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Research Article
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A STUDY TO ASSESS THE LEVEL OF ANXIETY ASSOCIATED WITH COVID-19 AMONG HIGH SCHOOL STUDENTS

Janula RAJU[1]*, Raju ASIRVATHAM[2]

[1]*College of Applied Medical Science, King Khalid University, Saudi Arabia*
[2]*St.Joseph's College of Pharmacy, Cherthala, Kerala, India*

Abstract: The COVID-19 pandemic has had a major impact on school children especially in their academic performance which may affect their mental health. The study aimed to assess the anxiety level of high school students. A cross sectional electronic study conducted among high school students in Tamil Nadu, the southernmost state of India. A total of 302 high school students participated in the study. A two sectioned self-structured questionnaire was used for data collection. The collected data were analyzed by using SPSS 16.0 version software. A chi-square test was used to investigate the level of anxiety among students and ANOVA test used to find the mean difference between groups, with significance set to $P<0.05$. Among 302 participants, 41.7% (n=126) were male, 58.3% (n=176) were aged 13-17 years. The study indicated that majority 132 (43.7%) of study participants reported that they had Mild anxiety which was followed by 77 (25.5%) no anxiety and 62 (20.5%) moderate anxiety. Factors such as age, gender, family members tested positive or being quarantined were having association with anxiety level. The researcher concluded that the high school children are experiencing a considerable level of anxiety during lock down of COVID-19 pandemic period. There was a strong association of anxiety level of students, if any family members are tested positive for corona virus or any one is being quarantined for the symptoms of corona virus.

Keywords: Anxiety, High school students, COVID-19, Corona Virus

***Corresponding author:** College of Applied Medical Science, King Khalid University, Saudi Arabia
E mail: janular@mail.com (J. RAJU)
Janula RAJU https://orcid.org/0000-0002-2903-1818
Raju ASIRVATHAM https://orcid.org/0000-0002-7939-4975

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1. Introduction

The COVID-19 pandemic, also known as the coronavirus pandemic, is an ongoing global pandemic of corona virus disease 2019 (COVID‑19), caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) (WHO, 2020). The outbreak was first identified in December, 2019, a series of pneumonia cases of unknown cause emerged in Wuhan, Hubei, China, with clinical presentations greatly resembling viral pneumonia (WHO, 2020).

A novel coronavirus was identified as the cause by Chinese authorities on 7 January 2020 and was temporarily named "2019-nCoV". On 11 March 2020, the rapid increase in the number of cases outside China led WHO Director-General Dr. Tedros Adhanom Ghebreyesus to announce that the outbreak could be characterized as a pandemic (WHO, 2020). As of 6 July 2020, more than 11.4 million cases of COVID-19 have been reported in more than 188 countries and territories, resulting in more than 534000 deaths; more than 6.16 million people have recovered.4 The announcement of Public Health Emergency of International Concern (PHEIC) by WHO on January 30, 2020 had stipulated the severity of the disease (Mahase, 2020).

In India, the disease was first detected on 30 January 2020 in Kerala in a student who returned from Wuhan (Ghosh et al., 2020). The cumulative number of confirmed infected people is 700724 till now across India as of 06 July 2020. A 76-year-old man who tested positive for SARS-CoV-2 was the first death from Covid-19 in India, reported from Kalaburagi, Karnataka, who was returned from Saudi Arabia (The Economic Times, 2020). On 24th March 2020, the Government of India Prime Minister Mr. Narendra Modi ordered a nationwide lockdown first time for 21 days, on order to limit the movement of the entire 1.3 billion population of India as a preventive measure against the COVID-19 pandemic in India (Gettleman and Schultz, 2020). The nation's capital New Delhi announced the primary schools remain closed from March 6th, to prevent the spread of corona virus to among children. The announcement comes after a new case of coronavirus was reported in Ghaziabad, taking the total number in the country was about 30 cases (ETO, 2020).

The southernmost state of Tamilnadu government announced for the closure of schools from March 17th, 2020. In a statement to the press, the Chief Minister said that while all schools will remain shut, only the board exams and practical exams for class X and XII and entrance exams will take place as scheduled (Bureau, 2020). So far as on July 06 2020 Tamil Nadu has reported

more than 1.1 lakhs of corona virus positive cases among Chennai the Capital city of TamilNadu has 68254 positive cases (Banerjea, 2020). The government also cancelled board exams for 11th standard, while making it clear that it will conduct exams for 12th standard students who could not attend the exams on March 24 and 26 at a later date (Sivapriyan, 2020).

Schools throughout the country have been closed and the students are facing unprecedented change and they stressed about their examination, results and the promotions. Majority private Schools in the state started to have virtual and online classes to refresh the knowledge of students. The sudden shift of class room education to virtual mode comes with several challenges. This may also affect the psychological state of school children. In addition to that if any of the family members is home-quarantined for suspected corona virus infection and it may affect the entire family members including children. So the researcher would like to explore the level of anxiety among school children associated with Covid-19.

2. Material and Methods

This study used a non-experimental, cross-sectional survey design to assess the level of anxiety among High school students in Tamil Nadu, South India. Potential participants received a link of Google form through social media such as Whatsapp and Facebook in late May and June 2020. The questionnaire contains two sections. The Google form included a brief description of the study and approximate time required to complete the survey. Electronic informed consent was shown on the initial page of the survey. Three hundred and two (302) high school students were consented to participate and filled out the questionnaire. All the participants were informed of the purpose of the study and were assured of confidentiality and anonymity before they start the questionnaire. The students were asked to not indicate their name anywhere in the survey form.

2.1. Data Collection Tool

A questionnaire with two sections was specifically designed for the study. The first section contained questions on the demographic variables, consisted of seven questions on socio demographic factors, such as age, gender, educational status, parents education, occupation, any family members quarantined?, any family members tested positive? The second part contains three point Likert scale of self-structured anxiety assessment scale used to measure the agreement. It contains 10 statements and each statement with three options like Not at all, sometimes and always. The total score ranges from 0-20. Total scores were classified as follows: 0-5 No anxiety, Mild anxiety 6-10, Moderate anxiety 11-15, and Severe anxiety 16-20. The higher score indicating higher anxiety level. Reliability of self-structured questionnaire was verified using Reliability statistics Cronbach's Alpha. The reliability was 0.88 and the questionnaire was found to be reliable.

2.2. Statistical Method for Analysis

For data analysis, the statistical software SPSS (Statistical Package for Social Sciences) version 16.0 was used, facilitating the process of organizing data into tables for the sake of better visualization of the results and their interpretation. Chi square χ^2 test was used to test the association between demographic variables and anxiety score. One way ANOVA tests and independent t test was used to compare the mean score between groups. Descriptive statistics were used to describe frequencies of variables. A significant P value was set at 0.05 at 95% confidence interval.

2.1. Ethical Consideration

The study was carried out with the permission of Local Research Ethics Committee (Protocol number: REC/2020/135-N) dated 03.05.2020.

3. Results

Among 302 high school students majority 176 (58.3%) of study participants were females and 126 (41.7%) were males. The mean age of study participant was 14.84 ± 1.45. The students from class 8th to class 12th were participated. Demographic status of the level of anxiety among high school students was given in Table 1. Association of demographic variables with anxiety score was tested by using χ^2 test, which was presented in Table 1. The χ^2 test stated that age, (χ^2=4.941, P=0.038<0.05) gender, (χ^2=1.362, P=0.000<0.05) family members tested positive (χ^2=1.506, P=0.000<0.05) and family members quarantined (χ^2=1.216, P=0.001<0.05) were having strong association with anxiety score. On the other hand educational level of students (χ^2=5.065, P=0.000>0.061), education of parents (χ^2=2.71, P=0.000>0.289) and occupation of parents (χ^2=2.437, P=0.000>0.251) were not having association with anxiety score.

Independent t test was used to compare the means of anxiety score with the gender. As presented in Table 2 the mean difference between two groups were statistically significant (P<0.05). This indicates that the females may have more anxiety than the males.

Figure 1 indicated that majority 132 (43.7%) of study participants reported that they had Mild anxiety which was followed by 77 (25.5%) no anxiety and 62 (20.5%) moderate anxiety. There were very limited number of participants 30 (9.9%) who experienced severe anxiety.

Overall anxiety score was given in Figure 2. One way ANOVA test was used to compare the mean differences of other variables. This test indicated that a significant difference between the mean score of students having different age. (F=18.48, P=0.000<0.05) Moreover the same test was used to analyze the mean differences of other variables which also confirmed that education (F=15.947, P=0.000<0.05), family members tested positive (F=11.333, P=0.000<0.05) and family members quarantined (F=16.748, P=0.000<0.05) were having significant difference between groups.

4. Discussion

Anxiety is an emotional state arising in situations of impending danger and manifested in expectation of unfavorable events (Nag et al, 2019). A survey report explained that during school closure increasing numbers of students say they feel overwhelmed, and not just about the health of their family and friends due to the coronavirus. Their parents might be newly unemployed, they can't see their friends, or they might be trapped at home for a long period of time (Jones, 2020).

Table 1. Frequency and percentage distribution of demographic variables and its association with the level of anxiety among high school students

Sl. No	Variables	Frequency (n=302)	Percentage (%)	χ^2 Value	P value
1	Age				
	13 years	74	24.5	4.941	0.038
	14 years	58	19.2		
	15 years	79	26.2		
	16 years	25	8.3		
	17 years and above	66	21.9		
2	Gender			1.362	0.000
	Male	126	41.7		
	Female	176	58.3		
3	Education			5.065	0.061
	8th std	85	28.1		
	9th std	47	15.6		
	10th std	76	25.2		
	11th std	24	7.9		
	12th std	70	23.2		
4	Education of parent			2.71	0.289
	School education	77	25.5		
	Undergraduate	127	42.1		
	Postgraduate and above	98	32.5		
5	Occupation of parent			2.437	0.251
	Govt employee	7	2.3		
	Private employee	121	40.1		
	Own bussiness	141	46.7		
	Others	33	10.9		
6	Any family members quarantined?			1.216	0.001
	Yes	62	20.5		
	No	240	79.5		
7	Any family members tested Corona Virus positive?			1.506	0.000
	Yes	7	2.3		
	No	295	97.7		

Table 2. Mean difference of Anxiety score between boys and girls.

Gender	Male	Female
Frequency	126	176
Mean	8.19	11.8
SD	3.71	5.53
Independent t value	5.60	
P value	0.000	

Anxiety among students is a common issue that students cannot ignore if they want to achieve academic success in school. If academic anxiety is not properly addressed, it can have many serious, severe and long lasting consequences (Mahato and Jangir, 2012). The study findings revealed that majority of students were reported some extend of anxiety during corona pandemic. This was confirmed by a study, stated that the majority of the students displayed anxiety during the entire period of lockdown (Baloran, 2020). This is confirmed the research

of Roy et al., (2020) reported that people were having sleep difficulties, paranoia about acquiring COVID-19 infection and distress related social media. Besides advised parents to create a schedule for their children to reduce anxiety induced by uncertainty (Lee, 2020).

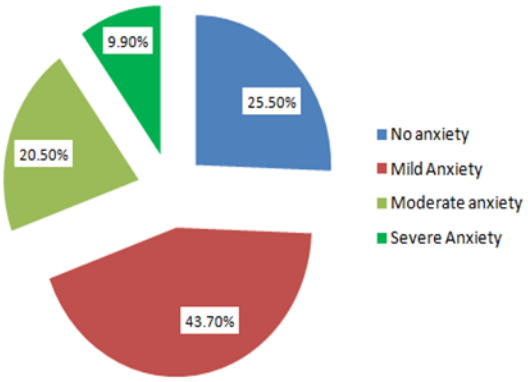

Figure 1. The overall anxiety level of high school students.

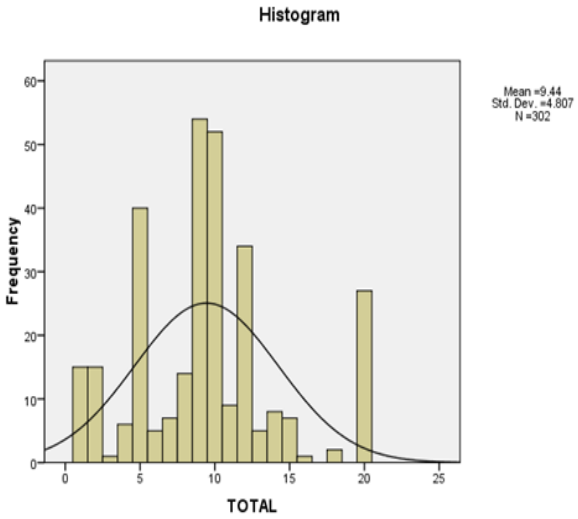

Figure 2. Revealed the histogram of anxiety score.

When comparing the mean differences of anxiety score among girls and boys it was identified there is a significant difference between groups. This was confirmed by a study done by Banga, (2014) and Nag et al., (2019) revealed that female students had severe anxiety than male students, and also stated that the anxiety level depends on the age group of students. Most of the study participants of current study said that they were getting anxiety when they have such symptoms of corona virus. During pandemic it is common there was increased anxiety felt by students regarding getting the COVID-19 infection (Baloran, 2020).

The study revealed that if any of the family members have tested corona positive the school students prone to get anxiety and stress. This is an anxiety-provoking and stressful time for everyone. While anxiety is a normal and expected reaction to the pandemic period (CMHA, 2020). The school students reported high level of anxiety if any of their family members are being quarantined due to COVID-19 symptoms. A review conducted by Brooks et al., (2020) Stated that Quarantine is often an unpleasant experience for those who undergo it and for their family members. During quarantine period the parents may get financial loss and they may be interrupted with their daily activities with no advanced planning. These factors may adversely affect their children also. It appears that in India, the present lockdown affects people differently with regards to their sex, profession, socioeconomic status or their residing place (Rehman et al., 2020).

Kumar and Nayar, (2020) have suggested that issues of mental health should be considered and also addressed as anxiety, stress, fear, trauma, helplessness and other psychological issues are experienced during a pandemic. Students of this study were reported that they were missing their friends and feeling lonely at home. The review report by Tian et al., (2020) stated that school closures are unprecedented globally. It is unclear how long countries can maintain tight suppression measures to prevent the spread of this disease. 24 So it may be additional stress for the students to be in home for a long period of time without meeting their friends.

5. Recommendations

The COVID-19 pandemic can be a stressful time for families, especially those with school going children who may be worried about a global health crisis. The study recommends that special attention to be given for the school students. Parents should speak in a moderate way to calm a child's anxiety. They can practice good hygiene, review safety steps about hand washing, and keeping the house clean. Parents and teachers should talk about how all school subjects are important in finding a solution to Covid-19. It is important to keep the children being touch with their school friends. Once the number of COVID-19 cases begin to fall, the Schools have begun to reopen and it will be essential for studies to monitor the effect of the reopening of schools. Policy makers and researchers should also look to other school social distancing interventions to maintain the control of this pandemic.

6. Limitations

To sum up, this research was done with certain limitations that should not be left unmentioned. These were like limited sample number and it was not a randomized samples. Since participation was voluntary, the data was completed by the students through electronic survey. Therefore, the measures were self-reported. This means that the accuracy of the responses could be compromised and the researchers are unable to verify the validity. There was a lack of time to extend the study as it is needed to be.

7. Conclusion

After the detailed analysis, the study leads to following conclusions: The researcher concluded that the high

school children are experiencing a considerable level of anxiety during lock down of COVID-19 pandemic period. There was a strong association of anxiety level of students, if any family members are tested positive for corona virus or any one is being quarantined for the symptoms of corona virus. So it is recommended to the parents to be kind with the students throughout the pandemic period.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Research Article
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A MACROANATOMIC, MORPHOMETRIC AND COMPARATIVE INVESTIGATION ON SKELETAL SYSTEM OF THE GEESE GROWING IN KARS REGION II: SKELETON APPENDICULARE

Gülseren KIRBAŞ DOĞAN[1]*, İsmet TAKCI[1]

[1]*Department of Anatomy, Faculty of Veterinary Medicine, Kafkas University, 36100, Kars, Turkey*

Abstract: The aim of this study was to comparatively, morphometrically and macroanatomically investigate skeleton appendiculare. A total of 24 goose cadavers were used. Scapula length was 107.31 ± 1.05 mm in female geese; it was 116.63 ± 0.65 mm in male geese. While the length of the os coracoideum was 78.5 ± 0.6 mm in female geese; it was detected as 87 ± 0.8 mm in male geese. The clavicula length was 66.90 ± 0.71 mm in female geese and it was 73.39 ± 0.59 mm in male geese and it was determined that both clavicula shaped furcula with a distinct curvature. While the length of the humerus in female geese was 175.02 ± 1.59 mm; it was measured as 191.28 ± 1.44 mm in male ones. While the mean ulna height was 162.60 ± 1.26 mm in female geese; it was determined as 178.84 ± 0.83 mm in male geese. The mean radius length was measured as 154.20 ± 1.63 mm in female geese, it was 169.75 ± 1.31 mm in male geese. It was detected that os carpi radiale was in a triangular shape, os carpi ulnare was in the form of a pipe. It was determined that the height of carpometacarpus was 94.57 ± 0.75 mm in female geese; 100.95 ± 1.03 mm was in male geese. Pelvis length was measured as 154.27 ± 1.81 mm in female geese; it was 169.91 ± 1.68 mm in male geese. While femur length was detected as 86.85 ± 0.98 mm in female geese; it was 93.87 ± 1.12 mm in male geese. While tibiotarsus length was 160.94 ± 1.88 mm in female geese; it was 174.20 ± 1.28 mm in male ones. While the tarsometatarsus length was 93.15 ± 1.19 mm in female geese; it was measured as 101.64 ± 0.88 mm in male geese. As a result, in this study, thought that obtained findings will contribute in scientific research, evaluations of sexual dimorphism, zooarchaeological studies and operations on poultry animals.

Keywords: Anatomy, Goose, Kars, Poultry, Skeleton appendiculare

***Corresponding author:** Department of Anatomy, Faculty of Veterinary Medicine, Kafkas University, 36100, Kars, Turkey
E mail: glsrn36@gmail.com (G. KIRBAŞ DOĞAN)
Gülseren KIRBAŞ DOĞAN https://orcid.org/0000-0003-3770-9956
İsmet TAKCI https://orcid.org/0000-0003-4795-3864

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1. Introduction

One of the main issues of people today is the need for basic food sources. Food from animals is of high importance within this need. The largest source for increasing species diversity in food production is poultry animals. Among waterbirds, geese are at a different position with their species that are raised for multiple purposes and the diversity they provide in production. Among the commercially and economically important yield features of geese are their meat, liver, fat, feathers and eggs (Aslan, 2013). In addition, according to a study (Kılıç et al., 2018), intramedullary mature goose radius as the intramedullary pin was used in the treatment of femoral fractures in puppies and rabbits as organic osteosynthesis material. With this application, which was found to be clinically, radiologically and histopathologically successful, a new usage area for goose bone emerged. Goose is the common name for the large species that constitutes the Anser strain of the Anatidae family from the Anseriformes tribe (Demirsoy, 1995; Tilki and Saatçi, 2013). In poultry, the locomotor system both contains the formations necessary for body balance and movement and reflects individual features. The skeleton constitutes the passive part of the locomotor system along with the joints and the skeletal muscles constitute the active part (Nickel et al., 1977). The most important feature of poultry is that they have pneumatic bones. These bones are in participation with the respiratory system through air sacs (sacci pneumatici). Non-flying poultry do not have pneumatized bones. There are three types of poultry bones: morphologically compact bones, cancellous bones, and medullary bones. In poultry, appendicular skeleton comprises of skeleton of the pectoral girdle, skeleton of the wing, skeleton of the pelvic girdle and skeleton of the pelvic limb. Poultry wing is equivalent to the front legs in mammals. Scapula, os coracoideum and clavicula (two clavicula merge form furcula) creates skeleton of the wing (cingulum membri thoracici) (Dursun, 2007). The wing is followed by these bones respectively; humerus, radius-ulna, carpus, metacarpus and ossa digiti (Nickel et al., 1977). Cingulum thoracic membrane of binding to the osseous in poultry while the body is in mammals muscle (Dyce et al., 1987, McLelland, 1990). Scapula and coracoid bones become smaller in good flightless birds,

even ostrich was lost (Kuru, 1987). The bones (sacrum, coxa) that make up the ossa cinguli membri pelvici in the mammals are fused as well as the vertebrae lumbicalis (Nickel et al., 1977, König et al., 2016). Ossa membri pelvici consist of; femur, patella, tibiotarsus, fibula, ossa pedis, ossa tarsi, ossa digitorum pedis and phalanges (N.A.A., 1993, Dursun, 2007).

2. Material and Methods

The cadavers supplied by breeders who butcher for food were brought to the laboratory of Kafkas University Faculty of Veterinary Science Anatomy Department and the study was conducted there.

A total of 24 goose cadavers; 12 of them female (1 old) and 12 male with average weights of 3.25 ± 0.15 kg (female) – 3.92 ± 0.21 kg (male) were used in the study. The weights of the goose cadavers were recorded with the help of digital precision balances (1g of unapproved sensitivity from 0-15 kg, and 2g from 15-30 kg, Baykon brand coded BCS21-6 MR). After the superficial muscles of 20 of the geese (10 female, 10 male) were dissected, the bones were revealed by maceration. After the superficial muscles of the geese whose bones are to be studied were dissected, boiling was performed for two hours in the water in which 10-15% sodium bicarbonate ($NaHCO_3$) was added (Taşbaş and Tecirlioğlu, 1965). The bones were thoroughly cleaned after the cooling procedure and soaked in a 10% hydrogen peroxide (H_2O_2) solution for two hours to whiten. After the last of the bones were thoroughly washed, they were left to dry in the sun (Taşbaş and Tecirlioğlu, 1965; Mussa et al., 2015). Measurements were taken from all goose bones with the help of a digital caliper and measuring tape in accordance with the method laid out by Von Den Driesch, 1976. Denomination was made in accordance with Nomina Anatomica Avium (N.A.A., 1993).

In order for pneumatic bones to be determined, 1 female and 1 male goose were injected acrylic (takilon), and 1 female and 1 male goose were injected a liquid rubber material (latex) colored with red fabric dye from the trachea for corrosion cast study. Each of the geese was injected with 120 ml of latex. They were then soaked in a 10% formaldehyde solution and dissected. The muscles were dissected and the pneumatized bones that the latex reached through air sacs were detected. Each of the geese was given 120 ml of an acrylic mixture containing 20% monomethyl-methacrylate and 80% plimetyl-methacrylate through the trachea. In order to ensure that this mixture solidified, cadavers were soaked in tap water for 24-48 hours. Then, examinations were made after the cadavers were soaked in a 30% potassium hydroxide (KOH) solution at a temperature of 60°C until the tissues were melted in order to make corrosion and cleaned. Thus, pneumatized bones were detected by means of both latex and acrylic. The mean and standard deviation values of all measurements and differences between genders were determined with the independent samples *t* test in the SPSS (version 20.0) packaged software.

2.1. Ethical Consideration

For this study, permission was obtained from Kars Provincial Directorate of Agriculture (dated 31.03.2017 and numbered E.791642) and KAÜ-HADYEK (KAÜ-HADYEK/2017-047).

3. Results

3.1. Skeleton Appendiculare

Geese skeleton appendiculare were examined in 4 parts; ossa cinguli membri thoracici, ossa alae, ossa cinguli membri pelvini and ossa membri pelvini.

