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Volume 2

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PROF. DR. CEM EVEREKLİOĞLU

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Academic Studies in Health Sciences - II

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Editor

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Chapter 30

THE EFFECTS OF TWO DIFFERENT BATHING METHODS APPLIED ON NEWBORNS WHO RECEIVE PHOTOTHERAPY ON THEIR BILIRUBIN LEVELS

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INTRODUCTION

Jaundice is known as a medical condition in which the sclera and mucosa acquire a yellow color due to accumulation of bilirubin in the epidermal tissues of the body.[1] When the level of bilirubin reaches 5-7 mg/dl, jaundice becomes observable on the skin of the newborn.[2] In the first week of life, jaundice –which is one of the most common problems seen in newborn babies- is seen in 60% of term babies and 80% of preterm babies.[3] It is also the most common reason of hospitalization in the first week after birth [4] Even though jaundice is accepted as a physiological illness in the newborn period which is mostly diagnosed as an innocent and temporary condition, the increased levels of bilirubin can result in potential neurotoxic effects which can cause irreversible brain damage (kernicterus).[5] Kernicterus, by definition, is a bilirubin encephalopathy with high morbidity and mortality caused by accumulation of bilirubin in the newborn's brain.[6] Although it can be prevented by early diagnosis and proper treatment, Turkey comes third among the countries with the highest rates of kernicterus by 16% . [7,8] The aim of treatment of jaundice is to prevent excessive increase in bilirubin levels, eliminate the risk of neurologic damage with proper treatment and eradicate kernicterus.[9] Today, the most common method is known as "phototherapy". In phototherapy, using the energy of light beams, bilirubin in cutaneous and subcutaneous tissues is detoxified by turning it into less lipophilic photo-products; the conjugating system of the liver is deactivated, and without any further metabolic processes, bilirubin is egested from the body.[10] With the American Academy of Pediatrics' latest guide on treatments coming into use, it was reported that the rates of hyperbilirubinemia decreased, while the use of phototherapy increased.[11,12] Being used in treatment of hyperbilirubinemia in newborns for 60 years as a reliable and effective method, phototherapy has some side effects such as skin rash, colitis, dehydration, hypocalcemia, damage on mother-baby relationship, allergic illnesses, bronze baby syndrome, DNA damage, epilepsy and infantile cancer. However, a treatment other than phototherapy with fewer side-effects and easily applicable processes has not been.[13,14,15,16,17] The skin of the babies that receive phototherapy should be observed regularly to evaluate their levels of tanning, skin rashes and jaundice. Furthermore, skincare is also important due to the risk of degradation in skin integrity caused by the pressure created on body tissues or liquid stool.[2] A special skincare is needed for the newborn's skin for its healthy development.[18] The situation of the skin of babies who are admitted for phototherapy treatment is evaluated by a nurse, and skincare practices are planned according to the nurse's evaluation. Therefore, bathing is an important medical nursing practice for skincare to ease the baby, regulate respiration and blood circulation, reduce pain, cause an increase in capillary permeability and intestinal peristalsis.[19,20]

It is assumed that excretion of bilirubin from the body is accelerated with the therapeutic effects of newborn bathing such as increasing blood circulation and lymph flow and increasing intestinal movements resulting from touching the skin during bathing. When the literature is searched about this topic, it was seen that, some studies found that passive movements and massage (swimming and touching), which were applied on babies, were observed to increase the frequency of defecation and decrease the levels of bilirubin.[21,22] In two studies which were conducted in Turkey, bathing was found to be effective in decreasing the bilirubin levels of newborns receiving phototherapy.[23,24]

According to this information, this study was conducted on babies diagnosed with hyperbilirubinemia as a clinical, randomized, controlled and experimental study to determine the effects of wiping or washing on bilirubin levels.

METHODS

Design and setting

This study was conducted as a clinical, randomized controlled, experimental and prospective study to determine the effects of eruptive bath and shower on newborns receiving phototherapy for hyperbilirubinemia on their bilirubin levels.

The study was performed at Düzce University Hospital's Neonatal Intensive Care Unit between April 2017 and May 2018. The intensive care unit where the research was conducted provides a tertiary intensive care for patients. Babies diagnosed with hyperbilirubinemia receive phototherapy without separation from the mother in the mother-baby room.

Sample and sampling procedure

A total of 90 patients were selected for the study in accordance with the study protocol and in an equal number in each group. 30 babies were included in the control group, 30 babies were included in the wiped bath group, and 30 babies were showered. These babies were randomly divided into these groups.

The inclusion criteria:

• Having a gestation period between 37 and 41 weeks

• Having a birth weight of 2500 g

• Observing phototherapy indication in the baby.

• Having the baby at least 2 days old and at most 10 days old

• Having no congenital anomaly (such as neural tube defection or gastroschisis)

• Having no health problems

• Having been fed in oral way only

• Having the parents' statements of approval

The exclusion criteria:

• Being diagnosed with a health condition other than indirect hyperbilirubinemia

• Having a major congenital anomaly

• Having a risk factor for hemolysis;

- Rh incompatibility,

- ABO incompatibility,

- Direct coombs positivity,

- Having a reticulocyte number bigger than 6%,

- Having a symptom that support hemolysis in peripheral smear,

- Glucose-6-phosphate dehydrogenesis deficiency.

• Being clinically diagnosed with symptoms of dehydration;

- Fontanel troughs,

- Low skin turgor,

- Dry mucosa,

- Tachycardia (Cardiac apex beat of >180/min),

- Weight loss in the process of hospitalization by >10%.

Data collection procedure

The materials and devices listed below were used to collect the data.

Data Collection Form: The form was composed of questions about the baby's sex, eating habits, mode of delivery, period of gestation, weight, bilirubin levels at time of hospitalization and at the 6th, 18th and 30th hours, daily nutrition levels, frequency of defecation, amount of urination and weight loss.

Application Tracking Form: The physiological parameters of the infants (pulse, respiration, oxygen saturation and body temperature) that were measured before, during and 10 minutes after were recorded on this form.

Phototherapy Device: As the phototherapy device, we used a "Blue Led Phototherapy" device which can emit blue light in the 450-470 nm

light spectrum and be applied for intense phototherapy in the scale of 0-50 µW/cm2/nm that can affect the baby from head to toe.

Non-invasive Bilirubin Measuring Device: To measure the bilirubin levels of the babies, a device which takes measurements from the skin via photometric reflectance without harming the baby was used. This "Bili-check" branded "non-invasive bilirubin measuring device" which was used in the experiment is a device which takes measurements by emitting white light in a safe frequency that does not harm the baby. It has a measuring scale between 0 and 340 µMol/ L and a precision value of 0-26 µMol/ L, and it can make measurements without being affected from skin pigmentation, skin thickness or hemoglobin levels. Before every measurement, the device was re-calibrated. Moreover, a 2.5-cm-diameter "BillEclipse protective guard band", which allows the values to be measured, was used to protect the area from the light.

The intervention

In the process of collecting data, firstly, the procedures that would be applied on the babies and the aim of the research were explained to the legal custodial parents. The signatures of those who wanted their babies to participate in the study were taken after having them read the form of approval. The newborns that fit the criteria were separated into groups of wiped baths, showering and control group. The infants were distributed into the groups via random selection by lot.

Control Group: The information about the baby such as sex, eating habits, mode of delivery and period of gestation were acquired via speaking to the mother and was recorded on the data collection form. The baby's clothes were taken off and its diaper was changed. Before beginning the study, the body weight of the baby was measured by the researcher. BillEclipse guarding band was located in the middle section of the baby's forehead. To determine the bilirubin levels before phototherapy, measurements were made with the non-invasive bilirubin measurement device from the middle section of the forehead, and these values were recorded into the data collection form. The eyes of the newborn were covered with a phototherapy mask, and the newborn was placed into the medical baby-cot. Then, the treatment started. In two-hour intervals, the position of the baby was changed (between face-down and supine positions) and a break from the phototherapy was taken to feed the baby each 3 hours. Bilirubin levels at the 6th, 18th and 30th hours of phototherapy were measured from the same area and recorded on the data collection form. Except for the routine clinical practices, no intervention was made for the infants in the control group.

Experiment Group: In difference to the control group, the newborns in the experiment group received wiped bath and showering interventions

applied by the researcher before the phototherapy treatment. The environment where bathing was going to take place and the materials that were going to be used were prepared, and suitable environmental conditions were provided. The ambient temperature was set to 26-28 ℃, and it was measured by a Loobex brand digital thermometer. The door of the room was kept closed to prevent heat losses due to air streams. All the equipment for the bathing process were prepared and placed at easily accessible places. The equipment which were used for the bathing process of the newborns were a suitable bathtub for the newborns, a bucket, a tankard, tap water with a suitable temperature, a thermometer to measure the temperature of the water, two towels, diapers and 90x100 mm cotton rags. All the equipment which were not disposable were disinfected after each patient.

To provide the least amount of heat loss for the newborn, tap water with a temperature very close to the body temperature (37 ℃) was used. The temperature of the water put into the bucket was measured with a Nuc brand bath-thermometer. In both groups, bathing was applied on the open bed, the bathing time was limited to 5 minutes, and the babies rested for 10 minutes wrapped in a warm towel.

The apex heart beat and saturation values were recorded via a suitable probe (y-sensor) placed on all newborns' feet in the experiment group, and their body temperatures were measured with a contactless thermometer. Respiratory values were counted for a minute. The vital signs of the newborns in both experiment groups were measured before treatment, during treatment and 10 minutes after treatment were recorded on the application tracking form.

Experiment Group 1 (Wiped bath group): A bucket, cotton rags and warm towels to dry the baby were placed on a moveable table near the open bed. Warm water at 37℃ was poured into the bucket. Soap and shampoo were not used since they were not advised in the first bath and can cause sensitivity or irritation in the baby's skin. The clothes of the baby were taken off when the baby was placed on the warm towels on the open bed. With covering the baby with warm towels, first, the eyes; second, the ears; and third, the whole face of the baby were cleaned with cotton rags. In each cleaning practice, only one cotton rag was used. Afterwards, the baby's neck, arms, body, back and legs were cleaned and wiped. Then, the baby's head and hair were cleaned. Each cleaned part of the baby was covered immediately with warm towels to prevent the baby from getting cold. Eventually, the diaper of the baby was removed, and the genital region was cleaned, following up with fastening a new diaper.

Experiment Group 2 (Showering): A bucket, cotton rags and warm towels to dry the baby were placed on a moveable table near the open

bed. Warm water at 37 ℃ was poured into the bucket. The clothes of the baby were taken off when the baby was placed on the warm towels on the open bed, and the diaper of the baby was changed including the cleaning of the genital region. The baby covered with warm towels was delivered to the mother's arms. The bathtub with a net was placed on the open bed. The baby was placed onto the net with its head facing up and held by the mother. Firstly, the baby's body, arms and legs, and then, the head were washed by pouring water slowly following up by drying the baby. Soap or shampoo were not used in showering.

Data analysis

The descriptive statistics (mean, standard deviation, median, minimum, maximum, interquartile range, percentage) for all the data were calculated. The quantitative variables' normality assumption was examined with Shapiro-Wilk test. The homogenization of the covariance matrix was tested with Box M test. For the comparisons between groups, Kruskal Wallis test was used. The Generalized Linear Modelling (Gamma with log link, post hoc: LSD) approach was used for comparison of the measurement values of variables that did not have normal distribution in different periods. For the relationships between categorical variables, Pearson's Chi-Squared and Fisher-Freeman-Halton tests were used. The statistical analyses were carried out in the SPSS 22 software. "$p<0.05$" was accepted as statistically meaningful.

Ethical considerations

To conduct the research, an ethical form of approval (2017-59, 20.03.2017; Supplement 4) and a formal approval were obtained from the Non-Invasive Medical Studies Ethics Committee at the Faculty of Medicine at Düzce University. Written approvals were also obtained from the parents of the newborns that were included after informing the parents on the objective of the study and the practices that were going to be applied. The parents were also informed on their right to participate in or quit the study at any time without paying a research expense to the social security institution and the policy of keeping their personal information secret to comply with the "principle of privacy".

RESULTS

Table 1. Comparison of Newborns' Sex, Delivery Method and Feeding Method According to Groups

		Group								
		Showering (n=30)		Wiped Bath (n=30)		Control (n=30)		Sum		
		n	C%	n	**C%**	**n**	**C%**	**n**	**C%**	**p**
Sex	**Female**	11	36.7	12	40.0	13	43.3	36	40.0	0.870*
	Male	19	63.3	18	60.0	17	56.7	54	60.0	
Delivery Method	**Normal**	14	46.7	11	36.7	12	40.0	37	41.1	0.800*
	Cesarean	16	53.3	19	63.3	18	60.0	53	58.9	
Feeding Method	**Breast Milk**	20	66.7	26	86.7	20	66.7	66	73.3	0.091&
	Formula	5	16.7	0	0.0	2	6.7	7	7.8	
	Breast Milk + Formula	5	16.7	4	13.3	8	26.7	17	18.9	

C%: Percentage in column, *Pearson Chi-Squared test, &: Fisher-Freeman-Halton test

In the extent of the research, the newborns' information about sex, delivery method and eating habits are shown in Table 1. According to these data, 43.3% of the newborns in the control group were female, while 56.7% were male, whereas 40% of the newborns in the wiped bath group were female, and 60% were male. There was no significant difference with regards to sex between the groups ($p>0.05$, Table 1).

When the feeding methods of the newborns were examined, 66.7% (n=20) of the newborns in the control group, 86.7% (n=26) of those in the wiped bath group and 66.7% (n=20) of those in the showering group were seen to be fed with breast milk. No significant difference in their feeding methods was seen between the groups ($p>0.05$ Table 1)

Table 2. Age, Gestation Period, Birth and Hospitalization Weight Comparisons According to Groups

		Group			p
		Showering (n=30)	**Wiped Bath (n=30)**	**Control (n=30)**	
Day Number	**Mean**	4.77	4.83	4.80	0.974*
	Std. Deviation	1.87	1.78	1.77	
	Median	4.00	4.00	4.00	
	IQR	3.00	4.00	3.25	
	Minimum	3.00	3.00	3.00	
	Maximum	9.00	9.00	9.00	
Gestation Period (weeks, days)	**Mean**	38.43	38.40	38.36	0.970*
	Std. Deviation	1.04	.96	.92	
	Median	38.25	38.30	38.05	
	IQR	1.88	1.80	1.43	
	Minimum	37.00	37.00	37.00	
	Maximum	40.00	40.00	40.00	
Birth Weight (grams)	**Mean**	3207.33	3194.33	3250.67	0.994*
	Std. Deviation	366.11	372.49	560.25	
	Median	3185.00	3175.00	3125.00	
	IQR	607.50	535.00	782.50	
	Minimum	2540.00	2530.00	2500.00	
	Maximum	3960.00	4000.00	5150.00	
Weight of the baby in hospitalization (gram)	**Mean**	3097.00	3105.67	3148.33	0.994*
	Std. Deviation	370.94	351.70	532.41	
	Median	3100.00	3100.00	3060.00	
	IQR	640.00	437.50	680.00	
	Minimum	2520.00	2530.00	2420.00	
	Maximum	3830.00	3850.00	4840.00	

Std: Standard, IQR: Interquartile Range, * Kruskal Wallis Test

Table 2 shows the comparison of age, gestation period, birth and hospitalization weight between the groups. No significant difference was found in these variables in the extent of the research ($p>0.05$).

Table 3. Pulsation Values According to Groups and Periods

Pulsation*							
Group	Period	n	Mean	Standard Deviation	Median	Min.	Max.
Showering	Before Bathing	30	133.37	19.38	133.50	102	188
	After Bathing	30	135.03	18.65	134.50	100	173
	10 min After Bathing	30	127.70	15.76	125.00	105	172
Wiped Bath	Before Bathing	30	135.13	14.83	136.00	108	160
	After Bathing	30	147.10	16.77	146.00	112	180
	10 min After Bathing	30	130.07	11.45	128.00	110	163

* Generalized Linear Modelling *min: minute *Min.: minimum *Max.: maximum

When the pulsation values of the newborns in wiped baths and showering processes before bathing, after bathing and 10 minutes after bathing were compared, it was seen that the differences between pulsation values were similar among the groups ($p=0.175$). The group and period fundamental effects were found to be significant ($p=0.027$ $p<0.001$). According to this result, the pulsation values for the showering group was found significantly lower than that of the wiped bath group ($p=0.005$). Moreover, the pulsation values in the wiped bath group before bathing was found significantly lower than the pulsation values after bathing ($p=0.006$).

In addition to these, body temperature, pulsation values and oxygen saturation values of the newborns examined in the extent of the research were also compared according to the wiped bath and showering practices. When the body temperatures before bathing, after bathing and 10 minutes after bathing were compared according to the groups, the wiped bath and showering processes were found to result in similar results ($p=0.529$). When the respiratory values before bathing, after bathing and 10 minutes after bathing were compared according to the groups, the differences between groups were found not to significantly vary based on different periods ($p=0.175$). When the oxygen saturation values measured in different periods in the wiped bath and showering groups were compared, the differences between oxygen saturation values were found to be similar in both groups ($p=0.718$).

Table 4. The Comparison of Defecation Frequencies According to Groups and Periods

Defecation*							
Group	**Period**	**n**	**Mean**	**Standard Deviation**	**Median**	**Min.**	**Max.**
Showering	1st day	30	4.27	1.89	4.00	1	9
	2nd day	30	5.73	1.87	6.00	3	12
	3rd day	30	6.33	1.12	6.00	4	8
Wiped Bath	1st day	30	4.33	1.65	4.00	2	8
	2nd day	30	5.63	1.50	6.00	2	8
	3rd day	30	6.40	1.25	6.00	3	8
Control	1st day	30	4.27	1.26	4.00	2	7
	2nd day	30	5.33	1.54	5.50	3	8
	3rd day	30	5.33	1.32	5.00	3	9

***** Generalized Linear Modelling

The defecation frequencies of the newborns for 3 days are shown in Table 4. When the group and period fundamental effects were compared, only the periodic effect was found to be statistically significant ($p<0.001$). According to this, in the showering group, the defecation frequency value measured on the 1st was significantly lower those on the 2nd and 3rd days ($p<0.001$ for each). In the wiped bath group, the defecation frequency value measured on the 1st day was also significantly lower than those on the 2nd and 3rd days ($p<0.001$ for each). In a similar way, in the control group, the defecation frequency value measured on the 1st day was also significantly lower than those on the 2nd and 3rd days ($p<0.005$ for each).

Table 5. Bilirubin Measurements According to Groups and Periods

Bilirubin*							
Group	**Period**	**n**	**Mean**	**Standard Deviation**	**Median**	**Min.**	**Max.**
Showering	**Before Phototherapy**	30	17.53	1.65	17.60	13.5	21.9
	6th hour	30	15.25	1.85	15.30	11.5	19.3
	18th hour	30	12.93	1.47	12.70	9.3	15.8
	30th hour	30	10.27	1.69	10.15	7.1	13.9

Wiped Bath	**Before Phototherapy**	30	17.69	1.74	17.70	14.5	23.0
	6th hour	30	15.59	1.59	15.60	12.8	19.0
	18th hour	30	12.79	1.66	12.50	9.3	16.8
	30th hour	30	10.78	1.69	10.40	8.3	15.1
Control	**Before Phototherapy**	30	17.72	1.72	17.85	13.7	20.0
	6th hour	30	15.71	2.35	15.95	10.2	19.7
	18th hour	30	13.79	1.72	13.90	9.3	17.3
	30th hour	30	11.64	1.63	12.00	8.3	14.6

***** Generalized Linear Modelling

When the bilirubin levels before phototherapy and after 6, 18 and 30 hours were compared according to the groups, the difference between wiped bath and showering groups was found not to change significantly in different periods ($p=0.158$). The group and period fundamental effects were significant ($p=0.001$; $p<0.001$). According to this result, bilirubin levels in the showering group at the 18th and 30th hours were found significantly lower than those in the control group measured in the same periods ($p=0.046$; $p<0.001$). Likewise, bilirubin levels in the wiped bath group at the 18th and 30th hours were found significantly lower than those in the control group measured in the same periods ($p=0.019$; $p<0.018$). Moreover, in all groups, bilirubin levels before phototherapy were significantly higher than the values measured at the 6th, 18th, 30th hours of phototherapy ($p<0.001$ for each).

DISCUSSION

In this section, the research conducted as a clinical, randomized controlled, experimental, and prospective study to determine the effects of eruptive bath and shower on newborns receiving phototherapy for hyperbilirubinemia on their bilirubin levels and its findings are discussed. In the study, there was no significant difference between the measurements which were made before bath, after bath and 10 minutes after bath in the groups, except in the showering group where the pulsation value measured after bath (135.03 ± 18.65) was found significantly lower than the pulsation value measured in the same period in the wiped bath group (147.10 ± 16.77) ($p=0.05$; Table 4). Furthermore, the pulsation value measured before bath in the wiped bath group was found significantly lower than the value after bath ($p=0.006$; Table 4). Additionally, it should be known that increasing heart rate is a response to pain or stress for newborns.[25] Thus, this may be interpreted as that wiped bath creates pain and stress in newborns. When the effects of different bathing methods were examined, Çaka and Gözen (2018) reported that the pulsation values of babies experiment-

ed with dipping in a bathtub were higher than that of babies experimented with wrapped bathing.[26] Fonseca Filho et al. (2017) studied newborns with wrapped bathing and showering, and it was observed that, as the pulsation values for wrapped bathing after bath were decreasing, the pulsation values for the showering group after bath were increasing.[27] Ar and Gözen (2015) also found no statistically significant difference between bathing in a tub and showering groups in terms of their pulsation values before and after bathing.[28]

Although the sample had no significant difference in the pulsation values after 10 minutes between groups, in each group, the pulsation values decreased after bathing (Table 3). Ar and Gözen (2015) also reported that the pulsation values in 10 minutes after bathing were lower than the pulsation values before bathing in all groups.[28] As it was reported by Kurtulan Bulut and Çimen (2009), the pulsation values after bathing were close to the values before bathing, and the change in heart rate was similar for each method of bathing.[29] In the measurements of Çaka and Gözen (2018) in 10 minutes after bathing, the babies who were studied with KDB reached the values before bathing whilst babies studied with SKDB had pulsation values even lower than the values before bathing.[26] According to the results acquired in this study, when the decrease in pulsation values in 10 minutes after bathing is evaluated, it may be seen that wiped bath and showering helped the babies relax and feel relieved by comforting them.

In this study, when the bilirubin levels before phototherapy and after 6, 18 and 30 hours were compared according to the groups, the difference between the wiped bath and showering groups was not significant($p=0.158$; Table 5). The group and period fundamental effects were significant ($p=0.001$ $p<0.001$; Table 5). According to this result, bilirubin levels in the showering group at the 18th and 30th hours were found significantly lower than those in the control group measured in the same periods ($p=0.046$ $p<0.001$; Table 5). Similarly, bilirubin levels in the wiped bath group at the 18th and 30th hours were found significantly lower than those in the control group measured in the same periods ($p=0.019$ $p<0.018$; Table 5). Moreover, in all groups, bilirubin levels before phototherapy were significantly higher than the values measured at the 6th, 18th, 30th hours of phototherapy ($p<0.001$ for each; Table 5).

In this study, while there was no significant difference among the showering, wiped bath and control groups at the 6th hour of phototherapy, the bilirubin level measurements made at the 18th and 30th hours for the wiped bath and showering groups were lower. The study by Çınar and Küçükoğlu (2014) on newborns receiving phototherapy observed that the bilirubin levels at the 6th hour of the study in the wiped bath group was lower than that of the control group.[23] Dağ and Yayan (2017) also reported

the effects of bathing and massaging on hyperbilirubinemia in newborns, and they observed that the bilirubin levels at the 6th and 12th hours for the bath-in-tub group, wiped bath group and massaging group were significantly lower than those in the control group.[24]

When the studies on bathing and massage in hyperbilirubinemia in literature were examined, similar hypotheses were seen. In both methods, the decrease in bilirubin levels was explained with driving bilirubin out of the body via increased bloodstream and lymph-stream. Additionally, it was stated that the increase in intestinal motility caused by the decrease in enterohepatic circulation with the vagal nerve stimulations created by touching the skin speeds up the egestion of bilirubin out of the body by defecation. [23,30,31,32]

Even though no statistically significant difference in defecation frequency between the control and experiment groups was found in this study, the mean defecation frequencies in the experiment groups were observed to be higher than that in the control group. The reason for this was thought to be the increased bloodstream and decreased enterohepatic circulation as a result of warm water and skin contact causing a decrease in bilirubin levels.

