$).

Conclusion: Nurses' level of mobile health applications acceptance in terms of using mobile applications was high in our study. Most of the nurses expressed positive views on the use of mobile applications in the hospital setting.

Keywords: Mobile health, mobile applications, nurse, health services.

1. INTRODUCTION

With many advances in technology, mobile devices have become an inevitable part of our daily lives. According to the 2018 Global Digital Report, the number of people in the world owning cellphones has reached 5.1 billion (1). The widespread use of cellphones has accelerated the utilization of Mobile Health Applications (MHA) (2–4). MHAs include portable medical devices, wearable technology, short health messages, smartphone health applications and gamification apps (5).

Mobile Health Applications are both easy to use and readily accessible. They save on time and energy, provide both voice and visual communication between patients and healthcare professionals (6–8), and make it convenient to send health data to patients and their close relatives (9). Additionally, MHAs make it possible to store a great quantity of data, are useful in determining individual health profiles, and developing strategies for regional and national healthcare investments (6, 10).

It is reported that 46% of patients with mental health problems have one or two MHAs installed on their smartphones and 64% use MHAs to access personal health data (9). In a study conducted with oncology patients, the authors indicate that 69.6% of the participants owned smartphones and 86.8% wished to use MHAs regularly to communicate with their healthcare professionals (11). For this reason, it is important that nurses follow up and learn how to use the latest technologies to improve patient care and advance their own professional development. MHAs are a means of providing speedier nursing care that is available to everyone (2–4, 7). However, in order to serve with the MHAs, the nurse must first accept the MSU and be willing to use it (12, 13). This study aims to examine nurses' mobile health applications acceptance. The research questions in this study are as follows:

What is the total mean score of nurses' Mobile Applications Acceptance Model (MAAM)?

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Are there differences in the MAAM scores according to the nurses' demographic characteristics (gender, age, education, working environment, years of work, and owning a mobile device)?

Are there differences in the MAAM scores according to the nurses' thoughts about mobile health applications?

2. METHODS

2.1. Design and Setting

This cross-sectional study was conducted in the Western Black Sea Region of Turkey, in a health practices and research center from March-July 2020. The universe of the research consisted of all nurses (N=432) in the hospital where the research was conducted, no sample selection was made. Those who were not willing to participate in the study, those who provided incomplete data, or could not be reached were excluded. A total of 321 nurses (participation rate: 74.3%) participated in the study.

2.2. Data Collection

Data was collected from the nurses by e-mail. The data collection instruments were "Personal Data Form" and "Mobile Applications Acceptance Model".

Personal Data Form

This form is made up of two parts. The first part queries sociodemographic data such as the nurse's age, education, working year and environment. The researchers prepared the second part on the basis of a literature scan (3, 16, 17). This part contains six statements about the thoughts of the nurses regarding MHAs. The nurses marked the statements that were appropriate for them.

The Mobile Applications Acceptance Model (MAAM)

This scale was developed by Uğur and Turan in 2016 to determine the factors affecting the adoption of mobile applications. Consisting of a total of 32 items (Cronbach's alpha=0.82), the instrument is a 5-point Likert-type scale. As the total score of the scale increases, the level of acceptance of the mobile application also increases. Needs, (items 1-7), Behavioral Intentions (items 8-13), Attitudes about Usage (items 14-19), Perceived Benefits (items 20-24), Subjective Norms (items 25-29) and Performance Expectations (items 30-32) make up the six sub-scales. Cronbach's alpha coefficients for the subscales are: Needs subscale, 0.861; Subjective norms subscale, 0.810, Attitudes about usage subscale, 0.816; Behavioral intentions subscale, 0.899, Perceived benefits subscale, 0.851, Performance expectations subscale, 0.788 (15). The Cronbach's alpha coefficients in this study were found to be 0.96 for total, 0.89 for "Needs subscale", 0.91 for "Subjective norms subscale", 0.92 for "Attitudes about usage subscale", 0.92 for "Behavioral intentions subscale", 0.91 for "Perceived Benefits subscale", 0.79 for "Performance Expectations subscale".

2.3. Data Analysis

The SPSS 22.0 package program was used to analyze the data. Descriptive data is indicated by numbers, percentages, means, and standard deviation. The data was checked for normal distribution with skewness and kurtosis, and parametric tests (one-way analysis of variance and student's t test) were used. The Bonferroni post hoc test was used to determine where the significant differences originated. Analysis results were analyzed at a significant level accepted at $p<0.05$.

2.4. Ethical Considerations

Written permission was obtained for the conduct of the study from an ethics committee of a university (Decision No. 2020/05) and a health practices and research center where the study was conducted. The researchers informed the nurses about the purpose of the research and the confidentiality of the data to be obtained, after which their verbal and written consent was obtained.

3. RESULTS

Of the nurses in the study, 88.2% were women, 53.3% were under the age of 30, 64.2% had a bachelor's degree, 49.8% worked at clinics outside of intensive care, 68.2% had worked for less than 10 years, and 96.9% used smartphones (Table 1).

A comparison of the nurses' personal characteristics in terms of the MAAM total score is given in Table 1. There was a statistically significant difference in terms of the total mean score of the MAAM according to age, working environment, and having a mobile device ($p<0.05$). The total mean score of the MAAM of nurses under the age of 30 is statistically and significantly higher compared to the nurses age 30-40 ($p=0.008$). The total mean score of the MAAM of the nurses who worked in intensive care units was higher compared to the nurses working in other clinical units ($p=0.02$). The total mean score of the MAAM of nurses who have a smartphone was higher compared to the nurses who did not have a smartphone ($p<0.001$). There was a statistically significant difference in terms of the total mean score of the MAAM according to gender, education, and working years ($p>0.05$) (Table 1).

The total mean score of MAAM of the nurses was 124.69±15.98, and the total mean score of the "Needs subscale" was the highest total mean score of the MAAM subscales (28.78±3.23) (Table 2).

Of the nurses, 97.2% thought that the use of MHAs should be made more widespread, 86.3% felt that MHAs were effective in accessing individual health data, 95.0% believed that using MHAs was easy and 83.2% thought that MHAs helped patients access health services without having to go to the hospital. Of the nurses, 89.42% wanted to help patients obtain health services without going to the hospital via their mobile health apps (Table 3).

The MAAM total mean scores were higher among those who thought MHAs should be made more widespread (p=0.04), who felt that MHAs were effective in accessing individual health data (p<0.001), who felt that using MHAs was easy (p=0.02), who believed that using MHAs helped patients access health services without having to go to the hospital (p<0.001) and who wanted to help patients obtain health services without going to the hospital (p<0.001) (Table 3).

Table 1. *Comparison of Nurses' Personal Characteristics and MAAM Total Scores (n=321)*

Characteristics			Mobile Application Acceptance Model total score	Statistical analysis	Significant difference (post hoc) †
	n	%	X̄ ± SD		
Gender					
Females	283	88.2	124.23 ± 16.18	p=0.161 t=-1.404	-
Males	38	11.8	128.10 ± 14.19		
Age					
<30	171	53.3	126.95 ± 1.22	**p=0.01* F=4.591**	**1-2 (*p* = .008)***
30-40	116	36.1	125.17 ± 3.20		
>40	34	10.6	121.20 ± 1.34		
Education					
High school	14	4.4	114.92 ± 3.43	p=0.09 t=2.151	-
Associate Degree	34	10.6	121.20 ± 2.64		
Bachelor's degree	206	64.2	125.39 ± 1.08		
Graduate degree	67	20.9	123.61 ± 2.16		
Working environment					
Intensive care	121	37.7	126.53 ± 1.37	**p=0.02* F=3.853**	**1-2 (p=0.02)***
Other departments	160	49.8	118.62 ± 2.31		
Administrative departments	40	12.5	124.81 ± 1.31		
Using a smartphone					
Yes	311	96.9	126.50 ± 0.52	**p<0.001** t=17.032**	-
No	10	3.1	124.63 ± 16.24		
Working year					
<10	219	68.2	125.66 ± 1.05	*p* = 0.26 *F*= 1.130	-
10-20	81	25.2	122.33 ± 1.96		
>20	21	6.5	123.66 ± 2.48		
Total	321	100.0	124.69 ± 15.98		

*Note. t, Student t test; F, one-way analysis of variance (ANOVA), †Significant difference: Bonferroni test, *p < .05, **p < .01*

Table 2. *Nurses' Mobile Application Acceptance Model Total Mean Scores (n=321)*

Mobile Applications Acceptance Model		$\bar{X}$ ± SD	Min-Max
Subscales	Needs	28.78 ± 3.23	21-35
	Subjective norms	18.34 ± 3.34	7-15
	Attitudes about usage	20.52 ± 5.34	8 – 30
	Behavioral intentions	25.38 ± 3.42	15-30
	Perceived benefits	19. 62 ± 3.17	12-25
	Performance expectations	12.03 ± 2.07	7-15
Total score		124.69 ± 15.98	88-160