3.1.1. Ossa cinguli membri thoracici (Bones of the pectoral girdle)

The wing bones was composed of scapula, os coracoideum and clavicula.

Scapula (shoulder blade)

Scapula was found to be curved in the middle and in the form of a sharper sword of the geese. The last vertebra cervicalis specialis (16-17) corresponds to the level of the thickened front end; it was determined that it joins with os coracoideum and furcula to form a joint pit involving the caput humeri.

In measurements taken (Figure 1), there is a significant difference in GL parameter in female and male geese; in male, the scapula length was found to be greater than that of females, while there was no significant difference in Dic parameter (P = 0.987) (Table 1).

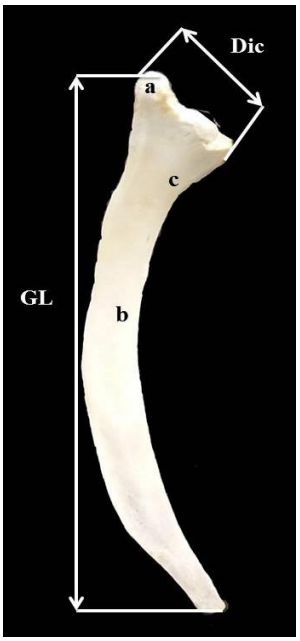

Figure 1. Measurements taken from scapula (GL= greatest length, Dic= greatest cranial diagonal, a= acromion, facies articularis clavicularis, b= corpus scapulae, facies lateralis, c= collum scapulae).

Table 1. Comparison of some parameters taken on scapula in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	107.31 ± 1.05	116.63 ± 0.65	<0.001
Dic	22.41 ± 0.46	22.43 ± 0.62	0.987

GL= greatest length, Dic= greatest cranial diagonal.

Os coracoideum (corvine bone)

Os coracoideum; clavicle, scapula and humerus that articulates with the narrow end of cranial, and caudal end of which articulates with the sternum was found to be wide (Figure 2). The strongest bone was found between the pectoral girdle bones. Saccus clavicularis was found to pneumatize the os coracoideum.

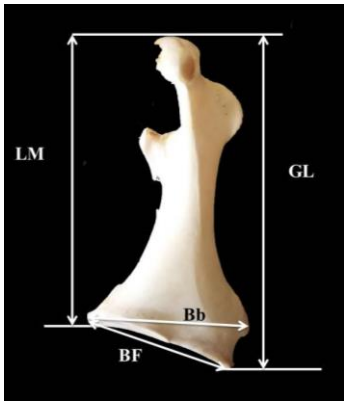

Figure 2. Measurements taken from os coracoideum (GL= greatest length, LM= medial length, Bb= greatest basal breadth, BF= breadth of the facies articularis basalis).

As shown in Figure 2, GL, Lm, Bb, and BF values were statistically different in male and female geese (P <0.001). In male, these parameters were found to be higher than females (Table 2).

Table 2. Comparison of some parameters on os coracoideum in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	78.5 ± 0.6	87 ± 0.8	<0.001
Lm	68.4 ± 0.6	75 ± 0.6	<0.001
Bb	32.76 ± 0.6	35.5 ± 0.4	0.001
BF	30.45 ± 0.4	33.6 ± 0.4	<0.001

GL= greatest length, Lm= medial length, Bb= greatest basal breadth, BF= breadth of the facies articularis basalis.

Clavicula (collar bone)

In the male and female geese, the clavicula was flat, while the cranial shaped curvature formed the furcula (Figure 3). The upper end of the clavicula was seen to be involved in the formation of the joint cavity of the art. humeri. It was determined that the lower end of the clavicula was formed in the synostosis on the middle plane with the other side and shaped the single bone called furcula. As seen in Table 3, the GL parameter was significantly different between male and female geese, it was longer in males than females. Clavicula was not found to be pneumatized.

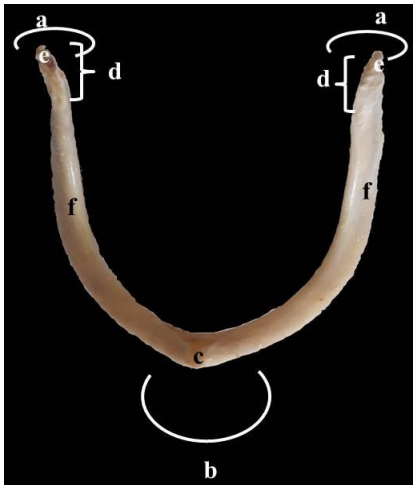

Figure 3. Furcula (Clavicula dexter and Clavicula sinister) (a= extremitas omalis claviculae (Epicleidium, extremitas scapularis), b= extremitas sternalis claviculae, c= apophysis furculae (Hypocleideum, lamina interclavicularis, proc. interclavicularis), d= facies articularis acrocoracoideae, e= proc. acromialis, f= scapus claviculae).

Table 3. Comparison of clavicula lengths in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	66.90 ± 0.71	73.39 ± 0.59	<0.001

GL= greatest length.

Ossa alae (Bones of the wing)

Ossa alae was found to be composed of humerus, skeleton antebrachii, carpus, carpometacarpus and digiti (Figure 4).

Figure 4. Ossa alae (1= humerus, 2= radius, 3= ulna, 4= ossa carpi, 5= carpometacarpale, 6= os metacarpale minus, 7= os metacarpale majus, 8= digiti II, 9= digiti IV, 10= phalanx I in digiti III, 11= phalanx II in digiti III)

Skeleton brachii (Humerus, arm bone)

The humerus was the strongest bone in the wing. It was seen that there was an oval structure (caput humeri) on the upper end of the medial. In the dorsolateral of the caput, it was found that the bump was shaped as a tuberculum laterally. In the ventromedial of caput, the tuberculum mediale was found; crista tuberculi medialis was found to extend downwards from the tuberculum mediale. Foramen (for) pneumaticum was seen in the mediodistal of tuberculum mediale. Trochlea humeri was detected at the distal end of the bone. It was found that the larger condylus ulnaris and smaller condylus radialis on trochlea. The trochlea had epicondylus ulnaris and epicondylus radialis (Figure 5). The length of the humerus in the geese was higher than the length of the antebrachium (radius and ulna). Saccus clavicularis was found to pneumatize the humerus.

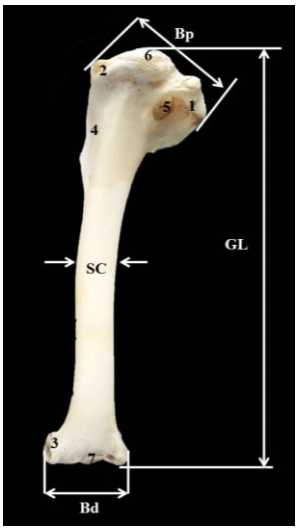

Figure 5. Measurements taken from humerus (1:= tuberculum ventrale, 2= tuberculum dorsale, 3= condylus dorsalis, 4= crista deltopectoralis, 5= for. pneumotricipitalis, 6= caput humeri, 7= fossa olecrani, GL= greatest length, Bp= breadth of the proximal end from the tuberculum laterale or dorsale to the tuberculum mediale or ventrale without the crista lateralis, SC= smallest breadth of the corpus, Bd= greatest breadth of the distal end).

GL, Bd, and Bp values were statistically different in male and female geese ($P < 0.001$). In other words, these parameters were higher in males than females. There was no significant difference between the sexes in terms of SC parameter ($P = 0.146$) (Table 4).

Skeleton antebrachii (Radius-Ulna, Forearm bones)

It was determined that the forearm skeleton consisted of thick ulna and thin radius (Figure 6-7). Radius and ulna were not found to be pneumatized.

Table 4. Comparison of some parameters of humerus in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	175.02 ± 1.59	191.28 ± 1.44	<0.001
Bp	37.73 ± 0.67	42.90 ± 0.79	<0.001
SC	11.84 ± 0.35	12.50 ± 0.27	0.148
Bd	25.56 ± 0.33	27.91 ± 0.40	<0.001

GL= greatest length, Bp= breadth of the proximal end from the tuberculum laterale or dorsale to the tuberculum mediale or ventrale without the crista lateralis, SC= smallest breadth of the corpus, Bd= greatest breadth of the distal end.

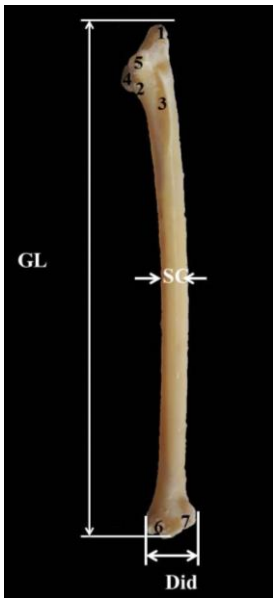

Figure 6. Measurements taken from the ulna (1= olecranon, 2= incisura radialis, 3= impressio brachialis, 4= cotyla dorsalia, 5= crista intercotylaris, 6= incisura tuberculum carpale, 7= sulcus intercondylaris, GL= greatest length, SC= smallest breadth of the corpus, Did= diagonal of the distal end).

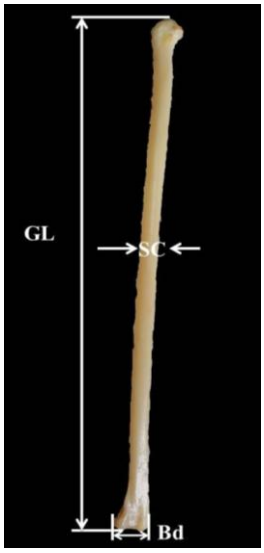

Figure 7. Measurements taken over radius (GL= greatest length, SC= smallest breadth of the corpus, Bd= breadth of the distal end).

Ulna (Elbow bone)

Ulna was thicker and longer than the radius. Corpus ulna was almost flat and papilla remigalies were found to be the basis for the adherence of the feather. It was determined that condylus distalis at the bottom was in contact with the radius when performing the art. radioulnaris. It was determined that condylus distalis at the bottom was in contact with os carpi ulnare and os carpi radiale when performing the art. carpoulnaris (Figure 6).

As shown in Fig. 6, GL, Dip, Bp, and Did values were statistically different between male and female geese (P <0.001; P = 0.001). In other words, these parameters were higher in males than females. There was no significant difference between the sexes in terms of SC value (P = 0.11) (Table 5).

Table 5. Comparison of some parameters taken from ulna in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	162,60 ± 1,26	178,84 ± 0,83	<0,001
Dip	21,40 ± 0,29	23,45 ± 0,40	0,001
Bp	10,53 ± 0,68	15,69 ± 0,24	<0,001
SC	8,72 ± 0,32	9,40 ± 0,24	0,111
Did	16,57 ± 0,23	18,81 ± 0,31	<0,001

GL: Greatest length, Dip: Greatest diagonal of the proximal end from the caudal border of the olecranon to the cranial border of the facies articularis lateralis (dorsalis), Bp: Greatest breadth of the proximal end from the facies articularis medialis (ventralis) to the facies articularis lateralis (dorsalis), SC: Smallest breadth of the corpus, Did: Diagonal of the distal end.

Radius (Rotary bone)

It was determined that the caput radii at the proximal end of the radius was articulated with the condylus radialis of the humerus.

As a result of the measurements (Figure 7), GL and Bd values were statistically different in male and female geese (P < 0.001; P = 0.001). In other words, these parameters were higher in males than females. There was no significant difference between the sexes in terms of SC value (P = 0.333) (Table 6).

Table 6. Comparison of some parameters belonging to radius in female and male geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	154.20 ± 1.63	169.75 ± 1.31	<0.001
SC	5.94 ± 0.21	6.28 ± 0.28	0.335
Bd	11.53 ± 0.24	12.69 ± 0.19	0.001

GL= greatest length, SC= smallest breadth of the corpus, Bd= breadth of the distal end.

Skeleton manus (Hand skeleton)

Ossa carpi (Hand wrist bones)

In the ossa carpi it was seen that only os carpi ulnare and os carpi radiale were on the antebrachial line. Os carpi radiale the two sides appeared to be a blunt edge as sharp as a plump triangle. Os carpi ulnare was found to be in the form of a pipe (Figure 8). The bones in the ossa carpi were not pneumatized.

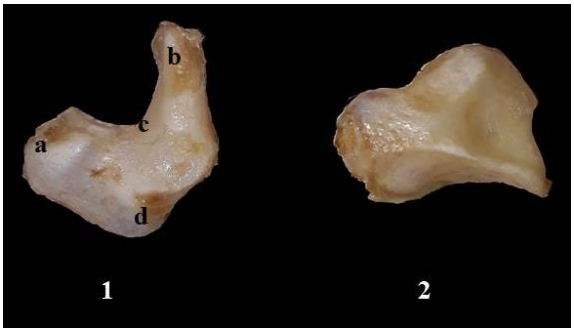

Figure 8. Os carpi ulnare and os carpi radiale (1= os carpi ulnare (Os cuneiform), a= crus breve, b= crus longum, c= incisura metacarpalis, d= proc. muscularis, 2= os carpi radiale (Os scapholunare)).

Carpometacarpus (Hand wrist-comb bone)

It was observed that carpometacarpus was composed of a total of 3 bones shaped by the distal order of ossa carpi and metacarpus 2, 3, and 4. It was determined that os metacarpale majus was longer and stronger and os metacarpale minus was shorter and weaker. Os metacarpale alulare was seen as rudimentary. It has 2 ends as extremitas proximalis carpometacarpi and extremitas distalis carpometacarpi.

In the measurements taken (Figure 9), GL, L, and Did values; male and female geese were found to be statistically different (P < 0.001; P = 0.009), while males in females carpometacarpus was determined to be higher than the length of these parameters. In addition, there was no significant difference between the sexes in BP value (P = 0.23) (Table 7).

Ossa digitorum manus (Hand finger bones)

It was determined that ossa digitorum manus consists of 3 fingers of different size and structure. Of these, 2 were formed from two phalanx. The third finger, the strongest finger, consisted of a total of 3 phalanx. The fourth finger is formed from a single. İt was found that the finger was attached to the proc. alularis located medially at 1.5-2 cm below the proximal end of the carpometacarpus.

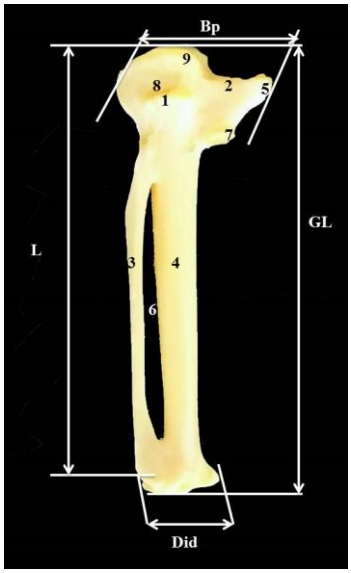

Figure 9. Measurements taken from carpometacarpus (Did= facies articularis digitalis major, 1= proc. psiformis, 2= trochlea carpalis, 3= os metacarpale minus, 4= os metacarpale majus, 5= proc. extensorius, 6= spatium intermetacarpale, 7= proc. alularis, 8= fossa infratrochlearis, 9= trochlea carpalis, GL= greatest length, L= length of metacarpus II, Bp= greatest breadth of the extremitas proximalis, Did= greatest breadth of the extremitas distalis).

Table 7. Comparison of some parameters on carpometacarpus in female and male geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	94.57 ± 0.75	100.95 ± 1.03	<0.001
L	93.50 ± 0.83	98.83 ± 0.82	<0.001
Bp	23.04 ± 0.25	24.74 ± 0.64	0.23
Did	12.98 ± 0.31	14.20 ± 0.26	0.009

GL= greatest length, L= length of metacarpus II, Bp= greatest breadth of the extremitas proximalis, Did= greatest breadth of the extremitas distalis.

3.1.3. Ossa cinguli membri pelvini (Bones of the pelvic girdle)

Os coxae (Hip bone)

Synsacrum and ossa coxae were combined to form the goose pelvis. It was determined that each of the hip bones consisted of the fusion of three bones, namely os ilium, os pubis, and os ischii. Os ischii and os ilii were determined to shape the acetabulum. Os ilium was the largest bone involved in the formation of coxae. Ala preacetabularis ili, ala postacetabularis ili and corpus ilii were found to consist of three parts. Os ischii was composed of two parts: corpus ischii and ala ischii. İncisura acetabularis, pila ilioischiadica and antitrochanter on corpus ischii were detected. Os pubis consisted of two parts: corpus pubis and scapus pubis.

In the measurements taken (Figure 10) GL, LS, LV, CB, SB, and BE values in male and female geese was statistically different (P <0.001, P = 0.006; P = 0.002) were seen. In other words, these parameters were higher in males than females. In addition, there was no significant difference between the genders in AA and DIA values (P = 0,147; P = 0,914) (Table 8).

Table 8. Comparison of some parameters on pelvis in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	154.27 ± 1.81	169.91 ± 1.68	<0.001
LS	151.44 ± 1.44	165.61 ± 1.76	<0.001
LV	137.24 ± 1.78	150.18 ± 2.47	<0.001
CB	35.31 ± 0.56	39.63 ± 0.52	<0.001
SB	26.92 ± 0.40	29.01 ± 0.53	0.006
AA	45.73 ± 1.74	48.62 ± 0.77	0.147
DİA	12.81 ± 0.32	12.87 ± 0.41	0.914
BA	50.85 ± 0.93	56.30 ± 1.20	0.002

GL= greatest length (without pubis), LS= length from the cranial border of the ilia to the spinaeilio-caudales, LV= length along the vertebrae, centrally, CB= cranial breadth, SB= smallest breadth of the partes glutaea, AA= breadth between the borders of the acetabulum, measured at the narrowest part, DİA= diameter of one acetabulum: greatest distance including the labium acetabuli, BA= breadth in the middle: breadth cross the two antitrochanter.

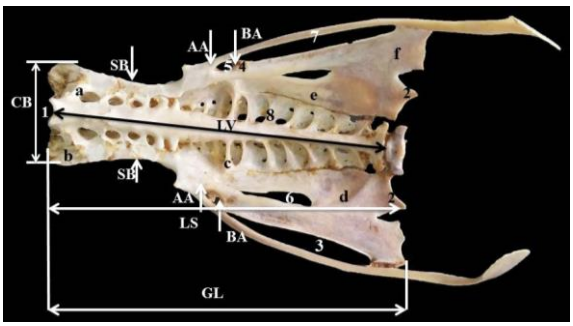

Figure 10. Ventral view of the pelvis (LV= sulcus ventralis synsacri, CB= extremitas cranialis synsacri, 1= margo iliocranialis, 2= spina iliocaudalis, Proc. marginis caudalis, 3= fenestra ischiopubica, 4= antitrochanter, 5= for. obturatum, 6= for. ilioischiadicum, 7= scapus pubis, 8= forr. intertransversariae, a= crista iliaca lateralis, b= ala preacetabularis ilii, c= proc. costalis, d= fossa renalis, e= ala postacetabularis ilii, f= ala ischii, GL= greatest length (without pubis), LS= length from the cranial border of the ilia to the spinaeilio-caudales, LV= length along the vertebrae, centrally, CB= cranial breadth, SB= smallest breadth of the partes glutae, AA= breadth between the borders of the acetabulum, measured at the narrowest part, BA= breadth in the middle: breadth cross the two antitrochanter).

3.1.4. Ossa membri pelvini (Bones of the pelvic limb)

Femur (thigh bone)

Caput femoris and trochanter major (femoris) were found to be almost the same level. Trochanter minor was

found in the distomedial of collum femoris (Figure 11). Trochlea femoris was seen on the anterior aspect of the distal end of the femur. Behind the distal end of the femur was found 2 condylus. Condylus medialis with the tibia and the larger condylus lateralis tibia and caput fibulae rudimentary with the joint was determined. Femur was not pneumatized.

As a result of the measurements (Figure 11), GL, Lm, Bp, Bd, and Dd values were statistically different in male and female geese ($P < 0.001$; $P = 0.01$). In other words, these parameters were higher in males than females. In addition, there was no significant difference between Dp and SC values ($P = 0.11$; $P = 0.07$) (Table 9).

Figure 11. Measurements taken from the femur (1= caput femoris, 2= trochanter major (femoris), 3= condylus medialis, 4= condylus lateralis, 5= condylus fibularis, GL= greatest length, Lm= medial length, Bp= greatest breadth of the proximal end, SC= smallest breadth of the corpus, Bd= greatest breadth of the distal end).

Table 9. Comparison of some parameters taken on femur in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	86.85 ± 0.98	93.87 ± 1.12	<0.001
Lm	82.26 ± 0.87	88.36 ± 1.01	<0.001
Bp	22.43 ± 0.26	24.66 ± 0.32	<0.001
Dp	17.00 ± 0.43	18.54 ± 0.33	0.11
SC	9.44 ± 0.13	10.09 ± 0.17	0.07
Bd	22.99 ± 0.35	24.94 ± 0.37	0.01
Dd	18.22 ± 0.32	20.05 ±0.32	0.01

GL= greatest length, Lm= medial length, Bp= greatest breadth of the proximal end, Dp= greatest depth of the proximal end, SC= smallest breadth of the corpus, Bd= greatest breadth of the distal end, Dd= greatest depth of the distal end.

Patella (Knee cap bone)

The patella; mm. femorotibialis and m. iliotibialis were found to be a small sesamoid bone within the common tendon and at the level of the trochlea femoris. The male and female geese were triangular.

Tibiotarsus (Foot-leg wrist bone)

It was determined that the goose skeleton consisted of the strong tibiotarsus and weak fibula, and tibiotarsus was not pneumatized. It was found that tibiotarsus was approximately 2 times longer than femur.

As a result of the measurements (Figure 12), GL, La, Dip, Bd, and Dd values were statistically different in male and female geese ($P < 0.001$; $P = 0.010$; $P = 0.001$). In other words, these parameters were higher in males than females. In addition, there was no significant difference between the sexes in terms of SC ($P = 0.270$) (Table 10).

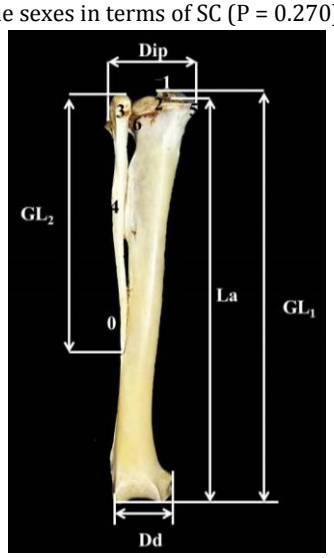

Figure 12. Measurements taken from tibiotarsus and fibula (0= spina fibulae, 1= proc. cnemialis, 2= tuberculum centrale, 3= caput fibulae, 4= corpus fibulae, Crista fibularis, 5= condylus medialis femoralis, 6= condylus lateralis femoralis, GL1= greatest length of tibiotarsus, GL2= greatest length of the fibula, La= axial length, Dip= greatest diagonal of the proximal end, Dd= depth of the distal end).

Table 10. Comparison of some parameters of tibiotarsus in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	160.94 ± 1.88	174.20 ± 1.28	<0.001
La	151.89 ± 1.87	167.64 ± 1.32	<0.001
Dip	28.36 ± 0.65	30.65 ±0.45	0.010
SC	8.48 ± 1.64	8.20 ± 0.18	0.270
Bd	18.55 ± 0.25	20.02 ± 0.14	<0.001
Dd	17.47 ± 0.75	20.63 ± 0.32	0.001

GL= greatest length of tibiotarsus, La= axial length, Dip= greatest diagonal of the proximal end, SC= smallest breadth of the corpus, Bd= greatest breadth of the distal end, Dd= depth of the distal end.