Because of these similar affecting mechanisms, the results on the effects of massaging on newborns receiving phototherapy are also included. When studies on the effects of massaging on the bilirubin levels of newborns receiving phototherapy were examined, it was observed that the measurements made at different times of treatment were found to significantly differ. Oa et al. (2017) reported that, after medical interventions, the TcB measurements made at 24th and 48th hours in the massage group were significantly lower, and the result of this study was accepted as similar because of the difference found at the 30th hour of phototherapy.[33] In contrast to our study, Lin et al. (2015) found no significant difference between the control and massage groups on the first and second days, while they reported that the bilirubin levels for the massage group was significantly lower than those in the control group on the third day.[30] Moreover, Eghbalian et al. (2017) reported no significant difference in bilirubin levels on the first and the second days, but on the third and fourth days, there was a significant decrease in bilirubin levels for the massage group.[31] Jalalodini et al. (2016) reported no significant difference in bilirubin levels on the first and second days between the massage and control groups.[34] Kianmehr et al. (2014) studied babies with an intervention of 15 minutes of massaging 3 times a day and reported significantly lower bilirubin levels on the 4th day in comparison to the control group.[32]

LIMITATIONS

The data were limited to term-born healthy newborns with a weight of over 2500 g. The results cannot be generalized to newborns who are preterm, have hemolytic conditions or low birth weight.

CONCLUSION

The results shown below were obtained in this study, which was conducted as a clinical, randomized-controlled, experimental and prospective study to determine the effects of eruptive bath and shower in newborns receiving phototherapy for hyperbilirubinemia on their bilirubin levels;

The babies who were included in the study were found to show homogeneous distribution in terms of their gestation periods, age in days, birth weight and weight of hospitalization ($p>0.05$).

When the physiological findings of the wiped bath and showering groups before bathing, after bathing and 10 minutes after bathing were compared, it was observed in both bathing methods that the body temperature decreased significantly after bathing and increased again in 10 minutes after bathing. In both bathing methods, the oxygen saturation values measured in 10 minutes after bathing were found significantly higher than the values measured before bathing. With no significant difference in the mean respiratory values, it was observed that the respiratory rate was increasing in the wiped bath group, it was not changing in the showering group, and it was also observed to hit lower values in 10 minutes after bathing. In the newborns subjected to wiped bathing, the pulsation values were found to be higher than those in the showering group in the periods after bathing. Even though the differences between defecation frequency values were not found statistically significant, it was recorded that the defecation frequency values which peaked on the 3rd day were to be higher in the wiped bath and showering groups than those in the control groups.

When the newborns' bilirubin levels were compared according to the groups, it was found in both bathing groups that the bilirubin levels measured at the 18th and 30th hours of phototherapy were found to be significantly lower than those in the control group ($p<0.05$).

IMPLICATIONS FOR PRACTICE

Bathing may be used as a therapeutic clinical routine nursing method to decrease the bilirubin levels of the babies who receive phototherapy faster. It is recommended that babies should be dried off well and wrapped up with warm towels to prevent heat loss by evaporation in the first 10 minutes after bathing. As wiped bath causes physiological symptoms indicating stress such as increase in respiration and heart rates, showering may

be preferred as a bathing method to comfort the baby by decreasing respiration rate. For babies that have bilirubin levels in the boundaries but no need for hospitalization, the parents may be educated on bathing practices. The results of this study may be utilized by administrations and medical professionals.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

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Chapter 31

SLEEVE GASTRECTOMY, SURGICAL TECHNIQUE, AND APPROACH TO COMPLICATIONS

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INTRODUCTION

Since the extensive application of endoscopic and laparoscopic interventions, bariatric-metabolic surgery (BMS) has gained tremendous momentum and become well known by all in the 2000s. However, surgical treatment of obesity in patients with high body mass index (BMI) has an almost 70-year history. This course, which began in the 1950s with the introduction of various types of intestinal bypass operations, has since developed and evolved to this day [1].

Today, many different BMS operations are performed all over the world. According to data from the American Society for Metabolic and Bariatric Surgery (ASMBS), 61.4% of operations performed in the USA in the year 2018 were sleeve gastrectomy (SG) operations. Not counting endoscopic procedures such as revision surgeries and intragastric balloon surgery, SG comprises almost 75% of primary cases [2]. SG rates are believed to be similar to this rate worldwide.

Sleeve gastrectomy was initially designed as the first step of two-step surgical procedures involving duodenal switch in high-risk patients. Roux-en-Y gastric bypass (RYGB) was later implemented for the same purpose. In other words, the original indication of SG was to ensure safety of the main procedure by allowing weight loss in super obese patients (BMI> 50 kg/m²) [3,4]. The Canadian surgeon, Michel Gagner, was the first to apply the procedure and enable its popularity.

Over time, it became understood that the procedure was highly successful in achieving weight loss while also resolving associated conditions and became accepted as a standalone method. Its superiority to medical treatment, especially in type 2 diabetes, and capability of providing outcomes similar to RYGB further increased its popularity [5]. SG is not only a simple restrictive procedure, its many effects on the hormonal level also play a significant role in its success [6]. Increased gastric emptying rate is an important factor for hormonal effect [7]. In addition to its successful results, the fact that it is technically easier and safer to apply compared to other BMS procedures is effective in making it the most preferred type of surgery all over the world today.

SURGICAL TECHNIQUE

Like all surgeries, the success of SG is directly related to the care and experience of the surgeon. Although it is a simple operation, the misguided perception that it can be performed by any surgeon and differences in technique, that is, lack of standardization, are the main causes of failure and complications in SG.

Start of Surgery: Sleeve gastrectomy, like all other BMS procedures, is applied with the laparoscopic technique. Open surgery plays no part in SG. While it varies according to the surgeon's preference, the first trocar can be directly inserted into the abdomen, though Veress needle use or open entry are other options. Depending on the surgeon's preference, 4-6 trocar is used. We prefer to insert the initial 12 mm trocar directly into the abdomen approximately 20 cm below the xiphoid, then inflate the abdominal cavity with carbon dioxide gas, raising the pressure to 13-15 mm/Hg, and use four more trocars with sizes ranging from 5-15 mm (Image 1). The port above the umbilicus is used for the camera, while the port on the left anterior axillary line is used for assistant traction. For liver retraction, the trocar in the subxiphoid or upper right quadrant can be utilized. Among these, the port that is not being used for liver retraction and the 12- or 15-mm port in the patient's left side are the entries used by the surgeon. Since it is easier to access the esophagogastric junction, especially in patients with high BMI, the upper right quadrant port is used for liver retraction, while the subxiphoid port is used by the surgeon for left-hand traction in the technique that we use. When all ports are entered, the patient is placed in the reverse Trendelenburg position to begin the operation.

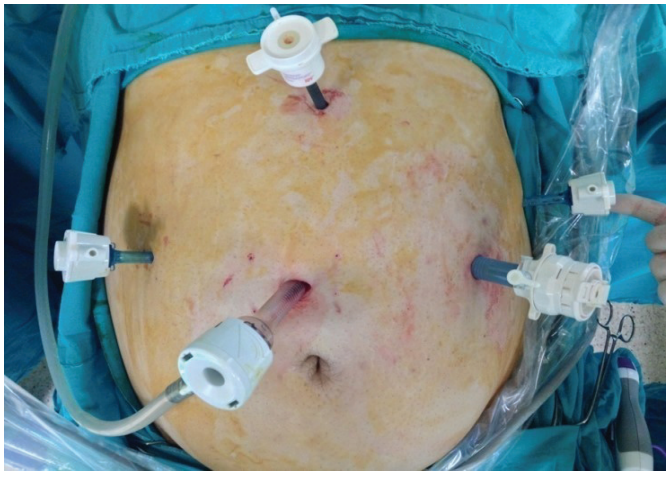

Image 1: Port placement in sleeve gastrectomy.

Greater curvature mobilization: After the stomach is emptied through a nasogastric (NG) tube, the gastrocolic ligament is divided close to the pylorus. The gastroepiploic vessels and short gastric arteries are divided with an energy device along the greater curvature, advancing towards the proximal (Image 2). By dividing the gastrosplenic and gastrophrenic ligaments, this dissection centers the Angle of His and left crus of diaphragm and continues until the gastric fundus is completely mobilized. At this step, if a hiatal hernia which was previously identified in endoscopy is revealed, suturing the diaphragmatic crura to close the defect, which is

a method that we frequently apply. In order to fully elevate the stomach, it is appropriate to first divide all adhesions, especially those on the anterior wall of the pancreas, thereby reducing the risk of gastric twist by preventing attachments to the posterior wall of the stomach. We also recommend complete mobilization of the gastroesophageal fat pad at the esophagogastric junction. We believe that this will enable full removal of the fundus and reduce the risk of leak by preventing thick tissue from being caught within the endoscopic staple jaws.

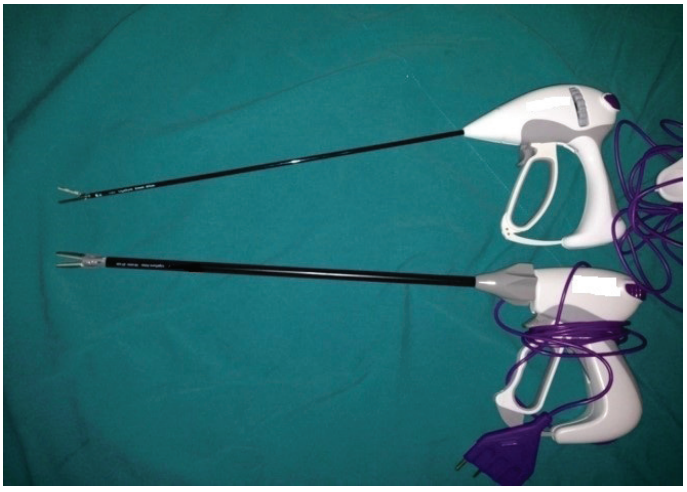

Image 2: Energy devices that are used.

For gastric resection, a 2-6 cm gap is appropriate while approaching the pylorus. When the distance to the pylorus is less than 5 cm at the resection line, some publications indicate there is increased risk of leak due to reduced antrum reserve and increased intraluminal pressure, while other meta-analyses state this is irrelevant [8,9]. In our practice, resection begins at a 2-3 cm distance from the pylorus. We believe that closer proximity may create risk of leak, while further distance will result in unnecessary stomach volume.

Placement of the calibration tube: First, the previously inserted NG tube is removed. Next, a bougie used for guidance when forming the tube-shaped stomach is orally inserted. During this step, it is important to act in a coordinated manner with the anesthesiologist and guide the bougie along the lesser curvature.

Various sized bougies are used for calibration. There are studies which state that the use of ≥40 French (Fr) bougies is associated with decreased leak rates [10]. However, many highly experienced surgeons recommend the ideal bougie size as <40Fr [11]. We implement 38-39 Fr bougies (Image 3). However, we also believe that proper application of surgical tech-

nique is more important than bougie size, and that bougie size does not account for a significant difference in terms of weight loss or leak risk.

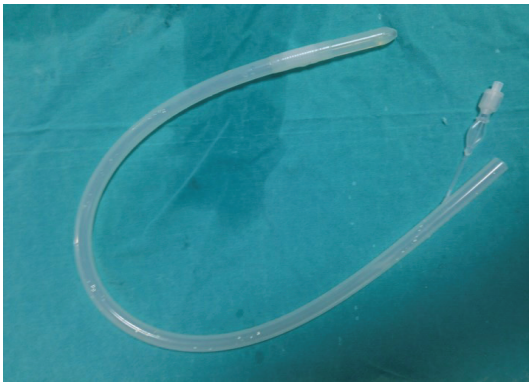

Image 3: 39 Fr calibration tube.

Gastric transection (Stapling process): For this procedure, 60 mm laparoscopic staplers are used. Staples begin at an appropriate distance to the pylorus advancing proximally along the bougie. As the thickness of the gastric wall decreases from the antrum to the fundus, the use of different cartridge heights for each region is especially important in preventing leak complications. While there is no standardization among surgeons in terms of choice of staple height, 81% of an international panel of experts determined that closed staple height must be at least 1.5 mm on any part of the SG[11]. In our operations, we prefer to use new generation stepped cartridges (Image 4). Staple heights of these cartridges are at least 3 mm for the antrum and corpus and at least 2 mm for the fundus. In the super obese, especially male patients, we use cartridges starting from 4 mm staples in the antrum. Accordingly, we believe that the risk of leak is reduced by making the most appropriate choice in the stomach wall, of varying distal to proximal thickness.

Image 4: Examples of cartridges with various staple sizes

Before stapling, the anesthesiologist should be reminded to administer a muscle relaxant. This is because a potential increase in intraabdominal pressure may compromise the reliability of the stapler line. Considering the curved shape of the stomach, it is appropriate to fire the first one or two staples from the trocar in the right side of the patient (this trocar may be replaced with a larger trocar if necessary). The next staples are fired from the trocar next to the camera port in the patient's left side. SGs with straight staple lines in the front and back walls of the stomach, at an even distance from the bougie, and with straight cartridge transitions without zigzagging have reduced risk of leakage as well as long-term risk of rotation. Therefore, it is important to ensure traction for proper position of the stomach before each firing. Special care should be taken to avoid narrowing of the incisura angularis region. If this region is not properly resected, proximal expansion and neo-fundus formation due to long term increase in pressure are expected. When the final staple is being fired, it is important to take caution to avoid the Angle of His by up to 1 cm in order to prevent leakage from this region. We also wait up to 30 seconds before each firing to ensure that the staples are well situated. We believe this will reduce the risk of leak and bleeding.

There is no consensus regarding staple line reinforcement in order to reduce leak rates. Apart from suture reinforcement, biological or synthetic reinforcements and adhesive materials can be used. Suturing in particular is a matter of controversy. There are contradictory publications on its impact on leakage rates. In the 2016 report by the Metabolic and Bariatric Surgery Accreditation and Quality Improvement Program (MBSAQIP), staple line reinforcement was associated with increased leak rates [12]. It should be noted that the most important factor in the success or failure of suturing the staple line is the skill and experience of the surgeon. The negative effects of these types of procedures on the operation time and on costs should also be considered. In our own practice, we prefer reinforcement of the staple line with fibrin adhesive as we believe this will help prevent leak forms of bleeding. We also believe that adhering the tube stomach to the underlying base will prevent rotation. In addition, we also adhere the fat pad on the previously mobilized fundus to the staple line (Image 5 A-B). We believe that this will be effective in preventing microleakage that may occur from the esophagogastric junction.

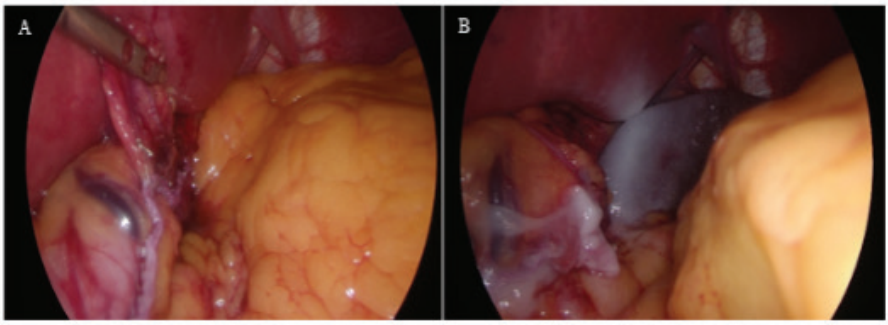

Image 5 A-B: Use of fibrin adhesive on the staple line and mobilized fat pad

Intraoperative leak test: According to the results of international consensus, intraoperative leak testing in SG is recommended and applied by almost half of surgeons [11]. While there are multi-center retrospective analyses which indicate that these tests have high failure rates in determining potential postoperative leaks [13], there are also large scale comprehensive studies which recommend testing to prevent postoperative complications [14]. After performing the transection, we retract the bougie to the esophagogastric junction and conduct an intraoperative methylene blue leak test. We recommend performing this procedure while paying close attention to the pressure formed within the staple line, since increased pressure may cause unnecessary strain in the transection line.

Final stage of the surgery: There are various opinions regarding the use of drains in BMC. While some studies recommend routine drain use [15], there are publications recommending the opposite [16]. After performing the methylene blue leak test and applying fibrin adhesive, we choose to place a silicone drain extending along the staple line because we believe that drains are helpful in the early diagnosis of complications such as leaks and bleeding and are also necessary in the follow-up of these complications as well as non-surgical treatments.

We recommend using an endobag when removing the specimen to prevent infection of the trocar site. We believe that closing the fascia of the site containing the 15 mm port for specimen removal is necessary in order to prevent trocar site hernia. For the other trocar openings, only the skin is closed and the surgery is concluded.

APPROACH TO COMPLICATIONS

A. Early Complications

Embolism: In bariatric-metabolic surgical procedures, embolism is not encountered as often as it is thought. The rate of venous thromboembolism, reported as 1.3% for all BMC surgeries, decreases to 0.25-0.30%

in operations such as SG, which takes considerably less time than other procedures in experienced hands [17].

In order to avoid embolism, precautions include the use of low molecular weight heparin (LMWH) at a proper dose starting one day before the operation, compression stockings after the patient is taken to the operating table, and mobilization of the patient 3-4 hours after the surgery. It is important that the compression stockings are not removed for up to 48 hours and that LMWH is continued throughout hospital stay and at least 10 days after discharge.

Hemorrhage: According to the literature, staple line hemorrhages occur at a rate of 2-2.5% [18]. Depending on the severity of the hemorrhage and the surgeon's judgement, reoperation or a conservative approach may be followed. There are ongoing discussions regarding the effect of staple line reinforcement on hemorrhage. However, most publications agree that sutures and other reinforcement materials reduce the rates of staple line hemorrhages. Combined use of these methods is considered the most effective approach [19]. In our own case series, hemorrhage complications were observed in 1.55% of cases. None of the patients required reoperation. Complete blood count was used to monitor patients who developed hemorrhage after SG. In clinical observation, transfusions of blood products were provided when necessary. In these patients, drains were kept longer than usual. Patients were discharged without requiring additional interventions.

Leak: Leak complications in BMS, especially with the widespread popularity of SG, have become increasingly relevant. This is because leaks are the most common cause of mortality and morbidity in SG. The literature reports leak rates of 2.4% [20].

Effective factors in leak development consist of: not using the ideal bougie, getting too close to the pylorus, incisura angularis, and Angle of His, incorrect firing of the stapler, stapling of the orogastric tube, thermal injury to the gastric tube with ultrasonic devices, and impaired vascularization due to aggressive dissection of the posterior wall of the gastric fundus. Leaks are mostly expected to occur at the esophagogastric junction. This is because increased intraluminal pressure causes strain to the proximal stomach and resections performed too close to the Angle of His disrupt blood supply to this region. In other words, surgical errors that cause ischemia and increased pressure are the main factor [21].

Despite its declining rates over the years, the most feared complication of SG is leakage. The basis of reducing leak rates lies in surgical experience and continuous emphasis on standardization in scientific meetings. It is clear that advancements in stapler and cartridge technology have contributed in reducing leaks rates [14,22].

There are contradictory opinions about performing routine upper gastrointestinal system contrast studies for leak detection after SG (Image 6). Considering that leak development is possible after discharge, it is evident that early postoperative leak tests will have low sensitivity. In addition, the accuracy of these studies may vary depending on the patient, the radiologist, the contrast agent used, and the region and extent of the leak. For these reasons, these applications should be left to the surgeon's judgement [22]. Rather than routine contrast studies, some publications recommend clinical monitorization of the patient and direct computed tomography when in doubt [23].

Image 6: Contrast study of upper gastrointestinal system after sleeve gastrectomy

The follow-up and treatment modalities of sleeve gastrectomy leaks are based on the onset, location, and size of the leak [24]. In cases detected early, primary repair is recommended. However, recurrence rates are high in such repairs. In leaks detected late, severe inflammation and abscess formation prevent primary repair. In these low molecular weight heparin cases, rinsing the surgical area and placing a drain is the best option [25].

The non-operative treatment of sleeve gastrectomy leaks requires a multidisciplinary approach which also involves the participation of the radiologist and gastroenterologist. Expandable intraluminal stent application provides effective treatment in proximal leaks and success rates range from 55-100%. The stent allows the patient to be orally fed, which is an important advantage of this treatment. Possible complications include stent migration, rotation, erosion, or patient intolerance. Endoscopic clip and biological adhesives are current procedures. Endoscopic clip placement provides successful results in experienced hands. Biological adhesives alone are often inadequate. These should be applied in conjunction with other

endoscopic treatment modalities [22].

In cases which cannot be treated despite all of these options, more aggressive and radical interventions should be considered. RYGB revision, or even total gastrectomy and esophagojejunal anastomosis may be necessary [24].

In our own clinical practice, we have only encountered leak once. In this case, the leak, contrary to expectation, occurred in the corpus of the stomach rather than the proximal stomach and was about 4-5 cm in size. We treated this complication, which was observed in one of our first patients, by endoscopic placement of a covered stent (Image 7 A-B).

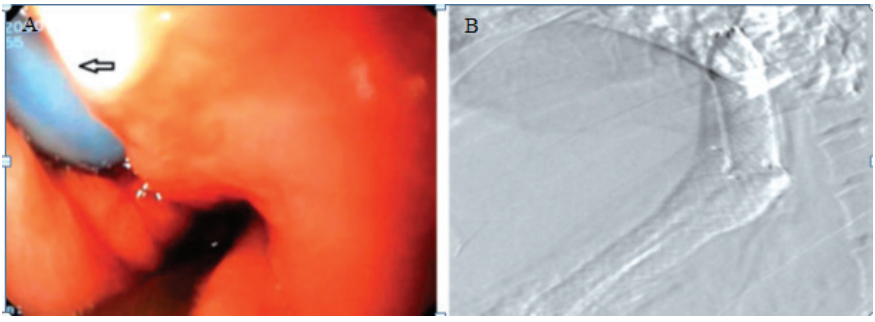

Image 7 A: Endoscopic view of a large leak in the corpus. The intra-abdominal drain appears to enter the lumen from this region. B: Radiological view of the coated stent applied in the treatment.

B. Late Complications

Stricture: Strictures are encountered in 3.5% of cases after SG. Its most common site is the incisura angularis. Early term symptoms include increased salivary secretion and persistent nausea and vomiting. As for late term symptoms, dysphagia, reflux resistant to medical treatment, and Dumping syndrome may be observed. In the acute period, it occurs secondary to edema or torsion due to technical problems. Diagnostic methods include contrast study and endoscopy of the upper gastrointestinal system (Image 8 A-B). Conservative approaches are usually sufficient in the treatment of acute stricture. In patients who present with chronic strictures, endoscopic balloon dilation or surgical treatments are recommended. In surgical treatment, laparoscopic seromyotomy, stricturoplasty, and revision to RYGB or mini gastric bypass may be preferred [26,27].

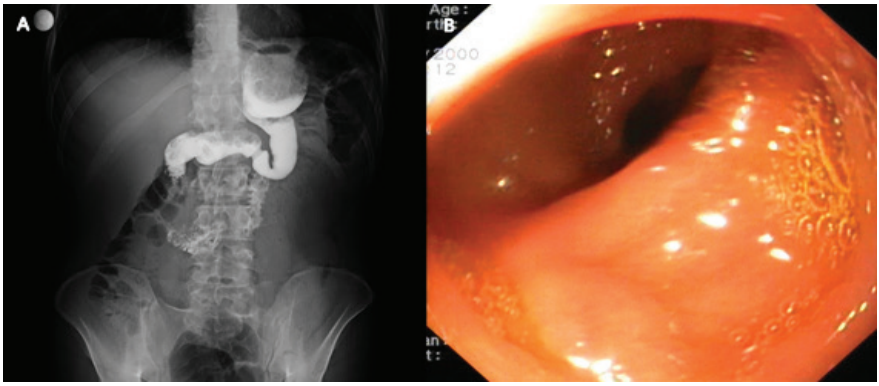

Image 8 A: Stenosis and associated neofundus formation due to twist after sleeve gastrectomy. B: Endoscopic view of the same patient.

Gastroesophageal Reflux: There are varying opinions regarding the association between SG and gastroesophageal reflux disease (GERD). It is stated that SG may be a remedial surgery for GERD for reasons such as reduced intraabdominal pressure due to weight loss after SG, reduced acid production due to fundus removal, and increased gastric emptying rate. However, factors such as reduced lower esophageal sphincter pressure, disrupted Angle of His, increased intragastric pressure increase, and development of hiatal hernia may be related to the development of GERD after SG [28]. The initial step in GERD treatment is the use of proton pump inhibitors. However, if the patient has symptoms resistant to medical treatment, revision of SG to RYGB is appropriate [29].

Nutritional Deficiencies: Like all BMS procedures, nutritional deficiencies may be observed after SG. Since SG is not an absorption-disruptive procedure like bypass procedures, nutritional deficiencies are less common. Furthermore, 57% of morbidly obese patients have existing nutritional deficiencies before surgery. Multivitamin supplements after SG are recommended to prevent potential defects. In addition, if deficiency of a specific micronutrient is identified, additional oral, parenteral, or intramuscular supplements should be given and followed up with consequent blood tests [30].