Table 3. *Differences in MAAM Scores According to The Nurses' Thoughts on Mobile Health Applications (n=321)*

The nurses' thoughts on mobile health applications		n	%	Mobile Application Acceptance Model Total score	Statistical analysis
Do you think the use of mobile health applications should be made more widespread?	Yes	312	97.2	125.00 ± 15.95	***p* = 0.04***
	No	9	2.8	113.88 ± 14.04	***t* = 2.066**
Do you think mobile devices are effective in reaching personal health data?	Yes	277	86.3	126.00 ± 16.47	***p* < .001****
	No	44	13.7	116.43 ± 8.94	***t* = 3.764**
Do you think using mobile health applications is easy?	Yes	305	95.0	125.16 ± 15.91	***p* = 0.02***
	No	16	5.0	115.75 ± 15.08	***t* = 2.310**
Do you think mobile health applications help patients receive healthcare services without having to go to the hospital?	Yes	267	83.2	126.94 ± 15.43	***p* < 0.001****
	No	54	16.8	113.55 ± 14.01	***t* = 5.901**
Would you want to help patients obtain health services without coming to the hospital via their mobile health apps?	Yes	287	89.4	126.27 ± 15.77	***p* < 0.001****
	No	34	10.6	111.32 ± 10.74	***t* = 5.376**

*Note. t, Student t test, *p < 0.05, **p < 0.01*

4. DISCUSSION

This study aimed to examine the acceptance of nurses regarding MHAs. The acceptance level of the nurses in the study regarding mobile health applications was high. Most of the nurses believed that MHAs should be made more widespread and MHAs helped patients to obtain medical services without having to go to a hospital. Most of the nurses wanted to help patients obtain health services via their mobile health apps without having to go to a hospital.

The total mean score of the nurses on MAAM was considerably high. Using a smartphone increases the usage of mobile apps and using smartphones regularly, continuously, and intensively can encourage the discovery of new mobile applications (11, 16). It is thought that the high level of nurses' acceptance of mobile health apps was related to the high rate (96.9%) of smartphone usage among the nurses in this study.

The total mean score of the "Needs subscale" is the highest total mean score of the nurses on the MAAM subscales. "Needs subscale" refers to the needs that drive an individual to use and satisfy the necessity of resorting to a mobile app (13, 14, 19). Another study conducted with nurses reported that 42% of nurses use mobile apps to meet their needs (the most frequent work-related smartphone activity was searching for work-related drug references) – and found that smartphones also helped nurses reduce work-related stress and improve unit cohesion and teamwork (12). In the same study, nurses emphasized that if smartphones are used properly, they are certainly helpful tools for improving patient safety. Nurses make use of mobile apps to evaluate and relieve pain (18, 19), apply therapy (20), provide postpartum care (21), deliver post-surgery care (22), promote healthy life behaviors (23, 24) and reduce aggressive behaviors in children with special needs (25). All of these factors (reducing work-related stress, improving unit cohesion and teamwork, evaluating and relieving pain, applying therapy, providing postpartum care, delivering post-surgery care, etc.) apply to the needs of nurses and patients. The fact that the nurses' "Needs subscale" total mean score was the highest emerged as a natural consequence of using Mobile Health Applications in this study.

In this study, the total mean score of the nurses on MAAM was higher for nurses under the age of 30 compared to those between the ages of 30-40. Clinical nurses below the age of 30 tend to use a smartphone more in their nursing practices for the purposes of efficient planning, team coordination, and checking up on medications. Nurses under 30 were statistically more likely to believe in the positive results that can be derived from the use of mobile apps in the clinical setting compared to their older colleagues (12). This finding can be a result of the high rate of mobile device usage among young people. Young nurses are more inclined to use smartphones and discover and use mobile applications faster, which can increase the acceptance level of mobile applications by young nurses.

This study showed that nurses working in the intensive care units had higher MAAM total mean scores compared to nurses working in other departments. Intensive care units are settings that restrict active communication between patients and their loved ones (26, 27). Relatives of patients under intensive care frequently need to obtain information about developments from health professionals (27). There is subsequently a need to communicate with healthcare personnel in order to continue with treatment and care after discharge from the hospital (28). In this case, MHAs can be an effective alternative method that nurses can use remotely to respond to the needs of patients and their relatives in intensive care units. Thus, it is thought that the mobile application acceptance scores of the nurses working in the intensive care units were higher in this study.

The study demonstrated that MAAM total mean scores were higher among nurses who believed that the use of MHAs should be made more widespread, that the apps

were effective in reaching personal medical data, and that the nurses felt they were helping patients to access health services without having to go to the hospital. Additionally, the MAAM total mean scores of nurses who wanted to help patients seek medical services without having to go to the hospital were higher. Using mobile applications will provide a different perspective to nursing care (8, 29). Remote nursing care can reduce the number of repeated hospital admittances as well as morbidity and mortality rates. This may be a result of the higher number of nurses who thought that MHAs were useful and effective.

4.1. Limitations

Limitations of the study are that the sample consists of the nurses at only one hospital, the results may not be open to generalization, and there may be different applications in other hospitals. The study can be repeated with a larger sample and with multicenter studies.

5. CONCLUSION

In this study, nurses' level of acceptance in terms of using mobile applications was high. The nurses working in the intensive care units who were younger and used smartphones had a higher level of acceptance regarding the use of mobile applications. Most of the nurses expressed positive views on the use of mobile applications in the hospital setting. It was determined that the nurses thought that the use of MHAs should be made more widespread, that the apps were effective in reaching personal medical data, and that they helped patients access health services without going to the hospital.

The acceptance of nurses in using such applications should be seen as an opportunity and nurses should be provided with training in this respect. Also, the areas of usage of mobile health applications should be expanded at the hospitals. We also believed that nursing services will be facilitated and made more accessible through the use of mobile health applications in hospitals and home medical services.

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Clinical and Experimental Health Sciences

Effectiveness of Pediatric Palliative Care Education Program in Strengthening Nursing Students' Palliative Care Practices and Attitudes Toward Care of the Dying

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ABSTRACT

Objective: The present study used a quasi-experimental design to determine the effectiveness of palliative care education program in strengthening nursing students' palliative care practices and attitudes toward care of the dying.

Methods: Using the GPOWER 3.0 statistical analysis software package, the sample size for each group was determined as 8 students. The study involved 39 nursing student of 4th year, including 17 students in the experimental group and 22 in the control group. A pediatric palliative care education program was applied to the intervention group through interactive learning methods. The education consisted of a 14-week program. It was delivered to the students as a 1-hour education session each week using the face-to-face teaching model. The measurements were made at the beginning of the education program and at 3 and 6 months using the data collection tools. The data was evaluated using multidimensional variance analysis, the Bonferroni modified t measure, and regression analysis.

Results: A significant difference was found among the experimental and the control group for total mean scores of the all scale. A high level, positive, and highly significant relationship was found between the education program and all scale.

Conclusion: The results of the study indicated that the pediatric palliative care education program was an effective method that could be used in educating nursing students. The study provides effective results for including pediatric palliative care nursing course to the nursing undergraduate education curriculum.

Keywords: Pediatric palliative care, interactive education, nurse, pediatrics

1. INTRODUCTION

Pediatric palliative care (PPC) refers to comprehensive care that begins from the time when the child is diagnosed with the disease and covers the period of grief (1). PPC is a necessary for pediatric nursing. For this reason, it is necessary to include topics related to PPC lessons to the curriculum starting from nursing undergraduate education (2). Moreover, regular evaluations should be performed to determine whether education programs for PPC in nursing education are effective. Such assessments are of significance in identifying deficiencies that arise during education, reorganizing the program, and achieving preparation for the profession (2). When nursing undergraduate education curricula in Turkey are examined, it is observed that certain universities have included PPC in their undergraduate nursing education; however, there is no standard in terms of duration and content. Furthermore the literature review indicates that study outcomes are limited in assessing the level of experience and practices of nursing students who include PPC (3). The study of Kudubes and Bektas (2019) suggests that web-based PPC program given to nursing students was effective in increasing the level of their knowledge and practices. The concept of pediatric palliative care is still confused with the concept of end-of-life care in our country. For this reason, it is very important to teach the concept of pediatric palliative care during the student period when the basics of nursing care are learned. It is thought that nursing students who learn this concept will maintain a higher quality of care in their professional lives. However, no other studies examining different methods in PPC education have been found. Thus, there exists a need for studies examining the effect of PPC education provided to nursing students on their knowledge levels and practices in our country.

One of the goals of PPC is to provide care for the child and the family during the last stage and to support the grieving time after death (4,5). While a person who is approaching death or dying is experiencing her/his own pain, those around the person also experience certain emotions. The healthcare team providing care to the children in the hospital environment, especially the nurse and nursing students

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who establish the longest relationship with the children, experience this situation more frequently. Therefore, the attitudes of nursing students toward the concept of death are extremely important (4,5). Although the number of studies centering on palliative care conducted in our country and abroad is high (4,5), there are no studies investigating the PPC knowledge level of nursing students, their PPC practices, and their attitudes toward the care of the dying altogether. This study was conducted to determine the effectiveness of PPC education in strengthening nursing students' PPC knowledge level, PPC practices, and attitudes toward the care of the dying.