Fibula (Calf bone)

It was observed that the fibula was proximal to the distal to a flat rod. It was determined that the tapered corpus of

the fibula was progressively tapering to the distal length of the tibia. It was observed that the fibula was bounded by two spatium interosseum, proximal and distal along its length. In the measurements taken as shown in Figure 12, it was determined that there was no significant difference between females and males in fibula length (P = 0.189).

Ossa pedis (Foot bones)

Ossa tarsi (Ankle bones)

Tarsal bones were not found independently. The proximal row of ossa tarsi was found to be fused with the distal part of tibiotarsus and the distal row of ossa tarsi with the proximal metatarsus.

Tarsometatarsus (Foot wrist-comb)

Tarsometatarsus was a composite bone composed of os metatarsale II, os metatarsale III, os metatarsale IV and ossa tarsi. Os metatarsale I and V were absent. The first finger in the distal part of the os metatarsal I was smaller and did not fuse with them. Hypotarsus was found to constitute the proximal boundary of os metatarsale III. It was determined that there were 3 crista hypotharsis in the hypotarsus of geese and the longest of crista medialis hypotarsi. The distal end of the one-piece metatarsus was divided into 3 trochlea with a very distinctive 2 notches. It was determined that there was a bone spur on the facies medialis aspect of extremitas distalis tarsometatarsi in male and female geese. This formation was better developed in males.

The statistical data generated as a result of the measurements taken in Figure 13; GL, Bp, SC, and Bd showed significant differences between male and female geese (P < 0.001; P = 0.001). In other words, all of these parameters were found to be significantly longer in males (Table 11).

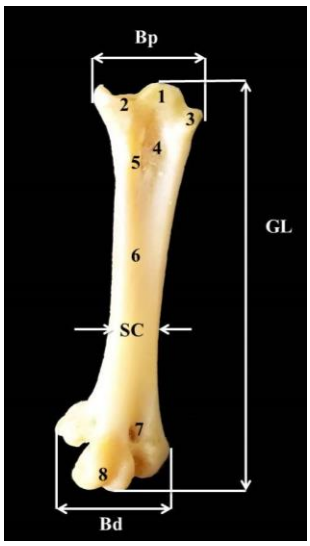

Figure 13. Measurements taken by tarsometatarsus (1= eminentia intercotylaris, 2= cotyla medialis, 3= cotyla lateralis, 4= sulcus extensorius, 5= tuberositas m. tibialis cranialis, 6= facies dorsalis, 7= for. vasculare distale, 8= trochlea metatarsi III, GL= greatest length, Bp= greatest breadth of the proximal end, SC= smallest breadth of the corpus, Bd= greatest breadth of the distal end).

Table 11. Comparison of some parameters of tarsometatarsus in male and female geese

Parameter	Female (n=10)	Male (n=10)	P value
GL	93.15 ± 1.19	101.64 ± 0.88	<0.001
Bp	19.05 ± 0.35	20.93 ± 0.30	0.001
SC	8.77 ± 0.11	9.41 ± 0.11	0.001
Bd	21.48 ± 0.39	24.03 ± 0.32	<0.001

GL= greatest length, Bp= greatest breadth of the proximal end, SC= smallest breadth of the corpus, Bd= greatest breadth of the distal end.

Ossa digitorum pedis (The foot toe bones)

There were four fingers on the goose leg. The first finger in the medial was caudal, while the 2nd, 3rd, and 4th fingers were directed towards the cranial.

Phalanges (Finger bones)

Two phalanxs were found on the first finger, three on the second finger, four on the third, and five on the fourth finger.

4. Discussion

While the length of the scapula was 61.8 mm (Özgel et al., 2002), it was measured as 116.63 ± 0.65 mm in male geese and 107.31 ± 1.05 mm in female geese. The diverticulum subscapulare of the saccus clavicularis in geese has been reported to pneumatize the scapula (Onuk, 2008). In this study, it was determined that saccus clavicularis had pneumatized scapula. It has been reported that the length of the bald ibis os coracoideum is 46-51 mm (Özgel et al., 2002). The length of the bone was 78.5 ± 0.6 mm in female geese and 87 ± 0.8 mm in male geese. It is reported that there is no pneumatization in the os coracoideum (Hogg, 1984a) in chicken, duck (Çevik Demirkan, 2002) and geese (Onuk, 2008) have been reported to be pneumatized. In the present study, it was determined that os coracoideum was pneumatized.

The forward curvature of the clavicula was less pronounced and stronger in the hen and rooster, and almost flat in the turkey (Gültekin, 1957). In male and female geese, the clavicula was found to be flat but with a distinct curvature, it was seen that two clavicles were joined together in synostosis. While clavicula was reported as a pneumatized bone in the duck (Çevik Demirkan, 2002), clavicle was not pneumatized in geese.

It has been reported that humerus is longer than antebrachium in chicken, domestic duck and quail (Çevik Demirkan, 2002). In pigeons, antebrachium was reported to be longer than humerus (Yıldız et al., 1998). In the study, it was found that humerus was longer than antebrachium in female and male geese. The length of the humerus is 51.80 ± 0.49 mm in partridge, 67.77 ± 0.55 mm in pheasant (Lök and Yalçın, 2007), in the long-legged buzzard 100-110 mm (Atalar et al., 2007), in goose 172.5 mm (Allison et al., 2006), 72.5 mm in the chicken, 87.4 mm in the domestic duck, 46.1 mm in the pigeon (Yıldız et al., 1998), 95.84 ± 1.63 mm in the female ducks, 106 in the male ducks. (Çevik Demirkan, 2002). In

this study, humerus length was 175.02 ± 1.59 mm in female geese and 191.28 ± 1.44 mm in male. The humerus length values we found were higher than those of the other birds, but similar to the Canadian geese. The humerus in the blue-headed parrot is reported to be pneumatized (McKibben and Harrison, 1986). It was reported that diverticulum humerale of saccus clavicularis in the geese had pneumatized humerus (Onuk, 2008). In our study, saccus clavicularis was found to pneumatize the humerus.

The average length of ulna is 29.7 mm in domestic quail, 29.4 mm in wild quail (Yaman, 1997), 49.74 ± 0.46 mm in partridge, 61.53 ± 0.50 mm in pheasants (Lök and Yalçın, 2007), 110-130 mm (Atalar et al., 2007) was reported as 100.1 ± 3.0 mm (Charuta et al., 2005) in males of the domestic duck. In our study, the length of ulna was 162.60 ± 1.26 mm in female geese and 178.84 ± 0.83 mm in male geese. Papillae remigalis, which were the basis for the attachment of feathers to ulna, were clearly seen. While the curvature of corpus ulnae is less pronounced in chickens and ducks, it is stated that it is more prominent in goose and duck (Nickel et al., 1977). There was a slight curvature in the corpus ulnae. Mean radius length was 44.54 ± 0.44 mm in partridge, 56.09 ± 0.47 mm in pheasants (Lök and Yalçın, 2007), female Pekin duck 94.9 ± 2.1 mm, male Pekin duck 91.0 ± 13.59 mm (Charuta et al., 2005). In this study, the mean radius length was 154.20 ± 1.63 mm in female geese and 169.75 ± 1.31 mm in male geese. It was observed that the poultry species compared with them were extremely large. It was determined that the radius and ulna were equal in length (Rezk, 2015) in the cattle egret and that the ulna was long in radius. The largest width at the distal end was 11.53 ± 0.24 mm in male geese and 12.69 ± 0.19 mm in female geese, whereas Charuta et al., (2005) in the study of Pekin duck in males 9.4 ± 0.6 mm female 9.0 ± 0.3 mm. It was reported that radius and ulna has not been pneumatized in the partridge, pheasant and long-legged buzzard from birds (King, 1957; Lök and Yalçın, 2007). Antebrachium was not pneumatized in geese.

It was reported that os carpi radiale is short and quadrangular in duck (Çevik Demirkan, 2002), it is almost rectangular in partridge and rectangular and bow tied with pheasants (Lök and Yalçın, 2007). In the study, it was observed that the two sides were shaped two blunt edge one sharp edge, pointed, triangular shaped. Os carpi radiale is generally similar to geometric shapes, but the reported figures and findings are not similar. Os carpi ulnare in duck (Çevik Demirkan, 2002), pheasant and partridge (Lök and Yalçın, 2007) V shaped, cattle egret (Rezk, 2015) U-shaped was stated. In this study, os carpi ulnare was seen as a pipe shaped. Os carpi ulnare's shape did not match the findings of other reported studies

Mean carpometacarpus length was 29.94 ± 0.31 mm in partridge, 34.82 ± 0.26 mm in pheasant (Lök and Yalçın, 2007), 59-71.2 mm long-legged buzzard (Atalar et al., 2007), male and female Pekin duck in 75.0 ± 1.7 - 73.3 ± 2.0 mm (Charuta et al., 2005). In the study, the length of carpometacarpus was determined as 94.57 ± 0.75 mm in female geese and 100.95 ± 1.03 mm in male geese. Carpometacarpus length was higher in geese compared to other studies.

It was observed that the apex pubis of the os pubis ended in bent to the ventromedian. It was reported that os pubis did not participate in the formation of acetabulum in chicken and duck (Dursun, 2007; Çevik Demirkan, 2002). It was observed that os pubis did not participate in acetabulum formation in geese. It have been reported that ilium, ischium and pubis to be pneumatized in the blue-headed parrot (McKibben and Harrison, 1986). Saccus abdominalis has been reported to pneumatize coxa (King and McLelland, 1975). There was no indication of pneumatization of coxa in chickens (Hogg, 1984b). In the wild duck (Çevik Demirkan et al., 2006) and the goose (Onuk, 2008), it was stated that saccus abdominalis pneumatized to the synsacrum. In this study, it was found that synsacrum was pneumatized by saccus abdominalis.

The average length of the femur was reported as 81.5 mm (Allison et al., 2006) in Canadian geese, 60.14 ± 0.49 mm in female ducks and 60.92 ± 0.93 mm in male ducks (Çevik Demirkan, 2002). In this study, femur length was 86.85 ± 0.98 mm in female geese and 93.87 ± 1.12 mm in male geese. The length of the femur was similar to that of the Canada goose while it was higher than the other poultry. It was reported that there was no pneumatization in the femur in the hens (Hogg, 1984a). Diverticula femoralia of saccus abdominalis was reported to pneumatize the femur in geese (Onuk, 2008). In the study, it was seen that the femur was pneumatized by saccus abdominalis.

It is reported that the patella has a triangular appearance in the partridges, and in pheasants it resembles the talus of mammals (Başoğul and Beşoluk, 2016), rectangular female ducks and square in shape male ducks (Çevik Demirkan, 2002). In our study, it was observed that patella was triangular in male and female geese.

The average length of tibiotarsus was measured as 150.5 mm (Allison et al., 2006) in Canadian geese, 94.9 ± 1.2 in female ducks and 102.5 ± 0.94 mm in male ducks (Çevik Demirkan, 2002). This study measured 160.94 ± 1.88 mm in female geese and 174.20 ± 1.28 mm in male. It has been reported that tibiotarsus in pigeons and chickens is longer than 1/3 of femur from femur (Dursun, 2007). As reported in the literature (Nickel et al., 1977; Çevik Demirkan, 2002) tibiotarsus was found to be 50% longer than the femur. As in the duck (Çevik Demirkan, 2002), it was observed that the fibula was extended to the distal part of the tibia length and to limit the two spatium interosseum, proximal and distal along its length.

In the study, it was determined that tarsometatarsus, which is the only one in the geese, was formed by the fusion of os metatarsale II, os metatarsale III (principal) and os metatarsale IV. In hypotarsus, it is reported that in some birds there is only one crista and sulcus, most birds have more than one crista and sulcus (N.A.A., 1993). In

the geese 3 cristae hypotharsia (crista lateralis hypotarsi, crista intermedia hypotarsi and crista medialis hypotharsia) was found and crista medianoplantaris (crista medialis hypotarsia) to be the longest. In extremitas distalis tarsometatarsia, trochleas (trochlea metatarsi II, troclea metatarsi III, trochlea metatarsi IV) belonging to each tarsometatarsus were seen. It was reported in some birds (N.A.A., 1993) that adjoint trochlea accessoria wasn't detected. Among the trochlea's were the incisura intertrochlearis lateralis and the incisura intertrochlearis medialis. The plantar of the trochlea's had a deep pit fossa supratrochlearis plantaris. Canalis interosseus distalis was not apparent, but for. vasculare distale was a big hole. The tallest bone of the tarsometatarsus was chicken and the shortest bone was reported as goose (Gültekin, 1966; N.A.A., 1993). However, in our study, we found that the tarsometatarsus length of larger other poultry tarsometatarsus (Zeffer and Lindhe Norberg, 2003) because of this information is contrary. In most birds, tarsometatarsus is shorter than tibiotarsus; two bones were reported to have approximately equal length in the rain bird (King and McLelland, 1984). It was determined that tibiotarsus length was greater than tarsometatarsus in geese. Tarsometatarsus length; 38.5 mm in common buzzard, 45.6 mm in mallard duck, 18.9 mm in sparrow, 17.9 mm in finch, 104.8 mm in gold eagle, 78.3 mm in diver bird (Zeffer and Lindhe Norberg, 2003). This length was 93.15 ± 1.19 mm in female geese and it was 101.64 ± 0.88 mm in male geese.

5. Conclusion

Goose (*Anser anser domesticus*) is one of the poultry animals shown as most examples in veterinary anatomy teaching. It is also the geographical sign of Kars region. Therefore, the bones of adult geese were examined in our study. As a result, in this study, morphometric and morphologic values of examined bones of adult geese were determined and contributed to the elimination of the information in this area. It is thought that obtained findings will contribute in scientific research, evaluations of sexual dimorphism, zooarchaeological studies and operations on poultry animals.

Author Contributions

All authors' contributions were equal.

Conflict of Interest

The authors declare that there is no conflict of interest.

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EFFECT OF POSTOPERATIVE ADMINISTRATION OF HYALURONIC ACID/TREHALOSE DROP ON CORNEAL HEALING AFTER PHACOEMULSIFICATION SURGERY

Hakika ERDOGAN[1]*, Orkun MUHSINOGLU[1]

[1]*Maltepe University, Faculty of Medicine, Department of Ophthalmology, 34844, İstanbul, Turkey*

Abstract: This study aimed to evaluate the efficacy of hyaluronic acid/trehalose drop for corneal epithelial and stromal healing after phacoemulsification surgery. It is a retrospective randomized comparative study. The patients had phacoemulsification surgery divided into two groups. These patients received either a mixture of sodium hyaluronate and Trehalose (Group 1) or sodium hyaluronate 0.15% (Group 2) postoperatively. The effect on corneal epithelial and stromal healing was evaluated using Oxford staining and the vertical scar length measured by optical coherence tomography. Preoperative and post-operative findings for Oxford staining, Schirmer's test, tear film break-uptime, and ocular surface disease index scores were also evaluated. The correlation between scar length and tear film parameters was examined. The effect of Trehalose on the epithelial healing and stromal scar formation was assessed. Group 1 and 2 each had 30 eyes. The two groups were similar according to age and sex distribution. In terms of epithelial healing, it was faster in the trehalose group, although there was no statistical difference between the two groups. The length of the stromal scar was not significantly different between groups. However, a smaller scar formation was observed in group 1 compared to those in group 2 at both one week and one month postoperatively. There was also no significant difference between the groups in tear film parameters. The stromal scar length was correlated with the Schirmer test on the 15th day. Although it was not statistically significant, the hyaluronic acid/trehalose may affect epithelial healing and stromal scar formation positively after phacoemulsification surgery. Larger and longer studies are needed.

Keywords: Hyaluronic acid/trehalose, Phacoemulsification surgery, Dry eye, Wound healing, Clear corneal incision

***Corresponding author:** Maltepe University, Faculty of Medicine, Department of Ophthalmology, 34844, İstanbul, Turkey
E mail: dr.gercek@yahoo.com (H. ERDOGAN)
Hakika ERDOGAN https://orcid.org/0000-0001-7749-2814
Orkun MUHSINOGLU https://orcid.org/0000-0002-0348-398X

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1. Introduction

With the help of the advancing technology, phacoemulsification surgery became one of the most comfortable ophthalmologic operations; however, complaints such as burning sensation, blurred vision, pain inside and around the eye, and foreign body sensation are still frequent in the post-operative period (Sutu et al., 2016). Since the prevalence of dry eye disease (DED) increases with aging, it is common in patients undergoing phacoemulsification surgery. Post-operative DED can be due to reasons such as topical anesthetics, cutting of corneal nerve, inflammation, and loss of goblet cells. Because of these abovementioned reasons, patient satisfaction can be lower than expected even though the operation was problem free (Cho et al., 2009; Pinto-Bonilla et al., 2015; Trattler et al., 2015). Since DED has both inflammatory and physicochemical components, it can be assumed that the mechanism behind dry eye following phacoemulsification surgery may be inflammation. Topical anti-inflammatory drops such as steroids are usually administered to manage post-operative inflammation and facilitate healing after phacoemulsification surgery. Increased ocular surface inflammation also has a role in increased tear osmolality (Aragona et al., 2014), and this may affect the corneal healing, as well it may lead to denaturation of the cell membrane and proteins. (Chen et al., 2009; Hill-Bator et al., 2014; Chiambaretta, et al., 2017).

Trehalose is a naturally occurring disaccharide that preserves cellular vitality and acts as a free radical scavenger. It can protect the cells that are in the same environment with it against dryness, high temperatures, dehydration, and oxidation (Mateo Orobia et al., 2017). It is a bio-protective (Hovakimyan et al., 2012) and osmoprotectant agent. It stabilizes the cell membrane and proteins by inducing proinflammatory cytokine secretion and prevents apoptosis (Aragona et al., 2014; Hovakimyan et al., 2012; Mateo Orobia et al., 2017). Thealoz Duo® is an artificial tear preparation which contains Trehalose and hyaluronic acid. Topical corticosteroids and/or nonsteroidal anti-inflammatory drugs are considered as a standard treatment after phacoemulsification surgery; however, the additive effect of topical Trehalose on the stromal healing process has not been studied.

This study aims to investigate Trehalose's effect on the healing process, changes, and scar formation in epithelium and stroma following phacoemulsification surgery. Secondly, the difference in tear film parameters by post-operative administration of Trehalose was evaluated.

2. Material and Methods

This retrospective randomized comparative study was conducted between January 2016 and December 2018. Our institutional review approved the study protocol that adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria to the study were; age between 60-75 years, no previous ocular surgery, no history of systemic diseases (Diabetes mellitus, hypertension, Sjögren syndrome, thyroid diseases, etc.), no record of DED, glaucoma, or any corneal pathology, and without a history of topical medication use for at least six months before cataract diagnosis.

All patients underwent phacoemulsification surgery and intraocular lens (IOL) implantation performed by the same surgeon (H.E) without intraoperative complications. Three-planed clear corneal incision (CCI) was done between 130 degrees and 140 degrees using a 2.2 mm metal blade (Alcon Laboratories, Inc.). A single-piece foldable acrylic IOL (Tecnis ZCBOO, Abbott Medical Optics, Inc.) was implanted. Minimal hydration was applied to the CCI using a balanced salt solution at the end of the surgery.

The patients were divided into two groups after surgery. The participants in group 1, trehalose 30 mg/mL, and sodium hyaluronate 1.5 mg/mL were applied four times a day on the eye had phacoemulsification surgery, and the participants in the second group only sodium hyaluronate 0.15% was used four times a day on the eye had phacoemulsification surgery. The standard post-operative anti-inflammatory treatment (prednisolone acetate 1.0%, Pred Forte) was administered to all patients every 2 hours on the day of surgery, one drop every 4 hours during the first post-operative week, and then drops stopped by reducing during the following three weeks. Findings for Oxford staining, Schirmer's test I, tear film break-up time (TBUT), and ocular surface disease index (OSDI) scores were examined preoperatively from the patients' field. Oxford staining was performed both post-operative 1st-day and 1st-month. Schirmer's test I, TBUT, OSDI tests were performed at post-operative 1st-month. Oxford staining was used both to evaluate epithelial healing and to evaluate DED findings (Bron et al., 2015).

The OSDI test is a 12-question questionnaire that evaluates the symptoms of ocular irritation and its visual-related functions to assess DED severity with scores ranging from 0 to 100 (Schiffman et al., 2000).

The Schirmer test measures the tear amounts on the eye surface by placing filter papers (SNO* Strips, Lab Chauvin, Aubenas, France) in the inferior fornix without topical anesthesia. The amount of wetting on the filter paper after 5 min is recorded as the test result. For our study, the results < 10 mm in the Schirmer test were accepted as positive for DED (McCarty et al., 1998).

In the TBUT test, the inferior fornix is touched using saline-soaked fluorescein sticks (Fluorescein, Haagen–Streit International, Koeniz, Switzerland). The patients are asked to blink and then abstain from blinking until told to do so. The time from the first blink to the detection of dry area formation on the cornea is recorded as the TBUT value. We used the TBUT values < 8 s to detect DED (McCarty et al., 1998).

The Oxford the scheme is a test to assess the state of the ocular surface using a fluorescein stick to stain the cornea, and the results are graded from 0 (no staining) to 5 (severe staining) (Bron et al., 2003).

The post-operative effect on corneal stromal healing was calculated using the vertical scar length measured with OCT, as previously described in the literature (Francoz et al., 2011). The OCT has a length measurement mode, and the device can automatically calculate the distance between two points. After the scar was detected, the upper and lower limit of the scar was manually marked, and the automatic measurement value was read. The measurement of the stromal scar formation at post-operative 15th day and 30th day was performed by determining the length from the shortest distance using the ruler mode, as shown in Figure 1.

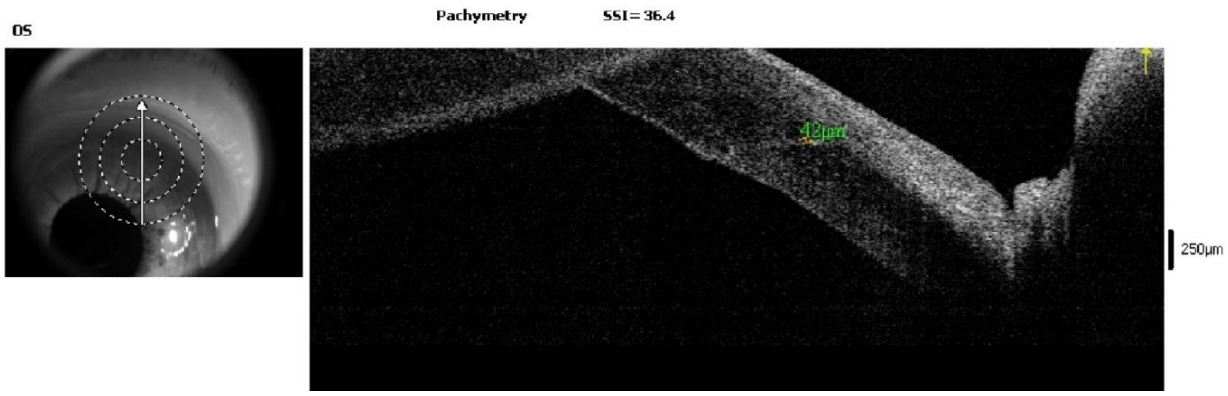

Figure1. Scar with the corneal OCT.