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Chapter 32

CURRENT DIAGNOSTIC APPROACH IN THE DIAGNOSIS OF THYROID NODULES

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Thyroid nodules are one of the most common diseases in the world. Especially in countries in the Mediterranean countries, it is more common. Approximately in every 5 people have goiter, in every goiter patients; one-fifth of thyroid nodules are seen. Approximately one in 20 thyroid nodule cases is thyroid cancer [1].

The diagnostic approach is very important in thyroid nodules in order not to unnecessarily operate many non-cancer cases of thyroid nodules and not to miss cancer cases.

We use many noninvasive (ultrasonography(USG), Doppler USG, elastography, magnetic resonance, analysis of molecular markers (in serum) and invasive methods (fine needle aspiration biopsy, core needle biopsy(FNAB), tru-cut needle biopsy, molecular analysis in tissue samples) in the diagnosis of thyroid cancers.

Molecular markers have a key feature not only in diagnosis but also in prognosis and treatment in thyroid cancers. When the molecular structure of cancer cells is revealed by the analysis of molecular markers in thyroid cancer cases, the way of personalized treatment is also opened. An important step has been taken so far in the preoperative diagnosis of thyroid cancers. With USG, Doppler USG, Elastography and FNAB, it is possible to largely diagnose thyroid cancers before surgery. However, due to the false or inadequate diagnosis, many cases of goiter without cancer are unduly operated, and many cases of cancer are delayed for the same reason.

Advances in research related to the molecular pathogenesis of thyroid cancers have taken an important step in the diagnosis of thyroid cancers [2]. Tissue molecular markers in the diagnosis of thyroid cancers have begin to be involved in algorithms related to the diagnosis of thyroid nodules. Providing high sensitivity and specificity rates in Bethesta III and IV nodules; commercially available products are used in Afirma Gene Sequencing Classifier (multigene expression), Interpace ThyGenX + ThyraMir (7 gene panel + 10 miRNA), CBLPath ThyroSeq version 3 [2]. However, tissue samples are needed to use tissue molecular markers in the diagnosis of thyroid cancers. This can only be achieved by invasive methods.

In the diagnosis of thyroid cancers, significant advances have been made in noninvasive methods. Combined 2D-SWE + conventional USG was performed on 31 thyroid nodules in 27 patients by Liu et al. resulting in 87.1% sensitivity [3].

However some authors has been reported that, multimodel diagnostic methods should be used in nodules smaller than 1 cm, since the sensitivity decrease when only USG is performed [3,4]. In a study by Ponti et al., cell-free DNA analysis was performed in non-plasma fluids (saliva, urine,

feces, etc.), and an increase was observed in many types of cancer, including thyroid cancers [5].

Today, many serum molecular markers are being researched for use in the diagnosis of thyroid cancers.

1. Etiology of Thyroid Nodules

Solitary or multinodular thyroid nodules are very common lesions. In autopsy studies, 30-60% of thyroid nodules were found. In approximately 40% of them, the diameter of the nodule was found to be larger than 2 cm.Although it is possible to detect nodules in millimetric sizes with modern USG methods, radiologists experienced in this field are more likely to detect small nodules. In palpation examination, the detection threshold of nodules is 1 cm. The rate of nodule detection by palpation in the community ranges from 5-20% [1].

The age of the nodules to be seen is usually 20 years old and the incidence increases with age. Although nodules with a diameter of more than 1 cm are seen in women about 10 times more than men, the incidence of cancer is approximately 2 times higher than in women when seen in men. Although thyroid nodules are unlikely to have cancer, the mortality rate in differentialized thyroid cancers is extremely low, approximately one in 100,000 deaths. Although very successful clinical results can be obtained in differentiated thyroid cancers, it is very important to follow the thyroid nodules and to put the indications carefully.

Suppression therapy in benign thyroid nodules is controversial. In recent years, the generally preferred approach is to monitor patients with normal hormone levels at certain time intervals without performing nodular suppression therapy which is estimated to be in this structure.

When the histological structure of thyroid nodules is examined, it is seen that most of them are colloid nodules, cystic or solid adenoma, showing different stages of nodule formation and degeneration within the nodular thyroid gland.

The most important etiologic factors in thyroid nodules are shown in Table 1.

Table 1. The most important etiologic factors in thyroid nodules.

Benign Lesions (90-95%)	**Malignant Lesions (5-10%)**
Adenomas (follicular, microfollicular, Hürthle cell)	Primary thyroid cancers:
Colloid nodule	Epithelial cancers: papillar, follicular, anaplastic.

Other reasons:	Other cancers: medullary thyroid cancer,
Chronic lymphocytic thyroiditis (Hashimoto)	primary thyroid lymphoma, other rare cancers.
Real epithelial cysts	Metastatic cancers to the thyroid gland:
infections (bacterial, tbc)	(renal, breast, lung, colon, melanoma and other cancers)
Subacute thyroiditis (de Quervain's thyroiditis)	Infiltrative (sarcoidosis, amlyoidoisis, lipomatosis)

Approximately 30% of thyroid nodules contain solid and cystic components. The incidence of cancer in the existing palpable thyroid nodules for a single or long time is approximately 10% [1]. Approximately 80% of these cancers are papillary thyroid cancer. The second most common is follicular cancers and the least common is anaplastic cancers. Nonepithelial thyroid cancers such as medullary thyroid cancer and thyroid lymphomas are less common.

Although thyroid cancers are common histopathologically in the thyroid nodules (occult carcinoma), it has been demonstrated that only 1 in 200-400 of these have clinically malignant biological behavior. However, since it is not possible to know in advance which thyroid nodule will be biologically malignant, it is even more likely to have cancer as well as large nodules in the thyroid nodules that are less than 1 cm and detected incidentally by USG.

2. Pathogenesis of Thyroid Nodules

2.1.Moleculer pathogenesis of thyroid nodules

In familial types of thyroid cancers, there is generally an autosomal dominant transition. In addition, there are many articles reporting that molecular factors play a role in the pathogenesis of thyroid nodules.

In a study by Paschke et al., It was reported that iodine deficiency or smoking causes oxidative stress in the formation of thyroid nodules, leading to DNA damage and accelerating tumorrogenesis [6].

Genetic mutations play an important role in the formation of thyroid nodules (RAS, BRAF-V600E, PIK3CA, TP53) [7].

These genetic changes lead to progressive irregularities in the signaling pathways and cancer formation. The developments occurring at the

molecular level in the diagnosis of thyroid cancers in recent years have also had important effects on prognosis and treatment.

Führer et al. Reported that telomerase reverse transcriptase (TERT) mutations are frequently seen in advanced thyroid cancers and are a bad prognostic factor [8].

2.2. Iodine Deficiency

Iodine deficiency is the most important factor in the formation of multinodular goiters in the world and concerns about 1.5 billion people[9]**.** However, this rate is decreasing gradually with the studies conducted in countries where iodine deficiency is intensive especially in recent years.

The pathogenesis of nodular goiter formation up to the last 10 years has been explained as the development of diffuse goiter in childhood and adolescence due to the increase in iodine due to iodine deficiency, and subsequent transformation into multinodular goiter.

However, in the last 10 years, this concept has been modified to change that the formation of thyroid nodules is a genetically transmitted disease, occurs as the age progresses, and factors that increase iodine deficiency and other THS stimulation factor accelerate the formation of nodules.

2.3. Radiation

External radiation to the neck in childhood is the most important environmental factor leading to the development of thyroid cancers. The incidence of thyroid cancer was found to be high in people exposed to radioactivity due to the Chernobyl accident in childhood [10].

Especially in childhood, women are more sensitive to external radiation. Approximately 40% of these people develop thyroid nodules after about 20-30 years, and it is observed that approximately 1/3 of them develop cancer as a result of pathological examination.

In the evaluation of nodules, thyroid function tests and TSH measurements are performed first, and determination of hypo or hyperthyroidism is important in terms of differential diagnosis.

Regardless of the results of invasive and non-invasive diagnostic tests, all cases of nodular goiter with clinical risk should be operated immediately. The same protocol should be applied for the nodules found incidentally (incidentaloma).

When fluorodeoxyglucose is used as an image transmitter, diffuse or focal thyroid involvement is frequently seen in all body positron emission tomographs (PET) used in the investigation of cancer in recent years (0.6-1.6%).Although diffuse involvement is generally seen in thyroiditis, opera-

tion is recommended because of the high rate (40%) of malignant cytology observed with FNAB performed in focal involvement.

3. Clinical Evaluation of Thyroid Nodules

The first criterion to be introduced in the clinical approach to thyroid nodules; whether nodules are malignant or not. In this regard, the size of the thyroid gland and the increase in growth rate, whether there is thyroid dysfunction and whether there are symptoms that occur due to the growth of thyroid nodules should be investigated.

Many nodular goiter cases are asymptomatic and detected by chance. However, especially in fast-growing nodular goiter cases, the thyroid nodule may be sensitive. This may be the result of enlargement due to hemorrhage or degeneration in a previously existing adenoma or cancer, or it may be a symptom of aggressive cancers such as anaplastic cancer or lymphoma.

Considering 5485 thyroidectomy cases performed in Ankara University Medical Faculty, thyroid cancer was detected in 433 cases and clinical risk factors for malignancy were revealed in nodular goiters [11]. According to the results of this research, no significant difference was found between the single and multinodular goiters in terms of malignancy risk.

Similar results were reported in a study by Belfıore and his colleagues. On 5637 thyroidectomy cases [12]. Although cancer is seen 4 times more frequently than men, the incidence of cancer in men is 2 times higher than women. Although the age of the occurrence of differentiated thyroid cancers is higher in young people, anaplastic cancers occur more frequently, especially after 70 years of age.

Hamming and his colleagues; divided clinical risks of thyroid nodules in terms of malignancy into three groups as high, medium and low[13]. In 169 thyroid cancer cases, they were also examined by FNAB, and patients were investigated, and 39 of them were detected (23%). It was found that 31 (71%) of the patients with cancer were in the clinically high-risk group.

As a result, it has been revealed that nodular goiter cases with thyroid cancer rarely show signs of malignancy, but surgical treatment should be performed when detected.

Clinical risk factors for malignancy are shown on nodular thyroid patients in Table 2.

Table 2.Clinical risk factors for malignancy on nodular thyroid patients.

Family history of thyroid cancer or thyroid cancer syndrome (MEN2,Werner, Cowden)
Rapid growing, hard nodule fixed to surrounding tissue,
Radiation to the neck area in childhood,
Over 45 years old and male sex,
Vocal cord paralysis, hoarsenes,
Cervical lymphadenopathy

4.Current diagnostic approach in the diagnosis of thyroid nodules

The most important dilemma of thyroid nodules; not to missing cancer cases and not operate the patient unnecessarily. Therefore, the methods used in the diagnosis of thyroid nodules; should be effective[sensitive), not to missing cancer cases(risk free) and

cost/benefit ratio should be low (cost-effective).

Although thyroid nodules are a very common disease, only 5-8% are thyroid cancer [1}.Fine needle aspiration biopsy (FNA) is the most commonly used method in the diagnosis of thyroid nodules today. It is a sensitive and relatively risk free method. However, there are some disadvantages of FNA.

In a study by Pinchot, he said that thyroidectomy should be performed directly in the nodules larger than 4 cm since FNA finds the false negative rate more than 50% [14]. Giles et al. compared the histopathological results of 323 thyroid nodules whose FNA results were benign. False negative diagnosis rate in all nodules; 11.7% , ın nodules between and 3-3.9 cm; 12.8, in nodules larger than 4 cm; 11% , and 4.8% in nodules of 3 cm or less [15].In a study conducted by Raad et al., in 738 cases who detected benign cytology in FNAB, the result of histopathological examination after the operation was found to be false negative in 5% [16]. In a study by Yoon et al., the FNA material from 661 thyroid nodules, he stated that the false negative rate increased to 2% in nodules larger than 3 cm. In the same study, when the cytological findings were compared with histopathological findings, the sensitivity rate; 96.7%, specificity; 85.9%, positive predictive value 76.6%, negative predictive value 98.2%, and accuracy 89.4% [17].

In FNA performed by 4077 nodules in 3767 patients by Choi et al., 16.1% of cases were insufficient cytology and 4.1% undetermined cytological results were obtained. In this study, the most important factor that increased the rate of obtaining insufficient cytological material was not the

diameter of the nodule, but the inexperienced physician performing the biopsy [18]. In a meta-analysis performed by Ospina et al. on 32 studies, the accuracy of the diagnoses were analyzed in the US-led FNAs [19]. The results obtained are explained practically as follows: 8 out of 10 cases with suspicion of malignancy in theultrasound (US) emerged. When FNA was performed in 10 cases where US findings were found benign, this number decreased to 3.Only one in 10 cases with low malignancy suspicion in the US was detected.

The probability of malignancy was found to be less than 1% in cases where FNA performed with US was found to be benign. Follicular cancer was found in 71% of patients with suspected follicular neoplasm in FNA.

In multi-centered papillary thyroid cancers, which is about 20%, there is a possibility of cancer in all nodules. Therefore, biopsy should be taken from all nodules and total or near total thyroidectomy should be preferred in these cases[1]. However, in a study by Kuo, if the tumor size is less than 0.5 cm, there is no change in prognosis compared to single-focus tumors [20].

One of the important problems in FNA is the high rate of cytological findings that cannot be determined.In a retrospective study by Alshaikh et al., cytological examination was performed in FNA made from 681 thyroid nodules.According to the Bethesta classification, 10.1% of the cases were found to be non-diagnostic [21].

In a study conducted by Labourier et al., It was shown that positive predictive values (PPV) can increase up to 97% in 109 cases with indeterminent cytological findings [22]. Duick et al. Stated that 74% of indeterminate nodules are benign in the histopathological examination at the end of the operation, and this rate can be reduced to 7.6% when gene expression classification tests are performed [23].

In a study of 358 thyroidectomy cases, Dedhia et al. revealed that when genetic expression profiling is performed in indeterminate nodules, the rate of diagnostic thyroidectomy can be reduced from 19 % to 7.2 % [24]. When a diagnosis of thyroid nodules is performed, if low risk of malignancy, uncertain cytology or insufficient cytological material is obtained 2 times in FNAB; tests with molecular markers in the tissue can be diagnostic. However, due to the common opinion of the European Thyroid Association and the Association of Clinical Endocrinologists, it is relatively expensive. So they recommend using molecular tests in tissue only in cases that have not been diagnosed with FNAB[25].

Expression of vascular endothelial growth factor (VEGF) increases in tissue in cancer cases. Salajegheh et al. Showed that VEGF A and C increased in 136 thyroid cancer cases [26].In a study by Gong et al. D2-

40, CK19, galactin-3, VEGF and EGFR expression were investigated and found high in 38 papillar thyroid cancer and 12 thyroid papillary hyperplasia cases [27].

In a study by Volirinsky et al. VEGF-C was found to be significantly higher in papillary thyroid cancers [28]. In a study by Luo et al. VEGF –C and D were found to be significantly higher in thyroid cancer cases [29].

Although cases with FNA also rarely occur; tumor formation, metastasis and death can be seen depending on the inoculation of cancer cells along the needle path. In a study by Hayashi et al. thyroid cancer (0.15%) located along the needle pathway as a result of biopsy from thyroid tumor in 22 of 11.745 patients who had FNA, metastasis in 10 cases (33.3%),ın 8 cases, thyroid tumor (0.37) located along the needle pathway from the lymph node needle biopsy was detected [30]. Disease-related death was observed in 4 of these patients. Cumulative tumor cell inoculation risk was found to be 0.34% in 11.745 patients who had FNA. In a study by Nandedkar et al. In India, 20% of the nodules undergoing biopsy reported that approximately 0.1 % of the population had thyroid cancer [31]. Considering that FNA is routinely performed in each thyroid nodule, It can be predicted that; tumor development due to cancer cell inoculation, the risk of metastasis and death may arise in a significant number of patients,

Increased expression of some miRNA types in serum as biomarkers in the diagnosis of thyroid cancers has emerged as one of the most promising noninvasive inexpensive methods.In a study by Rezai et al., With the serum of patients with papillary thyroid cancer,compared miR-222, miR-181a, miR-146a and miR-155-5p expression values in the sera of patients with benign thyroid nodules,found that the difference was significantly higher in papillary thyroid cancer cases [32]. In the study of Zhiyan et al. In 120 PTC, 29 nodular goiter and 131 healthy control groups; miR-346, miR-10a-5p and miR-34a-5p were statistically significantly higher in serum in patients with thyroid cancer [33].

Plasma expressions of 3 types of RNA were monitored dynamically in a study by Zhang et al. and significant overexpression of miR-222, miR-221 and miR-146b in patients with papillary thyroid cancer was determined compared to benign thyroid nodules [34].Therefore the authors stated that these 3 microRNAs may be a new noninvasive biomarker in the diagnosis, prognosis and monitoring of papillary thyroid cancers.

Fan and his friends in a study, serum samples collected from 57 thyroid cancer and other cancer patientsThey measured circMAN1A2 in serum samples taken from 121 healthy people as a control group, found that circMAN1A2 upregulation was significantly higher in cancer patients [35]. They stated that circMAN1A2 may be a new and important biomark-

er in early diagnosis of cancer. In a meta-analysis conducted by Li et al. On the diagnostic value of cir RNA as a biomarker in cancers; 64 single, 13 combined cirRNA analyzes were evaluated, and it was confirmed that combined cirRNA had an important place in cancer diagnosis [36].

In a multi-center study, it has been shown that the sensitivity and specificity rates of these features of nodules in the diagnosis of cancer occur as follows: malignant nodules were a taller than wide shape (sensitivity, 40%; specificity, 91%), a spiculated margin; (sensitivity, 48%; specificity, 92%), marked hypoechogenicity (sensitivity, 41%; specificity, 92%), microcalcification (sensitivity, 44%; specificity, 91%), and macrocalcification (sensitivity, 10%; specificity, 96%) [37].

In a comparative study conducted by Rosario et al with gray scala US (GSUS) and Doppler US (DUS) in 1502 solid thyroid nodules larger than 1 cm; They showed that when DUS was added to GSUS, the sensitivity increased to 89.4%.[38].

In a study by Rezavi et al. 3531 thyroid nodules(927 malignant,2604 benign);echogenicity, calcifications, edge properties, halo formation, image and color Doppler, elasticity and stres ratios were investigated. The respective sensitivities and specificities were as follows: elasticity score, 82% and 82%; strain ratio, 89% and 82%; hypoechogenicity, 78% and 55%; microcalcifications, 50% and 80%; irregular margins, 66% and 81%; absent halo sign, 56% and 57%; nodule vertical development, 46% and 77%; and intranodular vascularization, 40% and 61% [39]. In a study conducted by Cantizati et al, Doppler and strain ratio measurements were performed before operation in 315 indeterminent nodule cases. USG with strain ratio showed 90.6 % sensitivity, 93 % specificity, 82.8 % PPV, 96.4 % NPV, while US score yielded a sensitivity of 52.9 %, specificity of 84.3 %, PPV 55.6 % and NPV 82.9 %. According to the results of their studies, they emphasized that strain ratio findings are important especially in the separation of malignant nodules in indeterminent thyroid nodules [40].

In a study by Horvat et al. validation of the classification of TIRADS (Thyroid Imaging Reporting and Data System) made in 502 nodules was compared with histopathological examination of the surgically resected nodules. Malignancy was 0% (0/116) in TIRADS 2, 1.79% (1/56) in TIRADS 3, 76.13% (185/243) in TIRADS 4 (subgroups: TIRADS 4A 5.88% (1/17), TIRADS 4B 62.82 % (49/78), TIRADS 4C 91.22% (135/148)], and 98.85% (86/87) in TIRADS 5. In cases where FNAB was performed, in TIRADS 4 and 5; sensitivity 99.6% (95% CI: 98.9--100.0), specificity 74.35% (95% CI: 68.7–80.0), PPV 82.1% (95% CI: 78.0–86.3), NPV 99.4% (95% CI: 98.3–100.0), positive likelyhood ratio(PLR) 3.9 (95% CI: 3.6–4.2) and an negative likelyhood ration (NLR) 0.005 (95% CI:

0.003–0.04) for malignancy. According to the results of their study, they said that the TIRADS classification is a good method in the selection of cases requiring biopsy [41].

In a study conducted by Liu et al. In 331 nodules, they performed two-dimensional shear wave elastography (2D-SWE) after routine USG in order to separate malignant and benign nodules. According to the findings they obtained in their work, 2D-SWE, although nodules smaller than 1 cm are not very satisfying, they reported that they are effective in differentiating malignant cases in larger nodules [42].

Chang et al. Evaluated the results obtained in their US examination in 167 thyroid nodules according to ATA, BTA and TIRADS categories. The sensitivity in ATA is 98%, 95% in BTA, ın TIRADS, 94% were found [43].

Li and colleagues conducted a study taking into account the sensitivity and costs of molecular tests, and showed that performing molecular tests in patients with uncertain cytology at FNAB is less costly than performing FNAB every year [44].

Gomez and colleagues reported in a study that total thyroidectomy is more cost -effective than hemithyroidectomy in follicular thyroid cancer [45]. In a study by Bates et al., was reported that only 2 of the 71 cases who had reoperation due to recurrent papillar thyroid cancer had true recurrence, and 69 patients had persistent disease [46].

Considering that the probability of multicentric thyroid cancer is approximately 20% in cases of nodular goiters [20,46,47], performing a total or near total operation in all thyroid nodules at risk of malignancy will decrease the possibility of leaving tumor focus behind by eliminating the possibility of reoperation. Also eliminating the possibility of reoperation will reduce costs.

Molecular diagnostic methods have been placed in the algorithm created by Li et al for the diagnosis of thyroid nodules because it is cost-effective. However, in this algorithm, lobectomy has been suggested for suspected malignancy at many stages [44].

But, since there is a high risk of leaving tumor tissue behind in these cases, reoperation will be required and it will be risky and not cost-effective [45,46,47].

A new algorithm in the diagnosis of thyroid nodules is shown in (Figure 1)

In this algorithm we created for the diagnosis of thyroid nodules;

besides diagnostic methods, by selecting the type of surgeries to be performed, it is aimed to be more sensitive, risk free and cost-effective.

4.1.In nodules larger than 3 cm and in cases of substernal goiter:

The rate of false negative findings increases in nodules larger than 3 cm [14,15] Also, as the size of the nodules increases, the symptoms of compression on the trachea increase. In the study conducted by Eng et al, he was reported that the nodules larger than 1.5 cm had relief in the neck of the patient after thyroidectomy [48]. For these reasons, in patients with thyroid nodules larger than 3 cm and substernal goiter, thyroidectomy should be performed immediately after non invasive diagnostic methods are applied without FNAB (Figure 1).

4.2.In cases of nodular goiter smaller than 3 cm:

When multiple non invasive diagnostic methods are applied in thyroid nodules, sensitivity rates increase [32,33,34,35,36,37,38,39,40,41,42,43].

Therefore, in cases with high risk of malignancy in multipl non invasive diagnostic tests, eliminating the risk of tumor inoculation(0.34%), total or near total thyroidectomy should be performed without FNAB [20,29,30,46,47] (Figure 1).

4.3.In cases of nodular goiter with low risk in noninvasive diagnostic tests:

FNAB should be performed by an experienced specialist [16,17,18,19,20,21,31].When FNAB is performed by experienced specialist, insufficient cytological finding rate has been shown to decrease[18] (Figure 1).

4.4.In cases of noduler goiter with high risk of malignancy in FNAB:

In cases with high suspicion of malignancy in FNAB, total or near total thyroidectomy should be performed [20,46,47]. Because in most of these cases, the histopathological result will be malignant [16,17,18,19,20,21,31]. (Figure 1).

4.5.In cases with benign cytological findings in FNAB:

Periodic follow-up is sufficient every year in cases with benign outcome in FNAB.

The probability of malignangy is very low in these cases with non invasive multiple diagnostic methods and FNAB performed [16,17,18,19, 21,31,32,33,34,35,36,37,38,39,40,41,42,43] (Figure 1).

4.6.In cases with low risk malignancy and uncertain cytological results in FNAB:

When molecular tests, genetic expression tests and vascular endothelial growth factor (VEGF) expression studies were performed in cases with uncertain cytology and in cases with low risk malignancy in FNAB, the rates of diagnosis were increased. In these cases, since the cost of molecular tests and gene tests is high, VEGF tests should be performed first [26,27,28,29]. Total or near total thyroidectomy should be performed in cases with high risk of malignancy [20,46,47]. Molecular and gene tests in tissue should be performed in patients with low risk of malignancy in VEGF tests[22,23,24,25]. As a result of these tests, total or near total thyroidectomy should be performed in patients with high risk of malignancy. Periodic follow-up should be performed in cases with low risk of malignancy. After applying the multiple non invasive and invasive diagnostic methods mentioned above in these cases, the risk of malignancy will be extremely low [22,23,24,25,26,27,28,29] (Figure 1).