2. METHODS

2.1. Ethical Considerations

After obtaining permission from the institution where the study was conducted, ethics committee permission was obtained. The University Institutional Review Board (IRB approval number: 5162-GOA-2019/32–01) approved this study. In addition, by visiting and telling them about the purpose of the study, the written and verbal consent of students was included. Students voluntarily participated in the study. Students were involved in the study without any fear of results or retaliation

2.2. Study Design, Setting and Sample

The study planned a quasi-experimental design. The study was conducted between September 2019 and February 2020 with volunteering to nursing student of 4th year who were taking pediatric nursing elective courses at the nursing faculty of a university.

The calculation of the sample size required for the study was done using the GPOWER 3.0 statistical analysis software package. The calculation was based on the study of Bhatnagar and Patel (2018) and used the post-test scores of the experimental and control groups in the mentioned study with a level of significance of 0.05 and 99% power (6). The sample size comprised of 8 individuals for each group. Accordingly, 39 nursing students who were taking the pediatric nursing elective courses were recruited into the study. Of the 39 students included in the study, 17 constituted the experimental group and 22 the control group. To avoid contamination in the study, experimental and control groups were conducted in two different elective course classes. The study did not employ a randomization method. The study recruited nursing student of 4th year who were over 18 years and volunteering to participate in the study. In addition, control group conducted the pediatric emergency nursing elective course classes. They did not attend PPC nursing elective course classes.

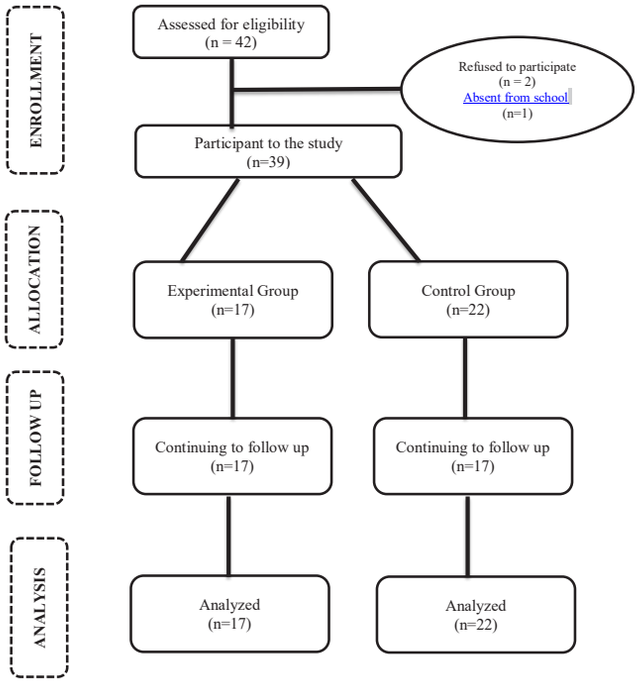

Figure I. *Participant flow diagram.*

2.3. Data Collection Tools

Students in the experimental group underwent a PPC education program that lasted 14 weeks. The measurements were made at the beginning of the education program and at 3 and 6 months using the data collection tools.

The information form consisted of four questions. The two questions collected socio-demographic information about students, such as age and gender, whereas the other two questions pertained to the students in getting information on PPC and their information sources for PPC.

The palliative care knowledge level questionnaire (PCKLQ) involves 15 questions and aims at measuring the level of students' knowledge. The questionnaire, which was designed by the authors, was created after a literature review on the PPC (4,5,7). The final form was obtained after necessary improvements. The sample group of the study was composed of 346 nurses and nursing students. The development study of the questionnaire consisted of 346 nurses and nursing students. Although the questionnaire has no cut-off point, higher scores show an increased level of student knowledge. The questionnaire was submitted to the opinions of six experts. The scale was finalized after the recommendations of the experts. The grades of the six experts were analyzed using content validity analysis; the content validity index (S-CVI) was found to be 0.99. There was a fit between the scores of the experts. Cronbach's alpha value of the questionnaire for the Turkish population was 0.793, Kaiser–Meyer–Olkin value was 0.886, and the Barlett test value was 1787.229 (8).

The palliative care self-reported practices scale (PCSPS) is a self-reporting scale that evaluates the status of nurses' implementation of palliative care practice in the clinical setting (9). The scale consists of 18 items and 6 subdimensions, including dying-phase care (items 1–3), patient and family centered care (items 4– 6), pain (items 7–9), delirium (items 10–12), dyspnea (items 13–15), and communication (items 16–18). PCSPS is a scale evaluated over total points. Higher scores received from the scale suggest improved procedures for palliative treatment. The Turkish scale validity and reliability analysis was performed in 2019 by Kudubeş et al. Cronbach's alpha (α) coefficients for the six sub-dimensions of the Turkish form were found to be 0.85, 0.91, 0.72, 0.89, 0.71, and 0.78, respectively. The α value for the overall scale was 0.91 (8).

The Frommelt Attitudes Toward Care of the Dying Scale **(**FATCOD) is a 30-item scale designed by Katherine H. Murray Frommelt in 1988. The scale contains an equal number of expression including desirable and undesirable attitudes. The total score is calculated by inverting the items containing negative attitudes and summing them with the scores of responses containing positive attitudes. The higher scores indicate increased positive attitudes. Çelik and Kav performed a validity and reliability review of the scale in Turkey in 2013 and α value was 0.73 (10).

The PPC Education Program which contains information about PPC nursing, was created by the researchers after a literature review on the topic (2,4,5,7). For its content, the education program was submitted to the opinions of experts including Department of Pediatric Oncology, Pediatric Nursing, Oncology Nursing.

The program consisted of a 14-week education module that contained introduction to PPC education program and administration of the pre-tests, definition, and purpose of the PPC, individuals involved in team, nursing and symptom management in PPC, (mucositis, changes in the blood, fatigue, sleep disorders, anorexia, nausea–vomiting, excretory changes, skin reactions, and psychosocial changes), communication in PPC, ethical and legal dimensions of PPC, spiritual care of the children and the family, end-of-life care, and complementary and alternative medicine (CAM) applications in PPC (Table 1).

2.4. Implementation of the Education Program

The education consisted of a 14-week program. It was delivered to the students as a 1-hour education session each week using the face-to-face teaching model. Interactive teaching methods, ensuring student participation, were used in each module. In each module, in-class group discussions on the topic were held, and concept maps were created with the nursing students. A machine-readable optical mark that provides information about the object to which it is connected is the QR code (Quick Response code). Using square codes, resources for PPC were shared with the students. Besides, for module centering on the roles and responsibilities of nurses working in the PPC unit, an interview was held with the nurse working in the PPC unit. In the module focusing on communication with the PPC patient and the family, an interview was conducted with the parent of the child who was receiving PPC. Participants in these interviews talked on the subject and answered the questions of students (Table 1).

2.5. Data Analysis

In order to determine the descriptive data, mean scores and percentage calculations were used. Two-way repeated measure ANOVA analysis was used in repeated measurements to compare mean scores on the PCKLQ, FATCOD, and PCSPS. In the advanced analysis of the difference between the mean scores of the PCKLQ, FATCOD, and PCSPS, one-way ANOVA analysis was used in repeated measurements to compare the mean scores of groups within the groups, and the Bonferroni adjusted t test were used to compare the control and experimental groups with each other. In order to analyze the data, the coding of the training program is "1" for the students in the experimental group and "0" for the students in the control group. The relationship between the education and PCKLQ and the relationship between PCSPS and FATCOD were analyzed using Spearman's correlation analysis. Linear regression analysis was employed to analyze the extent to which the education could predict the difference in PCKLQ, PCSPS, and FATCOD. Tolerance and variance inflation factor (VIF) values were employed to determine the existence of multicollinearity between the education and PCKLQ, PCSPS, and FATCOD, and to decide whether regression analysis could be administered to the variables. The power and the effect size of the study were calculated based on the regression analysis. G-Power 3.1 program was used to calculate the effect size with regression analysis and the calculated effect size was indicated with f^2. The significance level was accepted as 0.05.

3. RESULTS

Examination of descriptive characteristics of nursing students indicated that the mean age of the students in the experimental group was 21.53 + 0.94, and that of students in the control group was 21.36 + 0.65. Of the students in the experimental group, 88.2% were females, 64.7% were found to have received information about PPC, and 35.3% were determined to have learned about PPC from books and journals. On the contrary, 86.4% of students in the control group were females, 54.5% were determined to have received information about PPC, and 31.8% were found to have learned about PPC from books and magazines, congresses and seminars, and the Internet. Based on these analyses, the groups were observed to show no difference in terms of gender, receiving information about PPC, and information sources about PPC. Accordingly, the groups were determined to be homogeneous ($p > 0.05$).