2.1. Statistical Analysis

Above mentioned parameters were compared between two groups using the Mann Whitney U test. The correlation between the length of scar incision and tear film parameters was evaluated with the Spearman correlation coefficient. A P-value of less than 0.05 was considered statistically significant.

2.2. Ethical Consideration

The study ethics committee approval was approved by the Maltepe University Ethics Committee with the number 2019/900/06.

3. Results

Sixty patients were included in the study. The gender and age distribution of patients are shown in Table 1.

Table 1. Gender and age distribution for two groups

	Group 1	Group 2	P*
Age (year)	55.08±6.11	55.66±6.58	P=0.645
Gender	Female 16 Male 14	Female 18 Male 12	P=0.786

Group 1= trehalose 30 mg/mL and sodium hyaluronate 1.5 mg/mL, Group 2= sodium hyaluronate 1.5 mg/mL
*Mann Whitney U test

There was no statistical difference between the gender and age distribution of study and control.

Preoperative and post-operative dry eye parameters, Oxford shame scores, and post-operative OCT scar length are shown in Table 2. As shown in Table 2, there was no statistical difference between the groups regarding preoperative and post-operative dry eye parameters. However, significant improvement was achieved in tear film function parameters in both groups during the post-operative period and was a better trehalose group. Oxford staining score, which is used for corneal epithelial cellular viability, was higher in the trehalose group but not statistically significant. There was no statistically significant difference between the groups for stromal scar length at the post-operative 15th and 1st month. Still, it was smaller in the trehalose group at both time points.

In correlation analysis, only a significant correlation was found between the Schirmer test and incision scar length on the 15th day in the Trehalose group (r=-0.275; P=0.034). Other tear film parameters, oxford shame, and incision scar length at 15th and 30th had no significant correlations for two groups.

Table 2. Dry eye parameters, OSDI scores and CCI scar length measured by oct for two groups

Parameter	Group 1 Mean ± SD (Range)	Group 2 Mean ± SD (Range)	P value*
Schirmer test (mm)			
preoperative	9.40±5.60 (3-25)	7.46±2.75 (2-13)	0.240
postoperative	13.76±8.06 (3-30)	13.36±6.92 (3-27)	0.947
BUT (second)			
preoperative	6.20±4.46 (2-25)	4.36±1.79 (2-7)	0.060
postoperative	7.16±3.14 (2-17)	6.06±2.36 (3-12)	0.166
Oxford score			
preoperative	1.36±0.96 (0-3)	1.56±0.62 (0-3)	0.318
postoperative	0.53±0.73 (0-2)	0.76±0.89 (0-3)	0.310
OSDI			
preoperative	22,84±10,92(11.36-36,36)	25.24±12.38 (11.36-68.18)	0.131
postoperative	10,39±5,44 (6.25-20.45)	12.92±6.57 (6,25-54,55)	0.394
Incision scar (μm)(OCT)			
15 th day	39.50±4.95 (25-46)	41.10±4.90 (31-47)	0.86
30 th day	25.80±6.93 (11-35)	29.30±5.05 (18-38)	0.75

OSDI= ocular surface disease index; CCI= clear corneal incision SD= standard deviation; BUT= tear film break up time; OCT= optical coherence tomography
Group 1= trehalose 30 mg/mL and sodium hyaluronate 1.5 mg/mL, Group 2= sodium hyaluronate 1.5 mg/mL
*Mann Whitney U test

4. Discussion

Trehalose is a naturally occurring substance found in other organisms, but it doesn't occur in humans. It is secreted during times of stress to protect the organism against external factors. Trehalose had been predominantly used to address dry eye symptoms. However, many studies in the literature had shown that Trehalose also protects cornea wound healing, as well as it suppresses corneal scar formation and inflammation (Cejka et al., 2019).

Many researchers thoroughly studied Trehalose's therapeutic effect in dry eye treatment, but there are a limited number of studies that investigate its role in post-operative treatment. These studies that have looked into Trehalose's post-operative effect after other surgical approaches in both rabbits who were exposed to UV and corneas in a hypoxic environment. It has been found that Trehalose helped with better epithelial wound healing and decreased both scar formation and neovascularization (Cejkova et al., 2012).

Additionally, there were reports on trehalose use in glaucoma surgery. It has been speculated that Trehalose decreases fibrosis via it is effective over fibroblasts and protects from infiltration (Takeuchi et al., 2010; Takeuchi et al., 2011). In these two studies, bare cell cultures that were not protected by any barrier were used, and cells are in direct contact with Trehalose.

When compared to conventional post-operative treatment after Lasik surgery in patients treated with 3% trehalose, the study in which dry eye parameters were examined, the trehalose group had better results in terms of oxford staining for epithelial integrity and healing. However, there was no difference between the groups in staining lissamine green (Mateo Orobia et al., 2017).

Similar results were obtained in a study performed in keratoconus patients. Epithelial healing was significantly faster in the patients who had received Trehalose for cross-link treatment when compared to sodium hyaluronate only. However, in this study, there was no information about the stromal effect of Trehalose (Ozek et al., 2018).

In the present study, it was investigated Trehalose's effect on corneal epithelial healing after phacoemulsification surgery. It has a similar impact on corneal epithelial healing to previous studies (Takeuchi et al., 2010; Takeuchi et al., 2011; Mateo Orobia et al., 2017; Ozek et al., 2018).

Oxford staining score, which is used for corneal cellular viability, was higher after surgery and epithelial healing was faster in the trehalose group but not statistically significant. Stromal scar formation after phacoemulsification surgery

Apart from the corneal incision, exposure to light by a microscope lamp, the phacoemulsification device's vibration, and temperature can be additional factors that can create additional wound stress. Therefore, both the surgical incision and the factors mentioned above may affect scarring and epithelial healing (Trattler et al., 2009; Cho et al., 2017; Situ et al., 2019; Zaleska-Żmijewska et al., 2019). MMP 9 is an important factor in the formation of fibrosis and scarring, and it is useful in remodeling, and scar formation increases. MMP increases with inflammation in the post-operative period (Sambursky et al. 2013; Nakamura et al., 2008). For the MMP effect, the drug should be in contact with the surface for more than 15 days (Gabison et al., 2005). There was no significant difference between the groups for stromal scar formation, but the scar area was smaller at both early and late stages for the Trehalose group. Also, the absence of stromal healing and epithelial healing was significant, maybe due to the low number of participants. Although the epithelial defect closed rapidly, and the epithelium creates a barrier for the Trehalose to contact the stromal surface, the scar area was smaller in the Trehalose group. These results may suggest that Trehalose may also act to the subepithelium. It may be necessary to conduct studies in which the contact of Trehalose with the bare stroma is longer and supporting this situation with histological studies will be more useful to evaluate the stromal effect of Trehalose.

Almost all previous studies reported that Trehalose is an effective agent that can be used for the management of dry eye safely (Matsuo, 2004; Wozniak et al., 2017) as the present study. Articles in the literature have almost similar OSDI scores and dry eye parameters for similar age groups (Kasetsuwan et al., 2013; Garg et al., 2020; Rico-Del-Viejo et al., 2018), this is similar for the current study. However, in groups with phacoemulsification surgery that did not use artificial tears after the operation, dry eye parameters worsened over time (Kasetsuwan et al., 2013; Garg et al., 2020), while dry eye parameters increased to a better level in the present study. This result may be due to the use of artificial teardrops in both groups for the current study. In this study for the Trehalose group, dry eye parameters, especially Schirmer test results on day 15th, were found to be better.

If the current study had a group without artificial tears, a more accurate assessment would be possible. As the study was designed retrospectively, this has been a limiting factor in the present study. And the other limitation of the present study was the small number of patients included. More significant results can be obtained with a bigger population. Also, we were not able to reach many participants to evaluate their long-term results, which restricted our assessment. These may affect the value of the drawn conclusions.

As a result, Trehalose seems to be effective in improving dry eye symptoms. Although not statistically significant, scar formation and corneal epithelial cell viability were higher in the trehalose group. However, the effect of Trehalose on stoma due to epithelial defect that has closed seems to be limited. Perhaps the stromal effect may be even more positive in patients where the epithelium is not closed for a long time. Trehalose may be an alternative option for managing CCI reconstruction after phacoemulsification surgery since it minimizes the corneal scar formation and accelerates corneal epithelization. Trehalose assists in improving dry eye parameters after phacoemulsification surgery. Further studies are required to prove its effectiveness in more significant patient populations with longer follow up.

Author Contributions

HE; data collection and writing, OM; statistical analysis and language editing.

Conflict of Interest

The authors declare that there is no conflict of interest.

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INVESTIGATION OF RENAL ARTERY VARIATIONS WITH MULTIDETECTOR COMPUTED TOMOGRAPHY ANGIOGRAPHY TECHNIQUE

Gunes BOLATLI[1]*, Mahinur ULUSOY KARDENIZ[2], Mustafa KOPLAY[3], Musa ACAR[4], İsmail ZARASIZ[5]

[1]*Siirt University, Faculty of Medicine, Department of Anatomy, 56100, Siirt, Turkey*
[2]*Akşehir Community Health Center, 42550, Konya, Turkey*
[3]*Selcuk University, Faculty of Medicine, Department of Radiology, 42250, Konya, Turkey*
[4] *Necmettin Erbakan University, School of Health Sciences, 42090, Konya, Turkey*
[5]*Girne American University, Faculty of Medicine, Department of Anatomy Department, 99320, Girne, NCTR*

Abstract: It is necessary to have a comprehensive knowledge about renal vascular anatomy in order to be able to apply renal surgical procedures correctly. The renal artery is a pair to feed the right and left kidneys. Its variation is common. In this study, it was aimed to group the variations and to examine them clinically. Abdominal CT findings of 532 cases examined with MDCT device were included in the study. Variation was detected in 135 (25.3%) of 532 cases. Of these variations, 95 (17.8%) were early branching and 40 (7.5%) were extra renal artery variations. The presence of renal artery variation may cause complications in surgical procedures such as kidney transplantation. Knowing the variations before the intervention to be made in the region will be guiding.

Keywords: Renal artery, Variations, Renal transplantation, Anatomy

***Corresponding author:** Siirt University, Faculty of Medicine, Department of Anatomy, 56100, Siirt, Turkey
E mail: gunesbolatli83@gmail.com (G. BOLATLI)

Gunes BOLATLI	https://orcid.org/0000-0002-7648-0237	
Mahinur ULUSOY KARDENIZ	https://orcid.org/0000-0002-0647-8828	
Mustafa KOPLAY	https://orcid.org/0000-0001-7513-4968	
Musa ACAR	https://orcid.org/0000-0002-5646-693X	
İsmail ZARASIZ	https://orcid.org/0000-0002-0551-337X	

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1. Introduction

It is important for surgeons to have a comprehensive knowledge of renal vascular anatomy when performing renal interventions. Procedures such as renal transplantation, laparoscopic and partial nephrectomy, urological and vascular surgery require a good knowledge of vascular anatomy and variations (Kumar et al., 2010; Urban et al., 2001).

The kidneys are fed by the renal artery (RA), which is separated from the aorta abdominalis at the lumbar 1-2 vertebra level. RA is a pair of an artery supplying the right and left kidneys. The artery of the right side is longer and higher. Gives 4-5 segmental branches before entering the kidneys (Cinar and Turkvatan, 2016; Urban et al., 2001), and variations of these branches are common (Kumar et al., 2010; Urban et al., 2001). The most common variations early separation of RA into segmental branches (early branching) and multiple RA (extra renal artery) (Kumar et al., 2010; Urban et al., 2001). The early branching variation of RA is divided into two as the hilar renal artery and polar renal artery according to the entrance place to the kidney (Kumar et al., 2010; Ozkan et al., 2006).

Extra renal artery (ERA) variation is also divided into accessory renal artery (hilar artery) and aberrant renal artery (polar artery) (Koc et al., 2007; Urban et al., 2001). The accessory renal artery (ARA) is the most common and clinically important variation of the RA. The accessory renal artery divides from the aorta or iliac artery as a separate branch, and its prevalence has been reported between 25% and 30% in different study groups (Cinar and Turkvatan, 2016; Kumar et al., 2010; Ozkan et al., 2006).

The presence of ARA is important in preoperative evaluation interventional procedures such as laparoscopic or retroperitoneoscopic nephrectomy for kidney transplantation, in patients with kidney tumors and nephron-sparing surgery. This evaluation will prevent possible surgical complications and renovascular hypertension due to isolated ARA stenosis (Aytac et al., 2003; Budovec et al., 2010; Koc et al., 2007; Ozkan et al., 2006; Ugurel et al., 2010).

Multidetector CT (MDCT) angiography is a fast, reliable, and non-invasive method that comprehensively evaluates vascular structures (Ahn et al., 2014; Holden et al., 2005). MDCT angiography is preferred for evaluating kidney donors. It has replaced traditional angiography in many institutions. The accuracy of MDCT angiography in

the evaluation of renal vascular anatomy varies between 95% and 100% (Aytac et al., 2003; Budovec et al., 2010; Koc et al., 2007; Ozkan et al., 2006; Ugurel et al., 2010). Magnetic resonance angiography, another imaging method that allows non-invasive evaluation of renal vascular structures, remains in the background because it does not have a high spatial resolution as MDCT (Ramadan et al., 2011).

Kidney anomalies are among the most common organ anomalies, so the number of studies on RA is increasing. RA variations may be caused by the reason that the embryological development of the kidney is more complicated (Secen et al., 2014). Variations of RA have generally been done on healthy individuals. There is little variation study on cases such as kidney disease or hypertension patients (Secen et al., 2014). It is important to know whether the variations are associated with these diseases.

In this study, cases were grouped according to RA variations and examined clinically. The prevalence of cases with hypertension and kidney disease in cases with variations was evaluated.

2. Material and Methods

2.1. Patient population

Five hundred thirty-two cases who underwent abdominal CT examination with MDCT device for any reason were included in the study. Images were examined in terms of the presence of variation. As a result, the cases were divided into 3 groups.

1. Normal anatomy group (NA)
2. The Group with early branching variation (EB)
3. The group with an extra renal artery variation (ERA).

All groups were examined by gender. In addition, the rate of cases with hypertension and kidney disease in the variation groups was evaluated.

2.2. Abbreviations

RA= renal artery
EB= early branching variation
ERA= extra renal artery variation
HRA= hilar renal artery
PRA= polar renal artery
ARA= accessory renal artery
ABRA= aberrant renal artery

2.3. MDCT protocol

Axial CT images of the patients with a cross-section thickness of 3 mm were transferred to the workstation. Renal structures were evaluated morphometrically and morphologically on the axial, coronal, and sagittal plane images of these separated cases.

2.4. Statistical analysis

The data obtained in the study were analyzed using the SPSS (Statistical Package for Social Sciences for Windows 22.0) program. Number, percentage, mean and standard deviation were used as descriptive statistical methods in the evaluation of the data.

2.5. Ethical Consideration

This study was approved by the Mevlana University Faculty of Medicine Clinical Trials Ethical Committee. Approval date and number 12.03.2014 and 26857650/015.

3. Results

Variation was detected in 135 (25.3%) of the 532 cases evaluated in our study (80 men, 55 women). 95 of these variations, (17.8%) were EB, and 40 of these variations (7.5%) were ERA variations (Figure 1).

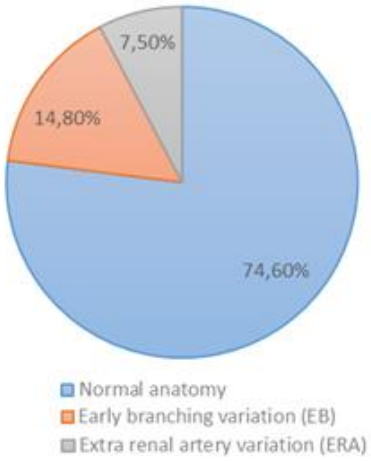

Figure 1. Incidence of variation.

Variation was observed in 95 cases, when we examine the EB variations (Figure 2). 80 of these cases (84.2%) were bilateral, 15 of these cases (15.8%) were unilateral early branching. 43 of the double-sided early branching variations were right and left hilar renal artery (HRA), 22 were right and left polar renal artery (PRA), 9 were right HRA left PRA, 6 were right PRA left HRA. Among the unilateral EB variations, right HRA was seen in 4 cases, right PRA in 1 case, left HRA in 5 cases, and left PRA in 5 cases. 41 of the 95 cases (43.1%) were female, 54 (56.9%) were male (Table 1).

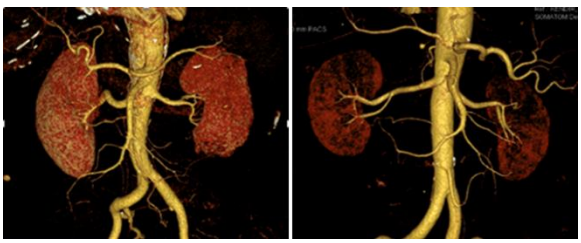

Figure 2. Early branching variation (EB).

29 of the 40 renal arteries with ERA variation (72.5%) were bilateral variation, 11 were unilateral (27.5%) variation (Figure 3). Right accessory renal artery (ARA) in 13 cases, right aberrant renal artery (ABRA) in 2 cases, left ARA in 10 cases, left ABRA in 4 cases, right and left ARA in 6 cases, right and left ABRA in 3 cases, right ABRA in 3 cases, left ARA in 2 cases detected. 15 of the 40 cases (37.5%) were female, 25 (62.5%) were male (Table 2).

Table 1. Early branching variations

	Bilateral early branching				Unilateral early branching				Total
	R-L HRA	R-L PRA	RHRA-LPRA	RPRA-LHRA	RHRA	RPRA	LHRA	LPRA	
Female	16	9	4	3	3	1	4	1	41
Male	27	13	5	3	1	0	1	4	54
Total	43	22	9	6	4	1	5	5	95
	8.1%	4.2%	1.7%	1.1%	0.7%	0.2%	0.9%	0.9%	17.8%

RHRA= right hilar renal artery, LHRA= left hilar renal artery, RPRA= right polar renal artery, LPRA= left polar renal artery

Table 2. Extra renal artery variations

	RARA	RABRA	LARA	LABRA	R-L ARA	R-L ABRA	RABRA-LARA	Total
Female	2	0	3	4	2	3	1	15
Male	11	2	7	0	4	0	1	25
Total	13	2	10	4	6	3	2	40
	2.5%	0.4%	1.9%	0.7%	1.1%	0.5%	0.4%	

RARA= right accessory renal artery, LARA= left accessory renal artery, RABRA= right aberrant renal artery, RABRA= left aberrant renal artery

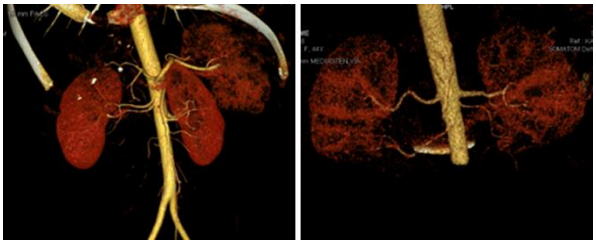

Figure 3. Early branching variation (ERA).

Patients with RA variations were also evaluated clinically. 11 of the cases with ERA variation (27.5%) had hypertension, 5 (12.5%) had kidney disease. In the cases with ED variation, 8 (8.4%) were hypertension patients.

4. Discussion

Detecting RA variations has gained importance in terms of renal transplantations, interventional radiological procedures, urological and vascular surgery (Khamanarong et al., 2004). Especially before renal transplantation surgeries, the morphological structure of the kidney should be examined. Extra renal artery and early branching variations are of particular importance for the decision on the site of nephrectomy (Hanninen et al., 2005). RA variations are a significant risk for anastomotic and postoperative complications (Hung et al., 2012; Shoja et al., 2008; Vazquez et al., 2010).

Accessory renal artery (ARA) is the most common and clinically important variation of the renal artery. It divides from the aorta, or iliac artery as a separate branch, and its prevalence varies (Cinar and Turkvatan, 2016; Kumar et al., 2010; Ozkan et al., 2006; Urban et al., 2001). Bleeding that may occur as a result of an invasive procedure performed on it may result in open laparotomy, or the absence of ARA may cause renal infarction in the recipient and hypertension in the donor (Saba et al., 2008). Obtaining information about the presence or absence of an accessory renal artery does not eliminate the need for conventional CT or MR angiography before attempts such as nephron-sparing renal tumor surgery or kidney transplantation. However, as this indirect information will show whether ARA is present, it may prevent additional investigations such as the search for isolated RA stenosis.

In the study conducted by Gümüş et al., RA variation was detected at a rate of 27%. When we look at the ERA variation; they stated that 15.5% of the EB variation was on the right side (11% ARA, 5.5% ABRA), 16.5% were on the left side (12.6% ARA, 3.9% ABRA), 26.7% was on the right side, 16.6% was on the left side, 6% was on both sides, 24% was female cases, and 30% was male cases among all variations (Gumus et al., 2012).

Oner and Oner (2019) found 50% of RA variation in their studies. 34.5% of these are 'ERA variation, 15.5% of these are EB variation. It is stated that 65% of the ERA variation is bilateral.

Ramadan et al. observed that one or more ARA were present at the rate of 22.8%. It was observed that the kidneys with an accessory renal artery were unilateral at the rate of 19.8% and bilateral at the rate of 3% (Ramadan et al., 2011).

Cınar and Turkvatan (2016) reported a 31.9% ERA variation. 11.5% of these were ar the right side (2.6% ARA), 12.9% of these are at the left side (3.7% ARA), 6.5% of these bilateral (0.2% ARA) and there are 24.6% female cases and 32.3% male cases in the whole population.

In this study, It was found that RA variations are quite common (25.3%), and the rate of variation in men is higher than in women. Our results are similar to the literature. On the contrary, it was determined that the incidence of EB variation was higher than the incidence of ERA variation. In addition, we made a comprehensive classification of RA variations, again different from the literature.

In some studies, it was stated that more than two ERAs were detected on the same side. (Bulic et al., 1996; Kuczera et al., 2009). This result was not found in our study.

Preoperative evaluation is important to evaluate renovascular hypertension, to prevent vascular

reconstruction and abdominal aortic aneurysms (Hanninen et al., 2005). In a study conducted on hypertension patients, it was reported that there was 7.8% ERA (46.1% left side, 45% left side, and 8.9% bilateral) (Secen et al., 2014). In another study, it was reported that there was 25.6% ERA (Kuczera et al., 2009). On the other hand, we found that 27.5% of the cases with ERA variation had hypertension, and 12.5% had kidney disease. In addition, 8.4% of the cases with EB variation were hypertension patients. The number of studies examining hypertension and RA variation is limited according to the sources we can reach. Therefore, we think that the results will contribute to the literature.

It has been stated that MDCT-Angiography is highly accurate in the diagnosis of RA variations compared to the conventional method (Gumus et al., 2012). It provides preliminary information to the radiologist, shortens the evaluation time, and speeds up the decision-making process (Aytac et al., 2003). We think that these variation rates, which we detected using the MDCT angiography method, may guide the evaluation of renal transplantation, essential hypertension, and kidney diseases.

Limitations

Our study was studied on a single race in a single hospital. The differences of the variations found according to the races could not be determined.