4.7.In cases with insufficient cytological results in FNAB:

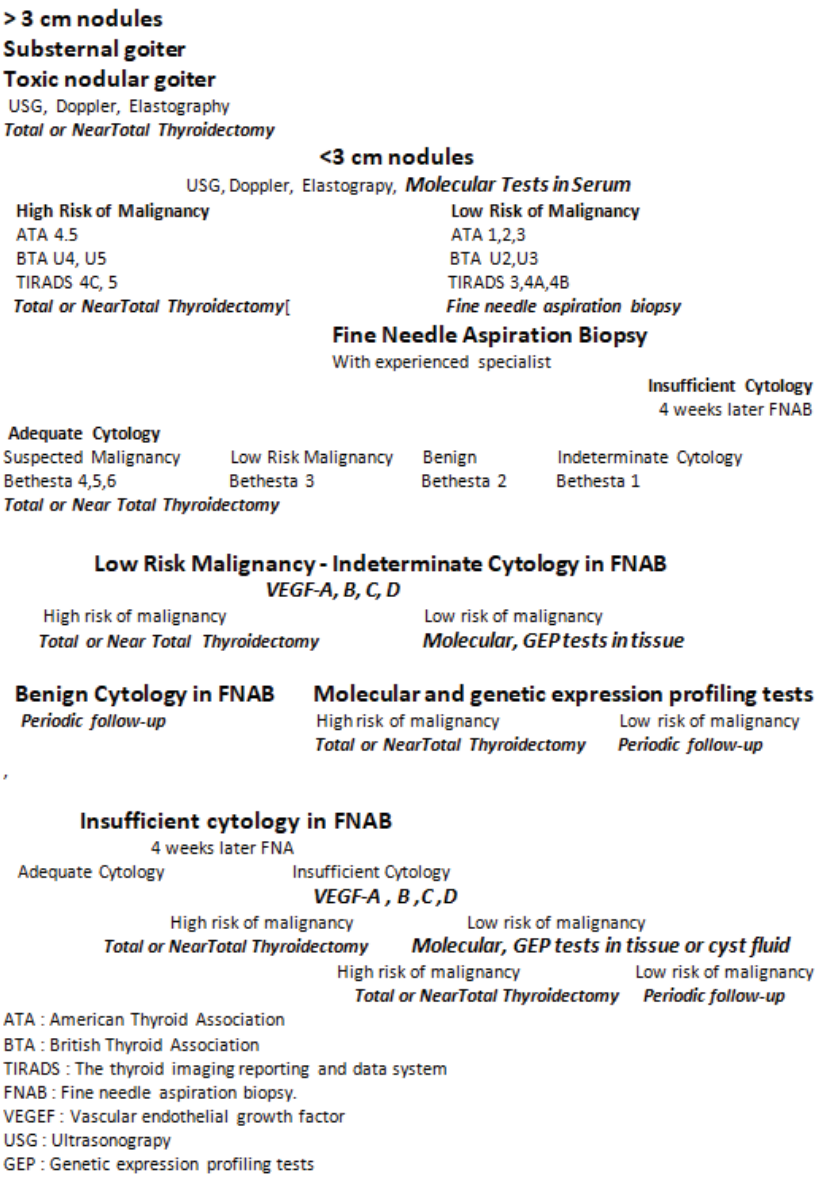
After a 4 week period, FNAB is performed again by experienced specialist under the guidance of US. If the result is undiagnostic or insufficient cytology, VEGF tests should be performed first. Total or near total thyroidectomy should be performed in cases with high risk of malignancy[20,46,47].Molecular and gene tests should be performed in patients with low risk of malignancy in VEGF tests. As a result of these tests, total or near total thyroidectomy should be performed in patients with high risk of malignancy. Periodic follow-up should be performed in cases with low risk of malignancy. After applying the multiple non invasive and invasive diagnostic methods mentioned above in these cases, the risk of malignancy will be extremely low [22,23,24,25,26,27,28,29] (Figure 1).

The difference and advantage of this algorithm over others; in order for the algorithm to be more sensitive, risk free and cost-effective, the most appropriate diagnostic methods are recommended at each stage, as well as which operations should be performed at which stage (Figure 1). Thyroidectomy should be performed after some noninvasive tests in nodular goiter larger than 3 cm and in substernal goiter. Primarily non invasive multiple diagnostic methods should be used in the diagnosis of thyroid nodules less than 3 cm.

FNAB is still present an effective, relatively inexpensive and low-risk method in the diagnosis of thyroid nodules. However, it should not be done in every case of thyroid nodules.

Irrespective of the size of the nodule and the kind of the diagnostic test; when a high risk of malignancy is seen, total or near total thyroidectomy should be performed.

Figure 1. Current algorithm in diagnostic approach to thyroid nodules



ATA : American Thyroid Association
BTA : British Thyroid Association
TIRADS : The thyroid imaging reporting and data system
FNAB : Fine needle aspiration biopsy.
VEGEF : Vascular endothelial growth factor
USG : Ultrasonograpy
GEP : Genetic expression profiling tests

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Chapter 33

USE OF 3D PRINTED MODELS IN DENTAL EDUCTIONS

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INTRODUCTION

The term of 3D printing is used to describe the manufacture of an object from a 3D digital model. The object is built one layer at a time, adding multiple layer to form object. This process is described as additive manufacturing.[1] This technology can basically be divided into four steps: creating a digital 3D model with a software using computed tomography data or intraoral scans, slicing and processing of the 3D model into many 2D layers, printing the 3D product layer by layer, post-processing of the printed object.[2] The most widely additive manufacturing methods include fused deposition modeling (FDM), selective laser sintering (SLS), stereolithography (SLA), bioprinting, and polyjet printing. FDM is the most affordable and the most commonly used 3D printing technology [2,3]

Digital technology is an important part of dental practice in today. Certain dental works can be performed in completely digital workflow such as digital smile design. In the last years, of 3D printing technologies is gaining very rapid potential for dental applications in nearly all dental fields.[4] Especially oral and maxillofacial surgery and prosthodontics and orthodontics, use of 3D printing has increased stunningly. The driving force behind advancement in 3D printing emerges from the possibility of creating individualized products, easily sharing and processing of patient image data and educational improving.[2,4]

In dentistry, technical procedures are an important part of the treatments carried out in dental office. Dental education is important the acquisition of technical skills for daily practice. In dentistry, until now only very simple artificial tooth models have been available for dental students' education in preclinical training. Most of simulation models are typodonts made by KaVo (Biberach, Germany) and Frasaco (Tettnang, Germany). These models have plastic, healthy and straight teeth. Students had to prepare these standardized teeth with no need to prepare them at all. Although models with simulated caries are produced by some industrials, the results are unrealistic. On the other hand, in reality, teeth are often rotated and bended, and have wedge-shaped defects, defective fillings and abrasion.[5,6] Students had no chance to experience different clinic situation these standard models and the learning effect started in the clinical education. Therefore, in a preclinical education, realistic hands-on training is needed to prepare students for their real-life patient. Variations in these models would lead to better diagnostics and improve the decision-making process. Recent advances in 3D printing technologies have enable the emergence educational tools for dental professionals.[7,8]

New 3D printing technology seems to be the most feasible way to develop individual tooth models for various training situations. With the

combination of cone-beam data and 3D scanners, 3D printers offer dental schools for new realistic models for education purpose.[9] The main problem for the creation of 3D printed models for dental training were the printing materials. But, today there are new materials available for 3D printers with more usable properties. Up to now, the use of 3D printed models and teeth in dental education were investigated in different studies in nearly all dental fields.[10,11]

Educational Approaches in Pediatric Dentistry

Marty et al[6] designed and printed a 3D model for pediatric dentistry training from the CT scan of a young patient. This study was the first 3D printed model for pediatric dentistry training. The design of training model was made in accordance with the pedagogical objective of the practical work session. Students were performed a pulpotomy and preparation for a stainless steel pediatric crown on mandibular second molar on both the industrial model and 3D printed model. While the students awarded both models high scores on learning potential and their applicability, the simulation of caries cavities in 3D models was appreciated. In addition, the students reported that 3D models provide a more realistic experience because the proximity makes it possible to understand the need for pulp therapy and provides a real educational advantage.

Educational Approaches in Dental Traumatology

Dental traumatology is mostly taught in theoretical lectures and less in practical course because there is no suitable available model, which offers the possibility of diagnosis and treatment of dental trauma. The students do not experience a real trauma case in the clinic. Accordingly, a correct treatment plan and clinical execution difficult for undergraduate students. Craniofacial traumas such as mandibular fractures and mechanical abnormalities caused by displaced fractures may be difficult to understand for the younger non-specialized students. Inadequate knowledge of newly graduating dentists, diagnosis and outcome of treatment can seriously jeopardize.[3,12,13]

Reymus et al[8] created a realistic model, designed based on the CBCT of the maxilla of a real patient that imitated several traumatic dental injuries (lateral luxation, complex crown fracture, horizontal root fracture and avulsion), for hands-on training using 3D printing technology. The model manufactured using the resin of the manufacturer mixed with barium sulphate powder for achieving radio opacity. In this way, this radiopaque model allowed the simulation of different traumatic dental injuries clinical and radiological evaluation for diagnosis. The model was considered highly realistic and all participants reported that the course offer additional

knowledge about dental traumatology. 97% of participants reported felt better prepared for treating traumatic dental injuries in the future. The authors reported that this resin trauma model can be used in the different treatment stages, for example the placement of Mineral Trioxide Aggregate (MTA) of the application of a titan-trauma splint.[8]

Nicot et al[3] introduced into a low-cost 3D printed model to education of craniofacial trauma for the oral and maxillofacial surgery teaching program of undergraduate students. Zygomatic fracture with an important displacement and two mandibular fractures were created using a Computed Aided Design and Manufacturing. The authors reported that 3D printed haptic model is an efficient teaching support in facial traumatology and randomized controlled trial comparing 3D printed models vs traditional education.

Educational Approaches in Endodontics

3D printed technologies has a very promising role in endodontic training and education. There has been an increasing trend toward replacing existing models (typodont teeth, extracted human teeth, animal teeth and simulated root canals in epoxy resin blocks, for example) with 3D printed tooth models with more realistic anatomical root canal structure.[2] Extracted human teeth have been used for many years to teach endodontic procedures, but, the risk of cross-infection, difficulties in obtaining them, nonstandard anatomy (especially for student assessments) and ethical factors are disadvantages. Many clinicians consider that transparent epoxy root canal simulators provide the visibility required to teach endodontic techniques and understand the biomechanics of root canal shaping. However, this models don't recapitulate a clinical setting (potential anatomical difficulties and pitfalls) and have disadvantages include high cost, limited selection of tooth types and delivery times [14,15]

3D printed models can be used as a teaching aid for dental students to improve understanding of tooth, root canal morphologies, and to simulate endodontic procedures such as access opening, canal shaping and cleaning, and obturation in endodontics.[16,17] The treatment of complicated endodontic cases in surgical and non-surgical endodontics, for example, difficult osteotomy sites, unusual root anatomy and curvature, thick cortical bone covering apical root and proximity to critical anatomical structures like nerves and blood vessels, can be challenging for most operators. These cases are more routinely encountered in specialist practice, but are less frequently performed in public health service systems. This situation may contribute to operator skill erosion and insufficient training opportunities. Therefore, the use of 3D printed models as learning aids may facilitate operator skill development. [2,16]

Robberecht et al[14] developed ceramic shaping technique to reproduce canal systems with standard lengths, diameters and degrees of curvature using a microporous hydroxyapatite-based matrix. Hydroxyapatite is the main mineral component of natural teeth and the aim of this study was to mimic chemical composition, microstructure, hardness and canal anatomy of natural teeth. New root canal model was fabricated by 3D printing (stereolithographic technique) and simple slip casting. Authors reported that anatomic root canal simulator is well suited for training students and conducting research on endodontics.

Reymus et al[15] produced artificial teeth for endodontic education using 3D printing technology to overcome the disadvantages of commercial tooth replicas. Students in the third and fourth years performed root canal treatment on printed molars and were evaluate experience with these compared to real teeth. According to the students, 3D printed replicas were very comparable to real teeth in terms of anatomic design, the preparation of the root canals was easier and 3D printed replicas were fair due to the standardization of the teeth.

Educational Approaches in Orthodontics

3D documentation in orthodontic disorders has been endorsed since the last decades. Therefore, plaster models have now been replaced by digital data and information. This digitalization opens a new horizon of education and research. 3D printed models have been used to relationships between alveolar area and the need for extraction. Additively manufactured dental models obtained with the cone beam computed tomography or intraoral scans of real patient can be used for training removable and fixed orthodontics to dental students.[2,18]

Educational Approaches in Periodontics

3D printing is used periodontology with the focus being on regenerative periodontology in research and 3D printed guides for gingival esthetic defects. In education, dental students have been trained using dental model for periodontal examination, scoring, and indexing procedures. Students often practice directly on patients, due to the limited capacity of the model. Students have found difficulties, leading to patient discomfort such as bleeding and pain as probing the patient for examination.[2,19] Therefore, different tissue components of the periodontium like bone, gingiva, and cementum can be simulated in 3D printed models. Periodontal defects created for training. 3D printed technologies also encourages printing models of patients with gingival esthetic defects to be trained using these models. [2,20]

Educational Approaches in Oral and Maxillofacial Surgery

3D printed anatomic models have been beneficial for diagnosis, pre

surgical planning, acting as a reference during surgery, and in the manufacturing process of custom implants in the field of oral and maxillofacial surgery. Although the training models are used in dental education, there is still a lack of exact training models to prepare a surgeon-in-training for a specific procedure. With the inclusion of additively manufactured anatomic models into the educational system, dental students and practitioners can avail from the progress in 3D printing.[2,21]

Werz et al[21] created new educational materials that directly transfer the practical skills obtained from an exact training replica to a real patient situation for two surgical procedures: the external maxillary sinus lift and the extraction of impacted mandibular third molars. They evaluated whether 3D models can be suitable to train surgical skills to dental students and maxillofacial surgeons using a questionnaire. All of the participants deemed the 3D models suitable or very suitable for surgical education. The results of this inquiry revealed that this methodology is suitable for basic and advanced surgical education.

Lambrecht et al[22] produced three-dimensional individual patient prototype models out of CBCT datasets for basic and advanced dental education. 3D models of segmental piece of mandible and wisdom tooth and the wisdom tooth in close anatomical relationship to the nervus alveolaris inferior demonstrated in the present study. Authors said that creating haptic models were highly suitable for demonstrate complex anatomic situations and instructing students and planning operations.

Hanisch et al[23] developed a method to create more realistic, an individualized 3D-printed surgical training model based on real patient data for root tip resection. Because in the typodont model used by the student to practice root tip resection, a sensitive periodontal ligament was not present, the apical granuloma was simulated in wax and anatomical variations could not be simulated. Students attending the surgical course compared with industrially manufactured typodont models and new 3D-printed surgical training model. The students reported that new model offer a realistic alternative to manufactured typodont models. But, for learning surgical incision and flap formation, models need to be improve.

Seifert et al[24] created 3D printed individualized patient models for oral surgery hands-on course. 4th year dental students practiced different operations (the dissection of a mucoperiosteal flap, the resection of a root tip, the dissection of a free mucosal graft and the osteotomy of impacted third molars) on cadaveric models and on 3D printed patient model and evaluated both models using a questionnaire. 3D printed models received positive feedback from students in a hands-on course. The models were regarded significantly more realistic with regard to the anatomical correctness, the

degree of freedom of movement and the operative simulation.

Educational Approaches in Prosthodontics

In the prosthodontics, computer-aided manufactured physical models on the basis of digital data obtain by intraoral scans of patients are increasingly used. Idealistic plastic typodont used in the training of dental students and professionals are replaced by more real-life 3D printed models.[2] The technique of 3D printing has successfully been used to create models in varied levels of hardness, replicating that of healthy enamel, dentin and caries.[25]

Cantin et al[26] created 3D printable models to learn to idetify the morphological characteristics of permanent teeth. The production process of the models was described in this study. Authors reported that these teeth can be used in addition to or instead of extracted teeth and they are anticipated to represent an improvement over plastic teeth in the teaching of tooth anatomy for dental students.

Kröger et al[5] created 3D printed simulation models based on patient situations for hands on practice. The visible part of patient's teeth, gingiva and teeth's position was acquired using an intraoral scanner. Thus more realistic tooth positions were achieved. Caries-like lesion structure are constructed as cavities by software. These cavities are filled with a softer material imitating demineralized tissue during printing. Three example models were presented: a prosthodontic model for veneer preparation, a conservative model for dental bonding practice and an interdisciplinary model featuring carious teeth and an insufficient crown. Voluntary fourth-year dental students evaluated the third model using a questionnaire. The model received very positive feedback, but some aspect such as the same color of gingiva and simulated teeth were criticized.

Höhne et al[10] planned to create 3D printed teeth model with internal preparation for crown preparation. Because the teaching and learning of a correct crown preparation is difficult for both supervisors and students, the 3D printed teeth was designed for supporting self-education and learning the ideal preparation. Fourth-year dental students evaluated 3D printable tooth using a questionnaire and compared with both a standardized artificial model tooth and a real tooth. The quality of the students' preparation was also evaluated five experienced dentists. The students rated the printed model as significantly better than the standard model. The expert group confirmed the usefulness of this models in dental education.

Höhne et al[7] designed 3D printed teeth with anatomical details such as realistic carious lesion and pulp cavity for use in preclinical education. Caries excavation, direct capping of the pulp, core-build-up, and crown

preparation were done by dental students in preclinical education. The students evaluated 3D printed teeth using a questionnaire and the printed teeth achieved a good result.

Lin et al[27] investigated the students' of using smartphones in their dental education and learning tooth preparation with the individually designed virtual 3D instructional models during the pre-clinical laboratory exercise. 3D model was created by dental laboratory scanner and open-source graphics software to provide instructional materials for learning tooth preparation. Students showed positive perception towards individually designed virtual 3D instructional models.

Boonsiriphant[1] used 3D printed tooth preparations in dental education to assist in teaching and learning of dental students in preclinical fixed prosthodontics course. Ideal full counter tooth preparations are scanned in the digital scan and print with a 3D printer using photopolymer resin. Authors reported that these printed tooth preparations facilitate 3D visualization as teaching and learning tools for prosthodontics courses.

Höhne et al[8] designed and established a 3D printed tooth with distinct layers of different hardness and color for enamel and dentin for education in crown preparation. Thus they thought that this tooth would help students gain experience in difference in hardness and color of a model tooth and would have benefits for education in full coverage crown preparation. Dental students and experienced dentists were trained during hand-on course and the benefits of the 3D printed tooth were evaluated by a questionnaire. The learning effect with 3D tooth model was rated as good on the questionnaire by both expert dentists and students.

Höhne et al[11] designed and printed 3D printable teeth for dentin post preparation. Because the preparation of a decayed tooth for dentin posts is difficult and risky and root perforation may occur. Voluntary pre doctoral dental students were trained in a hands-on course on similar printed teeth. The benefits of the 3D printed tooth were evaluated by the students and were rated as good to very good.

CONCLUSION

3D printing has a high potential for dental education in all the major disciplines of dentistry. The application of these technologies associated with 3D models in dental education is waiting to be exploited, once they have great potential to complement conventional training models in dentistry. New materials are also developed by the vendors with new mechanical and biological properties. This gives an opportunity to have difference shores, elasticities, strength on tooth models. A lot of different training models are possible with the help of 3D printed teeth in different fields.

Furthermore, 3D printed models can be produced at very low cost and we think many dental schools can produce these models at no charge for the students.

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Chapter 34

EVALUATION OF THE EFFECTS OF OZONE THERAPY ON VARIOUS ORGANS AND SYSTEMS

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Ozone was first described by Schönbein in Basel as a separate chemical compound (1). Schönbein, who described this compound that appeared in the cathode during the electrolysis of water as “the odor of electrical matter”, later gave the name ozone, which came from the Greek “ozein” (odorant) (1).

Ozone, which is the most widely known among the people, is trophospheric ozone, and it is known that long-term inhalation of this type of ozone, which is produced as precursor gas by many sources, damages many organs, especially the respiratory system (Figure 1). In addition to children living in contaminated environments, those with chronic airway diseases, smokers and the elderly, the normal population is also adversely affected (2). Ozone taken by inhalation causes chronic oxidative stress in the lungs and leads to the release of large amounts of reactive oxygen species (ROS), toxic lipid oxidation products (LOPs) and proinflammatory cytokines (3). With the transition of these products to the systemic circulation, chronic inflammatory processes can begin in many organs and/or systems (3). Especially these negative effects of ozone, which has a direct relationship between cumulative dose and toxicity, cause some people to suggest that ozone is completely harmful and should not be used for medical purposes.

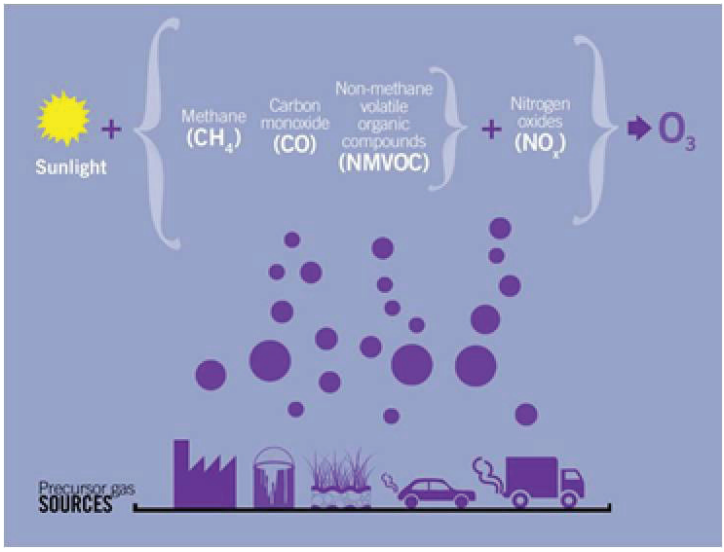

Figure 1: Schematic precursors and formation mechanism of trophospheric ozone (4)

However, when used properly, it has been reported in many studies that ozone has positive medical effects (5-7). It is also suggested that ozone, which is 10 times more soluble than oxygen, is the best water disinfectant to prevent epidemics (8). When we examine the use of ozone in terms of

medical use, we encounter the term “physiological ozone dose” (3). It is known that the basic mechanism of ozone, which is said to act as a real drug by Bocci, triggers acute and precisely calculated oxidative stress that can activate various biological processes (3). In chronic trophospheric ozone inhalation, unlike the toxic ozone dose to which the lungs are exposed, physiological ozone faces the organism with minimal oxidative stress with a series of biological products that ozone causes. This minimal oxidative stress acts as a training camp for the organism and allows the organism to be prepared for possible more intense stresses; therefore it provides less damage.Today, the physiological ozone, that increases the synthesis of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), which provides the organism’s self-protection against oxidative stress, without showing acute and/or chronic toxicity effects created by trophospheric ozone, is among routine medical treatment as complementary treatment.

The method of applying medical ozone produced from pure oxygen passed through the medical ozone generator with a voltage gradient of 5-13 mV is generally parenteral / intravenous (IV) autohemotherapy. However, especially in experimental studies, intraperitoneal application (IP) which is equivalent to IV use, rectal application or intracavitary applications, are among the methods of use. It is also applied as a method of local therapy, in the form of ozonated water or mixed with herbal oils. In autohemotherapy, ozone added to the person’s own blood is mixed gently and then returned to the donor patient as ozone blood. The circulation of blood containing ozone to all organs and systems regulates blood circulation and reoxygenation of ischemic areas is ensured, antioxidant systems are stimulated with low-dose oxidant effect and stimulates the immune system (5). Today, ozone is used medically for chronic osteomyelitis, pleural empyema, persistent fistulized abscesses, infected wounds, bed sores, chronic ulcers and initial gangrene, necrotizing fasciitis, diabetic foot, skin, mouth, vaginal and rectal bacterial and viral infections and burns (5-8). However, it is foreseen that the effects of ozone will be effective outside these routine areas. In this article, possible future uses of ozone in terms of musculoskeletal system, peripheral nervous system, cranial structures, skin, gastrointestinal system and liver will be discussed.