Table 1. *Education Modules*

	Module	Content	Methods
Module 1	Introduction to PPC training program and administration of pre-tests	Explaining the purpose of the training program	Presentation
Module 2	PPC and nursing	Introducing the purpose of PPC, Discussing PPC services and their current status in the world and in Turkey, Learning about the staff in the PPC team and their responsibilities	Presentation, In-class group discussion
Module 3	The roles and responsibilities of nurses working in the PPC unit-Interactive sharing	Defining PPC nursing, Discussing the duties and responsibilities of the PPC nurse	Presentation, In-class group discussion, Panel with the nurse working in the related unit
Module 4	Management of pain, fatigue, and sleep disorders in PPC	Identifying symptoms, Explaining the influencing factors and the etiology, Knowing the assessment methods of the symptoms, Discussing pharmacological and non-pharmacological approaches to symptoms, Determining appropriate nursing interventions toward symptoms	Presentation, Case scenario, In-class group discussion, Concept map, QR code application, Nursing care plan
Module 5	Mucositis, anorexia-cachexia, and management in PPC	Identifying symptoms, Explaining the influencing factors and the etiology, Knowing the assessment methods of the symptom, Discussing pharmacological and non-pharmacological approaches to symptoms, Determining appropriate nursing interventions toward symptoms	Presentation, Case scenario, In-class group discussion, Concept map, QR code application, Nursing care plan
Module 6	Nausea–vomiting, and diarrhea–constipation and management in PPC	Identifying symptoms, Explaining the influencing factors and the etiology, Knowing the assessment methods of the symptom, Discussing pharmacological and non-pharmacological approaches to symptoms, Determining appropriate nursing interventions toward symptoms	Presentation, Case scenario, In-class group discussion, Concept map, QR code application, Nursing care plan
Module 7	Neutropenia–thrombocytopenia–anemia, and management in PPC	Identifying symptoms, Explaining the influencing factors and the etiology, Knowing the assessment methods of the symptom, Discussing pharmacological and non-pharmacological approaches to symptoms, Determining appropriate nursing interventions toward symptoms	Presentation, Case scenario, In-class group discussion, Concept map, QR code application, Nursing care plan
Module 8	Skin reactions in PPC and nursing approach	Identifying symptoms, Explaining the influencing factors and the etiology, Knowing the assessment methods of the symptom, Discussing pharmacological and non-pharmacological approaches to symptoms, Determining appropriate nursing interventions toward symptoms	Presentation, Case scenario, In-class group discussion, Concept map, QR code application, Nursing care plan
Module 9	Psychosocial disorders and management in PPC	Identifying symptoms, Explaining the influencing factors and the etiology, Knowing the assessment methods of the symptom, Discussing pharmacological and non-pharmacological approaches to symptoms, Determining appropriate nursing interventions toward symptoms	Presentation, Case scenario, In-class group discussion, Concept map, QR code application, Nursing care plan
Module 10	Communication with PPC patient and family – Interactive Sharing	Learning to establish correct communication techniques with the patient and the family, Explain the points to be considered in communication	Presentation, Panel with the parents receiving care in the related unit
Module 11	Ethical and legal dimensions of PPC	Ethical considerations in pediatric palliative care, Legal aspects of pediatric palliative care	Presentation, Case scenario,
Module 12	Spiritual care of the PPC patient and the family	Learning the role of the nurse in spiritual care, Learning cases that may cause spiritual distress in the patient and the family, Discussing appropriate nursing interventions	Presentation, Case scenario, Concept map, Nursing care plan
Module 13	End-of-life care of the PPC patient and care of the family during the grieving process	Knowing nursing interventions to be implemented before, during, and after death, Knowing the role and importance of the nurse in family care during the grieving process, Learning the interventions to be applied to the family during the grieving process	Presentation, Case scenario, Concept map, Nursing care plan
Module 14	Complementary and alternative medicine (CAM) applications and nursing approaches in PPC	Purpose of CAM in pediatric palliative care, CAM methods used in pediatric palliative care	Presentation, In-class group discussion

PPC: Pediatric palliative care

Table 2 presents the comparison of mean total scores of nursing students in the intervention and control group for the PCKLQ, and the PCSPS and the FATCOD Scale. Multidimensional variance analysis was used in repeated measurements to determine whether there were any differences between the groups' total mean scores on the PCKLQ, PCSPS and FATCOD. Statistically significant differences were found between the mean scores, respectively (F = 48.961, p = 0.000; F = 14.809, p = 0.000; F = 84.756, p =0.000).

Table 3 presents the relationship between the education program and palliative care practices, PCKLQ and FATCOD. A high level, positive, and highly significant relationship was found between the education program and PCKLQ, FATCOD, and PCSPS (p <0.01).

Table 4 presents the difference in the level of attitudes with respect to nursing students' PCKLQ, their practices, and FATCOD. While the education program was found to explain 83% of the increase in the level of PCKLQ (R2 = 0.832), getting education was observed to increase the level of PCKLQ by 0.912 times (β = 0.912). Moreover, the education program explained 87% (R2 = 0.877) of the increase in the level of PCSPS, getting education increased the PCSPS by 0.936 times (β = –0.936). Besides, while the education program explained 95% of the increase in the level of FATCOD (R2 = 0.955), getting education increased the FATCOD by 0.977 times (β = –0.977). PPC education program was determined to be statistically significant in increasing nursing students' PCKLQ, PCSPS, and FATCOD (p < 0.001).

The power and effect size of the study was evaluated according to the regression analysis. The power of the study for the Palliative Care Knowledge Questionnaire was 0.99, and the effect size (f^2) was 0.846. The power for the PCSPS was 0.99, and the effect size (f^2) was 0.880. Moreover, for the FATCOD Scale, the power of the study was 0.99, and the effect size (f^2) was 0.960.

Table 2. *Comparison of Mean Total Scores of Nursing Students in the Intervention and Control Group for Palliative Care Knowledge Level Form and the Scale of Self-Reported Palliative Care Practices*

Time Group		Pretest X±SS	Posttest 1 X±SS	Posttest 2 X±SS	F	p
PCKLQ	**Experimental group**	27.35 ± 10.65	60.58 ± 6.87	59.00 ± 5.45	48.961	0.000
	Control group	25.59 ± 9.96	27.04 ± 9.73	27.45 ± 9.81		
PCSPS	**Experimental group**	28.58 ± 9.13	73.70 ± 8.65	72.82 ± 9.16	14.809	0.000
	Control group	28.50 ± 8.45	29.72 ± 8.59	29.72 ± 8.59		
FATCOD	**Experimental group**	46.47 ± 18.00	147.29 ± 16.13	143.82 ± 11.66	84.756	0.000
	Control group	45.90 ± 17.83	47.22 ± 16.82	48.54 ± 17.50		

PCKLQ: Palliative Care Knowledge Level Questionnaire; PCSPS: the Palliative Care Self-reported Practices Scale; FATCOD: the FATCOD Scale

Table 3. *The Relationship between Study Variables*

	1	2	3	4
1. The Palliative Care Self-reported Practices Scale	1.000			
2. The Frommelt Attitudes Toward Care of the Dying Scale	0.942	1.000		
3. The Palliative Care Knowledge Level Questionnaire	0.971	0.933	1.000	
4. Training	0.936	0.977	0.912	1.000

**Significant at p < 0.01.*

Table 4. *The Extent to which the Education Predicted the Difference in Palliative Care Knowledge Level, Practices, and Attitudes toward Providing Care to the Dying according to the Self-Evaluation of the Nursing Students*

Variable	Palliative care knowledge level					Palliative care self-reported practices					Attitude toward providing care to the dying				
	B	SH	β	t	p	B	SH	β	t	p	B	SH	β	t	p
Study*	32.781	2.419	0.912	13.551	0.000	43.890	2.704	0.936	16.234	0.000	99.505	3.546	0.977	28.062	0.000
R	**0.912**					**0.936**					**0.977**				
R^2	**0.832**					**0.877**					**0.955**				
F	**183.607**					**263.536**					**787.491**				
P	**0.000**					**0.000**					**0.000**				

**When coding the study program, the intervention group was coded as "1" and the control group as "0."*

4. DISCUSSION

The present research found statistically important variations between the cumulative scores of the experimental and control groups on the PCKLQ and PCSPS; the experimental group's post-test mean scores were higher than their pre-test mean scores (Table 2). In the literature, there are studies examining the effects of education models on nursing students' level of adult PCKLQ and PCSPS (11,12). However, literature reports limited studies investigating the effect of education models on nursing students' PCKLQ and PCSPS C. However, there were studies in the literature examining how interactive education models affect nursing students' level of PCKLQ and PCSPS (13,14). In nursing education, especially, interactive education models such as group discussion, and concept maps have been reported to help students understand the subject, reinforce learning, and ensure the permanence of information (13,15). Besides, case discussions and nursing care plan sharing are emphasized to help students use the knowledge to real-life (clinical practice, case-studies etc.) and enable them to grasp how to transfer theoretical knowledge into practice (16). In the literature, web-based PPC education was found effective in increasing PCKLQ and PCSPS (3). In addition, it determined that palliative care education provided to nursing students via videos, case studies, and role-play methods increased the knowledge level and practices of students in the literature (17). The findings of the literature were observed to be consistent with those of the study. In the current study, provision of PPC information to students, conduction of case discussions using case scenarios, discussion of students' questions through group discussions, and the ease of student access to related materials were thought to be effective in increasing PCKLQ and PCSPS levels and post – education mean scores of the experimental group.