Author Contributions

BG; data collection or management, data analysis, manuscript writing, corresponding author. KUM; project development, consultant, manuscript editing. KM; data collection or management. AM; project development, ZI; project development, manuscript editing.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Research Article
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DETERMINATION OF ANTIMICROBIAL ACTIVITY OF VARIOUS PLANT ESSENTIAL OILS ON VANCOMYCIN RESISTANT ENTEROCOCCI AND SOME PATHOGENIC MICROORGANISMS

Revza ÇELİK[1], Esin KIRAY[2]*, Ergin KARİPTAŞ[3]

[1]*Kirşehir Ahi Evran University, Institute of Science, Department of Biology, 40100, Kirsehir, Turkey*
[2]*Kirsehir Ahi Evran University, Vocational School of Health Services, Department of Medical Services and Techniques, 40100, Kirsehir, Turkey*
[3]*Kirsehir Ahi Evran University, Faculty of Medicine, Department of Medical Microbiology, 40100, Kirşehir, Turkey.*

Abstract: In this study, it was aimed to determine the anti-bacterial activities of plant essential oils such as thyme, rosemary, sage, linden, black seed, bay leaf, peppermint, parsley, cinnamon and oleagen on vancomycin resistant enterococci (VRE) and pathogenic microorganisms of clinical importance. In the study, the inhibition zones of the plant extract of 10 plants prepared at 2.5 and 5 mg / ml concentrations of VRE and various pathogenic microorganisms were detected by Disk Diffusion and Agar Well Diffusion Method. Following this, Minimal Inhibition Concentration (MIC) values of these extracts were determined. The activities of plant extracts on DNA and Gas Chromatography-Mass Spectrometry (GC-MS) analyzes were also performed. According to the data obtained, it was determined that the extracts of rosemary and sage plants showed high antibacterial effect against pathogenic bacteria. The rosemary extract was found to be the most effective extract on VRE strains, as well as medium-grade antibacterial effects on the VRE strains in oleaster, linden, thyme and mint extracts. It was also determined that extracts from different plants did not cause any change in the structure of DNA. In our study, it was determined that plant extracts that are especially effective on VRE strains have the potential to be used in the treatment of VRE. The results of this study should be taken into account in the research of drugs that can be used in the treatment of VRE and more in vitro and in vivo research should be carried out in this field.

Keywords: Vancomycin-resistant enterococci, Plant volatile oils, Antibacterial activity

***Corresponding author:** Kirsehir Ahi Evran University, Vocational School of Health Services, Department of Medical Services and Techniques, 40100, Kirsehir, Turkey
E mail: esin.kiray@ahievran.edu.tr (E. KIRAY)
Revza ÇELİK https://orcid.org/0000-0002-6733-617X
Esin KIRAY https://orcid.org/0000-0002-6908-5909
Ergin KARİPTAŞ https://orcid.org/0000-0001-6513-9589

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1. Introduction

Enterococci are found as natural flora bacteria in the mouth, vagina, bile ducts and urethra on the surface of the human intestine (Çaylan, 2004). In addition to having a low rate of disease ability, they can cause serious diseases (Shepard and Gilmore, 2002). While these bacterial groups, which are found intensely in the abdominal cavity, cause infectious diseases such as bacteremia and endocarditis, they may rarely cause meningitis, skin and soft tissue infections. Some members of the enterococcus species can grow in a wide temperature range (10 °C - 45 °C) outside the host organism, maintain their viability at different pH levels and even in the presence of detergents. (Lautenbach et al., 1999; Patterson, 2000; Shepard and Gilmore, 2002). It is resistant to dry conditions; therefore, Enterococci can remain in hospital settings for a long time and spread easily among patients. (Asgin, 2020). It may predominate the gut microbiota of long-term hospitalized patients, especially those who taking broad-spectrum antibiotics (Reyes 2016).

Vancomycin resistant enterococci (VRE) first appeared in the 1980s and a significant threat to patients in hospital settings today. The incidence of VRE infections has increased steadily in different regions in recent years. For this reason, VRE is currently considered by the World Health Organization as a high-level priority public health issue for the research and development of new therapeutic strategies (Correa et al., 2020).

Increased multi-drug resistance among microorganisms has made it necessary to search for new sources in order to require new antimicrobial compounds. (Akgül, 2014). In this context, determining the effective plant species against pathogenic microorganisms and the active substances contained in these species has become a field of intensive study in the world. Since essential oils have a complex structure containing different components, their biological effects are also observed. Many essential oils may show different antimicrobial effects depending on the properties of the active ingredient they contain. (Ertürk et al. 2010).

The large number of different groups of chemical

components in essential oils explains that their antibacterial activities depend on different mechanisms. Antimicrobial substances obtained from perennial plants can alter the enzymatic reactions of microbial metabolism, inhibit the uptake of nutrients in the environment, make changes in the structure of the cell membrane, inhibit enzyme synthesis at the ribosomal and nucleus level. (Toroğlu ve ark., 2006; Evren, 2011). When the antimicrobial properties of essential oils are evaluated, it is stated that gram-negative bacteria are more resistant than gram-positive bacteria and that this resistance of gram-negative bacteria may arise from the cell wall (Evren, 2011).

In this study, it was aimed to determine the antimicrobial activities of thyme, rosemary, sage, lime, black seed, bay leaf, mint, parsley, cinnamon and oleaster plant extracts grown in Kırşehir province by various methods against VREs and some pathogenic bacteria.

2. Materials and Methods

2.1. Bacterial Cultures

In order to determine the antimicrobial activity of plant extracts in our study, *Enterococcus feacalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 23857, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922, *Aeromonas hydrophila* ATCC 7966, *Shigella dysenteriae* ATCC 11835, *Listeria monocytogenes* ATCC 19111, *Proteus mirabilis* ATCC 29906 were obtained from the Microbiology Laboratory of the Faculty of Arts and Sciences at Kırşehir Ahi Evran University. VRE *Enterococcus faecium*, VRE1, VRE2, VRE3, *E. feacalis*, VRE ATCC 51299 strains were obtained from Kırşehir Ahi Evran University Training and Research Hospital Microbiology Laboratory.

2.2. Preparation of Plant Extracts

In this research, thyme, rosemary, sage, lime, black seed, bay leaf, mint, parsley, cinnamon and oleaster plants, which are very common among the plant varieties in our country, were left to dry in the laboratory environment (at room temperature). The Soxhlet device (isolab soxhlet NS 29/32 30 ml) was used to obtain plant extracts. Plants were treated with 200 ml of solvent for 8 hours (h). In the study, ethanol and n-hexane were used as solvents. The extracts obtained were evaporated until the plant extract remained on the rotary evaporator (Heidolph Hei-Vap Advantage ML / G1). The prepared extracts were kept at + 4 °C until use. Concentrated extracts were dissolved with DMSO (dimethyl sulfoxide acid) to obtain stock solutions with a final concentration of 2.5 mg/mland 5 mg / ml. Stock solutions were stored in the refrigerator at -20 °C until use (Figure 1).

2.3. Determination of Antimicrobial Activity by Agar Well Diffusion Method

Antimicrobial efficacy was determined by agar well diffusion method and disk diffusion method (Kalemba and Kunicka, 2003). In the antimicrobial activity test of plant essential oils, Tryptic Soy Broth (TSB) medium for pathogen test bacteria and sheep blood agar medium for VRE bacteria were used. After the at 37 °C 18 hours (h) incubation, the strains were spread homogeneously to the Tryptic Soy Agar (TSA) media and sheep blood agar medium with a sterile drigalski spatula so that the final concentration was 1 x 10^9 CFU / ml.

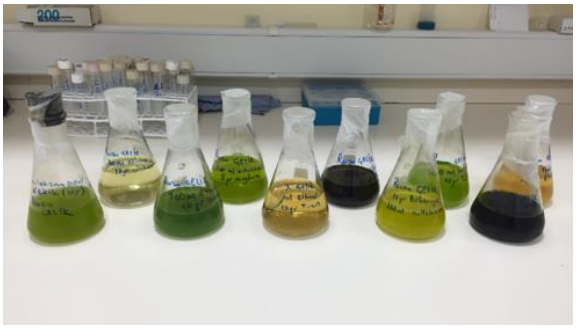

Figure 1. Plant extracts obtained from Soxhlet extraction.

After the plates were dried at room temperature for 2 hours, wells with a diameter of 6 mm were opened on them.

Plant extracts (rosemary, mint, oleaster, thyme, lime, black seed, parsley, sage, bay, cinnamon) obtained in the study were added to wells drilled in different concentrations (2.5 and 5 mg / ml) and incubated at 37 °C for 24 hours. Inhibition zone diameters of essential oils were measured in mm after incubation. Essential oil with a diameter of 12.0 mm and narrower does not have enough antibacterial effect on the existing bacteria (-), diameters at 12 and 21 mm limbs are moderately active (+), diameters from 21mm to 30 mm are active (++) On the other hand, diameters of 30 mm and above are considered to be very active (+++) (Djabou et al., 2013). Our study was carried out in duplicate for each microorganism and plant extracts.

2.4. Determination of Antimicrobial Activity by Disc Diffusion Method

In this method; pathogenic test bacteria activated overnight in TSB liquid medium spread to TSA solid medium (Sandri et al. 2007). Discs impregnated with plant extracts in concentrations of 5 mg/ml, previously prepared (6 mm) were placed in petri dishes containing pathogenic bacteria. The petri plates were left in 24-hour incubation at 37 °C. The zone diameters formed around the discs in the petri dishes after incubation were measured in mm.

2.5. Minimal Inhibition Concentration (MIC) Value Determination

Nutrient Broth (NB) broth was used for the production of bacteria and determination of antibiotic MIC values. Pathogenic test bacteria were activated in a 5ml NB liquid medium at 37 °C for 18 h in a 175 rpm shaking incubator. Activated bacterial cells were added in 50 ml NB medium, approximately 10^6 cells per 1 ml, in accordance with 0.5 McFarland turbidity standards. NB medium containing microorganisms and plant extracts of

different concentrations were mixed in an equal volume and then serial dilutions were made. Tubes were incubated at 37 °C for 24 h. The last tube without bacterial growth was determined as MIC value. MIC values obtained in the study were indicated as µg / ml.

2.6. Detection of DNA Cleavage Activity in Plant Extracts

Detection of DNA cleavage activity in plant extracts was evaluated in Amasya University Faculty of Education Microbiology Laboratory. Extracts obtained were performed using pBR322 DNA cleavage using agarose gel electrophoresis. Extracts were prepared by dissolving in DMSO at a concentration of 2mg/ml. The pBR322 plasmid DNA (0.25 ug / mL) was treated with each extract and the mixture was incubated at 37 °C for 4 h. The pBR322 plasmid DNA treated only with water (H_2O control) and 1 µl DMSO (DMSO control) was used as the DNA control. After the prepared incubation samples were loaded on 1% agarose gel, the electrophoresis was carried out at 120 V in 80 minutes TAE buffer (40 mM Tris / acetate and 1 mM EDTA, pH 8.0) and the obtained gels in EtBr (Ethidium Bromide) (1 mg / mL) was examined in the UV illuminator after staining (Figure 2).

Figure 2. Effect of plant extracts on DNA structure. 1= pBr322 DNA control H_2O, 2= pBR322 control DMSO, 3= Oleaster, 4= Cinnamon, 5= Nigella, 6= Thyme, 7= Sage, 8= Mint, 9= Linden, 10= Rosemary, 11= Bay leaf, 12= Parsley.

2.7. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The analysis of the essential oils obtained in this study was carried out using the Gas Chromatography Mass Spectrometer (GC / MS) system available at the Aksaray University Scientific and Technological Application and Research Center. Some of the extracts of sage, rosemary, thyme, peppermint and cinnamon plants were diluted in methanol, and thyme oil from industrially produced oils of the same plants was diluted in methanol and other oils were diluted in hexane and qualitative analysis of samples were performed.

3. Results

3.1. Antimicrobial Activity of Plant Essential Oils

It is about disc diffusion method, in which 10 different essential oils (thyme, mint, bay leaf, parsley, oleaster, ilamlam, rosemary, sage, black seed and cinnamon) have different effects (2.5 mg/ml and 5 mg / ml) for pathogenic microorganisms. Inhibition zone diameters obtained by agar well diffusion method are shown in Table 1, and zone diameters obtained by disc diffusion method are shown in Table 2. As seen in Table 1, all of the plant essential oils obtained in the study showed a high antimicrobial effect on various pathogenic microorganisms. However, as can be seen in Table 2, this effectiveness could not be determined exactly by the disk diffusion method. In this antimicrobial study, the zone diameters obtained in two different concentrations that we used as 2.5 mg/mland 5 mg/mlin agar well diffusion method increased proportionally with the concentration ratio (Table 3).

Table 1. Antimicrobial effect of plant essential oils on pathogen microorganisms at different concentrations (2.5 and 5 mg/ml) (Agar Well Method)

	E. coli ATCC 25922		*E. faecalis* ATCC 29212		*S. aureus* ATCC 29213		*P. aeruginosa* ATCC 27853		*E. aerogenes* ATCC 13048		*B. subtilis* ATCC 23857		*B. cereus* ATCC 14579		*A. hydrop.* ATCC 7966		*S. dysenteriae* ATCC 11835		*L. monocytogenes* ATCC 19111	
	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5
Silverberry	ND	11	14	15	ND	9	ND	11	10	12	13	13	10	16	15	11	12	12	13	13
Parsley	ND	ND	10	12	11	14	20	21	12	17	18	19	12	14	13	14	14	18	11	12
Rosemary	14	16	12	16	18	20	18	19	12	15	18	25	23	24	14	14	16	19	12	13
Linden	12	ND	ND	10	ND	ND	12	12	11	19	13	18	12	13	12	13	13	14	11	12
Black seed	13	15	11	11	ND	12	17	19	13	15	12	14	10	11	10	11	13	14	12	14
Sage	15	16	16	19	12	16	14	16	13	19	20	22	18	18	14	16	15	19	12	14
Thyme	ND	ND	13	14	14	15	11	12	12	12	14	18	12	12	14	15	12	14	10	10
Mint	13	13	12	14	11	12	13	14	12	14	15	20	13	13	12	12	12	15	10	12
Bay leaf	ND	ND	12	12	10	10	14	14	10	13	16	16	12	14	14	9	12	12	11	13
Cinnamon	13	15	12	12	20	24	15	16	14	15	20	22	19	20	14	16	17	21	13	14

ND= not detected, ATTC= American type culture collection

Table 2. Antimicrobial effect of plant essential oils on pathogen microorganisms at different concentrations (5 mg/ml) (Disc Diffusion Method)

	E. coli ATCC 25922	*E. faecalis* ATCC 29212	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*E. aerogenes* ATCC 13048	*B. subtilis* ATCC 23857	*B. cereus* ATCC 14579	*A.hydrop.*AT CC 7966	*S. dysenteri.* ATCC 11835	*L. monocy.* ATCC 19111
Silverberry	ND	ND	ND	ND	8	ND	ND	ND	ND	ND
Parsley	7	ND	ND	ND	8	ND	ND	8	8	7
Rosemary	7	ND	10	10	8	8	12	ND	ND	ND
Linden	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Black seed	9	ND	7	ND	7	ND	7	ND	8	ND
Sage	8	8	9	10	8	ND	ND	ND	10	ND
Thyme	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mint	10	8	ND	ND	ND	ND	ND	7	7	7
Bay leaf	ND	ND	ND	ND	9	ND	ND	ND	ND	ND
Cinnamon	ND	ND	ND	8	8	7	7	ND	ND	7

ND= not detected, ATTC= American type culture collection

In our study, VRE 1 concentration of oleaster, rosemary, sage, thyme, peppermint, bay leaf and cinnamon essential oils showed a moderate antibacterial effect. The oleaster essential oil showed higher antibacterial activity against VRE 1, rosemary and lime essential oil VRE 2 compared to other oils and formed the widest inhibition zone. While rosemary and lime essential oils prepared as 5 mg/ml in our study showed a very high effect on the development of VRE 2; Thyme, peppermint, cinnamon, oleaster, cinnamon showed low effects, while parsley, linden and bay leaf also had a moderate antibacterial effect. However, none of the essential oils impregnated with sterile discs showed any antibacterial effect against VRE 2 and did not form an inhibition zone.

Table 3. Antimicrobial zone diameters (mm) on VRE strains of plant essential oils. (2.5 and 5 mg/ml)

	VRE *E. faecium*			VRE 1			VRE 2			VRE 3 *E. feacalis*			VRE ATCC *51299*		
	2.5	5	D.D.M	2.5	5	D.D.M	2.5	5	D.D.M	2.5	5	D.D.M	2.5	5	D.D.M
Oleaster	ND	9	ND	14	18	ND	11	12	ND	12	13	8	12	12	ND
Parsley	10	11	7	ND	11	8	14	14	ND	10	11	ND	11	12	ND
Rosemary	12	17	ND	15	15	ND	15	20	ND	14	15	ND	14	14	ND
Linden	12	14	ND	10	11	ND	18	18	ND	14	14	ND	13	15	ND
Oleaster	11	11	8	11	11	7	13	14	ND	12	14	9	10	13	ND
Sage	14	17	10	14	14	8	11	13	ND	9	11	ND	11	10	ND
Thyme	11	11	ND	12	12	9	12	12	ND	10	12	ND	12	12	ND
Mint	16	19	7	12	14	9	12	12	ND	10	10	ND	12	13	10
Bay leaf	10	11	ND	14	15	ND	14	15	ND	10	11	ND	10	11	ND
Cinnamon	10	12	ND	11	12	ND	10	11	ND	11	11	ND	12	11	ND

ND= not detected

In our study, the concentrate prepared as 5 mg/ml had a moderate antibacterial effect on the VRE 3 *E. feacalis* of essential oil, lime, black seed and thyme essential oils. The rosemary essential oil VRE 3 *E. feacalis* showed higher antibacterial activity against VRE 3 *E. feacalis* than other oils and formed the widest inhibition zone. On the

other hand, essential oils that are impregnated with sterile discs, except for oleaster and black seed essential oils, do not show any antibacterial effect; although these essential oils did not have sufficient effect, they formed an inhibition zone. In our study, the essential oils of cinnamon, bay leaf and sage did not show sufficient effect on VRE ATCC 51299, while lime and rosemary essential oils showed high antimicrobial effects.

3.2. DNA Cleavage activity of plant essential oils

In this study, it was investigated whether the extracts obtained from different plants cause any change in the structure of DNA. As can be seen in Figure 1, samples 1 and 2 were used as controls. As a result of the study, the effects of the extracts obtained from plants on DNA were examined and it was determined that no plant extract caused any deterioration in the DNA structure of the plant, so it had no effect on the plasmid DNA.

3.3. Minimal Inhibition Concentration (MIC) Values

The MIC of plant extracts obtained in this study on various pathogenic microorganisms was determined. Stock solutions of the plant extracts used were prepared and used in DMSO. MIC values of plant extracts against pathogenic microorganisms are shown in Table 4.

Table 4. MIC values of plant extracts (ug/ml) against pathogenic microorganisms

	P. aeruginosa	*E. faecalis*	*E. aerogenes*	*A. hydrophila*	*E.coli*	*S. dysenteriae*	*L. monocytogen.*	*B. subtilis*	*S. aureus*	*B. cereus*
Bay leaf	1875	937	3750	3750	3750	3750	3750	937	1875	937
Thyme	234	58.5	234	468	234	468	234	234	117	58.5
Black seed	1875	468	3750	1875	1875	3750	3750	1875	937	58.5
Linden	1875	937	1875	1875	937	3750	3750	3750	3750	3750
Parsley	937	468	1875	937	1875	3750	3750	4000	4000	4000
Oleaster	937	1875	1875	1875	937	3750	1875	468	1875	1875
Rosemary	3750	1875	1875	3750	468	1875	4000	4000	7500	3750
Cinnamon	1875	937	1875	1875	3750	937	1875	468	937	468
Mint	1875	3750	3750	3750	3750	3750	3750	3750	3750	1875
Sage	3750	937	3750	3750	3750	3750	3750	3750	3750	3750
DMSO	>5000									

In this study, it was determined that the MIC values in the control samples were > 5000 µg/ml. In addition, when the MIC values of the extracts were analyzed, it was observed that this value varied between 58.5-4000 µg/ml. As can be seen from here, it is seen that some plant extracts have high antimicrobial effect on the life of bacteria.

As seen in Table 4, the extract from the bay leaf was found to have a moderate effect on *E. faecalis*, *B. subtilis* and *B. cereus* (937 µg/ml). Thyme extract appears to have a high antimicrobial effect on all microorganisms. It is seen that the plant extract obtained from Nigella is effective only on *E. faecalis* and *B. cereus* (MIC values are 468 µg / ml for *E. faecalis*, 58.5 µg/ml for *B. cereus*). Parsley only on *E. faecalis* (468 µg/ml), Spindle only on *B. subtilis* (468 µg/ml), Rosemary only on *E. coli* (468 µg/ml), and Cinnamon on *B. subtilis* and *B. cereus* (468 µg/ml) has been found to have significant effects.

3.4. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

In this study, GC-MS analysis of these extracts was performed by selecting 5 plant extracts (sage, rosemary, mint, thyme and cinnamon) that showed the most antimicrobial effects from 10 different plant extracts. As a result of GC-MS analysis of sage herbal extract shown in Table 5, four different components were determined and porphin (4.93%) was the most common chemical in the composition.

Table 5. GC-MS analysis results of sage herbal extract.

Name	Common name	Composition rate%
Sage		
	DMSO	94.21
9.12-Octadecadienoic acid	Linoleic acid	0.27
(9e.12e)-9.12-octadecadienoic acid	cis-Linoleic acid	0.59
	Porphin	4.93

As a result of GC-MS analysis of rosemary herb extract shown in Table 6, seven components were found and the most common chemical substances in their composition were cis-Linoleic acid (0.27%) Eucalyptol (Ökaliptaol) (0.18%). The only chemical substance in the composition of thyme herbal extract shown in Table 7 as a result of GC-MS analysis is o-Cymen-5-ol Thymol (1.14%). Porphin (4.96%) is the most common chemical substance among the four components encountered as a result of GC-MS analysis of peppermint herb extract shown in Table 8. As a result of GC-MS analysis of cinnamon plant extract shown in Table 9, six components were found. Its main components are specified as Cinnamaldehyde (2.75%) and Cinnamaldehyde Dimethyl Acetal (1.21%).

Table 6. Results of GC-MS analysis of rosemary herb extract

Name	Common name	Composition rate%
Rosemary		
	DMSO	99.30
1.3.3-Trimethyl-2-oxabicyclo[2.2.2]octane	Eucalyptol (Ökaliptaol)	0.18
Bicyclo[2.2.1]heptan-2-one, 1.7.7-trimethyl	Camphor (Kafur)	0.05
1,7,7-trimethylbicyclo[2.2.1]heptane	Borneol	0.04
2-(4-Methyl-3-cyclohexen-1-yl)-2-propanol	Alpha-terpineol	0.04
9.12-Octadecadienoic acid	Linoleic acid	0.13
(9e.12e)-9.12-octadecadienoic acid	Cis-linoleic acid	0.27

Table 7. Results of GC-MS analysis of thyme herb extract

Name	Common name	Composition rate%
Thyme		
	DMSO	98.86
3-Methyl-4-isopropylphenol	o-Cymen-5-olThymol	1.14

Table 8. GC-MS analysis results of peppermint plant extract.