In a study about ozone therapy, which is on the agenda of intradiscal use in the musculoskeletal system, especially in disc hernias, the effectiveness of intradiscal ozone application on pain secondary to disc hernia was evaluated. In this study, which examined the pain scores of 60 cases, it was reported that repetitive applications reduced pain, especially in single level bulging and protrusions (9). In another study on disc hernias, Bonetti et al. showed that the size of herniated discs, that did not change

over time, shrinked and the amount of material protrude to the spinal canal decreased with ozone therapy (10). Although it is a case report, there are publications stating that ozone application may have a complementary effect in the treatment of infection developed in surgical procedures applied to the spine (11). An experimental study for osteomyelitis, which is one of the infectious conditions in the musculoskeletal system, suggests that ozone application may be effective due to its positive effects in terms of inflammation, necrosis and abscess formation, which are also proven histopathologically (12). In rats with experimentally formed gout arthropathy, the decrease in active inflammation of IP ozone application points to the role of ozone therapy in inflammatory processes, not just infectious processes (13). It has been reported that ozone application has positive effects in terms of both physical and mental symptoms in the evaluation of patients who received 24 sessions of ozone therapy in the treatment of fibromyalgia, one of the diseases of the musculoskeletal system of rheumatology (14). It is thought that in the musculoskeletal system, it can find many areas where ozone application can be used effectively in both surgical and inflammatory processes, and ozone therapy will be among the routine treatment protocols by decreasing the symptomatology especially in chronic inflammatory conditions.

The positive effects of ozone application, whose effectiveness is evaluated especially in crush injuries in the peripheral nervous system, are shown in experimental and randomized controlled studies. It has been reported that ozone application in the facial nerve with crush injury in Winstar albino rats contributes to improvement in nerve function and in histopathological findings (15). In crush injury to the sciatic nerve, it has been reported that ozone application with methylprednisolone has positive effects on nerve regeneration (16). Similarly, in another experimental study evaluating acute sciatic nerve damage, administration of ozone at a dose of 0.5 mg/kg after peripheral nerve injury has been shown to reduce myelin and axonal injury (17). In another experimental study related to the peripheral nervous system, 1 mg/kg/day ozone application in the chemical radiculonoritis created in rats suppresses LC3B and Beclin-1 expression, inhibits autophagy and decreases apoptosis by blocking the NF-kB signal pathway (18).When human studies are examined, in a study investigating the effects of local ozone injection in 40 cases in Carpal tunnel syndrome; it was observed that symptoms and pain decreased in the ozone-treated group (19). With this rough literature review, it has been determined that ozone application positively affects the regeneration in peripheral nerves at the histopathological and functional level.

It is noteworthy that ozone application has been evaluated in various diseases and/or conditions in intracranial and extracranial neural tissue

related studies. In one of the studies related to intracranial neural tissue, ozone treatment in neonatal rats has been reported to decrease neuronal apoptosis and improve cognitive function in hypoxic ischemic brain injury (20). In studies on multiple sclerosis (MS), the efficacy of ozone therapy was evaluated with near-infrared spectroscopy (NIRS) as functional and vascular technique, and it was reported that ozone therapy increased brain metabolism and helped to improve the low activity levels in MS patients (21). Again in an experimental study on MS, it was reported that the ozone used with methylprednisolone was more effective than the separate use of the same agents, in MS findings in rats (22). When the experimental studies related to auditory and balance function are examined, it is seen that systemic ozone therapy is effective in the treatment of cellular damage in Cisplatin induced autotoxicity (23), and ozone application decreases the damage significantly in ischemia reperfusion injury in cochlea (24). Similarly, in a study on voice-induced mitochondrial damage and oxidative stress, it was observed that enzymatic and nonenzymatic antioxidant capacity increased in the rat brain and cochlea with ozone therapy (25). In human studies, the antioxidant and vasodilator effects of ozone application in acoustic trauma were evaluated, and it was reported that ozone therapy accelerated the reperation process (26). In 106 cases with sudden sensory-neuronal hearing loss, it was stated that the success of steroid replacement was low, but ozone or hyperbaric oxygen treatments added to the steroid increased the success (27). In a study conducted in 50 patients with peripheral vestibulocochlear syndrome, it was reported that ozone injected 20 sessions from C2-C3 level showed improvement in vertigo %90, hearing loss 80%, tinnitus 65% and nystagmus 100% (28). In Ménière's disease, ozone was found to reduce the frequency and severity of attacks and tinnitus (29). When the eye-related studies, which is described as the extension of the brain, are examined, one study investigating the effects of systemic ozone therapy in 140 cases with dry age related macular degeneration (AMD) suggests that ozone application may be a safe and effective treatment option for high-risk patients with AMD (30). In a study published in 2003, it was stated that the anti-hypoxic effect of ozone had a positive effect on the dynamics of functional retinal activity and that ozone therapy should be monitored by electroretinography (31). Similarly, ozone application has been found to affect retinal electrogenesis positively in total retinal detachment cases (32). In a study conducted to evaluate the place of ozone application in the treatment of uveitis, which can be associated with systemic autoimmune diseases, one of the inflammatory diseases of the eye; systemic ozone application was found to be associated with histologically proven reduced inflammation (33). Although there are many situations open to evaluation for the treatment of intracranial and extracranial lesions/diseases, ozone application alone or as a complementary

therapy in the treatment of hypoxic and/or vasospasm-related conditions, aging, oxidative damage induced diseases and inflammatory lesions, is believed to be useful.

In studies related to the skin, which has the largest surface area, both topical and systemic applications of ozone are included. In a study evaluating the effects of topical ozone application on atopic dermatitis, it was noted that skin exudation decreased and erosion improved after 3-5 days in 60 pediatric cases (34). Another study in which topical ozone therapy was applied was performed in lesions caused by S. Aureus and Methicillin Resistant S. Aureus (MRSA). The infection caused by MRSA observed in the skin with ozone application completely disappeared; in addition, 100% of S. Aureus and MRSA were reported to be eliminated in 1 minute with ozonated water (35). In another study, it was reported that ozone therapy promotes wound healing by a potential induction of vascular endothelial growth factor (VEGF) in digital ulcers, performed in 500 women with systemic sclerosis (36). When experimental studies were evaluated, it was found that the application of ozone in Doxorubicin (DXR) extravasation, which may cause mortality due to skin ulcer and necrosis, decreased the ulcer size significantly (37). In a study that investigated the effectiveness of ozone on maintaining the viability of skin flaps, it was observed that ozone therapy prolonged the flap life span, although histopathologically could not be proven (38). It is thought that ozone application can be used actively in skin diseases by mixing it with ozonized water or certain herbal oils, but studies on the subject are not sufficient.

The location of ozone application in the gastrointestinal tract (GIS) has been mostly investigated by lower GIS studies. In one of the few studies on upper GIS, ozone therapy has been reported to have a preventive effect on the development of fibrosis by reducing tissue damage and increasing antioxidant enzyme activity in the experimentally formed corrosive esophageal injury model (39). In another study, pre-treatment with ozonated sunflower oil in rats showed protective effects on ethanol-induced gastric ulcers (40). Peritonitis is one of the focuses of the subjects studied in the lower GIS publications. In one of these studies, which were planned especially considering the bactericidal and anti-inflammatory effects of ozone application, it was found that ozone therapy also contributes to tissue healing and increases vascularization in colon anastomoses after peritonitis (41). Another study in colon anastomosis after peritonitis demonstrated histologically proven tissue healing (42). One of the factors that adversely affect colonic anastomoses is radiotherapy (RT). The positive effects of ozone therapy on the healing of colon anastomoses after preoperative RT are among the experimentally proven findings (43). In a study of 12 patients with prostate cancer for proctitis and rectal bleeding

caused by radiation, it was found that ozone application reduced toxicity without causing serious side effects (44). When inflammatory processes are examined, it has been stated that ozone therapy has a positive effect in experimentally induced acetic acid-induced acute distal colitis by decreasing inflammation and edema, as well as increasing proliferation and vascularization (45). Similarly, another study reported that the therapeutic effect of ozone therapy in distal colitis is more effective than hyperbaric oxygen therapy (46). Ozone therapy has been found to have a protective effect on ileum by reducing tissue damage and increasing antioxidant enzyme activity in an experimental model of intestinal injury from Methotrexate (MTX) (47). Considering ischemic damage, experimentally, prophylactic ozone application in intestinal ischemia reperfusion injury has been reported to decrease cellular damage in relation to increased antioxidant enzymes and protection of cells from oxidation and inflammation (48). In another study, it was found that administration of naringin with ozone or ozone alone has a protective effect for mesenteric ischemia (49). It should be noted that ozone therapy, which plays a preventive and/or damage-reducing role in the pathophysiological mechanisms of many diseases, experimentally stimulates the intestinal cell cycle in the intestinal epithelium and parenteral ozone application plays an important role in this activity (50).

Studies in which the ozone application is evaluated in liver, which is the laboratory of the organism in mammals, are mostly experimental and toxicity studies have an important place in this field. In one of these studies, alcohol toxicity, which is the most common chronic toxicity in western societies, was evaluated. It has been reported that the application of ozone alone or in combination with selenium leads to a decrease in histopathologically observed damage in alcoholic liver damage, experimentally created in female rats (51). In another study on alcoholic liver injury, ozone and melatonin applications were compared and it was found that ozone therapy had more histopathological effectiveness (52). It has been emphasized that ozone application has positive effects on liver enzymes, as well as necrosis and inflammation, in liver damage induced by acetaminophen, another common toxicity (53). In experimentally induced chronic cadmium toxicity, ozone therapy has been reported to reduce the accumulation of cadmium in the liver and kidney and to slightly decrease the intensity of histopathological findings (54). In a study evaluating ischemia-reperfusion injury in the liver, it was stated that ozone therapy exhibited hepatoprotective effect by stimulating cell regeneration in ozone-treated rats (55). In another experimental study, the effect of ozone on hepatic fibrosis caused by biliary obstruction was evaluated; it was reported that AST and ALT decreased and bilirubin decreased statistically in the ozone applied group. In addition, histopathologically, fibrosis and inflammation were reported

to be significantly lower in the ozone application group (56). In Hydatid cysts caused by Echinococcus Granulosus, one of the most common parasitic diseases of the liver, ozone application has been reported to inactivate parasitic protoscoleces (57). In a human study, the effect of ozone application on HCV ribonucleic acid (HCV RNA) load and liver enzymes in 52 patients with chronic C hepatitis; it has been noted that application significantly improves the clinical symptoms associated with chronic hepatitis C, and that ozone causes the loss of HCV RNA from serum (for HCV RNA and PCR) in 25-45% of patients with chronic hepatitis C (58). In light of all these studies, it is predicted that ozone application will be routinely used in the future in many diseases of the liver, including toxicity, inflammation, infection, fibrosis and ischemia.

Considering the experimental and randomized controlled studies presented above, it is thought that the effects of ozone's multipotential effects on many diseases and / or lesions will be detected and their pathophysiological mechanisms will be explained. This article, which contains a very small part of the iceberg, has been prepared to create an idea of the future of ozone application.

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Chapter 35

THE RELATIONSHIP BETWEEN SLEEP PROBLEMS IN ADOLESCENTS AND THE USE OF ELECTRONIC MEDIA AND THE PERCEIVED STRESS

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Introduction

Adolecents have grown in technology area today. Mobile media has been developed day by day and teenagers can use many electronic devices at the same time. Thus, adolecents spend more time on the social media. According to the results of the household information technology usage survey, 83.8% of households had access to internet from home in April 2018, and this rate was 80.7% in the same month of 2017 [1]. Between 2009 and 2014, there was a need for media that increased by 1 hour 17 minutes per day for five years and the average use of electronic media in adolescents aged 8-18 years within this five years was 39% to 66% and use of cell phone increased from 18% to 76% [2].

According to data from TSI, Internet usage in Turkey in 2016, 56.6%, 66.8% in 2017, while in 2018 showed an increase of 72.9% [1]. When the literature is examined, it is seen that the frequency of the use of electronic media is mostly in the 11-18 age group and the use in the 0-6 age group is lower than the adolescent group [8]. Adolescents use electronic media to identify new individuals who are likely to enter their world, based on existing friendship or special interpersonal relationships in their social lives. Adocelents use new communication devices for improving their friendship environment [3,4]. These wider social networks can be used in a positive way to alleviate the burden of social anxiety against the society, as well as serve for negative purposes such as excessive use of sleep adversely affecting sleep quality, and increase in depression and anxiety levels [5]. When the aims of adolescents to use electronic media are examined, it is seen that electronic media use is the most for social media [6]. One of the negative side of the use of electronic media is excessive and problematic use. Although researchers did not think it was a real addiction, it was found that adolescence was affected in many ways [5]. Some results of excessive use of electronic media are internet addiction, greater use than intended, intensive use, inability to reduce use, disruption of school assignments, deterioration of interpersonal relationships, incompatibility with the environment, poor sleep quality, and increased anxiety and depression symptoms [7]. As seen that young people who use electronic media more often have problems such as education, family, physical health, depression, accumulated internet spending and substance use [5].

The Effect of Electronic Media Usage on Sleep Quality in Adolescent Period

Meaning of sleep quality is that you feel energic , positive and ready for the day [9]. . Watching tv, playing video games , and use of competer until late hours cause less sleeping and bad sleeping habits on adolecents and adults. In studies conducted in preschool children, not only the use of elec-

tronic media in the evening, but also video games cause deterioration of sleep quality and decrease sleep time [3]. Electronic media is an environment in which adolescents perceive themselves as a world that cannot be controlled by their environment, where an individual can express himself freely, cannot be intervened by anyone and has no sense of responsibility [10]. insomnia causes a decrease in daytime performance and negatively affects physical activities and daily life events and sleep quality on adolescents and adults [11]. In recent years, when the literature is examined, experiences think that the use of electronic media in adolescents should be avoided considering that poor sleep quality causes sleep habits [12]. There are many observational studies using cross-sectional or prospective approaches. In the studies, the connections between the use of electronic media (television, computer, video games, mobile devices, etc.) and various sleep parameters were examined. More than 90% of these studies found that children and adolescents with delayed bedtime had shorter sleep periods and spent more time in electronic media. The possible situation of this basis of observed relationship is as follows; time is displacement; The time spent in electronic media is in place of the time spent doing other things, including sleep [14,15]. In another study, it was observed that individuals with more than three electronic devices in their rooms sleep 45 minutes less than those without electronic devices in their rooms [16]. Many electronic media devices emit bright light in the dark, preventing sleep from starting and continuing. Moreover, as known that the increase in the use of electronic media is often associated with a decrease in physical activity [17]. In a study conducted with adolescents in the literature, inadequate sleep was caused by biological, psychosocial and environmental reasons, and among the environmental reasons, the non-controlled habit of watching television, mobile devices, computer and game consoles were listed [13]. Increasing use of the internet in recent years has led to a rapid increase in the number of publications and case reports that some individuals have reached a dependency dimension rather than the habit of using the internet. For this reason, researchers indicated that internet addiction is a behavioral disorder [18]. Sleep disorders are an important public health problem that affects the body's health, mental health negatively, and can cause decrease in academic success and work efficiency and increase in accidents [19,20]. The sleep problems change according to community and age of the group. Its percentage can be changeable between %5 and %71 [21]. Although it has been observed that sleep problems and physical, social and psychosocial effects of insomnia have been evaluated in adolescents, the relationship between insomnia and electronic media usage in adolescents has not been discussed in detail; prepared the ground. Through all this information, the research was carried out in descriptive and relationship seeker types in order to determine the connection between sleep problems seen in adolescents with the use of electronic media and perceived stress.

Methods

Aim and Design of The Research

The aim of this study is to investigate the relationship between views and sleep problems in adolescents with the use of electronic media and perceived stress. The research was planned in a descriptive and relationship seeking design. This study was conducted with 1380 high school students studying in public and private high schools in Düzce (excluding special education application centers and vocational education center schools). This research was supported by Düzce University BAP- Scientific Research Project numbered 2018.16.01.874.

In this study;

Determination of sleep hygiene in adolescents

Determination of the incidence of sleep problems in adolescents

Investigation of the relationship between sleep problems and the use of electronic media

to evaluate the relationship between sleep problems and perceived stress were proposed.

The study was done in between 13 and18 ages adolescents who, study in high schools in Düzce. The research was done between March 2018 and July 2018. Schools were divided into four categories as Private high schools, Straight high schools, Anatolian and Science high schools. The schools were selected and determined by random sampling method considering these four categorical distinctions.

Data Collection Tools

In the study, a questionnaire including socio-demographic characteristics, electronic media use questionnaire, sleep hygiene questionnaire, Pittsburgh Sleep Scale (PSQI) and Perceived Stress Scale (ASQ) were used as data collection tools.

Sociodemographic Characteristics Survey

A total of ten questions are asked in the form of sociodemographic characteristics, which will provide preliminary information about the person as a data collection tool, and the person's age, gender, class, economic status, type of school he reads, the presence of chronic illness and the use of drugs for this purpose are questioned.

Electronic Media Usage Survey

The electronic media survey examines how many hours a person is interested in electronic media before he goes to bed, how many hours he

spends with the electronic media device available at home, and for what purpose he uses the electronic media.

Sleep Hygiene Survey

In the sleep hygiene questionnaire, there were fifteen questions in total, and the total sleep time, whether there was any routine sleep habits, the total hours of sleep during the week and weekend, the time of going to bed at night, whether or not having caffeinated beverages during bedtime, and so on. questions were asked.

Calculation of Pittsburg Sleep Quality Index (PSQI)

PSQI is an index created by Buysse and Friends in 1989 [22]. . PSQI provides reliable, valid and standardized measurement of sleep quality. Thus, sufficient distinction can be made between healthy sleepers and bad sleepers. PSQI is an easy-to-use index for individuals and an easy-to-interpret index for clinicians. PSQI evaluates sleep quality in the last month. The Turkish validity and reliability of the scale was made by Agargun et al [23]. The Pittsburgh Sleep Quality Index is a questionnaire that evaluates sleep quality with seven main questions: subjective sleep quality, sleep latency, duration of sleep, habitual sleep activity, sleep disorders, sleep medication use, and daytime functions [24]. Questions are given a score of 0-3, high scores reflect poor sleep quality. Each of the seven main headings is previously evaluated within itself. Then the scores of the seven components are added. If the total score is 5 or above, it is considered as poor sleep quality. The Cronbach alpha coefficient of the Turkish validity of this scale was found to be 0.80 [25]. . In this study, the cronbach's alpha value calculated for the PSQI scale was 0.683. PSQI has seven components. These; Subjective sleep quality (component 1), Sleep latency (component 2), Sleep time (component 3), Conventional sleep activity (component 4), Sleep disorder (component 5), Sleep medication use (component 6), Daytime dysfunction (component) 7).

Perceived Stress Scale

In order to evaluate how stressful some conditions are in one's life, Cohen et al. (1983) was developed by Yerlikaya and Inanc (2007). The questions in the scale are aimed to evaluate the changes in the mood of the participants in the last month. The scale is a 5-point likert type (0: None, 1: Nearly None, 2: Sometimes, 3: Frequently, 4: Very often). The 4th, 5th, 7th and 8th questions in the scale are coded by reversing them. The total score to be taken from the scale is between 0-40. The higher the total score is meaning that the higher the stress. In this study, a 10-question form of the scale was used. Average answer time is 8-10 minutes. The internal consistency alpha coefficient calculated to test the reliability of the scale was

found to be 0.84 [26]. The cronbach's alpha value of the perceived stress scale calculated in our study was 0.735.

Evaluation of Data

Descriptive statistics of all data in the study were calculated. The evaluation of the questionnaires was made by entering the data into SPSS 23 (Statistical Package for Social Sciences) program on the computer and the data were summarized with mean, standard deviation, percentages, median and minimum-maximum. In order to compare continuous data, Independent Samples T Test and One Way Anova tests were used depending on the number of groups. Appropriate cross-table statistics were calculated in categorical data analysis.Pearson correlation analysis was used to investigate the relationships between continuous variables. Statistical analyzes were performed using SPSS 23 program, $p < 0.05$ was considered statistically significant. At the stage of data collection, questionnaires were completed on voluntary basis with permission from adolescents. Schools were evaluated in 4 groups as Science High School, Private High School, Anatolian High School and Vocational High School. It took 10-15 minutes for each adolescent to complete the questionnaire, and the questionnaires were checked upon receipt. Questionnaires with unanswered questions were filled with care.

Ethical Aspects of Research

In order to do the research, approval of the ethics committee dated 19.02.2018 and numbered 2018-34 from Düzce University Faculty of Medicine Non-Interventional Health Research Ethics Committee and permission of official institution from Düzce Provincial Directorate of National Education (Governorship dated 21/03/2018 and numbered 5882229) Gains). The disclosure principle for surveys was followed and it was stated that the research expenses would not be collected to adolescents or affiliated institutions and personal information would be kept confidential.

Limitations of Research

The findings of the research are limited to the adolescents who were educated in the schools of the Ministry of National Education in Duzce.

RESULTS

Table 1. Distribution of Adolescents by Descriptive Characteristics (N = 1380)

Characterist		n	%
Age	13-14	46	3.3
	15-16	879	63.7
	17-18	455	33.0
Gender	Female	542	39.3
	Male	838	60.7
Class	9th class	347	25.1
	10th class	349	25.3
	11th class	317	23.0
	12th class	367	26.6
Grades	First Honour	610	44.2
	Second Honour	426	30.9
	Passed	317	23.0
	Fail	27	2.0
School	Anatolian High School	350	25.4
	Science High School	331	24.0
	Technical High School	350	25.4
	Private High School	349	25.3
Family type	Small	1134	82.2
	Large	246	17.8
Ekonomic Situation	Good	524	38.0
	Middle	833	60.4
	Bad	23	1.7
Members of Family in the house	1-3	246	17.8
	4 and more	1134	82.2
Adolecent has their own room	yes	1146	83.0
	no	234	17.0
Adolecent has cronic ilness	yes	29	2.1
	no	1351	97.9

Adolecents use medicine	yes	23	1.7
	no	1357	98.3
Total		1380	100

The sociodemographic and some characteristics of the adolescents included in the study, such as age, gender, class, school success status and school, are presented in Table 4.1. According to the data, a total of 1380 adolescents participated in the study. According to age characteristics, 3.3% (46) of the people are in the 13-14 age group, 63.7% (879) are in the 15-16 age group and 33% (455) are in the 17-18 age group. In the distribution of adolescents according to gender, 39.3% (542) of them are female and 60.7% (838) of them are male. There are 25.1% (347) students in the 9th grade, 25.3% (349) in the 10th grade, 23.0% (317) in the 11th grade and 26.6% (367) in the 12th grade. When the distribution according to school success is examined, 44.2% (610) received appreciation certificate, 30.9% (426) received appreciation certificate, 23% (317) was only valid, 2% (27) was unsuccessful in degrees. It was determined that 25.4% (350) of them were educated in Anadolu High School, 24% (331) of them were in Science High School, 25.4% (350) of them were in Vocational High School and 25.3% (349) of them were in Private High School. It was seen that 82.2% (1134) of them lived in nuclear family and 17.8% (246) of them lived in extended family. It was found that 38% (524) of the families had good economic status, 60.4% (833) had moderate economic status of their family and 1.7% (23) had poor economic status. Among the adolescents, 17.8% (246) had one to three people living in their families, 82.2% (1134) had four or more people living in their families. It was determined that 83% (1146) of the adolescents had their own room and 17% (234) had no room of their own. When the chronic disease and drug use were examined, it was seen that 2.1% had chronic disease and 1.7% (23) of them were using drugs, while 97.9% had no chronic disease and 98.3% had no drug use. did not use.

Table 2. Mean of Sub-dimensions of PSQI Scale

PSQI Bottom Demention	Average	Standart deviation	Minimum	Maximum
Component1:Subjective Sleep Quality	1.20	0.70	0.00	3.00
Component 2 : sleep latency	1.10	0.82	0.00	3.00
Component 3: Sleep time	0.95	0.99	0.00	3.00
Component 4: Convential Sleep Activity	0.04	0.20	0.00	2.00
Component5: Sleeping disorder	1.35	0.69	0.00	3.00
Component6:Use of Sleeping pills	0.20	0.67	0.00	3.00
Component 7 Daytime Dysfunction	1.35	0.99	0.00	3.00

When the averages of the subcomponents of the PSQI were examined, the mean subjective sleep quality was 1.20 ± 0.70, the mean sleep latency was 1.10 ± 0.82, the mean duration of sleep was 0.95 ± 0.99, and the mean sleep activity was 0.04 ± 0, 20, mean sleep disorder 1.35 ± 0.69, mean sleeping drug use 0.20 ± 0.67, daytime function

The mean of the disorder is 1.35 ± 0.99. Since the mean scores of the PSQI components in this study were 6.43 in total (poor sleep quality of 5 points or more), the sleep quality of adolescents was found to be poor.