This study found statistically significant differences between experimental and control groups' total scores on the FATCOD, the post-test mean scores of the experimental group were higher than their pre-test mean scores and control group scores (Table 2). In the literature, there was no study examining the effect of PPC education applied to the nursing students on their FATCOD. However, there exist studies in the literature examining how education provided through interactive education methods affected nursing students' attitudes and anxieties toward death (4,5). In a study, interactive methods, such as case scenarios, concept map group work, and simulation, were stated to be employed in teaching how to provide care to the dying in nursing undergraduate education (12). In a study with nursing students, it determined that during palliative care education, case scenarios and interactive education methods provided an experimental opportunity in reducing students' anxiety about dying child (14). Another study emphasized that it was important for students to feel comfortable about providing care to the child who is dying; moreover, using several education methods to orient students to this situation was effective (5). The availability of palliative care education in undergraduate nursing education and the use of interactive education methods while teaching are emphasized to be significant in developing nursing students' PPC practices and effective clinical decision-making skills while caring for the dying child (4,5). The findings of the literature were consistent with those of the study. In the present study, discussion of care during the death and grieving process with methods, such as case scenarios, in-group discussions, were believed to positively affect students' attitudes. Furthermore, in the interview modules with a nurse working in the PPC unit and a parent whose child was receiving palliative care, the attitude scores of the students were believed to increase because students asked questions and joined group discussions by sharing their anxieties about providing care to a dying child.

In the current study, a high level, positive, and highly significant relationship was found between the education program and the PCSPC, the FATCOD Scale, and the PCKLQ (Table 3, $p < 0.01$). The education program explained 83% of the increase in the level of PCKLQ, 87% of the increase in the level of PCSPS, and 95% of the increase in the level of FATCOD (Table 4). While there are limited data in the literature regarding what percentage of changes in the level of PCKLQ and PCSPS levels were affected by PPC education programs (3), there was no information on the extent of change in attitude level related to providing care to the dying. When we examined literature revealing changes in the PCKLQ and PCSPS level brought about by PPC education programs, web-based education was found to be effective in increasing palliative care knowledge levels and practices (3). Moreover, several factors have been reported in the literature that affect both palliative care knowledge and practices including attitudes toward giving care to the dying (18,19). Therefore, the finding that the education program provided 83% increase in PCKLQ level of nursing students, 87% increase in their level of PCSPS, and 95% increase in their attitudes toward providing care to the dying suggested a good outcome in terms of PCKLQ and practices, which are affected by several factors, and in terms of caring for the dying. Furthermore, this finding reveals the effectiveness of the study. The effectiveness of the education program is believed to stem from the fact that it focused on nursing students; it covered all topics related to PPC; it included information about psychosocial areas such as spiritual care, death, and grieving in the context of PPC; it employed interactive learning methods; the content of the education centered on the learning needs of the nursing students, and it was nursing care plan-oriented.

The power and effect size were calculated according to the regression analysis. The study was found to be powerful in terms of both the level of PCKLQ, PCSPS and FATCOD. While the power shows the statistical significance of the study, the effect size provides information about practice/clinical significance (20). According to literature classification of effect sizes (f2), $0.02 \geq f2 < 0.15$ implies small effect size, $0.15 \geq f2 < 0.35$ implies a medium effect size, and $0.35 \geq$ implies a large effect size (20). Considering these values, the present study was observed to have a large effect size in all dimensions. In literature, studies indicating the power and effect size of education programs related to PPC are

limited (3). In the study of Kudubeş and Bektaş (2019), the web-based PPC education program was determined to have a small effect size for palliative care knowledge level and large effect size for palliative care practices. In the present study, the effect size for all variables was greater than 0.80, which indicated that the application significance of the study was high. The application significance of the face-to-face interactive education conducted with nursing students was believed to be higher than that of the web-supported education method. These results revealed that the study was feasible and effective.

4.1 Limitations

The investigators developed the PCKLQ used in the analysis. To ensure the reliability and authenticity of the assessment form, expert opinions were collected and the material validity index was determined.

5. CONCLUSION

In conclusion, it was found that the PPC education curriculum was successful in enhancing the skills and activities of nursing students and their constructive attitudes towards the treatment of the dying infant, adding to the literature and nursing education of the nation and world. This study showed that the PPC education program could be effectively used in educating nursing students. Furthermore, assessment of cognitive and psychomotor skills and conducting future clinical evaluation studies that include PPC education programs would provide clearer results.

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Clinical and Experimental Health Sciences

Salivary Levels of IL-21 as a Potential Marker of Stage III Grade C Periodontitis

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ABSTRACT

Objective: The onset, severity and progression of periodontal diseases are mainly related to the inflammatory host response against periodontal pathogens. The aim of this study was to evaluate salivary interleukin (IL) – 1β, IL-13, IL-21 and IL-33 levels in patients with stage III grade C periodontitis and compare it with periodontally healthy individuals.

Methods: A total of 58 individuals, including 28 periodontally healthy and 30 stage III grade C periodontitis patients were included in this study. Periodontal parameters including plaque index, gingival index, bleeding on probing, probing depth and clinical attachment level were measured. Saliva samples were obtained from all patients. Salivary interleukin (IL) – 1β, IL-13, IL21, IL-33 levels were assessed using enzyme-linked immunosorbent assay.

Results: All clinical parameters were significantly higher in periodontitis patients compared to healthy individuals (p<0.001). Elevated salivary IL-1β and IL-21 levels were found in the periodontitis group compared to healthy ones (p=0.009 and p<0.001, respectively). However, IL-13 and IL-33 levels were similar in both groups (p= 0.92). IL-1β was significantly correlated with both clinical and biochemical parameters but IL-21 was correlated with only clinical parameters.

Conclusion: This study showed that elevated salivary IL-21 and IL-1β levels are associated with periodontitis and might be used as a marker for the diagnosis of periodontitis.

Keywords: Periodontitis, interleukin-1 beta, interleukin-13, interleukin-21, interleukin-33

1. INTRODUCTION

Periodontitis is a common infectious condition with the presence of gingival inflammation, alveolar bone resorption, and attachment loss (1). Host response against pathogenic microorganisms in dental biofilm is a main factor for pathogenesis of periodontitis (2). Cytokines are the messenger molecules between cells that regulate this response. Moreover, dysregulated production of cytokines is associated with the initiation and progression of several infective and inflammatory diseases such as periodontitis (2-4). Cytokines can act antagonistically or synergistically and are classified according to their functions as pro-inflammatory or anti-inflammatory molecules (3). The pro-inflammatory cytokine IL-1ß, act as a critical mediator of inflammation and tissue destruction. It plays a vital role in regulating inflammatory and immunological events, such as leukocyte chemotaxis, monocyte/macrophage activation, production of matrix metalloproteinases (MMPs), prostaglandins and T cell activation (5). Elevated levels of IL-1ß were detected in saliva, serum, gingival crevicular fluid (GCF) and gingival tissue of patients with periodontitis (6-8).

Anti-inflammatory cytokines promote physiological health by stimulating the protective antibodies production and diminishing the levels of destructive inflammatory cytokines (9, 10). IL-13 is an anti-inflammatory cytokine activated by T helper 2 cells (Th2) (11). It has been shown that IL-13 inhibits pro-inflammatory cytokine synthesis and osteoclastogenesis (12, 13). Although, there are several studies about IL-13 levels in GCF and serum, there is no study that compares the salivary levels of IL-13 in both healthy and periodontitis groups. Miranda et al. showed similar serum IL-13 levels in both periodontally healthy and periodontitis patients (14). On the other hand, Elabdeen et al. found lower plasma IL-13 levels in aggressive periodontitis patients compared to the healthy controls (15). In GCF, Gorgun et al. showed lower IL-13 levels in aggressive periodontitis patients than chronic periodontitis and periodontally healthy groups (16). Moreover, three studies have evaluated IL-13 levels in

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periodontitis before and after periodontal treatment. While two of these studies found significantly elevated GCF IL-13 levels after treatment (16, 17), the other showed no change (18).