Name	Common name	Composition rate%
Mint		
	DMSO	94.37
(E)-3-phenylprop-2-enal	Cinnamaldehyde	0.17
9.12-Octadecadienoic acid	Linoleic acid	0.31
	Porphin	4.96

4. Discussion

The most notable feature of enterococci is that they are resistant to many antimicrobial agents used in the treatment of gram-positive bacterial infections. Multiple antibiotic resistance in enterococci in Turkey as well as the whole world emerges as an important problem. In other words, the antibiotic alternatives that can be used in the treatment of enterococcal infections are quite limited (Berzeg, 2005; Karagöz, 2005). In our study, the antimicrobial activites of various plant essential oils on VREs and various pathogenic microorganisms isolated from patients by Kırşehir Ahi Evran University Training and Research Hospital Microbiology Laboratory was investigated.

Table 9. GC-MS analysis results of cinnamon extract

Name	Common name	Composition rate%
Cinnamon		
	DMSO	95.58
(E)-3-phenylprop-2-enal	Cinnamaldehyde	2.75
[(E)-3.3-dimethoxyprop-1-enyl]benzene	cınnamaldehyde dımethyl acetal	1.21
[(E)-3-phenylprop-2-enyl] acetate	Cinnamyl Acetate	0.14
9.12-Octadecadienoic acid	Linoleic acid	0.1
(9e.12e)-9.12-octadecadienoic acid	cis-Linoleic acid	0.22

Turkey is rich in plant ingredients and potential, there are a large number of plant species used medicinally. Researches conducted both abroad and in Turkey found that many of these plants have antimicrobial effects. In this study, extracts of 10 plants (peppermint, thyme, rosemary, lime, cinnamon, oleaster, bay leaf, black seed, parsley and sage) grown in our country and which we can easily find on the market are used.

In this study, it was observed that the plant-specific extracts studied had antimicrobial activity against VREs. The most susceptible bacterial strain to extracts is VRE 2. It is possible to use the plant extracts that we use in our study as antimicrobial agents of modern drugs produced for the treatment of infectious diseases. It is seen that the spindle essential oil showed higher antibacterial activity against VRE 1 and the rosemary and lime essential oil showed higher antibacterial activity against VRE 2 compared to other oils.

While the rosemary and lime essential oils prepared as 5 mg / ml in the study showed had a very high effect on the development of VRE 2; thyme, peppermint, cinnamon, oleaster, cinnamon showed low effects. In addition to these, parsley, linden and bay leaf have a moderate antibacterial effect. The rosemary essential oil, which was prepared as 5 mg / ml, showed higher antibacterial activity against VRE 3 *E. feacalis* compared to other oils and formed the widest inhibition zone. The rosemary essential oils at the concentration of 5 mg/ml showed a

high antimicrobial effect on VRE ATCC 51299. As a result, rosemary essential oils have been identified as the most effective vegetable oil on VRE bacteria. Samy, 2011 investigated the antimicrobial activity of 11 different essential oils on *E. coli* O157:H7 and VRE and he determined that the most powerful antimicrobial activity of thyme oil was the result of the study. In our study, it was found that thyme oil is effective on various pathogens and VRE. However, in our study, it was seen that especially rosemary oil was more effective. Studies on plant essential oils have found a limited number of plants with antimicrobial activity on VRE microorganisms. One of them is Lavender essential oil. It has also been found that it has antimicrobial activity on (VRE) species (Roller et al., 2009).

It is concluded that the antibacterial activity does not depend on a single mechanism and that there are many targets in the cell, considering the excess of different groups of chemical compounds in essential oils (Toroglu et al., 2006). The antimicrobial effect of essential oils can be seen in the cytoplasm membrane with changes in structure and function. Antimicrobial substances obtained from higher plants can stop the enzymatic reactions of microbial metabolism, prevent the intake of nutrients in the environment, change the structure of the membrane, and prevent enzyme synthesis at the core and ribosomal level (Uçan, 2008). According to GM-MS analysis in our study, some plant extracts had a great effect on the life of bacteria. In particular, thyme herb extract appears to have a significant antimicrobial effect on all microorganisms. The antimicrobial properties of plant essential oils come from phenolic (thymol, kavrachrol, eugenol, etc.) and terpenoid components in their structure, and plants rich in these components are also used for therapeutic purposes. The important thing here is to obtain these components without creating any damage and use them in appropriate doses and combinations (Faydaoğlu and Rideroğlu, 2013).

In various studies on thyme oil, it has been determined that it shows high bacteriostatic activity on both Gram-positive and Gram-negative bacteria (Winska, 2019). In a study investigating the in vitro antimicrobial activity of *Thymus vulgaris* essential oil against oral pathogens, its effectiveness on p-hemolytic *Streptococci* ssp. strains (Fani and Kohanteb, 2017), Salehi et al. determined that oregano oil inhibited *S. aureus* ATCC 25923 and *K. pneumoniae* ATCC 13882 strains (Salehi, 2019). In many reviews, it has been stated that the antimicrobial activity of *T. vulgaris* is due to its high thymol and carvacrol content, which can form hydrogen bonds with the active site of many enzymes (Marchese, 2016).

In general, the antimicrobial properties of vegetable essential oils come from phenolic (thymol, kavrachrol, eugenol, etc.) and terpenoid components, and plants rich in these components are also used for treatment. The important thing here is to obtain these components without causing any damage and to use them in appropriate doses and combinations (Benefitoğlu and Rideroğlu, 2013).

It is thought that sage, cumin, rosemary oils and their main components are ineffective or less effective against Gram (-) bacteria, the reason of which is the external membranes of Gram (+) bacteria, which are also composed of lipopolysaccharide and protein in Gram (-) bacteria. The lipopolysaccharide structure limits the diffusion of hydrophobic compounds (Burt, 2004; Delamare et al., 2007). According to some research results, Burt 2004 stated that there is no difference between Gram (-) and Gram (+) bacteria, and according to some studies, the responses of Gram (-) and Gram (+) bacteria may differ. According to these results, the important factor in antimicrobial activity is not only the properties of the target microorganism, but also the chemical composition of the essential oil is different (Ouattara et al., 1997; Sağdıç and 2003).

In our study, it was seen that peppermint essential oil, which has a strong antimicrobial effect especially on VRE *E. faecium*, has a small effect on other pathogenic microorganisms. In other studies, it has been pointed out that although peppermint essential oil alone has a weak antibacterial activity, it may have a synergistic effect with other essential oils or substances. One of the popular preparations used to treat headaches, colds, cough, mild spinal gastrointestinal complaints and relieve local muscle pain is Olbas ® Tropfen (Olbas). It contains essential oils such as peppermint essential oil (5.3 g), eucalyptus (2.1 g), cajuput and to a lesser extent juniper essential oil (0.3 g). Antibacterial activity has been demonstrated against many strains, including Olbas ®, methylcine-resistant *S. aureus* (MRSA) and VRE (Hamoud, 2012).

In our study, it was stated that cinnamon essential oil, which is weakly effective on VRE strains but has a strong antimicrobial effect on *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 23857 and *B. cereus* ATCC 14579 strains, has also shown a strong antimicrobial effect in other studies. In particular, they showed that cinnamon essential oil has a better effect compared to essential oils such as clove, lemongrass, tea tree, ginger, basil and has very good activity against *S. aureus*, *E. coli, A. baumannii* and *P. aeruginosa*. (Chouhan, 2017). These results are similar to our study.

Various plant essential oils have been reported to show high antiproliferative effects on pathogenic microorganisms. In one study, eucalyptus, juniper, peppermint, rosemary, sage, clove and thyme oils were found to be the most effective essential oils against VRE and *E. coli* O157: H7 strains with multi drug resistance. (Selim, 2011). These data are similar to the results in our study. Also, in our study, it was investigated whether the extracts obtained from different plants cause any change in the structure of DNA. As a result of the study, it has been determined that the extracts obtained from plants do not cause any deterioration in the DNA structure, therefore they have no effect on plasmid DNA.

4. Conclusion

It is a known fact that bacterial resistance to antibiotics and widespread hospital infections pose a great danger to public health and our future. Therefore, it is so important to research and develop new classes of antimicrobials that are effective on pathogenic microorganisms today. For this reason, it should be aimed to know the antimicrobial properties of plants that exist in nature and to obtain plant extracts suitable for this purpose. Considering all microorganisms and plant extracts, it is seen that not all plant extracts have an antimicrobial effect and are not effective on all strains of strains with antimicrobial effect. For this reason, further research should focus on microorganisms affected by plant extracts. In the studies conducted, it can be said that especially rosemary, sage, thyme and cinnamon have high antimicrobial effects. It has been proved in national and international studies that the plants used in this study have many biological activities. In this study, this efficacy has been demonstrated and some plant extracts have also been found to be effective on VRE strains. When all the data obtained are evaluated, it can be seen that these plants can be used for different purposes in the fields of pharmacy and medicine and therefore it is worth to study more.

Author Contributions

EK, RÇ and EK; concept, EK; design, supervision, data collection and/or processing, EK, RÇ and EK; materials, RÇ, EK and EK; data analysis and/or interpretation, EK and RÇ; literature search and writing.

Conflict of Interest

The authors declare that there is no conflict of interest.

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STUDENT OPINIONS ON THE EFFECT OF THREE DIMENSIONAL DIGITAL SOFTWARE PROGRAMS FOR SHOWING HUMAN ANATOMY ON ANATOMY EDUCATION

Erengül BODUÇ[1]*

[1]*Kafkas University, Faculty of Medicine, Department of Anatomy, 36100, Kars, Turkey*

Abstract: The aim of this article is to investigate the effect of three-dimensional digital images on anatomy education through student views. The data collection form prepared with a five-point Likert test was sent to the first and second year students of the Kafkas University Medicine Faculty on the web. 133 students in total answered the questions. When the answers given to the questions were examined, it was observed that there was a significant difference between the answers given by the first and second graders in the third (P=0.013) and ninth (P=0.002) questions (P<0.05). It is thought that the results and opinions obtained from the study will guide the future of anatomy education. Thanks to the student views, it is expected that the study will raise awareness among educators.

Keywords: Human Anatomy, Cadaver, Digital software programs, Likert test

***Corresponding author:** Department of Anatomy, Faculty of Medicine, Kafkas University, 36100, Kars, Turkey
E mail: erenboduc@gmail.com (E. BODUÇ)
Erengül BODUÇ https://orcid.org/0000-0001-8872-1993

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1. Introduction

Cadaver is a very important teaching tool in anatomy education (McLachlan et al., 2004). Technological developments in recent years have brought many innovations to the field of anatomy (Lewis et al., 2014). Three-dimensional course software, digital atlas and tablets have been tools that students are interested in and use (Peker et al., 2012; Hoyek et al., 2014). However, the replacement of cadavers by these teaching tools is a highly controversial issue (Murgitroyd et al., 2015).

Anatomy education using cadavers is actually a laborious education model that requires infrastructure (Estai and Bunt, 2016). Laboratory infrastructure should include cadaver tanks, stretcher vehicles carrying cadavers and personnel (Cornwall and Stringer, 2009). In addition, solutions that serve to embalm the cadaver should prevent the risk of infectious diseases and infections (Balta et al., 2019). It is necessary to have done many cadaver dissections in order to be able to master the cadaver and to give lessons with all the details (Granger, 2004). For this reason, having knowledge about cadavers is a success that requires a lot of effort and opportunity (Older, 2004).

When it comes to three dimensional software tools; in recent years, they have benefited greatly from anatomy education (Sugand et al., 2010). Especially, cross-sectional and three-dimensional anatomy of the structures has positive effects in increasing the level of knowledge (Gunderman and Wilson, 2005). Sometimes, dissection areas that are not suitable for the cadaver are learned quite clearly with three-dimensional software (Lazarus et al., 2012). However, three-dimensional software cannot provide the perception of reality sufficiently (Kotze et al., 2012). A virtual object of the realness acquired in the cadaver cannot afford much (Lazarus et al., 2012; Biasutto et al., 2006).

In this study, students' views on the state of digital software that has emerged in recent years in anatomy education are included. Education can follow a more dynamic path with student views and the views of young generations can direct education.

2. Material and Methods

The study was carried out on the first and second-year medical students of Kafkas University Medicine Faculty in the 2019–2020 academic year. The data collection forms used in the study were prepared on the web and sent to the students by e-mail. One hundred thirty three students (75 from the first class, 58 from the second class) answered the questions in the data collection form. Data collection questions were prepared with a five-point Likert scale (totally agree, agree, undecided, disagree, and totally disagree) (Bahsi et al., 2020). The answers given by the students were automatically analyzed through the web system and the results were obtained through the software. The answer options given were calculated automatically on the system with the calculation of frequency and percentage. The answers are given by the first year and second-year students to the questions were statistically compared with the chi-

square test.

2.1. Statistical Analysis

The first and second years were compared according to the answers given by the students. Statistical analysis was carried out using SPSS 22.0 version software program for Windows. Descriptive statistics for categorical variables are expressed as frequency and percentage values. A Chi-square test was used in the analysis of categorical data (). The results were evaluated at a 95% confidence interval, and a p-value of <0.05 was considered significant.

2.2. Ethical Consideration

This study was approved by the ethics committee of the Medicine Faculty of the Kafkas University (Approval number: 2019/12; Decision: 14). The study was performed following the aid of the ethical standards down in the 1964 Declaration of Helsinki and its later amendments.

3. Results

The first and second years were compared according to the answers given by the students. Statistical analysis was carried out using SPSS 22.0 version software program for Windows. Descriptive statistics for categorical variables are expressed as frequency and percentage values. A Chi-square test was used in the analysis of categorical data. The results were evaluated at a 95% confidence interval, and a p-value of <0.05 was considered significant. The answer 'I strongly agree' to the first question is higher for the second graders (62.1%) than for the first graders (46.7%). The sophomore students have marked quite decisively. In the second question, the answer 'I strongly agree' is higher in the second grade (79.3%) compared to the first grade (66.7%). In the third question, the answer 'I strongly agree' is higher in the first grade (53.3%) compared to the second grade (37.9%). In the second year, although the rate of 'I absolutely agree' (37.9%) is the highest, 'I strongly disagree' (32.8%) and 'I am indecisive' (27.6%) responses are also quite high. The answer I strongly agree on the fourth question is almost equal in first (40%) and second grade (39.7%). In the fifth question, the rate of undecided students is highest in both the first and second year (Table 1, Table 2). According to the statistics of the Chi-square test of first and second-grade answers, there is a significant difference only in the third (P=0.013) and ninth (P=0.002) questions (P<0.05). Other P values are given in Table 3.

Table 1. Percentage of frequency (f) that first-year students gave to the questions

First year of the medicine faculty students n:75	**5**	**4**	**3**	**2**	**1**
Three-dimensional software tools including anatomy education are the future of anatomy education.	46.7	1.3	13.3	28	10.7
Anatomy education can be supported by three-dimensional software tools.	66.7	-	2.7	24	6.6
Anatomy education can be supported with three-dimensional digital anatomy software, but cannot replace cadavers.	53.3	1.3	6.7	33.3	5.4
Learning by touching (tactile sense) is a must in anatomy education. Reality perception cannot be achieved with digital images.	40	-	22.7	28	9.3
Learning by touching (tactile sense) is essential in anatomy education. Reality perception can be provided with digital images.	10.7	5.3	34.7	21.3	28
Since the fixative solution has an odor in the cadaver, if a digital transparent cadaver is developed instead, the lesson can be learned just like in the cadaver.	14.7	9.3	28	22.7	25.3
Cadaver education I do not want it because of the irritant effect of solutions that protect the cadaver on respiration and skin.	4	18.7	25.3	9.3	42.7
The structures in the cadaver are complex, I have difficulty in perception, I think it will be more comfortable to learn and perceive anatomy with digital three-dimensional software.	10.7	5.3	45.3	22.7	16
Anatomy lesson should be learned from models and digital three-dimensional software.	18.7	2.7	33.3	17.3	28
I think that instead of learning anatomy lessons entirely from mock-up and three-dimensional digital software, mock-up and three-dimensional software should be supportive materials in anatomy education.	57.3	2.7	6.7	30.7	2.6

5= Totally agree (%), 4= Agree (%), 3= Undecided (%), 2= Totally Disagree (%). 1= Disagree (%)

Table 2. Percentage of frequency (f) that second year students gave to the questions

Second year of the medicine faculty students n:58	5	4	3	2	1
Three-dimensional software tools including anatomy education are the future of anatomy education.	62.1	-	10.3	27.6	-
Anatomy education can be supported by three-dimensional software tools.	79.3	-	3.4	17.1	-
Anatomy education can be supported with three-dimensional digital anatomy software, but cannot replace cadavers.	37.9	-	27.6	32.8	1.7
Learning by touching (tactile sense) is a must in anatomy education. Reality perception cannot be achieved with digital images.	39.7	1.7	31	17.2	10.4
Learning by touching (tactile sense) is essential in anatomy education. Reality perception can be provided with digital images.	25.9	8.6	27.6	24.1	13.8
Since the fixative solution has an odor in the cadaver, if a digital transparent cadaver is developed instead, the lesson can be learned just like in the cadaver.	27.6	8.6	24.1	25.9	13.8
Cadaver education I do not want it because of the irritant effect of solutions that protect the cadaver on respiration and skin.	10.3	24.1	24.1	13.8	27.6
The structures in the cadaver are complex, I have difficulty in perception, I think it will be more comfortable to learn and perceive anatomy with digital three-dimensional software.	19	6.9	24.1	27.6	22.4
Anatomy lesson should be learned from models and digital three-dimensional software.	22.4	-	24.1	44.8	8.7
I think that instead of learning anatomy lessons entirely from mock-up and three-dimensional digital software, mock-up and three-dimensional software should be supportive materials in anatomy education.	53.4	1.7	12.1	31	1.8

5= Totally agree (%), 4= Agree (%), 3= Undecided (%), 2= Totally Disagree (%). 1= Disagree (%)

Table 3. P values of first and second year students' answers to each question with chi-square test

Questions	P
Three-dimensional software tools including anatomy education are the future of anatomy education.	0.070
Anatomy education can be supported by three-dimensional software tools.	0.147
Anatomy education can be supported with three-dimensional digital anatomy software, but cannot replace cadavers.	0.013
Learning by touching (tactile sense) is a must in anatomy education. Reality perception cannot be achieved with digital images.	0.431
Learning by touching (tactile sense) is essential in anatomy education. Reality perception can be provided with digital images.	0.073
Since the fixative solution has an odor in the cadaver, if a digital transparent cadaver is developed instead, the lesson can be learned just like in the cadaver.	0.270
Cadaver education I do not want it because of the irritant effect of solutions that protect the cadaver on respiration and skin.	0.280
The structures in the cadaver are complex, I have difficulty in perception, I think it will be more comfortable to learn and perceive anatomy with digital three-dimensional software.	0.146
Anatomy lesson should be learned from models and digital three-dimensional software.	0.002
I think that instead of learning anatomy lessons entirely from mock-up and three-dimensional digital software, mock-up and three-dimensional software should be supportive materials in anatomy education.	0.843

P: Chi-square test of 1st and 2nd grade students 'P' values.

4. Discussion

The innovation and benefits of three-dimensional digital development to the field of anatomy are quite high (Nicholson et al., 2006). In addition to classical systematic anatomy, nowadays, ultrasonographic anatomy, laparoscopic anatomy and cross-sectional anatomy can be taught (Gunderman and Wilson, 2005; Ten Brinke et al., 2014). This is perhaps one of the most valuable and life-saving advantages that technology provides to the medical world (Tan et al., 2012). The issue discussed among anatomists is that the digital system can replace the cadaver (Theoret et al., 2007). There are many articles put forward on this subject. Some anatomists have argued that the digital world is very effective and useful, while others have stated that the digital world will very difficultly replace education with cadaver and reality perception (Theoret et al., 2007; Triepels et al., 2020; Saltarelli et al., 2014; Turhan, 2020).

In this article, the subject was presented to the students' opinions with important questions, using the comments and the literature on the effects of digital education on the field of anatomy. Both first and second year students agree to the idea that digital systems will be the future of anatomy education. Even if the number of those who agree with the view that 'anatomy education can be supported by digital 3D software but cannot replace the cadaver' is high in the first and second year, the number of those who do not agree is almost close to the value of those who agree. This may indicate that cadaveric education is still supported by students today, but the trend towards digital education will be more in the following years. 'Learning by touching (tactile sense) is essential in anatomy education. The view that perception of reality cannot be achieved with digital images' participation in the first and second classes is almost equal and more. However, the number of students who are undecided is also quite high. This situation may show that the importance of the concept of learning with touch perception is not adopted by some students.

In order to clarify the uncertainty created by some of the students, the fourth question was posed with a little change, and important values were obtained. 'Learning by touching (tactile sense) is essential in anatomy education. Reality perception can be achieved with digital images.' the rate of those who agree with the opinion is very low. This is desirable. However, the highest rate is undecided students in both the first and second year students. In addition, the total of those who do not participate and definitely do not participate is higher in both the first and second years. In this question, as in the fourth question, the fact that there are undecided people shows that the approach that digital education is adopted more than cadavers is not clear yet.

In the sixth and seventh questions, the desire for digital software due to the irritating effect of the fixative solution in the cadaver was examined. While a small portion of the students are affected by irritant solutions, in the first grade, despite irritant solutions, the desire for cadaveric education is higher than the second graders. Apart from that, although the classroom environment with digital cadavers instead of cadavers with irritant solution is mostly rejected by the first graders, the students from the second grade who want this situation are higher than the first grade. The reason why the second graders' approach to digital cadavers is a bit too much may suggest that some students experience exam stress in cadaveric education.

Since the questions asked form a thinking process in the students' minds, the questions were asked in a certain order. As a matter of fact, in the eighth question, the thinking area in the sixth and seventh questions was expanded and clarity was sought. At the end of this situation, it was concluded that the answers given to the eighth question both in the first grade and in the second grade are not certain that digital education will actually provide an easier understanding or replace it than cadaver. The rate of undecided students in the opinion that 'anatomy lessons should be learned from models and software' is high in both first and second year. This is an indication that the new generation is suspicious of the adoption of digital education instead of cadavers. It is observed that students are not willing to learn medicine from digital software instead of a real cadaver.

When it comes to the last question, a question was asked that would actually be the summary of the study. Almost all of the first and second year students strongly agree with the view that 'Anatomy lesson can be a supportive material in anatomy education instead of learning from model and 3D digital software'. It is understood from this that the new generation students have a very positive view of digital technological educational tools. However, the interest in cadavers and the effectiveness of cadavers in the lesson still maintain their place. Therefore, new technology can be useful and effective if integrated with cadaveric education (Pereira et al., 2007; Kharb and Samanta, 2016).

5. Conclusion

Cadaver is a very important tool in anatomy education. An effective and useful education model can be created only when the digital course tools offered by the developing technology are integrated with the cadaver.

Limitations

The study is limited with the answers of students who participated in the survey. The collected information is assumed to be true; the subjects involved in the study were assumed to give their answers honestly. The questionnaire was not a standardized instrument and could be validated in future studies.

Author Contributions

All task have been made by the single author.

Conflict of Interest

The author declared that there is no conflict of interest.