Table 3. Comparison of PSQI Mean Means by Adolescents' Answers to Electronic Media Use Questionnaire

Charecteristic		PSQI			
		n	Average	Standart Deviation	P
Total electronic media usage time during weekdays	2 hours and less	404	6.19	2.96	<0.001#
	2-3 hours	482	5.99	3.07	
	4-5 hours	313	6.83	3.01	
	5 hours and more	181	7.48	3.62	
Usage internet time in a day	2 hours and les	479	6.18	2.91	0.001#
	2-3 hours	464	6.09	3.10	
	4-5 hours	261	6.89	3.15	
	5 hours and more	176	7.36	3.60	
Aim of usage electronic media	research	157	6.40	3.40	<0.533*
	game	230	6.17	3.32	
	chat	190	6.55	2.93	
	Social media	803	6.49	3.09	
Total usage electronic media time in weeekdays	2 hours and less	381	5.92	2.96	<0.001*
	3-5 hours	459	6.54	3.14	
	6-8 hours	196	6.33	3.05	
	8 hours and more	344	6.92	3.32	
How long before stopped using electronic media	2 hours and less	1182	6.40	3.09	<0.050*
	3 hours	113	6.42	3.29	
	4 hours	28	5.79	3.47	
	5 hours and more	57	7.49	3.68	
Having electronic device in sleeping room	yes	1102	6.54	3.10	0.011&
	no	278	6.00	3.26	
Turning off electronic device before sleeping situation	I turn off	339	6.10	3.36	0.098*
	Sleeping mood	139	6.26	3.00	
	Taking soudness mood	355	6.61	3.03	
	Turning on	547	6.56	3.10	
Using a phone alarm as an alarm in the morning status	Yes	895	6.63	3.14	0.002&
	No	485	6.07	3.12	

(*:Anova, #:Welch Test,&:Indipendent Samples T,test)

In Table 3, the PSQI mean scores of adolescents were compared according to their answers to the questions of electronic media use questionnaire. Daily electronic media usage averages are 6,19 ± 2,96 for those who

use two hours or less, 5,99 ± 3,07 for those who use two or three hours, 6.83 ± 3,01, five for those who use four or five hours. and those who use more than one hour is 7.48 ± 3.62. It was determined that the higher the use of electronic media received by users who use a maximum of four hours or more on average, the more the sleep quality was affected and the difference was significant (p <0.001).

The average time spent on the internet for the day was 6,18 ± 2,91 for those with two hours and less, 6.09 ± 3,10 for those with two-three hours, 6,89 ± 3,15, and 5 hours for those with four-five hours. the average of those who are more than 7.36 ± 3.60. It was found that the duration of daily internet usage was similar to the electronic media and it affected the sleep quality as the duration of use increased (p = 0.001).

When evaluated according to the purpose of use of electronic media, the average of those who use it for research is 6,40 ± 3,40, 6,17 ± 3,32 for playing games, 6,55 ± 2,93 for chat, and 5,53 ± 2,93 for social media. and the mean value was 6,49 ± 3,09. According to the purpose of use, it was determined that those who spend time on social media and chat were the most average (p <0.533).

When the total media usage time averages are examined during the weekday, the average of those who use two hours or less is 5.92 ± 2.96, the average of those who use three-five hours is 6.54 ± 3.14, and the average of those who use six or eight hours is 6.33 ±. It was found that the average of those who use 3.05, eight hours and more is 6.92 ± 3.32, and the maximum is composed of those who use electronic media for eight hours and more. As the duration of electronic media usage increased during the week, sleep quality deteriorated and there was a significant difference between them (p <0.001).

The average PSQI score of the adolescents who stopped using electronic media until two hours and before bedtime was 6.40 ± 3.09, those who stopped up to three hours were 6.42 ± 3.29, and those who stopped up to four hours were 5.79 ± 3.47, five hours or more. mean value of the patients was 7.49 ± 3.68 (p = 0.050). The mean PSQI score of the adolescents who had electronic devices in the sleeping room was 6.54 ± 3.10, and more than half of the adolescents had electronic devices in the sleeping room (p = 0.011).

When the average time spent in bed before sleep is examined, the average of those who stay awake for half an hour before bed is 5.77 ± 2.94, the average of those who stay awake for one hour is 7.07 ± 3.13, the average of those who stay awake for two hours is 7.58 ± 2.92, the average of those who remained awake for two hours or more was 8.17 ± 4.01. When the adolescents were awake in bed before sleeping, the highest mean score

was found to be more than two hours. At least those who remained awake were awake for half an hour. As a result, the most ideal time spent awake before sleeping in bed was found to be half an hour ($p < 0.001$).

When adolescents were compared to postpone their sleep hours to spend more time in electronic media, the average of those who postponed their sleep hours was found to be 7.06 ± 3.14. Therefore, it was found that adolescents postponed their sleep hours later to spend more time in electronic media and in this case, it affected sleep quality adversely ($p < 0.001$).

Table 4. Comparison of PSQI Mean Means by Adolescents' Answers to Electronic Media Use Questionnaire

Charecteristic		PSQI			
		n	Average	Standart Deviation	P
Spending time on the internet via phone, computer, tablet, 2 hours before going to sleep	Always	383	7.12	3.27	<0.001#
	Mostly	495	6.42	3.19	
	Sometimes	304	5.95	2.56	
	Rarely	133	5.76	3.13	
	Never	65	6.08	3.77	
Prefer one device in electronic media use	Always	367	6.63	3.15	0.560*
	Mostly	427	6.33	3.08	
	Sometimes	250	6.48	3.08	
	Rarely	166	6.20	3.01	
	Never	170	6.43	3.49	
Prefer two devices in the use of electronic media	Always	126	7.63	3.59	<0.001#
	Mostly	188	6.58	3.14	
	Sometimes	278	6.74	3.06	
	Rarely	366	6.39	2.86	
	Never	422	5.85	3.17	

Prefer three devices in the use of electronic media	Always	54	7.56	3.52	0.001#
	Mostly	84	7.64	3.94	
	Sometimes	143	6.71	3.19	
	Rarely	196	6.49	2.84	
	Never	903	6.20	3.05	
Prefer four devices in the use of electronic media	Always	44	7.55	4.01	0.001#
	Mostly	47	8.23	3.98	
	Sometimes	96	6.69	3.37	
	Rarely	93	7.15	3.65	
	Never	1100	6.23	2.95	

(*:Anova #:Welch Test)

In Table 4, according to the answers given by adolescents to the questions of electronic media usage questionnaire, PUKI score averages were compared. In the table, there are averages of those who spend time on the internet via phone, computer, tablet two hours before bedtime, averages of those who use one device in electronic media, two devices at the same time, three devices at the same time and four devices at the same time. The average score of adolescents who spend time on the internet via phone, computer, tablet almost two hours before going to sleep is 7.12 ± 3.27, and the average of those who spend time is 6.42 ± 3.19. It has been determined that spending time with electronic media tools before sleep has a bad effect on sleep quality and there is a significant difference between them ($p < 0.001$).

The average of those who prefer only one device in electronic media use is almost always 6.63 ± 3.15, the average of those who prefer two devices is 7.63 ± 3.59, 7.56 ± 3.52 for three devices, and four devices at the same time. It was found that the average of those who preferred was 7.55 ± 4.01 and it was found that the use of multiple devices (using two or three or four electronic media tools at the same time) negatively affect the sleep quality and there is a significant difference between them ($p = 0.001$).

Table 5. Comparison of Perceived Stress Scale with Mean and PSQI Mean Scores

	Average	Standart deviation	Minimum	Maximum
Mean percieved stress scale score	20.43	6.61	0.00	40.00
PSQI average score	6.43	3.14	0.00	19.00
The total mean score of the perceived stress scale was 20.43 ± 6.61, and the mean total score of the PSQI was 6.43 ± 3.14. The average score was <5.0 = 29.7% (410 students) and ≥5.0 = 70.3% (970 students). The perceived stress level of adolescents was found to be moderate (20.43 ± 6.61)				

PSQI Components	Perceived Stress Scale Average Score	
	r	p
Component 1st Subective sleep quality	0.286	<0.001
Component 2nd Sleep Latency	0.231	<0.001
Component 3rd Sleep Time	0.031	0.254
Component 4th Conventional Sleep activity	0.091	<0.001
Component 5th Sleeping Disorder	0.250	<0.001
Component 6th Usage sleeping pill	0.054	0.044
Component 7th Daytime Dysfunction	0.355	<0.001
Total PSQI Score Average	0.341	<0.001

When Table 5 was examined, it was found that there was a positive weak correlation between perceived stress, subjective sleep quality, sleep latency, habitual sleep activity, sleep disturbance and daytime dysfunction among the PSQI components. Subjective sleep quality, sleep latency, habitual sleep activity, sleep disturbance and daytime dysfunction affect the perceived stress level. While there was a weak negative correlation between sleep medication use and perceived stress, no correlation was found between sleep duration and perceived stress. In order to determine the connection between sleep problems seen in adolescents with the use of electronic media and perceived stress, the following results were obtained in a descriptive study.Turkey in the majority of adolescent sleep quality is poor. It was determined that the majority of the students included in the

study had electronic devices in the sleeping room and this situation had a negative effect on sleep quality and duration. It has been found that as the duration of daily internet usage increases, sleep quality of students is adversely affected. It was concluded that the discontinuation of the use of electronic media four hours before sleep had a positive effect on sleep quality, that adolescents postponed their sleep hours in order to spend more time in electronic media and this situation had a negative effect on sleep quality. Adolescents were using a multimedia device until two hours before sleep and this caused poor sleep quality. It was evaluated that the stress level of adolescents who spend more time in electronic media is higher and the stress level of adolescents which delay the time of going to bed using electronic media tool and cause shortening of sleep time was reached. It is important to inform adolescents about how the use of electronic media affects sleep quality, to provide training on stress management and to gain healthy living habits. It is recommended that interventions involving school-based interventions to address individual, familial and societal risk factors should be planned, and that researchers should carry out studies with control groups showing the effectiveness of these interventions because of the many etiological factors affecting sleep quality deterioration

Discussion

The use of electronic media is increasing in children and adocent period day by day and technologies play an important role in their lives. Recent studies have shown that 97% of adolescents have at least one electronic device and use of these devices is at the highest level and affects adolescents' sleep duration and sleep quality in a negative way [27]. Since daily life and long-term use have a detrimental effect on sleep quality, studies have shown that the use of electronic media, especially social media addiction, is related to sleep habits[28]. In this study, it was determined that 35.8% of adolescents had four to five hours of daily use of electronic media. In addition to the use of electronic devices, the presence of adolescents in their rooms and the location in which they leave them before going to sleep are highly effective on sleep quality and duration. 79.9% of the adolescents sleep in the room was found to have electronic devices. Similar to our study, Excelmans et al. Found that 60% of adolescents had mobile phones as electronic devices in their sleeping rooms[29]. Lange et al. (2017) study on the use of electronic media in 7533 adolescents living in Germany and its effect on sleep quality. Sleep quality was found to be adversely affected as the duration of use increased ($p < 0.05$) and is similar to our study[30]. Approximately half (49.7%) of the adolescents who participated in this study stated that they spend hours on electronic devices for only a few minutes of work and more than half of adolescents postpone their sleep hours

to spend more time on electronic media (52.4%). Despite the increased need for sleep during adolescence, total daily sleep time is shortened [31]. In the studies, it was found that adolescents connected to the internet not only by mobile phone, but also by computer, tablet, etc… they used two and more electronic media at the same time. In this study, it is observed that almost half of the adolescents (63.7%) spend time on the internet with more than one electronic media (phone, computer, tablet, etc.) two hours before bedtime. respectively. Winneke A. Van der Schuur et al. (2018) reported that adolescents prefer to use more than one electronic media device instead of single device and that the quality of sleep is affected worse in television, computer and computer. , mobile phone and adolescents mostly whatsapp, snapchat to communicate with using, usually use of electronic media to listen to music, it is determined that girls use electronic media more than boys [32]. When the mean scores of the PSQI subscales were examined (1. subjective sleep quality, 2. sleep latency, 3. sleep duration, 4. habitual sleep activity, 5. sleep disorder, 6. sleep medication use, 7. daytime dysfunction) total average scores of the components In our study, it was found that the sleep quality of adolescents was poor, because it was 6.43 (total 5 points and above) (Table 2). In this study, it was determined that 70% of adolescents had poor sleep quality and 30% had good sleep quality. In the study of Tekeli S. (2009), sleep quality of all adolescents according to PSQI was evaluated as good sleep at 28.2% (n = 98) and bad sleep at 71.8% (n = 250) [33]. In the study conducted by Aysan et al. (2014) on sleep quality in university students, the mean PSQI score was 6.15 ± 1.90 and the rate of students with a mean sleep quality score greater than 5 was 59%. Mean distributions of PSQI sub-components were examined; 1.12 ± 0.62 for subjective sleep quality, 1.1 ± 0.85 for sleep latency, 1.45 ± 0.7 for sleep duration, 0.14 ± for conventional sleep activity 0,45, 1,19 ± 0,42 in "sleep disorder 0,2, 0,25 ± 0,55 in"sleep medication use ve and 0,91 ± 0,45 in "day dysfunction ve. poor sleep quality of students [35]. Shochat (2014), in his study on the use of electronic media and sleep quality in Israeli adolescents, constituted more than 50% of the adolescents who sleep between 23:00 and 01:45, and that the average use of electronic media during the week and at the weekend was 10 hours or more. late night stay and long stay in electronic media have a negative effect on sleep duration and sleep quality and impaired sleep quality of adolescents [34].

At the same time, it was determined that the majority of adolescents were interested in telephone (19.8%) compared to tablets (2.4%) two hours before bedtime. Schweizer (2017), in his study, found that those who have smartphones had significant sleep problems especially in school days and the result is similar to our study [36]. Lemola et al. (2015) were asked questions about how the use of telephone, internet, television, playing and

chatting affects the sleep quality of adolescents before going to sleep. negative effects, causing depressive symptoms, and the literature was found to support and support the findings of our study [39]. Poor sleep quality and insomnia will cause tension during the day, increase stress levels and cause difficulties in daily activities [37]. In this study, the perceived stress scale average score of adolescents was evaluated. The total score average of perceived stress scale was 20.43 ± 6.61. According to the results, the perceived stress level of adolescents was found to be moderate (20.43 ± 6.61). When the perceived stress levels according to PSQI subscales were compared, there was no significant relationship between sleep duration and sleep drug use and stress levels in adolescents ($p = 0.254$, $p = 0.044$). however, there was a negative relationship between daytime dysfunction and stress levels (Table 5; $p < 0.001$). When the effects of electronic media usage on adolescents' stress levels were examined, it was found that there was a significant difference between the time spent on the internet, the duration of daily electronic media usage and the purpose of using electronic media negatively. Daily electronic media usage time was more than five hours and it was found that the average of chatting and spending time in social media was higher than the researching and the stress scores of adolescents were higher ($p < 0.001$). When the results were evaluated, it was found that adolescents with high stress levels spent more time on electronic media, chatted more and spent time on social media. In the study of Jocelyne Matar Boumosleh et al. (2017) examining the relationship between smartphone use and depression and anxiety among university students, it was found that the presence of daytime sleepiness in 35.9% and decrease in sleep quality in 38.1 %38 [38]. In our study, similar to this study, it was found that there was a significant relationship between telephone attention and perceived stress level in adolescents before sleeping and that stress level was negatively affected ($p < 0.001$).

Conclusions and Recommendations

The following results were obtained in a descriptive type of research conducted to determine the relationship of sleep problems seen in adolescents with the use of electronic media and perceived stress. In Turkey the majority of adolescent sleep quality is poor. It was determined that the majority of the students included in the study had an electronic device in the room where they slept, and this situation adversely affected the sleep quality and duration. As the daily internet usage time increases, it has been determined that students' sleep quality is affected negatively. It was concluded that stopping the use of electronic media four hours before going to sleep positively affects the quality of sleep, adolescents delay their sleep

hours in order to spend more time in the electronic media and this situation has a bad effect on the quality of sleep. It was found that adolescents used multimedia devices up to two hours before sleeping and this caused poor sleep quality. It was concluded that the stress level of adolescents, who spend more time in electronic media, is higher, and the stress level of adolescents, which delays the time to go to bed using electronic media, and causes shortening of sleep time.

Results Of The Researchs:

It is important to inform adolescents about how electronic media use affects sleep quality, to provide training on stress management and to gain healthy living habits. Because many etiological factors affect the deterioration of sleep quality, it is recommended to plan initiatives involving school-based interventions in addressing individual, familial and social risk factors, and researchers to conduct studies with control groups demonstrating the effectiveness of these interventions.

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Chapter 36

EVALUATION OF THE EXTRACTION DECISION OF THE THIRD MOLAR TEETH ACCORDING TO THE CURRENT PARAMETERS

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1. Introduction

Surgical removal of the affected third molar tooth is one of the most common oral and maxillofacial surgical procedures. Historically, dentists often recommend extracting these teeth away so as not to cause problems later in life. This is called a prophylactic tooth extraction. In 2000, the National Institute of Health and Care Excellence (NICE) reviewed this practice and recommended that these teeth should not be removed if they do not cause discomfort to the person (1). Venta et al. state that extraction of a third molar tooth that is completely covered with bone and without symptoms constitutes an unreasonable risk for the general or local health of the patient (2).

Periodic check-ups are recommended at regular intervals to evaluate these patients for pathologies associated with third molar. The decision to prophylactically extract an asymptomatic third molar tooth has been a matter of debate for many years. Some authors advocate third molar teeth retention until clinical findings, and state that withdrawal is necessary for the presence or suspicion of symptoms. In addition, some also advocate that it should be extracted in the early period in terms of prophylactic removal. Complications and problems that may arise during and after extraction should be well evaluated in order to decide the prophylactic extraction of the tooth. On the other hand, the issues that may be encountered as a result of keeping the asymptomatic teeth in the mouth should also be evaluated thoroughly.

2. Third Molar Tooth Evaluation

Although impacted third molar teeth can remain asymptomatic in patients without causing any problems, they might have a potential to cause a number of problems. When third molar teeth stay in the mouth, it can cause some potential discomfort as time progresses. The most common problem is that, due to the cleaning and hygiene difficulty of the area, the potential for caries to occur in the third molar teeth and in the adjacent teeth is higher compared to others. In the case of mesio-angulation of third molar or insufficient space for eruption of third molar, it may cause an increase to the interface caries of the adjacent teeth (3,4). In addition, they can cause pericoronitis and other periodontal diseases (5,6).

Statistically, the highest incidence in terms of dental embedding is due to third molar teeth and is more common in the lower mandibular third molar teeth than in the upper ones. Third molar teeth usually show symptoms between the ages of 17 and 26 (7). In the study of Chaffin et al. (8), third molar tooth pain was shown as the second leading cause of dental emergencies. In the study of Langsten et al. (9), it is stated that approximately one of the five dental emergencies is associated with pain related to mandibular third molar.

3. Third Molar Tooth Extraction Evaluation Parameters

The parameters that are effective in the evaluation of the third molar tooth for extraction and the most common symptoms are the development of recurrent or severe pericoronitis, the presence of a periodontal pocket of 5 mm or more in the distal of the second molar tooth, the untreated dental caries affecting the third molar tooth, the extraction of the third molar tooth to facilitate the restoration of the second molar tooth in the presence of an advanced dental caries, presence of pathological changes associated with the third molar tooth, swelling, trismus, orthodontic evaluations, bleeding on probing, defects in the underlying bone, internal or external resorption that may occur in the surrounding teeth, evaluation of the follicular space, presence of intense plaque and other periodontal problems that may develop due to oral hygiene deficiency, and smoking.

4. Factors That Can Affect Third Molar Tooth Extraction

Boer et al. (10) reported that the total ratio of complications was 10.6% in the third molar tooth extraction. Many factors that depend on the patient, may affect complications and the course of these complications. According to the literature, the most impactful issues are the position of the third molar tooth in alveolar bone and the advanced age of the patient (11). Berge and Boe (12) reported that some variables that existed before the operation may affect the problems that may occur during and after the operation. It is reported that inflammation factors existing in the region prior to tooth extraction, the patient's gender, the stage of an eruption of the third molar tooth, the angulation of the tooth in the sagittal plane, the presence of pericoronitis, the duration and difficulty of the operation, the time of the day of the operation, the use of oral contraceptives, the use of tobacco and alcohol, can significantly affect the complications the postoperative response. However, it should be noted that after considering all these factors, there may be other factors that are still not specified. Yuasa et al. (13) state that the difficulty of the extraction is related to the depth of the affected third molar tooth in the bone tissue. In addition, the distance between the second molar and the mandibular ramus; and the diameter of the largest root of the third molar tooth are also effective factors. The difficulty of the operation usually results in more bone tissue removal in the periodontal area. Besides all these variables and parameters, it can be said that the complications resulting from third molar tooth extraction are generally rare and tolerable (14). However, third molar tooth extraction is so common that even if the complication rate is low, the number of patients experiencing possible complications can increase significantly. After the surgery, edema and reduced function are typical, but these symptoms normally go away a few days after the procedure (15). Concerning complication is mostly related

to lingual and inferior alveolar nerve damage, which can be seen with a mandibular third molar tooth extraction.

5. Effects of Age on Third Molar Tooth Extraction

With the advancement of age, the alveolar bone can become concentrated, and calcified may even cause ankylosis of the tooth. Therefore, under tooth extraction forces, bending resistance may decrease and bone flexibility may be lost. As a result, it should be remembered that it may be necessary to remove more bone tissue in order to complete the extraction surgically, and that the risk of mandibular angulus fracture may increase (16). Chiapasco et al. (17) showed that the complication rate was three times higher in patients over 25 years old compared to patients below the age of 25. Similarly, Chuang et al. (18) state that the complications that occur after third molar tooth extraction for patients over the age of 26, can be 1.5 times higher compared to patients below the age of 26. The increase in the complication rate potential, the duration of the procedure, and the recovery time in older patients; the possible necessity of intervention that might occur due to the symptoms or pathologies that are initially asymptomatic and may surface later are all reasons for early intervention. In addition, young patients are known to have a high incidence of caries and infections due to poor oral health education. These patients may have a mouth environment that triggers the cariogenic environment, such as high sugar consumption and a lack of daily brushing habits. For these reasons, early tooth loss due to caries can be observed more frequently in young people (19). I t would be appropriate to extract the third molar teeth at the early ages for cases where the extraction is considered to have the potential to become a necessity at older ages.

6. Third Molar Tooth Inferior Alveolar Nerve Relationship and Postoperative Complications

Some of the complications that can be encountered during the extraction of third molar teeth include bleeding, injury to the wound of the tooth, fracture of the root of the tooth, damage to the adjacent tooth, and fracture of the lingual bone plate. In the literature, it is stated that without prophylactic extraction in positions where the mandibular third molar is close to the mandibular canal, the number of patients that may develop numbness in the tongue and lip due to inferior alveolar nerve damage will significantly decrease. The incidence of both inferior alveolar and lingual nerve damage is high and can be permanent. Nerve damage, temporary or permanent labial or lingual paresthesia or anesthesia are the major surgical risks of the extraction. The rate of patients with temporary inferior alveolar nerve damage due to third molar tooth extraction varies between different

studies. Alling et al. (20) reported a value between 0.5% and 5% in his study. In the study conducted by Stevao and Bath (21), inferior alveolar nerve and lingual nerve damage occurred in 1.5 to 3% of cases. Similarly, inferior alveolar nerve damage rate has been reported as 0.5% to 5% and lingual nerve damage rate has been reported as 0.6% to 2% in other studies (22). In some studies, a total incidence of 13.4% has been reported for patients with affected nerve damage due to permanent injury of the lingual nerve after removal of the mandibular third molar (23). The most common postoperative complication is reported to be alveolitis. According to the literature, alveolitis cases can occur in 3 to 30% of patients (21). In another study, it has been reported that alveolitis occurs in 25.9% of all cases after mandibular third molar extraction (24). Infection, which is one of the complications that can be encountered in the postoperative period, can occur at the rate of 3% and secondary bleeding at the rate of 0.5% (25). Complications related to third molar tooth extraction should not be underestimated. Pain, swelling, and trismus after surgery are almost universal after this procedure. 50% of patients may have complaints of pain, trismus, edema, and dysphagia related to extraction in the first four days postoperatively (26). Problems with adjacent teeth and periodontium can occur in 3% of all cases (25). Ruvo et al. (27) report that the lifestyle and daily behavior have increased significantly delayed clinical recovery after the third molar surgery.

Fracture formation in the jawbones following third molar tooth extraction is among the rare complications. There is a tendency for fracture in the mandibular angulus region during the extraction of the mandibular third molar. This issue poses a risk especially for adolescent patients and young adults dealing with contact sports (28). In addition, fractures can occur in the mechanically weak parts, condylar region, and on both sides of the mentum. Condylar fractures are generally more severe, more difficult to treat, and have the risk of causing longer complication durations compared with angulus fractures (29). It is known that there may be fractures in the tuber maxilla during maxillary third molar extraction. These fractures can be seen as less frequent in younger patients than in older patients due to having less sclerotic bone in the region and having a more flexible bone structure. Another complication that can be seen in the maxilla is oro-antral perforations. It is more likely to occur in older patients following maxillary third molar extraction and may lead to chronic sinus problems (30). In elderly patients, periodontal damage to the distal of the second molar tooth may not improve after third molar tooth extraction and postoperative periodontal problems may occur (31,32). In 2005, Threlfall et al. (33) stated the extraction of third molars may cause temporomandibular joint disorders. They showed that the anterior disc displacement with reduction in the temporomandibular joint increased after third molar extractions. In

addition to all these, systemically dangerous medical condition and advancing age, which can usually go together, should be taken into consideration when deciding to follow for asymptomatic third molars.