IL-21 is predominantly released by Th17 cells and acts as a pro-inflammatory cytokine. It targets a broad range of immune cells (19, 20). A growing body of evidence shows that T-cell responses associated with inflammation and tissue destruction are improved by IL-21 (21-25). Moreover, it suppresses the production of the anti-inflammatory IL-13 cytokine produced by Th2 cells (26). There is a bidirectional activating relationship between IL-21 and IL-1ß. While IL-1β increases IL-21 secretion by inducing Th17 cells, IL-21 has the ability to upregulate IL-1β (27-29). There are limited and contradictory results regarding IL-21 levels in periodontitis compared to healthy controls. A study showed elevated salivary IL-21 levels in periodontitis compared to healthy controls (30). However, Gumus et al. found no difference in serum or salivary IL-21 levels between groups (31).

IL-33 plays a crucial role in inflammation. Since IL-33 has an effect on both increasing Th2 derived anti-inflammatory cytokines and stimulating mast cell degranulation or production of pro-inflammatory cytokines, it acts as an immunoregulator (32, 33). Although, elevated salivary levels of IL-33 in periodontitis patients compared to healthy controls has been reported (31), there are also studies that revealed no difference between these groups in saliva and GCF (34, 35).

The objective of the study was to compare the salivary IL-1β, IL-13, IL-21, and IL-33 levels in patients with stage III grade C periodontitis and healthy controls.

2. METHODS

2.1. Study Population

A total of 58 individuals (29 male and 29 female) were recruited from the Department of Periodontology, Faculty of Dentistry, Marmara University, Istanbul, Turkey. A medical and dental histories were recorded. All individuals were systemically healthy, non-smoker, aged over 20 years and had at least 20 teeth (except third molars). The individuals that were included in the study met the following criteria; they were not pregnant or lactating, had not received periodontal treatment and were not using any antibiotics, immunosuppressive or nonsteroidal anti-inflammatory drugs in the past 6 months.

The participants were categorized into healthy or stage III grade C periodontitis groups according to the consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions (36). The periodontally healthy group was defined as presence of intact periodontium, no sites with attachment loss and radiographic evidence of alveolar bone loss, no history of periodontitis, bleeding on probing (BOP) <10%, probing depth (PD) <3mm, while stage III periodontitis patients had at least 4 interdental sites clinical attachment level (CAL)> 5 mm due to periodontitis, radiographic bone loss reaching to the mid-third of the root or beyond, teeth loss less than 4 teeth due to periodontitis.

Grade assessment was carried out according to the ratio between radiographic bone loss (%) and age. Since the bone loss (%)/age values were >1.0, all periodontitis patients were defined as grade C.

The clinical research ethics committee of Marmara University, Faculty of Medicine, Istanbul, approved the present study protocol (12.06.2020/ 09.2020.652). All patients were informed about the study, and a written informed consent form in compliance with the 1964 Helsinki Declaration and its later amendments was obtained.

2.2. Clinical Measurements

A single calibrated examiner (NGG) carried out a full mouth periodontal examination of all participants. Before clinical measurements, intra-examiner calibration was performed by measuring PD and CAL values twice on five patients with one day interval resulting in intraclass correlation coefficients were 0.92 for PD and 0.90 for CAL.

Plaque index (PI) (37), gingival index (GI) (38), BOP, PD, CAL measurements were recorded at six sites of each tooth except third molars. All clinical values were examined using a UNC15 probe (Hu-Friedy, Chicago, IL). Self-reports of the patients were used as a basis for assessing existing tooth loss due to periodontitis.

2.3. Collection and Analyses of Salivary Samples

Unstimulated saliva samples were collected from all individuals a day after clinical measurements. All samples were obtained between 9 am and 10 am to decrease the effect of circadian rhythm on biomarker levels. The participants were asked not to brush their teeth, floss, chew gum, eat or drink within the last 3 hours. During saliva collection, they were requested to accumulate the saliva in the mouth for 5 min and spit into sterile 2 ml Eppendorf tubes (Safe-Lock Tubes 1.5 ml, Sigma, Hamburg). Then all saliva samples were stored immediately at − 80 °C before assays.

Salivary concentrations of IL-1β, IL-13, IL-21 and IL-33 were determined using specific ELISA kits (Elabscience, Houston, TX, USA and Bioassay Technology Laboratory, Shanghai, China). The manufacturer's guidelines were followed for each assay, using saliva samples. The minimum detection thresholds for IL-1β, IL-13, IL-21 and IL-33 were 4.69 pg/mL, 0.2 ng/L, 2.46 ng/L and 2.61 ng/L, respectively.

2.4. Statistical Analyses

The minimum sample size was determined based on a study investigating salivary IL-1β levels in a similar group design (39). This analysis indicated that the minimum required

sample size was 14 for each group at α=0.05 significance level and with a power of 90%.

All statistical analyses were assessed using a statistical software package (SPSS 22.0 for Windows, Chicago, IL). Descriptive statistics such as median, minimum-maximum, mean and standard deviation values were used to present age, gender distribution, clinical and biochemical data. Difference in gender distribution was analyzed by Chi-square test. Normality analyzes of all data were performed using Kolmogorov Smirnov test. Intergroup comparisons were carried by either Students' t-test or Mann Whitney U test, depending normality of the distribution. Correlations between clinical and biochemical parameters were determined by Spearman rank correlation test. For testing the possible utility of IL-1β and IL-21 in periodontitis diagnosis, receiver operating characteristics (ROC) and area under the curve (AUC) analyses were constructed. Statistical significance was accepted as $p<0.05$.

3. RESULTS

Demographic variables are presented in Table 1. The median age and gender distribution were similar in both groups ($p=0.103$ and $p=0.525$, respectively). Significantly higher values of clinical periodontal measurements were detected in the periodontitis group compared to periodontally healthy ones ($p<0.001$) (Table 2).

Table 1. *Age and gender distribution pattern of healthy individuals and periodontitis patients*

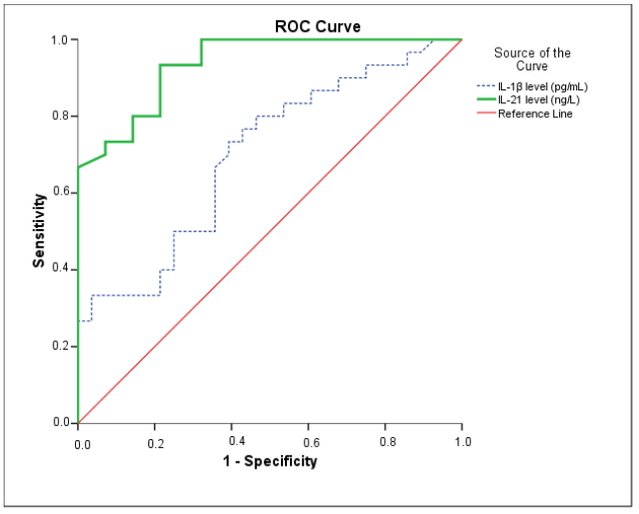
		Healthy N=28	Periodontitis N=30	p^*	p^{**}
Age	Median Min-Max (Mean+SD)	31.00 23-48 (33.50±7.63)	35.50 25-59 (36.23±7.94)	0.103	
Gender N (%)	Female Male	15 (53.6) 13 (46.4)	14 (46.7) 16 (53.3)		0.599

*SD: Standart Deviation. *Mann Whitney-U test, **Chi-square test; p<0.05*

The saliva levels of IL-1β, IL-13, IL-21, IL-33 in both groups are shown in Table 3. Elevated salivary levels of IL-21 and IL-1β were found in the periodontitis group compared to the periodontal health ($p<0.001$ and $p=0.009$, respectively). However, saliva IL-13 and IL-33 levels did not differ between healthy and periodontitis groups ($p= 0.932$ and $p=0.926$, respectively).

Correlations of salivary interleukin levels with clinical periodontal parameters and with each other are presented in Table 4. IL-1β levels correlated positively with both clinical and biochemical parameters ($p<0.01$ or $p<0.05$) but IL-21 levels only with all clinical parameters ($p<0.01$). Moreover, positive correlation was found between IL-13 and IL-33 ($p<0.01$).

AUC values and ROC curves for IL-1β and IL-21 in discriminating periodontitis patients from healthy controls are shown in Figure 1. IL-1β and IL-21 provided larger AUC values than 0.5 (0.699 and 0.937, respectively).