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OBSTETRIC BRACHIAL PLEXUS PALSY AND REHABILITATION PROCESS: CASE PRESENTATION

Sevda CANBAY DURMAZ[1]*, Selma SOLGUN[1], Davut ÖZBAĞ[2], Ali CANBAY[3]

[1]Inonu University, Institute of Health Science, Anatomy, 44280, Malatya, Turkey
[2]Inonu University, Medical Faculty, Department of Anatomy, 44280, Malatya, Turkey
[1]Elbistan Government Hospital, Orthopedics and Traumatology Department, 46300, Kahramanmaraş, Turkey

Abstract: Obstetric brachial plexus palsy (OBPP) is a clinical situation induced by the damage to the plexus brachialis and the branches originating from its location. While the diagnosis of OBPP can be made with a detailed examination performed right after the baby is born, it may limit the daily life activities of the child throughout his/her life when the situation noticed at a late stage. Treatment for OBPP can be grouped as conservative treatment, palliative surgery during conservative treatment, surgical therapy and late period treatment. In addition to all these, the physical therapy and rehabilitation processes are initiated for the patient from the moment the patient is diagnosed. The aim of the study is to reveal the importance of the early physiotherapy and rehabilitation program and the significance of the regular exercise program not neglected after a series of operations, in OBPP treatment. In this case presentation, we will present a 9-year treatment program, physiotherapy and rehabilitation period of a female patient with OBPP diagnosed at the time of birth, and her condition before and after this process.

Keywords: Brachial plexus, Obstetric brachial plexus palsy, Physiotherapy

***Corresponding author:** Inonu University, Institute of Health Science, Anatomy, 44280, Malatya, Turkey
E mail: sevdacnby@hotmail.com (S. CANBAY DURMAZ)
Sevda CANBAY DURMAZ https://orcid.org/0000-0002-7792-5306
Selma SOLGUN https://orcid.org/0000-0003-2827-2158
Davut ÖZBAĞ https://orcid.org/0000-0001-7721-9471
Ali CANBAY https://orcid.org/0000-0001-6695-4235

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1. Introduction

Plexus brachialis is the neural network that consists of all anterior branches of the 5th-8th cervical and 1st thoracal spinal nerves and the anterior branches of the 4th cervical and 2nd thoracal nerves, and which spreads to the upper extremity (Arıncı and Elhan, 2006). The spinal nerves join and from the truncuses which also join and form the fasciculus (Arifoğlu, 2017). Among those which form the plexus brachialis, C5 and C6 form truncus superior, C8 and T1 form truncus inferior and C7 forms truncus medius (Özbağ, 2019).

Brachial plexus can be damaged as a result of pulling the arms above the head level in breech babies during labor. Strained and damaged spinal nerves may cause severe function loss by affecting the small muscles of the hand (Frade, 2019).

OBPP was defined by William Smellie, an obstetrician, for the first time in 1764. Later, French neurologist Guillaume Duchenne and German neurology professor Wilhelm Erb described the condition clinically known as Erb-Duchenne Paralysis in which C5-C6 root injuries and sometimes C7 injuries may accompany (Mollberg, 2018; Tandon and Tandon 2005). Approximately 10 years later, French neurologist Augusta Klumpke revealed the Klumpke Paralysis on which C8-T1 inferior root injuries and ophthalmological findings reported by Friedrich Horner can be seen together with the Horner Syndrome which is related to the involvement of sympathetic fibers (Suziki et al., 1984; Klumpke, 1885).

Although there are various reasons related to OBPP mechanisms, the most common reasons are direct or indirect compression or traction. Conditions such as compression neuropathy induced by asphyxia, oligohydramnios and intrauterine malposition can be regarded among the congenital factors (Yücetürk, 1994). Being a large baby of a diabetic mother or a breech baby are also important risk factors for OBPP (Kaplan and Başar, 2014).

Generally, OBPP has different incidences, and the incidence rate is reported as 5.1/1000 in developed countries (Uysal et al., 2007). The incidence rate in Turkey is reported as 0.9/1000 live birth (Yücetürk, 1994). Factors that may cause OBPP have been better understood in time. Despite increased cesarean births and developed technology, the fact that the incidence frequency of OBPP does not change is due to the babies' birth weights and mothers' metabolic diseases (Zafeiriou and Psychogiou, 2008; Alfonso 2011).

While the diagnosis of OBPP can be made with a detailed examination performed right after the baby is born, it may limit the daily life activities of the child throughout their life if noticed late (Van Der Sluıjs et al., 2004).

Although it is not difficult to make the diagnosis in the first place, it is necessary to benefit from the clinical observation and diagnostic procedures such as Moro reflex, tonic neck reflex and the condition of the extremity in a resting position within two weeks after birth (Gilbert, 1995). If the injured arm in the upper extremity is in total flexion and if no muscle activity is observed, then total involvement should be considered. The sensory is affected, and there is no reaction to a painful stimulus. There is also faintness and heat loss in the whole arm due to vasomotor involvement (Al Quattan et al., 2019). In patients with proximal involvement, arm is in internal rotation, elbow in semiflexion, fingers in flexion and there are no active movements (Cosmos, 2019).

Treatment for OBPP can be grouped as conservative treatment, palliative surgery during conservative treatment, surgical therapy and late period treatment (Cosmos, 2019). In addition to all these, the physical therapy and rehabilitation process is started for the patient from the moment the patient is diagnosed. If there is an OBPP condition noticed at the time of birth, then physiotherapy practices should be started after one week, if possible, and within three weeks at the latest. Shoulder abduction and elevation should not exceed 90° in the exercises made within the first three weeks. The patient should be evaluated every 2-4 weeks and the treatment program should be adjusted accordingly (Ramos, 200).

The primary goal of the treatment in the early period is to prevent contractures as well as to preserve the joint range of motion at the highest possible level (Kuran et al., 2007).

In this case presentation, we will show approximately 9 year-long treatment modalities and physical therapy and rehabilitation period of the female patient diagnosed with OBPP in birth and whose treatment process started very early and progressed for a long time in a multidisciplinary way, her condition before and after this process.

The aim of the study is to present the importance of the early physiotherapy and rehabilitation program and the significance of the regular exercise program, which was not neglected after a series of operations, in OBPP treatment. Additionally, we are in the opinion that early diagnosis and multidisciplinary work increased the success of the treatment and may prevent possible new complications that may appear.

2. Case Presentation

We will present the physical therapy and rehabilitation process of a female patient who was born with normal birth on 08.06.2008 and weighed 4750 grams and was diagnosed with left OBPP. The patient is the third child of a diabetic mother and has a difficult birth history. Follow-up was recommended after the examination during birth, and the family consulted to a physician after noticing immobility in the left arm of the baby when she was two months old, and physiotherapy was primarily started. Although minimal benefit was seen in the patient who received physiotherapy for five days a week within approximately eight months. She was brought to our center when she was two years old and her treatment still continues.

2.1. Physical Findings

According to MRI result of the patient, it has been reported that the left glenoid rim appears to be dysplastic and has a smaller glenoid cavity than normal.

As a result of the electromyography (EMG) taken on 09.07.2009, while there was a decrease in the compound muscle action potential (CMAP) and compound sensory action potential (CSAP) amplitudes in left n. ulnaris and n. medianus, mild chronic denervation in m. supraspinatus and moderate chronic denervation in m. biceps brachii were observed. The patient was reported to have degenerations compatible with the brachial plexus inferior truncus involvement and C8-T1 root avulsion.

When the patient started the physical treatment and rehabilitation program in our center, the arm was in flexion in the direction of internal rotation, the elbow was in a slight flexion position and the fingers were in the flexion position. The extremity was pale, sweaty, and there was sensory loss. There was minimal ptosis in the left eyelid. There were no active movements except minimal shoulder abduction and flexion (Figure 1).

Figure 1. Before treatment.

The patient underwent a shoulder tendon transfer operation in a university hospital for her left OBPP diagnosis on 02.04.2012. In this operation, m. latissimus dorsi and m. teres major tendons were transferred as external rotators. Shoulder myotomy (m. subscapularis myotomy), shoulder tenotomy (m. pectoralis major tenotomy) procedures were also performed in this operation. The patient received a plaster splint application for eight weeks and then, intense physical treatment application was started. There was a minimal increase in the shoulder, arm, hand and wrist movements of the patient at this stage, but there was a mild loss of strength and limitations.

2.2. Treatment Program

Our goal in this treatment is to eliminate limitations, ensure the mobility of joints, increase the joint range of motion, strengthen weak muscles, increase hand skills and ensure postural smoothness.

In this regard, electrotherapy practices took part in our treatment plan. Acupuncture TENS electrical stimulation was performed for 15 minutes in 1000 ms intervals with 50 ms current duration and in the form of a square current to ensure sufficient muscle contraction, and for 15 minutes in case of pain from time to time after the operation. Mostly superficial warmers (hot pack) among heat agents are preferred.

In the rehabilitation program, in the first periods before the surgical operation, massage therapy was applied to reduce edema and increase blood circulation together with passive exercises, and later interferential and diadynamic currents were applied.

Readjustments were made on the treatment plan after the surgical operation and the family was informed in every stage. While the electrotherapy practices continued, friction massage for the post-op scars and superficial massage applications also continued to increase the circulation. The exercise program included the Proprioceptive Neuromuscular Facilitation (PNF) (hold relax and repeated contractions) techniques, Codman exercises, mobilization techniques (scapular and glenohumeral), isometric exercises (for m. serratus anterior). Later on, when we reached the painless period, resistance exercises and progressive resistance exercises were included in the treatment program. Roller systems, TheraBand, springs, finger ladders and arm wheels were used. Kinesiological taping practices were applied when needed. The Southern California Sensory Integration Tests and sensory training bags with different structures were used for sensory training. Occupational therapies were also included. The joint range of motion of the patient increased after the study (Table 1).

Table 1. The patient's joint range of motion measurements

	After surgical treatment 2020	Before surgical treatment 2012	First pre-treatment evaluation 2010
Left shoulder			
Flexion:	70°	34°	15°
Extension:	5°	weak	0
Abduction	118°	55°	weak
Adduction:	20°	5°	0
Internal rotation	77°	weak	0
External rotation	70°	weak	0
Left elbow			
Flexion:	90°	24°	weak
Extension:	90°	27°	0
Pronation Supination	weak	0	0
Left wrist			
Flexion:	14°	3°	weak
Extension:	16°	7°	weak
Radial deviation:	weak	weak	0
Ulnar deviation:	weak	weak	0

The patient has started to be independent in daily life activities and is clinically better than before treatment (Figure 2). Considering the general evaluations, there is no serious improvement in fine motor movements in hands and fingers; thus, surgical and physical therapy applications will continue. Tendon transfer operations for the hand and wrist are in the planning stage.

2.3. Ethical Consideration

The study was conducted after obtaining the ethical committee approval of the İnönü University Health Sciences Non-Interventional Clinical Studies Ethics Committee with the decision number 2020/928. The family was informed about the content of the study and the Informed Consent Form was signed.

Figure 2. After treatment.

3. Results and Discussion

Babies with high birth weight and difficult birth history are at the risk of OBPP. While making the diagnosis in the early period increases treatment options and success, it is quite important to know the anatomical localization of the involvement (Benson et al., 1996).

While recovery is observed in some patients in the early postpartum period, some cases may have life-long permanent disabilities (Nehme and Kany, 2002).

Total plexus involvement, comorbid Horner syndrome, and severe sensory loss indicate a prognosis that will not go well (Leblebicioğlu, 2005). Our case also had a difficult birth history with high birth weight and her prognosis was bad due to total involvement.

Related studies have reported that the right-side extremity is affected more due to traction between the shoulder and neck since births usually take place from the left side, and the right shoulder enters the pelvis first and trips (McDaid et al., 2002). Unlikely, our case had a left-sided involvement.

Physical therapy and rehabilitation process is quite important in OBPP cases. The success rate is higher in cases who started the treatment early. A relevant study found that 53.12% of the patients had a full recovery after treatment and 25% had partial recovery after treatment. In this regard, 78.12% of the patients had partial or full recovery due to physical treatment and rehabilitation (Demir et al., 1999).

With the early onset of physical therapy and rehabilitation period and later directing the patient to surgery, the treatment of our case continued with a correct course. Physical therapy and rehabilitation program was applied to the patient before and after the surgery and was not interrupted. In line with our treatment goal, the joint range of motion was preserved and increased, the muscle strength was increased, flexibility was provided in soft tissues, in case of edema, edema was decreased, and the functionality of the patient was increased. At the same time, inaccurate use of extremity and deformity development were prevented.

It should be noted that especially multidisciplinary work is very useful to obtain such results in patients. We are in the opinion that an accurate physical therapy and rehabilitation program before and after the operation will accelerate the independence of not only the patients who have OBPP but all orthopedic and neurological cases in daily life activities.

Author Contributions

All authors have obtained the necessary data by evaluating our case in detail.

Conflict of Interest

The authors declared that there is no conflict of interest.

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NON-EXTRACTION ORTHODONTIC TREATMENT WITH DAMON SYSTEM: TWO CASE REPORTS

Muhammed Hilmi BÜYÜKÇAVUŞ[1]*

[1]*Department of Orthodontics, Faculty of Dentistry, Suleyman Demirel University, 32260, Isparta, Turkey*

Abstract: Self-ligating braces offer shorter orthodontic treatment, more comfortable follow-up and less chair time. Passive braces are more preferred in self-ligating systems as they cause less friction. Damon system is also one of the most popular passive self-ligating braces. In addition to protrusion of incisors, it is one of the most important advantages of this bracket system that it provides expansion in dental arches. The purpose of these case reports is to present the treatments of two patients treated with Damon System. It can be concluded as; thanks to the Damon braces system, moderate and severe crowding cases were successfully treated in appropriate cases. Treatment times were also shorter compared to average orthodontic treatments. Instead of stripping and tooth extraction, which are among the methods of gaining space in orthodontics, success can be achieved in suitable cases.

Keywords: Damon System, Orthodontic treatment, Expansion, Orthodontic brackets

***Corresponding author:** Department of Orthodontics, Faculty of Dentistry, Suleyman Demirel University, 32260, Isparta, Turkey
E mail: mhbuyukcvs@gmail.com (M.H. BÜYÜKÇAVUŞ)
Muhammed Hilmi BÜYÜKÇAVUŞ https://orcid.org/0000-0003-2184-1549

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1. Introduction

Today, with the advancing technology in orthodontic practice, many brackets, arch wires and material options for connecting them have emerged. Steel and elastomeric ligature wires are often used to connect the arc wire to the bracket. With the introduction of self-ligating brackets in orthodontic applications, it has brought many advantages in terms of ligaturing (Harradine, 2003).

The advantages of self-ligating brackets include the ability to securely connect the arch wire, less friction, fast and easy insertion and removal of the arch wire, and shorten the time spent at the patient (Damon, 1998; Eberting et al., 2001; Harradine, 2003). Manufacturers claim faster treatment, less pain, and fewer appointments (Miles, 2009). Self-ligating brackets are divided into three categories as active, passive or interactive according to the basic differences in their designs. The purpose of the active system is to insert the arc wire into the bracket slot for effective rotation and torque control. In the active system, the cover of the bracket narrows the lumen of the housing, causing more friction to occur. Since the actively placed angular arc wire will touch the cover, the friction will increase further (Karataş et al., 2013).

In passive brackets, after the slide is closed, the bracket becomes a tube and rotation and crowding is corrected with large diameter flexible wires filling the slot. Damon braces system is the most popular self-ligating bracket with passive system (Ormco Corp., 1332 South Lone Hill, Ave., Glendora, CA, USA). It is a passive self-ligating system introduced by Dwight Damon in 1996 (Damon, 2004). A distinctive feature of this system is that these low profile brackets do not require auxiliary elastics and steel ligatures like traditional brackets or clips holding the arch wire in the bracket as in active brackets. Damon talked about a new expansion method provided by the brackets he designed. This method is called "Damon bracket system"; the straight wire technique is based on the principle of using super elastic NiTi wires together with passive self-ligating braces. Damon argues that the light forces created by the arch wires cannot overcome the lip muscles and cause posterior expansions. Studies have emphasized that the orbicularis oris and mentalis muscles create a 'lip bumper' effect, preventing the proclination of the anterior teeth and widening the posterior segment (Birnie, 2008).

The purpose of these case reports is to present the treatments of two patients treated with Damon System.

2. Case Reports

2.1. Case 1

A 12-year-old girl presented to our clinic with complaints of dental crowding and the appearance of high canines. In the detailed clinical examination of the patient, it was observed that the discrepancy of maxillary arch (-7.5 mm) and the patient was found to have Class II subdivision malocclusion with 2 mm overjet and 1.5 mm overbite (Figure 1). In cephalometric measurements, it was found that skeletally Class I (SNA: 79.6°, SNB: 77.7°, ANB: 1.9°) malocclusion was found, vertical direction dimensions (SN / GoGn: 32.8°) were within normal limits and the inclinations of the upper incisors (U1 / PP: 107.8°) was found to be in a retrusive position relative to

the cranial base.

Figure 1. Initial photographs of Case 1.

Orthodontic treatment without extraction was planned for our patient with Damon braces system. Braces were placed in the first session and treatment was started with 0.014 NiTi (Figure 2).

Figure 2. Damon braces system (Damon Q2, ORMCO, Glendora, Calif)

0.016, 0.014x0.025, 0.016x0.025, 0.018x0.025 NiTi wires and finally 0.019x0.025 SS were used. Session breaks are planned as 8 weeks. The total treatment time is 11 months.When the cephalometric parameters after treatment are examined, it is seen that the skeletal Class I relationship at the beginning is preserved (SNA: 80.4˚, SNB: 78.7˚, ANB: 1.7˚). It has been determined that the teeth approach the ideal inclination with the protrusion in the lower and upper incisors (U1 / PP: 110.2˚; IMPA: 94˚). A minimal increase was observed in vertical direction parameters (SN / GoGn: 33.1˚).

At the end of the treatment, ideal overjet and overbite were provided. Class I canine and molar relationships were obtained in the patient (Figure 3 and 4).

Figure 3. Final photographs of Case 1.

Figure 4. Initial and final radiographs of Case 1.

2.2. Case 2

A 21-year-old female patient applied to our clinic for orthodontic treatment due to the complaint of crowding. No systemic or dental problems were encountered in the patient that would interfere with orthodontic treatment. In the intraoral examination, the patient was found to have Class I malocclusion with 1.5 mm overjet and 1.5 mm overbite (Figure 5).

In cephalometric measurements, it was found that skeletally Class I (SNA: 80.3˚, SNB: 78.2˚, ANB: 2.1˚) relationship was found, vertical direction dimensions (SN / GoGn: 33.4˚) were within normal limits and the

inclinations of the upper incisors (U1 / PP: 109.6˚) was found to be in a retrusive position relative to the cranial base.

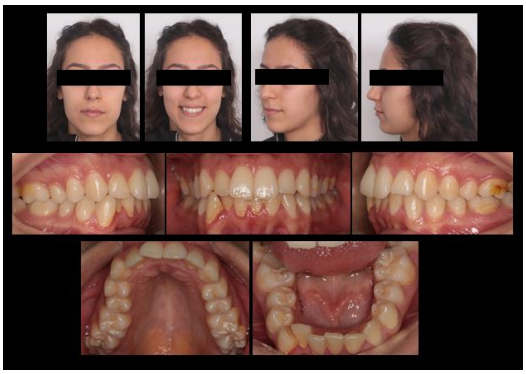

Figure 5. Initial photographs of Case 2.

Fixed orthodontic treatment was planned for our patient to ensure ideal overbite, overjet and Class I molar and canine relationship. Damon braces system (Damon Q2, ORMCO, Glendora, Calif) was preferred to provide dental expansion as well as protrusion of incisors. Brackets were attached in the first session, and treatment was started with 0.014 NiTi archwires. 0.016, 0.014x0.025, 0.016x0.025, 0.018x0.025 NiTi archwires and finally 19x25 SS wires were used respectively (Figure 6). Session breaks are planned as 8 weeks. After treatment, cephalometric values increased, SNA and SNB angles increased and ANB angles decreased compared to baseline (SNA: 80.9˚, SNB: 79.2˚, ANB: 1.7˚). As a result, it is seen that skeletal Class 1 relationship is preserved. Protrusion has also occurred in the upper and lower incisors (U1 / PP: 111.4˚; IMPA: 95˚). A minimal increase was observed in vertical direction parameters (SN / GoGn: 34 ˚).

Figure 6. Damon System brackets (intraoral view).

End of the treatment, Class I molar and canine relationship was established. As a result, in the case with moderate perplexity in the anterior mandible, a good occlusion with normal overbite and overjet was achieved with dental Class I relationships after 10.5 months of treatment (Figure 7 and 8).

2.3. Ethical Consideration

All procedures followed during the publication of the case report were in accordance with the ethical standards (institutional and national) of the committee responsible for human experiments and the 1964 Helsinki Declaration and its later versions. Informed consent was obtained from the parents for the Case 1, and from the patient for the Case 2. In addition, a letter was received stating that the treatment materials can be used in scientific publications.

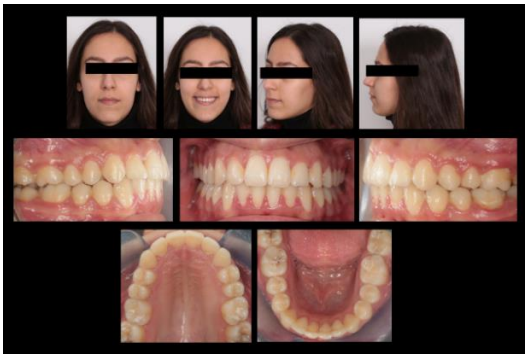

Figure 7. Final photographs of Case 2.

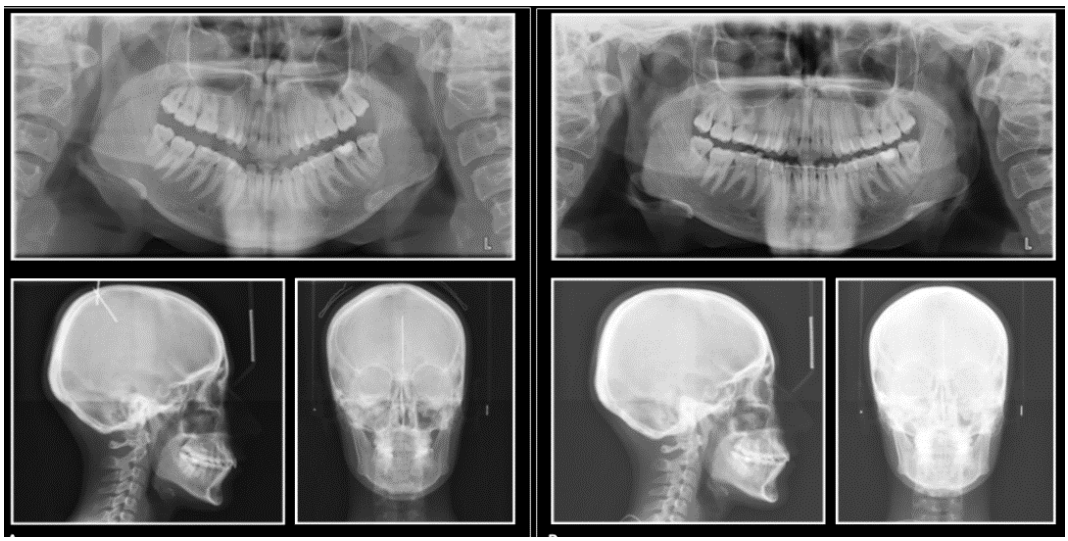

Figure 8. Initial and final radiographs of Case 2.