Keith et al. (34) reported a caries incidence of 1.6% in third molars. Alattar et al. (35) and Mourshed et al. (36) reported 1% and 1.4% incidence of cyst development in third molar teeth. Guven et al. (37) reported a 2.31% incidence of cyst formation from third molars. The possibility of developing tumors such as ameloblastoma and epidermoid carcinoma is another indication for prophylactic removal of these teeth. It has been reported that the incidence of ameloblastoma development associated with third molar teeth is between 0.14% and 2% (38). In the literature, the development of odontogenic tumors associated with third molars is seen in the mandible in most of the cases (92%) (37). However, when all the data are examined in the literature, it is observed that the cysts and tumors develop in a relatively small minority.

7. 3. Molar Tooth Extraction Decision

According to the study of Lopes et al., (39) the average number of inactive days for patients who could not continue their work and do their daily activities after third molar extraction was 3 and this period varies between 0 and 10 days. A decision to follow-up after extraction would reduce the days that patients will lose due to the need for rest. In addition, complications that may occur during and after the surgery and may result in death will decrease. In addition, the lawsuits experience to be filed due to the malpractice may also decrease. Unfortunately, it is possible for oral surgeons to see it as a threat not to conduct prophylactic extractions considering this might lead to a decrease in their potential sources of income. It should not be forgotten that the point to be emphasized here is the extra time gained and the less stressful lifestyle. Venta et al. (40) argued that it is not possible to prophylactically remove all third molars from a practical point of view. It is always accepted in this regard that third molar teeth should always be removed if they are symptomatically affected, even in the case of patients with terminal diseases. Regarding prophylactic extraction, Laskin (41) states that third molar teeth that were initially asymptomatic may frequently be involved in pathological processes throughout an individual's lifetime, and a preventive extraction may be beneficial in preventing pathologies. Regarding the time of extraction, he stated that it should be extracted as soon as the insufficient area is detected in the eruption of the third molar. Stephens et al. (42) stated that the main reason for prophylactic surgery is the prevention of lesions such as dentigerous cyst, ameloblastoma, epidermoid carcinoma, infections, and root resorption in the adjacent tooth. However, the decision to extract should be made as the

relapse of pericoronitis can cause incidence of infection, and crowding of the lower anterior teeth.

8. Conclusion

Each patient should be evaluated individually when deciding on a third molar tooth extraction. The removal of these teeth is based on many factors that depend on patients and the clinical experience of the physician. Oral surgery specialists should review the risks and benefits of both alternative treatment approaches. The evaluation should be completed by informing the patient, and the decision to extract the tooth should be made.

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Chapter 37

SURGICAL MANAGEMENT OF PANCREATITIS

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ACUTE PANCREATITIS

In the last 20 years, the incidence of acute pancreatitis has increased. Many of the patients develop a mild and self-limited course; however 10-20% of the patients have a rapidly progressive inflammatory response, which is associated with long-term hospitality period and important morbidity and mortality. Patients with mild pancreatitis have a mortality rate which is below 1%; however this rate increases to 10-30% in severe pancreatitis. The most frequent cause of death is multiple organ failure in this patient group. Mortality has a two-mode distribution in pancreatitis: in the first 2 weeks – it is also known as the early stage -, multiple organ failure is the final consequence of an intense inflammatory cascade, which is triggered with the pancreas inflammation initially. The mortality, which occurs in late stage, is generally caused by the septic complications (20th ed.; Sabiston Textbook of Surgery, 2017).

Pathophysiology

Activation of intra-acinous pancreatic enzyme induces the auto-digestion of normal pancreatic parenchyma. In response to this first action, the cells secrete proinflammatory cytokines such as tumour necrosis factor-alpha acinar (TNF-α) and interleukin (IL) -1, IL-2, and IL-6. These mediators do not lead to pancreatic injury, they disseminate the response locally and systemically. As a consequence, TNF-α, IL-1, and IL-7, neutrophils and macrophages accumulate in pancreatic parenchyma, and they cause more prostaglandin release. The local inflammatory response damages the micro circulation of pancreas and exacerbates the pancreatitis more by increasing the permeability further. The inflammatory cascade limits itself approximately in 80-90% of the patients. Intense release of inflammatory mediators into systemic circulation occurs in very few patients. The mortality, which is seen in the early stage of pancreatitis, is the result of this permanent inflammatory response (Bhatia et al., 2005, p. 132).

Biliary or Gallbladder Stone Pancreatitis

There are strong evidences regarding that gallbladder stones initiates a pancreatitis attack through the temporary or permanent obstruction of the distal common biliary duct vent in the ampulla of Vater, however the definitive pathogenesis of the subsequent pancreas inflammation remains uncertain. The treatment shall depend on the severity of the attack and the presence of obstructive jaundice and/or acute cholangitis, after the gallbladder stone pancreatitis diagnosis is made (Uhla et al., 2002, p. 565).

Alcohol-Induced Pancreatitis

Alcohol has harmful impacts on pancreas. It triggers proinflammatory pathways such as nuclear factor κB (NF-κB), which increases the production of TNF-α and IL-1. Furthermore, it increases the expression and ac-

tivity of the caspases. Caspases are the proteases, which mediates apoptosis. In addition, alcohol reduces the pancreatic perfusion, induces the sphincter of oddi spasm, and obstructs the pancreatic ducts via the precipitation of the proteins within the ducts (10th ed.; Schwartz's Priciples of Surgery, 2015).

Anatomical Obstruction

AP has been defined in patients having pancreatic tumours, parasites, and congenital defects. Pancreatic divisum is an anatomical variation, which is present in 10% of the population. Its relation with AP is controversial. The patients with this variation, have 5-10% risk for AP development caused by the relative outlet obstruction in the minor papilla. Endoscopic retrograde cholangiopancreatography (ERCP) with minor papillotomy and stenting may be beneficial for this type of patients. Ascaris lumbricoides infection and annular pancreas are the rare anatomical obstacles which are linked with AP (Heyries et al., 2002, p. 376).

Endoscopic Retrograde Cholangiopancreatography Induced Pancreatitis

AP is the most common complication following ERCP, and it is seen in 5% of the patients. When diagnosis methods are compared, AP is more common in patients, who have undergone treatment procedures. It is more common in patients, who have multiple cannulation interventions, Oddi sphincter dysfunction, and abnormal imaging of the secondary pancreatic ducts following the injection of contrast agent. The clinical course is mild in 90-95% of the patients (Elmunzer et al., 2012, p. 1414).

Drug-Induced Pancreatitis

2% of AP cases are caused by drugs. The most common are sulphonamides, metronidazole, erythromycin, tetracycline, didanosine, thiazide, furosemide, 3-hydroxy-3-methylglutalyl-coenzyme A (HMG-CoA) reductase inhibitors (statins), azathioprine, 6-mercaptopurine, 5-aminosalicylic acid, sulfasalazine, valproic acid, and acetaminophen. More recently, the antiretroviral agents, which are used in the treatment of AIDS, have been shown in AP (Jones et al., 2015, p. 45).

Metabolic Factors

It is envisaged that hypercalcemia leads to pancreatic injury, causing ductal obstruction and afterwards pancreatitis attacks through the activation of intraductal precipitation of trypsinogen, trypsin, and calcium. AP is developed approximately in 1.5-13% of the patients having primary hyperparathyroidism (Khoo et al., 2009, p. 2115). AP is more common in patients having Type I, II, or V hyperlipidaemia. Patients having a triglyceride level higher than 1000 mg/dL should be suspected. A triglyceride level higher than 2000 mg/dL verifies the diagnosis. AP is not induced by

hypothyroidism, diabetes mellitus, and alcohol-induced hypertriglyceridemia typically (Kyriakidis et al., 2006, p. 259).

Miscellaneous Conditions

The blunt and penetrating abdominal trauma may be related with AP respectively at a rate of 0.2% and 1%. Long-term intraoperative hypotension and excessive pancreatic manipulation during the abdominal surgery may also result with AP. Pancreatic ischemia along with the acute pancreatic inflammation may be developed following the splenic artery embolization. Scorpion toxin and perforated duodenal ulcers are among the other rare causes (Debi et al., 2013, p. 9003).

Clinical Symptoms

The cardinal symptom of AP is epigastric or periumbilical pain, which spreads to the back. Typically, continuous pain, nausea, or vomiting are seen approximately in 90% of the patients. The pain is stable, therefore, if the pain disappears or wears off, then another diagnosis must be considered. Dehydration, weak skin turgor, tachycardia, hypotension, and dry mucosa may be seen in patients with AP. Excessively dehydrated and older patients may also display changes in mental state. The rare findings include lateral and periumbilical ecchymosis (respectively, Grey Turner's and Cullen's signs). Both of them are the indicators of retroperitoneal haemorrhage associated with severe pancreatitis. In patients having concomitant choledocholithiasis or oedema compressing the intrapancreatic section of the common duct in the head of pancreas, it may occur with jaundice. Decrease in the left respiratory sounds or less frequently pleural effusion secondary to AP in the right hemithorax may be seen (Cappell, 2008, p. 889).

Diagnosis

The keystone of AP diagnosis is clinical findings plus the increase of ribonuclease levels in plasma. A three-time or greater amylase or lipase increase verifies the diagnosis. The serum half-life of amylase is shorter than that of lipase. Determining the lipase levels is a more precise indicator in order to make a diagnosis, in patients, who do not apply to emergency service within the first 24-48 hours following the onset of symptoms. Furthermore, lipase is a more specific AP indicator, because serum amylase levels may increase in a range of cases such as disease of peptic ulcer, mesenteric ischemia, salpingitis, and macroamylasemia. Patients with AP are typically hyperglycaemic; besides they may have an abnormal increase in their leucocytosis and liver enzyme levels. Increase of alanine aminotransferase levels in serum within the context of AP, which is confirmed with high pancreatic enzyme levels, have a positive predictive value of 95% in the diagnosis of acute biliary pancreatitis (Kiriyama et al., 2010, p. 24).

Imaging Studies

Although the simple abdominal radiographies are not helpful for the diagnosis of pancreatitis, they may help to exclude other cases such as perforated ulcer disease. The benefit of ultrasonography regarding the diagnosis of pancreatitis is limited with the intraabdominal fat and increasing intestinal gas. However, an increase in combined liver transaminases and pancreatic enzyme levels, and the presence of gallbladder stone in the ultrasonography have a greater sensitivity (97%) and specificity (100%) regarding the diagnosis of acute biliary pancreatitis. Today, the contrast-enhanced computerized tomography (CT) is the best method in the assessment of pancreas, particularly if it is carried out with a CT scanner having multidetector. The most valuable contrast phase of pancreatic parenchyma assessment is the portal venous phase, which enables the assessment of viability of the pancreatic parenchyma, amount of peripancreatic inflammation, and intraperitoneal abdominal free-air or fluid collections (after 65-70 seconds following the injection of contrast agent). Unenhanced CT scan may be valuable in patients having renal failure, by defining the fluid collections or extraluminal air. Abdominal magnetic resonance imaging (MRI) is useful in assessing the degree of necrosis, inflammation, and free fluid presence. Magnetic resonance cholangiopancreatography (MRCP) has an important role in the assessment of the patients with unexplained or recurrent pancreatitis; because it enables a complete scan of the biliary and pancreatic duct anatomy. In addition, administration of intravenous (IV) secretin increases the pancreatic duct secretion, which causes temporary disintegration of the pancreatic duct. Endoscopic ultrasonography (EUS) may play an important role in the persistent choledocholithiasis assessment, in the formation of gallbladder stone pancreatitis (Surlin et al., 2014, p. 16544).

Assessment of the Severity of Disease

The earliest scoring system, which has been designed in order to assess the severity of AP, was introduced by Ranson et al. The Ranson score has a low positive predictive value (50%) and a high negative predictive value (90%). Therefore, it is mainly used to exclude severe pancreatitis or to estimate the mortality risk (Bedirli et al., 2003, p. 97).

The severity of AP may also be addressed with Acute Physiology and Chronic Health Assessment (APACHE II) score. Its primary advantage is being able to be used during admission and being repeatable at any time. APACHE II has a positive predictive value of 43%, and a negative predictive value of 89% (Aliosmanoğlu et al., 2012, p. 500).

Balthazar and his partners have created the CT pancreatitis severity index, by using the imaging features. This index links the CT findings with the result of the patient. Computerized Tomography Severity Index (CTSI) in Acute Pancreatitis: CTSI 0-3, mortality 3%, morbidity 8%; CTSI 4-6,

mortality 6%, morbidity 35%; CTSI 7-10, mortality 17%, morbidity 92% (Leung et al., 2005, p. 6049).

C-reactive protein (CRP) is an inflammatory indicator, which peaks 48-72 hours after the initiation of pancreatitis, and which is related with the severity of the disease. A CRP level of 150 mg / mL or more defines a severe pancreatitis. The greatest limitation is being not able to be used during admission (Khanna et al., 2013, p. 1).

Treatment

Regardless of the cause or severity of the disease, the keystone of AP treatment is aggressive fluid resuscitation with isotonic crystalloid solution. The rate of administration should be personalized and adjusted on the basis of age, comorbidities, vital signs, mental state, skin turgor, and urination. The patients, who do not respond to the initial fluid resuscitation or who have significant kidney, cardiac, or respiratory comorbidities, generally require an invasive monitoring having central venous access and with a Foley catheter. In addition to fluid resuscitation, continuous pulse oximeter is required in patients with AP, because one of the most common systemic complications of AP is hypoxemia, which is caused by acute pulmonary damage associated with this disease. Patients should receive additional oxygen in order to keep the arterial saturation over 95%.

Particularly morphine, which is among the narcotics, is preferred usually in order to ensure an efficient analgesia. An increase in Oddi sphincter pressure is one of the effects, which is explained after the systemic application of morphine. Notwithstanding, there are not any evidences regarding that narcotics have a negative impact on the results of the patients with AP. It has not any proven benefit in the treatment of AP with antiproteases (for instance; gabexat mesylate, aprotinin), platelet activating factor inhibitors (for instance; lexipafant), or pancreatic secretion inhibitors.

Nutritional support has a vital importance in the treatment of AP. Oral feeding may not be possible due to ileus, pain, or intubation. In addition, recurrent pain is developed in 20% of the patients with severe AP, shortly afterwards the restart of the oral path. Enteral nutrition and total parenteral nutrition (TPN) are the basic options in providing this nutrition support (TPN). Despite there are not any differences in mortality rates between these two forms of nutrition, enteral nutrition is linked with less complications, and it reduces the need for pancreatic surgery. Although TPN provides the most nutritional need; it is associated with mucosal atrophy, decrease in intestinal blood flow, risk of excessive bacterial growth in small intestine, antegrade colonization with colonic bacteria, and increasing bacterial translocation. Enteral nutrition should not be used instead of TPN, when applicable.

Routine antibiotic prophylaxy is not recommended in severe AP, in order to prevent the complications caused by the infection. Some benefits of routine antibiotic prophylaxy in selected intestinal decontamination in preventing the infection were demonstrated. Probiotic prophylaxy is not recommended (Besselink et al., 2013, p. 1).

Special Topics:

***Endoscopic retrograde cholangiopancreatography:**

Early ERCP, with or without sphincterotomy, was firstly supported in order to reduce the severity of pancreatitis; because the obstructive theory of AP indicates that pancreatic injury is a result of the obstruction of pancreatic duct. However, three randomized studies have demonstrated that ERCP was only useful for the patients having severe acute biliary pancreatitis. The groups of United Kingdom and Hong Kong, which have undertaken the first randomized controlled studies of ERCP and endoscopic sphincterotomy (ES), supported the early intervention in severe acute pancreatitis, independent from the presence of obstructive jaundice and/or acute cholangitis. The results of the 3 randomized controlled studies from Germany, which were conducted in acute pancreatitis associated with gallbladder stone, have demonstrated that ERCP and ES must be limited with the patients having biliary sepsis or obstructive jaundice. There are studies, which have reported that immediate and early ERCP was successful in 95% of the patients having biliary pancreatitis clinically. In these studies, choledocholithiasis was diagnosed as 69%, and ES was performed with a morbidity of 3% in all of them (Oría et al., 2007, p. 10).

Routine use of ERCP is not indicated in the patients having mild pancreatitis; because obstruction of bile duct is generally temporary, and it is recovered within 48 hours following the onset of the symptoms. The recent randomized clinic studies demonstrate that the sphincterotomy, which is applied in cases of moderate acute biliary pancreatitis, have a positive impact on the prognosis of the disease at a significant level. ERCP in severe acute biliary pancreatitis is indicated for those developing cholangitis and having persistent bile duct obstruction, which is displayed with the other imaging methods such as EUS. In old-aged patients, who have low performance status or severe comorbidities hindering the surgery, ERCP with sphincterotomy is a safe alternative in order to prevent recurrent biliary pancreatitis (Behrns et al., 2008, p. 629).

***Laparoscopic cholecystectomy:**

In patients, who had pancreatitis, the rate of recurrent pancreatitis development ranges between 32-61%. It has been reported that the pancreatitis was more complicated in the recurrent pancreatitis attack. Therefore,

cholecystectomy after the symptoms of pancreatitis are subsided and choledoch exploration when necessary are recommended during the hospitalization, for the patients having mild biliary pancreatitis. In severe biliary pancreatitis, it is recommended to carry out endoscopic sphincterotomy at the time of hospitalization of the patient and to delay the gallbladder surgery afterwards the severity of the biliary pancreatitis is subsided (Girgin et al., 2011, p. 141).

Complications:

Sterile and Infected Peripancreatic Fluid Collections:

The presence of acute abdominal fluid during AP attack is identified in 30-57% of the patients. Fluid collections are not surrounded by epithelial or fibrotic capsule, unlike the pseudocysts and cystic neoplasia of pancreas. The treatment is supportive, because most of the fluid collections shall be absorbed by the peritoneum automatically. Fever, leucocytosis, and stomach ache indicate that this fluid is infected, and if there is infection, then percutaneous drainage and IV antibiotic administration must be carried out (Banks et al., 2013, p. 102).

Pancreatic Necrosis and Infected Necrosis:

Pancreatic necrosis may reveal itself as the non-pancreatic parenchymal or peripancreatic fat involvement. Pancreatic necrosis is developed in 20% of the patients with AP. Contrast-enhanced CT is the most reliable technique in diagnosing the pancreatic necrosis. Infection is the primary complication of pancreatic necrosis. The risk is directly related with the amount of necrosis; the infection risk is 22% in patients having pancreatic necrosis, which involves less than 30% of the gland. The risk increases up to 37% in those, whose pancreatic necrosis involves 30-50% of it, and up to 46% when more than 70% of it is affected. This complication is generally associated with the bacterial translocation, which involves the enteric flora (Petrov et al., 2010, p. 813).

Conducted studies recommend intense conservative treatment, suggesting that the chance of the necrosis be infected within the first 14 days is very low. The surgical intervention to be performed in this period may increase the current inflammatory response in the patient. However, in some patients performing a surgical intervention may be necessary in also this phase. These are the patients, whose APACHE II scores are increased, general condition is worsened, and findings of multiple organ failure (MOF) are not recovered, or number of MOF is increased, despite the intense conservative treatment. Furthermore, the patients, in which severe necrosis issues are developed (arterial haemorrhage, organ puncture, etc.), are the candidates for immediate surgical intervention (Nieuwenhuijs et al., 2003, p. 111).

The most reliable method in determining whether the infection is developed or not in necrosis is entering into the necrotic tissue with a needle, taking the material, and performing its bacteriological examination (culture or direct staining) with the guidance of CT or US. The sensitivity of this method has been reported as 88%, and its specificity as 90%, and false negative rate as up to 4%. However, infection investigation must be carried out in necrosis immediately via this method, in persons, which more than 50% of the organ is necrotic, particularly in those, whose MOF findings are exacerbated, which the sepsis findings are detected, there are not any descriptor causes regarding the worsened general condition, whose APACHE II score and C-reactive protein examination are increased. Considering that the chance of being infected after the 15th day following the necrosis shall be increased, this investigation may be repeated at one-week intervals (Van Baal et al., 2014, p. 442).

The infected pancreatic necrosis must be suspected in patients having prolonged fever, leucocytosis, or progressive clinical worsening. The evidence of the air within the pancreatic necrosis, which is seen in CT scan, verifies the diagnosis, but it is a rarely seen finding. It is estimated that the infected pancreatic necrosis shall be developed approximately in one-third of the patients, who have suitable activity spectrum against the microorganisms penetrating into the pancreas and generally existing in necrotizing pancreatitis. With the increasing use of antibiotics, there has been a relative change, in which the rate of infection with gram-positive bacteria and fungi, was higher, 2-3 weeks after the initiation of antibiotic treatment mainly from the gram-negative bacteria. After the infection is demonstrated, IV antibiotic must be administered. Carbapenems are the initial treatment option, as they penetrate into the pancreas, and involve the spectrum. The alternative treatments include quinolones, metronidazole, third generation cephalosporins, and piperacillin (Büchler et al., 2000, p. 619).

Regarding the timing of the surgical intervention intended for the necrosis; it is recommended to delay the necrosectomy intervention afterwards the 15th day, preferably to the 3rd-4th week, as long as the general condition of the patient allows. This approach reduces the haemorrhage risk, and minimizes the loss of vital tissue related with the surgery, which is prone to the endocrine and exocrine pancreatitides caused by the surgery. Resective procedures should be avoided (pancreatectomy, etc.). They have high mortality and morbidity risks (Hollemans et al., 2018, p. 253).

Three methods are utilized in applications intended for the treatment of remaining small necrosis focuses and new necrosis areas which may be formed, following necrosectomy. These are simple passive drainage, continuous closed lavage, and zipper method. The traditional passive drainage is a simple drainage through a permanent drainage tube. Continuous

closed lavage is consisted of a continuous local irrigation with physiological saline of 6-8 litre/day via a pair of permanent tubes with lumen, and continues until the necrotic tissue discharge is observed. Open drainage is carried out directly through the open wound on the abdominal wall. The same procedure is repeated as it is planned (in every 2-3 days) by packing the retroperitoneal space and less pouch with gauze patch and until the necrotic tissue is no longer observed. The studies have demonstrated that the patients, who had been treated with continuous closed lavage, had the best results. In another article related with continuous closed lavage, laparotomy / debridement is applied 12 or 13 times again to the approximately 35 of the patients, and therefore the reported results may not be accepted as a pure assessment of the effect of continuous closed lavage. It is difficult to reach to a conclusion from the current evidences, whether the continuous closed lavage or open drainage (or zipper method) is better, and in the recent years one of these drainage techniques has been selected by the surgeons freely according to the size of the infected necrotic tissue. When the necrotic tissue is placed around the pancreas, then continuous closed lavage is selected, and open drainage is recommended in case the necrotic area expands to the right and/or left paracolic gutter from the stem of the mesocolon (Isaji et al., 2006, p. 48).

In recent days, attempts of new approaches are being seen in the treatment of pancreatic necrosis. In the beginning of 1996, Gagner defined the laparoscopic debridement and necrosectomy for the treatment of necrotizing pancreatitis with three different minimal invasive approaches: (1) transgastric drainage, (2) retrogastric retrocholic debridement, and (3) a completely retroperitoneoscopic technique (2). It has been demonstrated that the percutaneous approach to the infected pancreatic necrosis was safe and applicable in multiple retrospective case series. Laparoscopic necrosectomy may ensure a better access to the fluid collections, which are not suitable for endoscopic approach. This may facilitate a more comprehensive debridement of the cavity. It has been demonstrated that the laparoscopic approach was also safe in a several small case series. However, there is a significant injury potential in intra-abdominal organs or vascular structures during laparoscopic-aided necrosectomy. Despite the pre-election of the patients, it has demonstrated a very high rate of complication, including the fistula (20-60%) and haemorrhage (15%) (Bello & Matthews, 2012, p. 6829).

In 1998, Freeny et al. have reported a series of patients with infected acute necrotizing pancreatitis, for the first time, only through CT-guided percutaneous catheter drainage. Freeny et al. have developed the percutaneous drainage technique, which also includes the necrotomy (28 F), not only by discharging the infected necrosis passively, but also at the same

time adding aggressive irrigation through the percutaneous catheters with large vents (28 F). 37 patients with necrotizing pancreatitis and uncontrolled sepsis were treated (Baudin et al., 2012, p. 192).