Biochemical parameters	AUC	%95 (CI)	Cut-off	Sensitivity	Specificity	p
IL-1β (pg/mL)	**0.699**	0.564-0.833	18.595	0.633	0.643	**0.009**
IL-21 (ng/L)	**0.937**	0.881-0.993	240.045	0.800	0.786	**<0.001**

Figure 1. *Receiver operating characteristics (ROC) curve of IL-1β and IL-21 in regard to periodontitis with area under the curve (AUC), 95% confidence intervals, cut off, sensitivity, specificity and p values.*

Table 2. *Clinical Measurements of Study Groups*

Periodontal Parameters	Healthy N=28 Median Min-Max (Mean+SD)	Periodontitis N=30 Median Min-Max (Mean+SD)	p
PI	0.11 0.01-0.33 (0.12±0.07)	1.85 1.05-3.39 (1.88±0.51)	**<0.001***
GI	0.07 0.01-0.18 (0.06±0.04)	1.90 0.41-2.22 (1.75±0.46)	**<0.001***
BOP (%)	5.75 1.19-10.50 (6.11±2.52)	93.64 20.51-100 (82.24±23.45)	**<0.001***
PD (mm)	1.91 1.52-2.32 (1.91±0.16)	4.28 3.12-5.82 (4.30±0.63)	**<0.001**#
CAL (mm)	1.92 1.52-2.32 (1.93±0.17)	4.76 3.22-5.99 (4.73±0.69)	**<0.001**#

*SD: Standart Deviation; PI: Plaque Index; GI: Gingival Index; BOP: Bleeding on Probing; PD: Probing Depth; CAL: Clinical Attachment Level. *Mann Whitney-U test, #Student's t-test; p<0.05*

Table 3. *Saliva Levels of Interleukins in Study Groups*

Biochemical Parameters	Healthy N=28 Median (Mean±SD)	Periodontitis N=30 Median (Mean±SD)	*p**
IL-1β (pg/mL)	10.02 2.32-61.62 18.17±16.40	26.30 2.37-368.56 54.91±76.09	**0.009**
IL-13 (ng/L)	18.79 11.01-23.65 17.76±3.82	18.12 9.82-23.13 17.86±3.43	0.932
IL-21 (ng/L)	200.78 127.65-255.01 197.33±37.76	261.49 223.37-312.65 265.43±24.73	**<0.001**
IL-33 (ng/L)	319.99 121.92-466.96 300.14±82.77	303.76 140.64-433.81 306.72±65.59	0.926

*SD-Standart deviation. *Mann Whitney-U test; p<0.05*

Table 4. *Correlation of Biochemical and Clinical Parameters*

		IL-1β (pg/mL)	IL-13 (ng/L)	IL-21 (ng/L)	IL-33 (ng/L)
PI	r	**0.326***	0.090	**0.668****	0.104
GI	r	**0.406****	0.050	**0.684****	-0.011
PD	r	**0.407****	0.139	**0.679****	0.126
CAL	r	**0.414****	0.161	**0.677****	0.167
BOP (%)	r	**0.406****	0.062	**0.716****	0.054
IL-1β (pg/mL)	r	-	**0.612****	**0.441****	**0.455****
IL-13 (ng/L)	r		-	0.210	**0.837****
IL-21 (ng/L)	r			-	0.228

*PI: plaque index; GI: gingival index; BOP: bleeding on probing; PD: probing depth; CAL: clinical attachment level, r: Correlation coefficient. Spearman's Rank Correlation Test; *p<0.05, **p<0.01*

4. DISCUSSION

The present study is the first study that investigated salivary IL-1β, IL-13, IL-21 and IL-33 levels in patients with stage III grade C periodontitis and healthy controls. Various cytokines have been studied in the literature to understand the host-mediated nature of peridontitis. Although, periodontitis is diagnosed according to the clinical measurements and radiographic findings, these parameters do not provide information about disease activity and early diagnosis. Thus, it is important to explore a reliable biomarker of periodontal tissue destruction with high specificity, sensitivity and utility (40).

Periodontal diseases are associated with increased levels of particular pro-inflammatory cytokines in saliva due to repeated insult of dental biofilms (41-43). IL-1β is a pro-inflammatory cytokine that involves in inflammation, immune regulation and bone resorption in periodontitis. Considered number of studies showed the well-established role of IL-1β in pathogenesis of periodontitis (6, 7). In line with earlier findings, the present study resulted in significantly higher salivary IL-1β levels in patients with periodontitis compared to healthy ones (6, 7). Furthermore, IL-1β is a strong biomarker in discriminating periodontitis patients from periodontally healthy individuals with its high AUC value (0.699). The positive relation between clinical parameters and IL-1β found in the present study supports the knowledge about the pathogenic role of IL-1β in periodontitis.

Levels of anti-inflammatory cytokines are expected to be lower in periodontitis than healthy individuals (10). Unexpectedly, in the present study, the salivary levels of IL-13, an anti-inflammatory cytokine did not differ between groups. To our knowledge, there is no study available regarding to the salivary IL-13 levels in periodontal health or disease. Future studies are needed to better understand its role in periodontitis.

In an in vitro study, it was shown that IL-1β enhances Th2 differentiation and IL-13 production (44). In accordance with this study, the positive relation was found between IL-1β and IL-13 in the present study. However, future studies are needed to establish the relationship.

IL-21 is a pro-inflammatory cytokine that affects the functions of various immune cells and exaggerates the host-immune response. It involves the development of Th17 cells and suppresses the Th2 cell differentiation and function (26, 45, 46). Thus, IL-21 have a critical function in the pathogenesis of periodontal disease. The present findings demonstrated elevated salivary IL-21 levels in periodontitis compared to healthy individuals (p<0.001). In accordance with present results, several studies showed higher IL-21 levels in periodontitis (30, 46, 47). Similar to our findings, Lokhande et al. found higher serum and salivary IL-21 levels in patients with periodontitis than healthy controls (30). Dutzan et al. found overexpressed IL-21 in periodontitis-affected tissues than healthy ones (47). Since cytokines that highly expressed in gingival tissues during inflammation, are spilled over eventually to saliva, this finding may explain the higher salivary levels of this interleukin. On the other hand, a single study revealed similar IL-21 levels between periodontitis and healthy controls (31). Furthermore, the diagnostic accuracy of IL-21 was found to be good in the present study with its high AUC value (0.937). Thus, IL-21 can be an important mediator for periodontal disease. The positive and significant correlation between IL-21 and IL-1β levels seen here may be attributable to the fact that IL-21 upregulates the IL-1β expression and IL-1β stimulates IL-21 production, as mentioned earlier (27-29). Although, IL-1β is a well-known biomarker that differentiates health and disease, there are very limited data on the role of IL-21 in periodontitis. Our results demonstrated that IL-21 might be a crucial marker for the diagnosis of periodontitis similarly to IL-1β.

Early evidence demonstrated that IL-33 is released from damaged endothelial cells and functions as an alarmin. It induces the production of anti-inflammatory cytokines like IL-13 as a result of stimulating Th2 cells, but at the same time it increases mast cell degranulation and the synthesis

of pro-inflammatory cytokines such as IL-1β (32, 33). Results of the present study revealed similar salivary IL-33 levels in both healthy and periodontitis patients which is consistent with other studies by Saglam et al. (34) and Buduneli et al. (35). However, Gumus et al. found elevated salivary IL-33 levels in periodontitis group compared to healthy individuals (31). Moreover, in an experimental animal study higher expression was found in periodontitis compared to healthy ones (48). Future studies are needed to understand its role in periodontitis.

There was a positive relation between IL-33 and IL-1β. The positive relation between salivary IL-33 and IL-1β levels supports the knowledge about the inductive effect of IL-33 on mast cell degranulation and therefore, IL-1β production (33). Furthermore, we found positive relation between IL-33 and IL-13. This finding may be explained by the inductive effect of IL-33 on Th2 cells and IL-13 mentioned before (32).

The limitations of the present study were investigation of the cytokines in a single body fluid and a cross-sectional study design.

5. CONCLUSIONS

In conclusion, IL-21 levels like IL-1β, were detected higher in the periodontitis patients than healthy ones suggesting a crucial role in periodontitis pathogenesis. Follow-up studies including different body fluids like GCF and/or serum, are needed to confirm that IL-21 could be used as a salivary biomarker of peridontitis.

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Clinical and Experimental Health Sciences

Nursing Care in Postpartum Atypical Hemolytic Uremic Syndrome: A Case Report

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ABSTRACT

Pregnancy-associated atypical hemolytic uremic syndrome (P-aHUS) is a systemic disease associated with high morbidity and mortality rates, caused by dysregulation of the alternative complement pathway, leading to uncontrolled complement activation resulting in thrombotic microangiopathy. In this case it's reported patient care management of a P-aHUS patient which developed in postpartum period with renal failure, anasarca edema, hypertension and important laboratory signs of the syndrome. Patient was effectively treated by hemodialysis and eculizumab therapy, which controls complement activation and discharged without signs of hemolysis. The patient was discharged to home from the hospital after 42 days. At follow-up 2 weeks after discharge, all signs and symptoms of renal failure had resolved. The patient has not had any inpatient readmissions to the hospital to date. There are no case report in P-aHUS nursing care. Discussion of this case contributes the literature describing nursing interventions associated with caring for patients with P-aHUS.

Keywords: Postpartum, Hemolytic uremic syndrome, Nursing

1.INTRODUCTION

Pregnancy associated atypical hemolytic uremic syndrome (P-aHUS) is a rare, life-threatening complication in pregnancy. It is seen in about one in 25.000 pregnancies and it's associated with a significant perinatal or maternal morbidity and mortality (1-3). P-aHUS is a severe, systemic disease, first described by Robson in 1968 (4), associated with uncontrolled complement alternative pathway activation, leading to hemolytic anemia, usually accompanied by thrombocytopenia, hypertension and acute renal failure (5). Here it's reported nursing care management of a severe P-aHUS case in the postpartum period, through timely diagnosis and treatment resulted in healing and remarkable renal recovery. The aim of this case report is; to describe a rare severe illness and to summarize the nursing care in these cases.