3. Results and Discussion

Damon thought that the best way to achieve biologically appropriate forces was through a self-locking bracket system. In the early years of orthodontic applications, physicians thought that passing an arch wire through a tube was more advantageous than connecting it to a bracket. One of the first tube systems, "twin-wire cap" and "channel" (Johnson, 1976) appliances shed light on the self-ligating systems used today. Difficulties in placing the covers, lack of memory wires, preset bracket slots and modern mechanics caused these appliances not to be used over time.

The development of self-ligating brackets has accelerated after 1995 and active and passive brackets have been introduced to the market. Damon braces system is the most popular self-ligating bracket with passive system. Damon system is based on the principle of applying only enough force to initiate tooth movement. So the threshold force is sufficient. The force at the threshold value is the force that prevents the occlusion of the blood vessels in the periodontal membrane and allows the transport of cells and necessary biochemical transmissions in the region of bone resorption and apposition. Thus, tooth movements will occur faster (Karataş et al., 2013).

Damon system creates natural strength systems compatible with normal growth and development in every phase of treatment. Although Damon appliances are defined as a bracket system, it is actually a tube system and consists of tubes with wings. It has been reported that when the Damon passive SL bracket is closed, it behaves like a tube that provides rotation control (Damon, 2004).

Damon believes that posterior expansion causes the tongue to be positioned above and further back, allowing a new balance with the cheeks and lips. This expansion causes an increase in arc length. Damon thinks that during the development of this new balance, the teeth will expand physically. While the mechanics of the Damon bracket system enable expansion, this new balance also provides the stability of the expansive arch. In our cases, this expansive effect of the Damon system was used rather than the protrusion of the incisors to gain a place in the treatment of dental crowding. The fact that the U1-PP and IMPA angles did not increase much in the cephalometric analysis results and the increase in interpremolar and intermolar distances in model analysis also support these results.

In the Damon system, the treatment time is shorter than the traditional treatment method, and fewer appointments are required during the treatment. Harradine et al. reported in the results of their study that the treatment period was shortened by 4 months and the average number of appointments decreased by 4 months in the group treated with the Damon system compared to the traditional technique (Harradine et al., 2006). Eberting et al, in their study comparing traditional fastening systems with Damon SL, examined the duration of treatment and the total number of appointments of patients treated with 108 Damon SL and 107 traditional brackets (Eberting et al., 2001). As a result of the study, they stated that the duration of treatment decreased from 31 to 25 months in patients using Damon SL, and the total number of appointments decreased from 28 to 21. Thorstenson et al., (2001) reported that the treatment with Damon System lasted 7.2 months shorter in 2001 in a clinical study in which they compared traditional systems with the Damon System. In our cases, the orthodontic treatments of the patients were also completed in less than 1 year.

4. Conclusion

Thanks to the Damon braces system, moderate and severe crowding cases were successfully treated in appropriate cases. Treatment times were also shorter compared to average orthodontic treatments. Instead of stripping and tooth extraction, which are among the methods of gaining space in orthodontics, success can be achieved in suitable cases.

Author Contributions

All tasks have been done by the single author.

Conflict of Interest

The author declared that there is no conflict of interest.

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KIRIM-KONGO KANAMALI ATEŞİ LABORATUVAR TANI YÖNTEMLERİNE GENEL BAKIŞ

Laleh BAHRIKAREHMI[1]*, Serbülent YİĞİT[2]

[1]*Ondokuz Mayıs Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji ve Genetik Anabilim Dalı, 55139, Samsun, Türkiye*

[2]*Ondokuz Mayıs Üniversitesi, Veteriner Fakültesi, Veterinerlik Genetiği Anabilim Dalı, 55139, Samsun, Türkiye*

Özet: Kırım-Kongo kanamalı ateşi (KKKA) Bunyaviridae familyasından kene kaynaklı bir virüsün neden olduğu potansiyel olarak ölümcül bir zoonoz hastalıktır. KKKA virüsü, virüs taşıyan kenelerinin ısırmasıyla, KKKA hastaların ve enfekte hayvanların kanı veya dokuları ile temas yoluyla insanlara bulaşır. İnsan enfeksiyonları spesifik olmayan ateşli semptomlarla başlar, ancak vaka fatalitesi olan ciddi bir hemorajik sendroma ilerlemesi % 2-50 oranındadır. Hızlı ve kesin tanı yaklaşımı hastaların kontrolü, tedavisi ve tespiti için kritik olduğundan epidemiyolojik çalışmalar için yararlıdır. KKKA'nın laboratuvar tanısı üç yaklaşıma dayanmaktadır: Virüs İzolasyonu, serolojik testler (IFA, RPHA (Ters pasif hemaglütinasyon testi) ve ELISA) ve antijen tespit testleri (ELISA, PCR). Dünyadaki ve Türkiye'deki çalışmaları, tüm veri tabanları üzerinden tarayarak KKKA'de biyogüvenliği, örnekleme ve tanı yönlerinin çeşitlerini incelemeyi amaçladık.

Anahtar kelimeler: Kırım-kongo kanamalı ateşi, Laboratuvar tanı, Teşhis

An Overview of Crimean-Congo Hemorrhagic Fever Laboratory Diagnostic Methods

Abstract: Crimean-Congo hemorrhagic fever (CCHF) is a potentially deadly zoonotic disease caused by a tick-borne virus from the Bunyaviridae family. The CCHF virus is transmitted to humans by the bite of its virus-bearing ticks, through contact with the blood or tissues of CCHF patients and infected animals. Human infections begin with non-specific febrile symptoms, but their progression to a severe hemorrhagic syndrome with case fatality is 2-50%. The rapid and precise diagnostic approach is useful for epidemiological studies, as it is critical for the control, treatment and detection of patients. Laboratory diagnosis of CCHF is based on three approaches: Virus Isolation, serological tests (IIF, RPHA and ELISA) and antigen detection tests (ELISA, RT-PCR, Real Time RT-PCR and Microarray). Studies in the world and Turkey Biosecurity in CCHF by scanning through all databases, we aimed to examine the types of sampling and diagnostic aspects.

Keywords: Crimean-congo hemorrhagic fever, Laboratory diagnosis, Diagnosis

***Sorumlu yazar (Corresponding author):** Ondokuz Mayıs Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji ve Genetik Anabilim Dalı, 55139, Samsun, Türkiye
E mail: laleh.bahri.lb@gmail.com (L. BAHRIKAREHMI)
Laleh BAHRIKAREHMI https://orcid.org/0000-0003-4105-5991
Serbülent YİĞİT https://orcid.org/0000-0002-1019-3964

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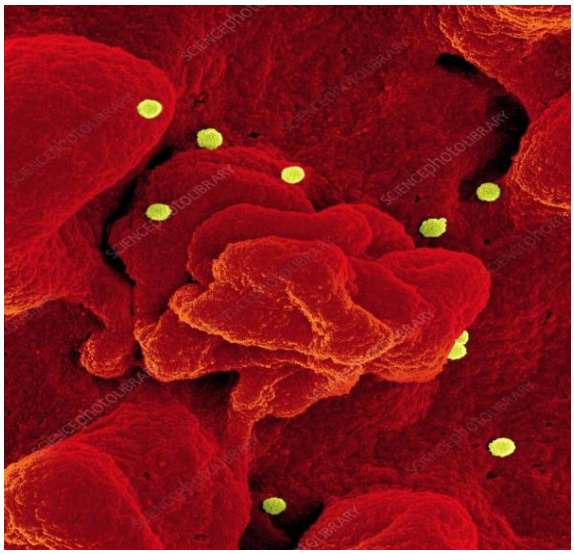
1. Giriş

Kırım Kongo Kanamalı Ateşi (KKKA) (Şekil 1) birçok organ ve sistemleri etkileyen, kanamalar ve karaciğer fonksiyon bozuklukları ile karakterize, mortalite oranı yüksek olan (%5-30) zoonotik enfeksiyondur (Bodur, 2004).

Türkiye'de yapılan çalışmalarda takip edilen olgulardaki mortalite oranı % 2-12 arasındadır. Enfekte olanlarda hastalık gelişme olasılığı yaklaşık %22'dir. Aslında enfekte olan her beş kişiden birinde hastalık gelişmektedir (WHO, 2008). KKKA'ya neden olan virüs insanlara genellikle vektör olan Hyalomma (Şekil 2) ve Amblyomma cinsi kenelerin ısırması ile bulaşır. Virüs, birçok evcil ve yabanî hayvanı enfekte etmekte ve hastalık hafif seyretmektedir. Birçok kuş türü, virüse karsı dirençli iken, virüsün yayılmasında önemli rol oynarlar. Hayvanlardaki hastalık enfekte kenelerin ısırması ile başlamaktadır. Daha çok hayvancılıkla uğraşanlarda, mezbaha çalışanlarında, kırsal kesimde yaşayanlarda enfekte hayvanların kan ve dokuları ile temas sonucu ya da süt içimi ile bulaş olabilmektedir. Nazokomiyal epidemilerin, ağır kliniği olan hastalara ait kan veya kanlı sekresyonlar ile direkt temasla ve hava yolu ile de oluşabileceği belirtilmektedir. Hastalık Afrika, Güney Doğu Avrupa ve Asya'daki birçok ülkede endemik olup Türkiye'den ise ilk defa 2002 yılında Tokat'tan bildirimi yapılmış ve 2013 yılı itibariyle yaklaşık 8000 olgu ve 400 ölüm saptanmıştır.

Türkiye'nin pek çok ilinden bildirimler var olmakla birlikte olguların çoğunun (%95) Mart-Ekim ayları arasında, Karadeniz Bölgesinin iç kısımları, Orta ve Doğu Anadolu'nun kuzey ve orta bölgelerinde görüldüğü tespit edilmiştir (SB, 2004). Bu hastalığın semptomlarının başlaması ani olarak ve yaklaşık 1 ila 7 gün arasında sürer. Hastalığın ilk belirtileri gribe benzemektedir. Hastada şiddetli baş ağrısı, ateş, titreme, eklem ağrısı, kas ağrıları, baş dönmesi, boyun ağrısı ve sertliği, göz ağrısı ve ışık korkusu gibi semptomlar gözlenir. Bulantı ve

kusma, bazen ishal, karın ağrısı ve iştah kaybı ile birlikte, hastalığın erken evrelerinde, boğaz ağrısı ortaya çıkabilir. Ateş sıklıkla 3 ila 16 gün sürer. Yumuşak ve sert damakta ve boğazda leke şeklinde lezyonları, yüz, boyun, göğüste hafif kanamalar ve kızarıklık gözlenmesi yaygındır. İnek, koyun ve keçi gibi rumninantlar virüs ile enfekte olduktan sonra bir hafta boyunca kanlarında virüs saptanabilir ve bu süreçte hiçbir belirti göstermeyebilirler (Ergonul, 2008).



Şekil 1. Kırım-Kongo kanamalı ateşi (KKKA) virüsü. Bir hastadan kültürlenmiş epitel hücrelerinin yüzeyinden tomurcuklanan KKKA viral partiküllerinin (sarı) renkli taramalı elektron mikrografı (SEM) (https://www.sciencephoto.com/media/945621/view/crimean-congo-hemorrhagic-fever-virus-sem).

Şekil 2. Hyalomma (https://www.woidmo.org/alerts/crimean-congo-hemorrhagic-fever-russia/).

Hastalığın hızlı teşhisinin önemi nedeniyle bu çalışmada, KKKA hastalığının laboratuvar tanı yöntemlerini inceleyeceğiz. İnsanlarda, laboratuvar testleri genellikle kan, plazma, serum, vücut sıvıları veya biyopsiler üzerinde yapılır. Şüpheli KKKA numunelerinin transferi, yüksek riskli enfeksiyon örneklerinin taşınması ile ilgili uygun spesifik protokoller ile yapılmalıdır. Numune ile birlikte bir klinik bilgi formu ve hasta epidemiyolojik bilgileri gönderilmelidir. KKKA virüsü izolasyonu amacıyla hücre kültürü yapmak için 4'üncü seviye biyolojik güvenlik özellikleri gösteren uygun laboratuvar şartları gerekir. 4. seviyede uygun biyolojik güvenliğe sahip olmayan laboratuvarlarda çalışma yapılacak ise tanıdan önce örneğin geçerli yöntemlerle inaktif hale getirilmesi gerekir. Numuneyi etkisiz hale getirmek için hemorajik ateşlerin mevcut kılavuzları arasında örnek manipülasyonundan önce ısı kullanımı, gama çözülme ve triton X100'le muamele yer alır (Ergonul ve Whitehouse, 2007). KKKA virüsü diğer Bunyaviride ailesi gibi yağlı çözeltilere ve iyonik olmayan deterjanlara karşı hassastır. Serum örneği 60 °C'de 60 saniye ısıtılarak inaktive edilmelidir. Doku örnekleri, virüsü teşhis etmek için kullanılabilecek %10 formalin veya diğer doku stabilizatörlerine yerleştirilmelidir. Ayrıca 4. seviye biyolojik güvenliği olan (BSL4) laboratuvarında kullanılan tüm teşhis reaktifleri kullanımdan önce ultraviolet ile steril edilmelidir (Ergonul ve Whitehouse, 2007; Morshed ve ark., 2007).

2. Örnekleme

İnsanda KKKA hastalığının şüpheli, olası ve kesin olarak üç sınıflandırılmış tanımı bulunmaktadır;

a) Şüpheli tanım: hastalığın ani başlaması ateş, kas ağrısı, kene ısırması, hemorajik belirtiler (peteşi döküntüsü, oral mukoza ve burun kanaması, kusma, hematüri, epidemiyolojik semptomlardan biri (kenelerin ısırması, taze kan veya enfekte olmuş hayvanların diğer dokularıyla doğrudan temas, kesin veya şüpheli KKKA hastası ile doğrudan temas, çiftlik hayvanlarıyla temas etme olasılığı bulunan kırsal bir bölgede kalmak veya seyahat etmek).
b) Olası tanım: lökopeni veya lökositoz ile ilişkili olabilecek şüpheli trombositopeni vakaları.
c) Kesin tanım: olası vakalar ve pozitif serolojik testler veya virüsü şüpheli KKKA olan birinden izole etmek (Majidzadeh ve ark., 2011).

3. Virüs İzolasyonu

Virüs izolasyonu, viremi yüksek olduğunda hastalığın ilk 5 günü boyunca başarılıdır. SW-13, Vero, CER, LLC-MK2 veya BHK-21 gibi çeşitli hücre kültürleri kullanılabilir. Sitopatik etki (CPE) genellikle görülmez; bu nedenle virüs izolasyonu, kantitatif RT-PCR'de titrelerin arttırılması veya kontroller olarak bilinen antikor pozitif serum örnekleri kullanılarak dolaylı immünofloresan analizi (IFA) ile doğrulanabilir. Hücre kültüründe KKKA virüsü izolasyonu hastalığa karşı bağışıklık tepkisini bir antijenik madde ile tetikledikden 1-6 gün sonra yapılır. Virüs ayrıca yeni doğan farelerin beyin dokusu içine inokülasyonu ve ardından izolasyonu ile elde edilebilir. Bu prosedür hücre kültüründen daha duyarlıdır ve 5-10 gün sürer (Shepherd ve ark., 1986; Papa, 2019) . Plak azaltma nötralizasyon testleri, KKKA nötralizasyon

antikorlarının seviyesini tahmin etmek için uygulanabilir. 4. seviye biyolojik güvenliği olmayan laboratuvarlarda, virüse karşı serum nötralizasyon aktivitesi psödotipli bir virüs kullanılarak ölçülebilir (Berber ve ark., 2013). Anti-KKKA virüsü ile problanan enfekte olmuş hücrelerin enzim katalizli renk gelişimine dayanan sahte plak indirgeme nötralizasyon testi antikorlar da uygulanabilir (Ackermann-Gaumann ve ark., 2019).

4. Moleküler Yöntemler

Enfeksiyondan sonraki ilk günlerde, laboratuvar teşhisi esas olarak klinik örneklerde viral RNA'nın ters transkriptaz polimeraz zincir reaksiyonu (RT-PCR) ile saptanmasıyla sağlanır. PCR için kullanılabilecek örnek tipleri arasında tam kan, plazma, serum, beyin-omurilik sıvısı, idrar, tükürük, biyopsi ve nekropsi örnekleri bulunur. Viremi, hastalığın onsekizinci gününe kadar tespit edilebilir; bununla birlikte, PCR testi antikorların üretiminden önce en başarılıdır (semptomların başlamasından sonraki ilk 5 gün boyunca). İlk RT- PCR protokolü 1996 yılında tarif edilmiştir, KKKAV (kırım-kongo kanamalı ateşi virüsü) RNA tespiti için yaygın olarak kullanılmıştır ve halen kullanılmaktadır. Ayrıca, ticari RT-PCR platformlarının sayısı artmaktadır. RT-PCR protokolleri, lokal KKKAV suşlarını veya virüsün birden fazla genetik soyunu tespit etmek için tasarlanmıştır. Farklı virüs suşları göçmen kuşlar veya hayvan ticareti yoluyla taşınabileceğinden geniş spektrumlu bir PCR testi kullanılması tercih edilir. viral yükün hastalığın şiddeti ve sonucu ile önemli ölçüde ilişkili olduğu düşünüldüğünde, nicel gerçek zamanlı RT-PCR, hastalığın seyri için yararlı bir gösterge sağlayabilir. Uzun süreli viremi raporları olmasına rağmen, virüs genellikle hastalığın ilk haftasında tespit edilebilir. Pozitif sonucu olan hastalarda, ölümcül sonuçları olan vakaların aksine virüs temizlenir (Hasanoğlu ve ark., 2018). Virüsün tüm genom dizisini elde etmek amacıyla KKKA kültür süpernatanları ve KKKA pozitif keneler üzerinde de novo next generation sequencing (NGS) kullanımı hakkında raporlar vardır. WGS'lerin analizi, virüs gelişimi, rekombinasyon ve yeniden sıralama olayları üzerine yapılan çalışmaların temelini oluşturur ve etkili teşhis araçlarının tasarlanmasını sağlar. De novo NGS'nin doğrudan klinik örneklerde tanısal kullanımı, yüksek viremili vakalarda başarılıdır. Amplifikasyon hedeflendiğinde, virüs düşük konsantrasyonlarda bile tespit edilebilir (Dinçer ve ark., 2017).

5. Serolojik Yöntemler

Serolojik testler antijenik varyasyona duyarlıdır, ancak genellikle genetik varyasyondan daha az etkilenir. Çoğu test, insanlarda erken, güçlü ve uzun süreli bir bağışıklık tepkisine neden olan KKKAV N proteinini hedefler. Aktif KKKAV enfeksiyonu, semptom başlangıcından 4-9 gün sonra, enfeksiyonun akut fazını takiben IgM veya IgG titresinde belirgin bir artış ile tespit edilebilir; bununla birlikte, ciddi ve ölümcül vakalar sıklıkla saptanabilir bir antikor yanıtı oluşturmaz. Anti-KKKA IgG'nin saptanması, mevcut veya çözülmüş enfeksiyonu gösterebilir (enfeksiyondan yıllar sonra) ve sürveyans epidemiyolojik çalışmalarında yararlı olabilir. KKKA için ELISA testi IFA veya nötralizasyon testinden daha iyidir. Virüs nötralizasyon analizleri teşhis için daha az yararlıdır, çünkü KKKA, nispeten düşük seviyelerde nötralize edici antikorlar ortaya çıkarır, ancak epidemiyoloji ve aşı araştırmaları için yararlı olabilir. KKKAV nötralizasyonu genellikle plak indirgeme nötralizasyonu kullanılarak gerçekleştirilir ve 5-7 gün için de sonuçlar elde edilir (Mazzola ve Kelly-Cirino, 2019).

6. Moleküler Epidemiyoloji

KKKA virüsü, GenBank'taki izolatlar arasında S, M ve L segmentleri için %20, %31 ve %22 sapma ile yüksek derecede dizi çeşitliliği gösterir. Viral S segmentinin bir analizine dayanarak, virüsün Afrika'dan Avrupa'ya, Orta Doğu'ya ve ardından Asya'ya uzun bir coğrafi yayılma geçmişi olduğunu gösteren altı veya yedi viral soy tanımlanmıştır. Genom çeşitliliği, kene içinde memeli konakçıdan daha fazla olabilir. KKKAV'nin geniş dizi çeşitliliği muhtemelen suşların dolaşım ve yeni coğrafi bölgelere adaptasyonu ile arttırılan genetik yeniden düzenlenmelerden kaynaklanmaktadır (Mazzola ve Kelly-Cirino, 2019).

7. Tartışma

KKKA'nın laboratuvar tanısı üç yaklaşıma dayanmaktadır: virüs izolasyonu, serolojik testler (IIF, RPHA ve ELISA) ve antijen tespit testleri (ELISA, RT-PCR, Gerçek Zamanlı RT-PCR ve Mikroarray). Bu hastalığın erken teşhisi, hasta tedavisi, potansiyel nozokomiyal enfeksiyonların önlenmesi için kritik öneme sahiptir. Ayrıca, tekrar olan bir enfeksiyonu ve tekrar olmayan bir enfeksiyonu ayırt etme yeteneği prognostik değere sahip olabilir. ELISA ve IFA testleri gibi yaygın olarak kullanılan serolojik testler ile hastaların serumunda virüse karşı oluşan IgM veya IgG tipi antikorlar belirlenerek tanı konulabilmektedir. IgM ve IgG antikorları hastalığın 6-7. gününden itibaren serumda belirlenebilmektedir. Özgül IgM antikorlar 4 ay kadar, IgG antikorlar ise en az 5 yıl hasta serumlarında saptanabilmektedir. Ölümcül seyreden olgularda ve genellikle hastalığın ilk birkaç gününde saptanabilecek seviyelerde antikor yanıtı gelişemeyebildiğinden bu dönemde tanı koymak için kan veya diğer doku örneklerinden RT-PCR virüs izolasyonu ve antijen tespiti ile tanı konulabilmektedir. Son yıllarda RT-PCR ve real-time RT-PCR gibi spesifikliği ve duyarlılığı yüksek moleküler tanı yöntemleri ile virüs RNA'sı daha hızlı tespit edilebilmektedir. KKKA hastalarında viremi 10-12 gün kadar sürdüğünden, bu dönemde hasta serumunda PCR ile viral RNA gösterilebilmektedir. Viral kültür en etkilidir ve yüksek viremi düzeylerinin en yaygın olduğu semptomların başlamasından sonra erken uygulandığında pozitifliğe daha hızlı ulaşma olasılığı yüksektir. KKKA teşhisi için

viral kültür kullanmanın bir avantajı, çok çeşitli KKKAV suşlarını tespit etme kabiliyetidir (Raabe, 2020). Moleküler tanı testleri: Hızlı sonuç alınması hem hastanın tedavisinin planlanması hem de hasta izolasyonu açısından avantaj sağlar. Antijen testleri: Rekombinant antijen temelli antikor ve antijen tespit testleri geliştirilmiştir. Bu testlerin avantajı biyogüvenlik düzeyi-4 olmayan laboratuvarlarda da kullanılabilmesidir (Eren Gök, 2016).

Katkı Oranı Beyanı

Tüm yazarlar aynı oranda katkıya sahip olup, tüm yazarlar makaleyi incelemiş ve onaylamıştır.

Çatışma Beyanı

Yazarlar bu çalışmada hiçbir çıkar ilişkisi olmadığını beyan etmektedirler.

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