2. CASE PRESENTATION

A 29-years old female, free of previous medical history except tonsillectomy and migraine. She has 3.25 degrees myopia and astigmatism. She is non-smoker. Her elder sister was diagnosed with P-aHUS following by her first labor in March of 2017. Similarly P-aHUS symptoms was presented in postpartum period in our patient. Her pregnancy had been without complication and she followed routine antenatal appointments including light thrombocytopenia, iron deficiency and anemia (hemoglobin=8-9g/dL). She was transfused two units of blood just before the cesarean (Hgb=6.6g/dL) and four units more after the labor. She was admitted in March 2018 for a planned cesarean section 38 week and 5 days. One week after cesarean section, she presented hypertensive crisis, anasarca edema, oliguria, hematuria and she was hospitalized postpartum day 13. Diagnosis process and initiation of treatment were quickly because of her elder sister P-aHUS history.

Schistocytes were detected 3-4% on peripheral blood smear, while elevated lactate dehydrogenase (LDH)=1805 IU/L (90-240), total bilirubin (TBIL)=3.36mg/dL (0.3-1.5), direct bilirubin=0.62 (0-40), serum creatinine=1.88mg/dL (0.40-1.40), blood urea nitrogen (BUN)=33mg/dl (5-24), as well as decreased platelet count (PLT)= 41×10^9/L ($173\text{-}390\times10^9$/L), red blood cell (RBC)= $2.66\text{x}10^6$(3.92-5.08), hemoglobin=8.3g/dL(11.9-14.6) were recorded. Haptoglobulin levels were low

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at 0.315g/L (0.3-2), and direct Coombs testing was negative. ADAMTS-13 activity was normal %99.82 (40-130).

Glomerular filtration rate (GFR) was calculated as 15ml/dk/1.73m^2. Protein value in urine analysis was ≥300(0-25). She was treated with antibiotic, diuretic, beta blocker, antihypertensive, corticosteroid, antiemetic and antiasid. In the present case also occurred tonic-clonic seizures twice in first week her inpatient stay and antiepileptic therapy (epanutin) 2x100mg is added to treatment by neurologist. There was no seizure in the follow up. Daily plasma exchange was initiated on day 14 postpartum but there were no improvement in thrombocytopenia and signs of hemolysis. The case was immediately administered a meningococcal vaccine and prophylactic ciprofloxacin treatment before starting of anti-C5 eculizumab therapy. Renal function of the patient started to deteriorate gradually and on the 24th postpartum hemodialysis was performed. One week later eculizumab was started following an induction schedule at a dose of 900 mg intravenously (IV) per week for 4 weeks. The patient's need for hemodialysis gradually decreased within 22 days after eculizumab treatment and was discharged on the 42nd day with full recovery. After discharge eculizumab was followed by 1200 mg every second week for 7 months. And she is still going on receiving 1200 mg eculizumab monthly for 6 months and antihypertensive, beta blocker and antiepileptic treatment. In our case, symptoms and treatment options were similar to worldwide cases (6-11).

Our patient could not breastfeed and interact with her newborn baby because of her hospitalization and medication process. During our interview with her medical history she was upset sometimes. She did not want to remember that days. She has no more pregnancy plan because of morbidity risk.

2.1. Nursing Management

Our patient was treated in the intensive care unit with severe hemolytic uremia that developed suddenly after cesarean section. During this period, hypertension, diffuse edema, anuric renal failure, liver dysfunction, anemia, thrombocytopenia, convulsions and anxiety are among the main problems. In addition, the patient experienced deterioration and delay in all functions expected after cesarean section. The main nursing diagnoses (ND) related to childbirth in this process can be listed as inadequacy in mother-infant attachment, maternal role performance, breastfeeding, family relations, and ineffective coping. In the care plan, current and potential risk diagnoses (risk for /electrolyte imbalance, impaired skin integrity, bleeding related thrombocytopenia, infection related with eculizimab therapy and immunosupression, ineffective cerebral tissue perfusion, sensory and cognitive alterations and confusion related with convulsion and uremic toxins) some have been addressed according to NANDA (North American Nursing Diagnosis Association) and NIC (Nursing Intervention Classification) (12).

2.2. Nursing Care Plan

Nursing Diagnosis 1. Excess fluid volume related with acute renal failure

Expected Outcome: The fluid-electrolyte balance of the individual will be maintained.

Interventions:

- Follow the peripheral, sacral, pretibial and periorbital edema
- Follow fluid intake and output
- Daily weight tracking
- Measure abdomen or extremity circumference
- Check laboratory results related to fluid retention (urea, creatine, sodium, potassium)
- Follow fluid overload symptoms (dyspnea, hypertension, CVP increase, neck vein engorgement)
- Restrict salt and fluid in the diet
- Check skin integrity
- Observe the effects and side effects of drugs given for edema (12-15).

Nursing Diagnosis 2. Impaired physical activity/Activity intolerance related with anemia, uremic toxins and cesarean operation

Expected Outcome: Optimal values are reached in laboratory results related to anemia and uremia.

Interventions:

- Provide a bed rest
- Evaluate the patient's motivation and reactions (saturation, heart rate) to the activity
- Help self-care requirements. Patient participation is encouraged as much as possible.
- Provide sufficient energy source
- Follow the patient's sleep pattern
- Follow treatments and laboratory findings for anemia and acute renal failure (12-15).

Nursing Diagnosis 3. Imbalanced nutrition: less than body requirement related to anorexia and nausea

Expected Outcome: The patient will eat and maintain a balanced diet.

- Apply the desired antiemetic before the meal.
- Elevate the head of the bed or place the patient in a position to avoid aspiration.
- Keep patient and bedding clean when vomiting occurs
- Immediately remove odor-causing substances (eg bedding, food)

- Avoid procedures that cause pain and nausea near mealtimes
- Carry out oral care after vomiting
- Apply a cool, damp cloth to the patient's wrists, neck, and forehead.
- Recommend that cold foods and other foods be less fragrant (12-15).

Nursing Diagnosis 4. Anxiety related to lack of knowledge of diagnostic tests, disease process and therapeutic regimen

Expected Outcome: She will make necessary decisions to change the negative situation around her and to take appropriate actions in accordance with her decisions.

Interventions:

- Assess the level of anxiety, the factors that influence the onset of anxiety.
- Evaluate patient level of understanding of diagnosis.
- Provide actual information about diagnosis, action, and prognosis.
- Provide a comfortable relaxed environment to express their feelings, fear, anger and perception.
- Provide opportunity for questions and answer patient and family members honestly.
- Encourage the family to accompany the client
- Assess the client's expectations to treatment and care.
- Teach relaxation techniques to reduce anxiety (12-15).

Nursing Diagnosis 5. Risk for bleeding related with thrombocytopenia

Expected Outcome: Uterus is felt to be firm on postpartum palpation.

- Follow up bleeding for wound and incision areas
- Follow the wound healing process
- Follow up the bleeding of the dressings
- Vital signs, especially blood pressure and heart rate, as indicated at risk level.
- Check hemoglobin and hematocrit values
- Follow the coagulation factors
- Follow the uterine height and stiffness regularly
- Observe the lochia for bright red bleeding and clotting
- Avoid injection (12-15).

Nursing Diagnosis 6. Risk for impared skin integrity

Expected Outcome: Skin and mucous membranes be structurally complete and show normal physiological function.

- Use a risk assessment tool (eg. Braden, Norton Scale)
- Evaluate the skin at first admission and every day
- Assess skin for pallor or redness
- Assess the individual's ability to move around in a chair or bed
- Assess nutritional status
- Determine if there is urinary or stool incontinence
- Turn the patient every 1-2 hours as appropriate
- Turn the patient carefully to avoid injuries to sensitive skin (eg avoid shear forces)
- Teach in-bed exercises
- Consult a dietitian about increasing the intake of high-energy foods (12-15).

3. DISCUSSION

P-aHUS can occur during pregnancy, but as confirmed by the data from the French Cohort (6) most cases occur in the post-partum period (>75%), when inflammation, the release of fetal cells into the maternal circulation, infections, and hemorrhage may trigger systemic complement activation. Similarly our case and her elder sister were P-aHUS cases as in the majority. It's important to detect genetic predisposition for the family. We informed the patient and family about the treatment, prognosis, complications, emergency and social transmitted infections and a new pregnancy that can trigger the relaps of the disease. It's important of a conscious, true, thorough, complete, and holistic assessment made by the nurses in the postpartum period, as well as the true diagnosis, and true nursing interventions that reduce the risk of morbidity and mortality.

4. CONCLUSION

As in the present case, the possibility of syndrome should be considered in such symptoms occurring in the postpartum period. It is thought that discussing care and treatment of this rarely seen case report will contribute to the literature.

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