In 2010, the Holland Pancreatitis Study Group conducted a randomized study, which assesses the open necrosectomy and percutaneous drainage, following the minimal invasive retroperitoneal drainage for necrotizing and infected pancreatitis. The results demonstrated that the long-term complications and mortality rates were better, when compared with the open necrosectomy group. Percutaneous retroperitoneal necrosectomy was combined with the disruption of necrotic pancreas and peripancreatic tissue with a trap catheter and Dormia baskets, continuous lavage of the cavity, and recurrent endoscopic imaging of the cavity fragment. The approach has the theoretical advantage of not transmitting intraperitoneal infection from the necrotic area, when compared with laparoscopy. This technique provides access to the areas, which are not reached with endoscopy, and to the potential for removing all necrotic tissues within the region (Van Santvoort et al., 2011, p. 1254).

In the recently published study of PANTER (The Minimal Invasive Step-up Approach and Maximal Necrosectomy in Patients with Acute Necrotizing Pancreatitis), the approach is a "step by step" approach, in which the first step is the percutaneous or endoscopic transgastric approach. With this study, a decrease from 69% to 40% in mortality and major complications, a decrease from 38% to 16% in long-term complications, and a decrease at a rate of 12% in the medical cost of each patient have been determined (Van Santvoort et al., 2010, p. 1491). 3D is important in this approach:

Delay: It is the period of medical follow-up with the support of antibiotics and nutrition, until the necrosis is encapsulated.

Drain: Percutaneous catheter drainage (PCD) and Endoscopic transluminal drainage (ETD).

Debridement: Video-aided retroperitoneal debridement (VARD), retroperitoneal percutaneous necrosectomy (MIRPN), Laparoscopic transgastric necrosectomy (LTN), and Endoscopic transluminal necrosectomy (ETN).

At least, 30% of the patients with infected necrotizing pancreatitis are being treated only with catheter drainage successfully. Today, it is not possible to foreseen that which patients are in need of necrosectomy. Hollemans et al. have conducted a predictive study in relation with this matter. In this study, it has been found that the male gender, multiple organ failure, increase of pancreatic necrosis percentage, and heterogeneity of the collec-

tion were the negative determinants for the success of the catheter drainage in infected necrotizing pancreatitis (Hollemans et al., 2016, p. 787).

In the study of mini-invasive surgical intervention in infected pancreatic necrosis, conducted by Li et al., the treatment was divided into four successive stages: percutaneous catheter drainage (PCD), mini-incision drainage (MID), video-aided debridement (VAD), and open surgery. 18 of 54 cases (33.3%) were treated after PCD; 13 of them with uncontrolled infection (24.1%) were treated after MID; and the remaining 19 cases (35.2%) were treated after VAD. Open surgical intervention was not carried out. The overall mortality was 7.4% (4/54), and the incidence of complication was 12.9% (7/54). In this study, it is foreseen that the gradual mini-invasive techniques may be accepted as the first option (Li et al., 2016, p. 508).

Minimal invasive and open surgery techniques in necrotizing pancreatitis were compared in the study conducted by Wroński et al. Primary open necrosectomy was carried out in 22 of 70 patients, and 48 patients were treated with minimal invasive techniques (percutaneous and endoscopic drainage). Rates of postoperative new-onset organ failure and staying in intensive care unit were significantly lower in the minimal invasive group; gastrointestinal fistulas have occurred more frequently after open necrosectomy. As a consequence of the study, the idea regarding that the minimal invasive treatment should be preferred instead of open necrosectomy in the initial treatment of necrotizing pancreatitis was accepted (Wroński et al., 2017, p. 22).

The most extensive study, which compares the minimal invasive and endoscopic techniques with open necrosectomy for necrotizing pancreatitis, is the study conducted by Van Brunschot and Hollemans. In this study, individual data of 1980 patients have been collected and analysed. They combined 15 original data, which has been obtained from the published and unpublished patient cohorts (51 hospitals, 8 countries), and newly collected data. It was found that it is related with low mortality rates, when minimal invasive surgery and endoscopic necrosectomy are compared with open necrosectomy in the patients with necrotizing pancreatitis and high risks (Van Brunschot et al., 2018, p. 697).

In another study, surgical set-up approach and endoscopic set-up approach were compared. In this study, the endoscopic ultrasonography-guided transluminal drainage and percutaneous catheter drainage monitored with video-aided retroperitoneal debridement were compared. As a result of the study, it has been proven that the endoscopic step-up approach was not superior to the surgical step-up approach in reducing the major complications or mortality in patients with infected necrotizing pancreatitis.

However, pancreas fistulas and hospitalization periods were determined lower in the endoscopy group (Van Brunschot et al., 2018, p. 51).

In the study of PANTER, which was published in 2010 (2005-2008), it has been suggested that a surgical set-up approach reduced the mortality or major complications, when compared with open necrosectomy, and 35% of the patients were treated successfully only through a simple catheter drainage, for infected necrotizing pancreatitis. However, a concern exists regarding that the minimal invasive treatment increases the need of re-intervention for residual peripancreatic necrotic collections and for other complications in long term. Therefore, Hollemans et al. have conducted a long-term follow-up study (2008-2015). In long-term follow-ups, it has been seen that incisional hernia, exocrine or endocrine pancreatic failure were developed at significantly lower rates in the patients within set-up approach group. Any differences were not seen in additional drainage procedures, or pancreatic surgery, or acute pancreatitis recurrence, or formation of chronic pancreatitis, or Izbicki pain score, between the both two groups. As a result of this study, it has been found that necrotizing pancreatitis was superior to open necrosectomy, without the increased risk of re-intervention (Hollemans et al., 2019, p. 1016).

Pancreatic Abscess

It is the most common complication, which requires intervention following necrosectomy. Mostly, the pancreatic abscess is the consequence of a residual or recurrent infection. In general, pancreatic abscesses are developed during the disease or afterwards (usually after five weeks). Minimal invasive treatment strategies may be carried out easily in many of these cases, due to their less aggressive behaviours and limited localizations. A new aggressive endoscopic approach includes the use of N-butyl-2-cyanacrylate for the impermeability of pancreatic fistula after cystogastrostomy or cystoduodenostomy balloon dilatation, daily endoscopic necrosectomy, and lavage of saline solution, following the synchronous EUS-guided multiple transmural and/or transpapillary drainage procedures, in the patients having pancreatic necrosis and pancreatic abscess (Seewald et al., 2005, p. 92).

Pancreatic Pseudocysts

Pancreatic pseudocysts are seen in 5-15% of the patients having peripancreatic fluid collection after AP. As a definition, the capsule of pseudocyst is consisted of collagen and granulation tissue, and it is not covered with epithelium. Typically, at least 4-8 weeks is required for the development of fibrotic reaction. Symptoms are developed in 50% of the patients having pancreatic pseudocysts. Permanent pain, early satiety, nausea, weight loss, and increased levels of pancreatic enzymes in plasm suggest this diagnosis. The diagnosis is verified via CT or MRI. The characteristic

features of the pancreatic pseudocysts include high amylase levels, which are associated with the absence of mucin and low carcino-embryonic antigen (CEA) levels (Habashi & Draganov, 2009, p. 38).

The following are the generally accepted indications for the drainage treatment of pancreatic pseudocysts:

(1) concomitance of symptoms such as stomach ache;

(2) complication due to infection or haemorrhage;

(3) increase in size during the observation period;

(4) a diameter of 6 cm or above;

(5) no-tendency for a decrease in size at least during the observation period of 6 weeks.

In current studies, there are not any reports in contradiction to the indications from (1) to (3); however (4) and (5) "criteria of 6 cm and 6 weeks" are not considered as the absolute indications for drainage, as the pseudocysts with a diameter of 6 cm or above may be dissolved spontaneously after an observation period lasting 6 weeks or more.

Percutaneous, endoscopic, and surgical drainage (primarily cystoenteric anastomose) are among the drainage procedures of pancreatic pseudocysts. It is considered that the percutaneous drainage, which is the least invasive and which has a cure rate of 80-100%, is a promising alternative to the surgical drainage. A prospective controlled study provided a single-stage cure rate of percutaneous drainage and surgical drainage respectively as 77% (20/26) and 73% (18/26), there are not any significant differences between them (Varadarajulu et al., 2013, p. 583).

However, there are increasing evidences regarding that the transgastric and transduodenal endoscopic drainage are the safe and efficient approaches for the patients having pancreatic pseudocysts in close contact with stomach and duodenum (defined as <1 cm). In addition, EUS concomitant transpapillary drainage may be tried in pancreatic pseudocysts communicating with the main pancreatic duct. Endoscopic dilatation and stent placement are carried out in patients, which the stenosis of the pancreatic duct and pancreatic pseudocyst is associated (Lerch et al., 2009, p. 614).

Surgical treatment is indicated in patients, who do not respond to conservative treatment, percutaneous drainage, or endoscopic drainage, and which infection and/or haemorrhage accompany with. The definitive treatment depends on the location of the cyst. The pancreatic pseudocysts, which are tied close to the stomach, should be treated with cystogastrostomy. The pancreatic pseudocysts, which are in close contact with duodenum and located in the head of pancreas, are treated with cystoduodenostomy. Finally, some

pseudocysts are not in contact with the stomach or duodenum. The surgical treatment for these patients is a Roux-en-Y cystojejunostomy. The surgical cystoenterostomy is successful in ensuring the immediate cyst drainage in over 90% of the cases (Hauters et al., 2004, p. 1645).

Pancreatic Acids and Pancreaticopleural Fistulas

Although it occurs very rarely, the complete disruption of the pancreatic duct may cause fluid accumulation at a significant level. This must be suspected in patients, who have AP attack, and which abdominal distension is developed at significant degree and which free intraabdominal fluid is developed. Typically, high amylase and lipase levels are seen in paracentesis. The treatment is consisted of the abdominal drainage, which is combined with the endoscopic placement of pancreatic stent. Failure of this treatment requires surgical treatment (Gómez-Cerezo et al., 2003, p. 568).

The frequency of pancreatic fistulas is low. Only 0.4% of the patients have this complication following the acute attack. However, the incidence of this complication increases in patients having other complications after AP: it is seen in 4.5% of the patients having pancreatic pseudocysts, and in 40% of the patients having infected necrosis following the surgical debridement. The treatment is conservative for most of the patients (Zerem, 2014, p. 13879).

Pancreatic duct fistula to the pleural space is rarely described. Dyspnoea, stomach ache, cough, and chest pain may be listed among the symptoms, which indicate this situation. The diagnosis is verified with the chest radiography, thoracentesis, and CT scan. The amylase levels above 50.000 IU in pleural fluid verify the diagnosis. It is more frequently seen after the alcoholic pancreatitis, and it is associated with pancreatic pseudocysts in 70% of the patients. The initial treatment requires chest drainage, parenteral nutrition support, and octreotide administration. 60% of the patients give response to this treatment. Endoscopic sphincterotomy and stent placement is necessary in the permanent treatment (Machado, 2012, p. 1).

Vascular Complications

Haemorrhage into the gastrointestinal system, retroperitonea or peritonea space, which threats the life, is seen only in 1-3% of the patients. The most common vein, which is affected, is the splenic artery; however it has been found that superior mesenteric artery or cystic artery were also affected. It was suggested that pancreatic elastases damages the veins, and causes formation of pseudoaneurysm. Spontaneous laceration causes massive haemorrhage. Clinical findings include sudden onset of abdominal pain, tachycardia, and hypotension. Selective angiography is the “golden standard”, which is used in order to localize the active haemorrhage due to the vascular necrosis. Angiographic approach of catheter embolization

may be life-saving, while it was necessary to manage the vascular complications through the surgical methods in the past. The mortality rate ranges between 28-56% (Balachandra & Siriwardena, 2005, p. 489).

Pancreatic inflammation may also produce vascular thrombosis; usually the affected vein is the splenic vein, however portal may expand to the venous system in severe cases. In imaging, splenomegaly displays gastric variations and splenic vein obstruction. Thrombolytic has been defined in the acute early phase, but many of the patients may be treated conservatively. Recurrent upper Gastrointestinal haemorrhage attacks, which are caused by venous hypertension, should be treated with splenectomy (Heider et al., 2004, p. 876).

Compartment Syndrome

Severe multiple organ dysfunction syndrome and high mortality exist in the patients having severe acute pancreatitis and abdominal compartment syndrome, which is treated with the surgical decompression. Surgical decompression may improve the kidney or respiratory functions. Early surgical decompression is associated with the reduction of the mortality in the patients having severe acute pancreatitis, early multiple organ dysfunction syndrome, and abdominal compartment syndrome. It is explained that, particularly the placement of drainage catheter into the abdomen is the first stage treatment, thus it enables us both to remove the intraabdominal fluid, and reduce the intraabdominal pressure (Mentula et al., 2010, p. 764).

CHRONIC PANCREATITIS

Unlike AP, the histological feature of chronic pancreatitis (CP) is the permanent inflammation associated with the atrophy of pancreatic parenchyma, and irreversible fibrosis. These histological features are associated with the chronic pain and insufficiency of endocrine and exocrine, which reduce the life quality of these patients significantly. The frequency of chronic pancreatitis affects 3-10 / 100,000 persons (Etemad & Whitcomb, 2001, p. 682).

Risk Factors

Extreme alcohol consumption, chronic duct obstruction, trauma, pancreatic divisum, cystic dystrophia of duodenal wall, hyperparathyroidism, hypertriglyceridemia, autoimmune pancreatitis, tropical pancreatitis, hereditary pancreatitis, and idiopathic chronic pancreatitis are among the risk factors (Coté et al., 2011, p. 266).

Clinical Symptoms

Pain is the primary symptom of chronic pancreatitis. The life quality of these patients is affected significantly due to the reduction of oral intake, interaction with the daily activities, and dependency to the narcotic

analgesic drugs. Nausea and vomiting are not common in the early stage, however they may emerge as the disease progresses. Pancreatic inflammation and fibrosis not only affect the pancreatic ducts, but also they reduce the number and function of the acinar cells. At least 90% of them must be non-functional before steatorrhea, diarrhoea, and other malabsorption symptoms are developed. Diseases, which are associated with the deficiency of fat soluble vitamin, such as haemorrhage, osteopenia, and osteoporosis, are developed in severe cases. Exocrine deficiency is seen in 80-90% of the patients having chronic pancreatitis, which continues for a long period. Furthermore, chronic pancreatitis also affects the populations of islet-cell. As a result, 40-80% of the patients shall have the clinical symptoms of diabetes mellitus. Typically, diabetes mellitus emerges years after the onset of stomach ache and pancreatic exocrine deficiency. Jaundice or cholangitis is seen in 5-10% of the patients, due to the fibrosis of the distal common bile duct. The dense scar on the head of the pancreas may cause severe nausea, vomiting, and stomach ache, by clogging also the duodenum. The secondary upper gastrointestinal haemorrhage into the portal or splenic vein thrombosis is the rare indication of chronic pancreatitis (Braganza et al., 2011, p. 1184).

Diagnosis

Imaging Studies

The diagnosis of chronic pancreatitis may be difficult during the course of the disease; because the correlation between the symptoms and the structural changes displayed in imaging studies is weak. The CT findings, which are seen most frequently in chronic pancreatitis, include dilated pancreatic duct (68%), parenchymal atrophy (54%), and pancreatic calcifications (50%). The other findings are peripancreatic fluid, focal pancreatic expansion, biliary duct dilatation, and irregular pancreatic parenchyma contour. CT has a sensitivity of 56-95% and a specificity of 85-100% in the diagnosis of chronic pancreatitis. In addition to making a diagnosis, CT is particularly useful in assessing the complications such as the disruption of the pancreatic duct, pseudocysts, portal and splenic vein thrombosis, splenic and pancreaticoduodenal artery pseudoaneurysms (Nair et al., 2007, p. 1679).

MRI is a reliable alternative in order to assess the patients with chronic pancreatitis. Sensitivity for the diagnosis of pancreatic calcifications is lower, however MRI is useful for detecting the changes, which indicate the chronic inflammation such as the intensity of pancreatic parenchyma, pancreatic atrophy, and the irregularities in the contour. In addition, MRCP with secretin injection is particularly useful in the assessment of the intraductal stenosis and disruption of pancreatic duct (Choueiri et al., 2010, p. 114).

ERCP should be considered as a therapeutic method in the patients, which pancreatic duct complications suitable for the endoscopic treatment such as stricture, stone, pseudocysts, and biliary stenosis, are developed. It is suggested that it may be the golden standard technique in diagnosing the chronic pancreatitis cases, which may not be diagnosed with Endoscopic Ultrasonography (EUS) imaging methods. Furthermore, it has an important place in also diagnosis and treatment of the cystic lesions of pancreas. A panel, which is consisted of endosonographs, has defined the criteria required for the diagnosis of the chronic pancreatitis, which is known as Rosemont criteria. Histological inflammation, atrophy, and fibrosis evidences are the golden standards for the diagnosis of chronic pancreatitis (Catalano et al., 2009, p. 1251).

Functional Tests

Measurement of faecal elastase 1 level is a non-invasive study, which is preferred in order to diagnose the pancreatic exocrine deficiency (Gok et al., 2008, p. 213).

Treatment

Medical Treatment

The primary purpose in the treatment of these patients is to alleviate the symptoms. An optimal treatment requires the multidisciplinary team to follow a systematic and well-structured treatment plan. Patient consultancy is an important component; because the current evidences suggest that this disease is irreversible, however progress of disease may be delayed. The patients should be encouraged strongly to quit drinking alcohol and smoking cigarettes. Besides, the other risk factors such as hypertriglyceridemia must be treated, and modification of diet (thus, low-fat diet) may be beneficial for some patients. Selection of the analgesic is the keystone of the treatment, as the most patients develop pain during the natural course of the disease. Non-steroidal anti-inflammatory medications is the first stage of the treatment. A moderate pain, which does not give response to non-steroidal anti-inflammatory medications, should be treated with tramadol or propoxyphene. Patients, who do not give response to these suggestions and who have severe pain, should be treated with strong and long-term narcotics (De-Madariaa et al., 2013, p. 18).

Interventional Therapy: Endoscopic Treatment

ERCP is the primary method in the treatment of symptomatic pancreatic duct obstruction through dilatation and polyethylene stent placement. It must be noted that pancreatic cancer is present in the differential diagnosis of pancreatic duct stenosis. However, the probability that the endoscopic treatment of the malign diseases should be considered has been excluded

completely, after a comprehensive assessment, which includes CT, MRCP, or EUS. The surgical resection must be carried out, if a concern of malign disease is present. Endoscopic stone extraction should be considered for the patients having secondary pain to the pancreatic Stones and pancreatic duct dilatation. Extracorporeal shock wave lithotripsy and ERCP, which is preferred afterwards, may be necessary for the treatment of the affected large stones. The rate of success ranges between 44-77% in this technique. Stenting the pancreatic duct along with the stone extraction may provide benefit, by alleviating the obstruction (Cahen et al., 2007, p. 676).

Endoscopic Treatment of Chronic Pancreatitis: Clinical Guide of European Society of Gastrointestinal Endoscopy (ESGE):

European Society of Gastrointestinal Endoscopy (ESGE) recommends extracorporeal shockwave lithotripsy / ERCP as the first stage intervention option for the treatment of painful, uncomplicated chronic pancreatitis. Clinical response should be assessed in 6-8 weeks; if it is not satisfactory, then the state of the patient should be discussed in a multidisciplinary team again. Surgical options should be considered in patients, which bad results are foreseen, particularly following the endoscopic treatment (Recommendation degree B). ESGE recommends extracorporeal shockwave lithotripsy as a first stage, whether combined with the extraction of the spalls endoscopically or not, depending on the proficiency of the centre, for the treatment of chronic pancreatitis associated with radiopaque stones of ≥ 5 mm, which obstructs the main pancreatic duct (Recommendation note B).

ESGE recommends the stent change within 1 year, along with the placement of a single 10-Fr plastic stent, for the treatment of the chronic pancreatitis associated with the dominant stenosis of the main pancreatic duct (Recommendation degree C). ESGE recommends the discussion of the current options (for instance, endoscopic placement of multiple pancreatic stents, surgical) in a multidisciplinary team, in the patients having ductal stenosis continuing after the removal of 12-month single plastic stent (Recommendation note D).

ESGE recommends endoscopic drainage as a first stage treatment in order to treat the chronic pancreatic pseudocysts, which are open to endoscopic access, and uncomplicated (Recommendation degree A).

The selection between the endoscopic and surgical treatment for the treatment of the biliary stenosis associated with chronic pancreatitis should depend on the local proficiency, comorbidities of the patient, and the expected patient adaptation with the recurrent endoscopic procedures (Recommendation note D). In case endoscopy is selected, ESGE recommends the temporary placement of multiple, collateral, plastic biliary stent (Recommendation class A) (Dumonceau et al., 2012, p. 784).

Surgical Treatment

The most frequent surgical indication is the pain in the patients with chronic pancreatitis. Furthermore, the other indications are the obstruction of the common bile duct and duodenum, pseudocysts along with the pancreatic duct pathology, resistant pancreatic fistulas, and the suspicion of malignancy. Selection of the surgical procedure depends on the symptoms, which require palliation, and the presence or absence of the pancreatic ductal dilatation. In general, patient having an expanded pancreatic duct (which the diameter is defined as > 7 mm) require a decompression procedure; and the patients having an undilated pancreatic duct or small duct disease require a resectional procedure (Malya et al., 2018, p. 15).

The surgical interventions intended for chronic pancreatitis are classified under two main titles. These are 'drainage" and "resection" procedures. The pancreatic duct dilatations, which we encounter frequently in chronic pancreatitis patients, are resolved with the drainage procedures. The decompression procedures are frequently applied in chronic pancreatitis cases, which the pancreatic duct is dilated. In 1958, Puestow and Gilleby have defined Roux-en-Y pancreaticojejunostomy longitudinally. In 1960, Partington and Rochell have defined collateral longitudinal pancreaticojejunostomy without resection, and this procedure is known as Modified Puestow. Pain control is ensured in 61-91% of the patients in short term with this procedure, which can be applied with low mortality and morbidity rates; However pain emerges again approximately in 30% of the patients in 3-5 years (Van Loo et al., 2010, p. 1079).

Procedures with resection are applied in patients, whose pancreatic duct is not wide, and which malignancy suspicion is present, and conservative methods are inefficient. Pancreaticoduodenectomy, pylorus-preserving pancreaticoduodenectomy, pancreatic head resection, in which duodenum is protected (Beger), and Frey operation, in which longitudinal pancreaticojejunostomy is applied along with the duodenum-preserving pancreatic head resection are the primary techniques, which are applied for this purpose (Zeytunlu et al., 2005, p. 23).

Pancreaticoduodenectomy, which is procedure having a high morbidity, ensures the pain control in 71-89% of the patients in chronic pancreatitis. Pylorus-preserving pancreaticoduodenectomy, in which the diet is more efficient, has been another option. The most important disadvantage of these methods is the significant disruption in the pancreatic endocrine and exocrine functions with the resection of duodenum. Total pancreatectomy is an option, which may be applied to the patients, whose findings do not regress, and who have severe function losses, despite the surgical procedures. However, severe disruption in endocrine functions is the most

important disadvantage of this method (Jimenez et al., 2000, p. 293).

Although the resection procedures are efficient in the pain control, hybrid methods have been defined due to the function loss. Duodenum-preserving pancreatic head resection, which was defined by Beger, in 1980, is the most well-known among these methods. The Frey procedure, which was defined in 1987, is the modification of Beger and Partington methods. In this method, the pancreatic cervix section is preserved, and excessive haemorrhage is avoided. At the same time, drainage of the pancreatic duct is ensured in pancreatic body and tail sections (Strate et al., 2005, p. 591).

Hamburg procedure is the modification by combining the drainage and local resection, in order to treat the small ducts within the Frey procedure. The secondary and the tertiary ducts are drained by means of the lateral pancreaticojejunostomy with V-formed incision made on the ventral pancreas. Elimination of the permanent pain (pain score was reduced 95%), improvement in life quality (life quality improvement index was 67%), 0% hospitality mortality, and 15.4% morbidity was reported with Hamburg procedure. Izbicki et al. have conducted a prospective, randomized study, which compares Frey procedure and pylorus-preserving pancreaticoduodenectomy. In this study, patients who had underwent Frey procedure, had a low morbidity rate (19%), and a better life quality score (71%) (Yekebas et al., 2006, p. 940).

Farkas et al. have conducted a study, which compares Berne modification and pylorus-preserving pancreaticoduodenectomy. In this study, they have reported shorter operation period, less intraoperative blood loss, lower postoperative morbidity, shorter hospitalization period, and better life quality in the patients, to whom Berne modification has been applied (Farkas et al., 2003, p. 29).

Farkas et al. have described pancreatic head resection and longitudinal pancreaticojejunostomy, without applying lateral pancreaticojejunostomy. This approach has been described by Gloor et al. as the Berne modification. However, if inflammation is present in the great duct or in the mucosa of the duct, extension of the longitudinal pancreaticojejunostomy may be performed in order to prevent the recurrent stricture (Gloor et al., 2001, p. 21).

Aspelund et al. have compared the Frey procedure and duodenum-preserving pancreatic head resection method, and any differences regarding the pain control and major complications have not been found between the two procedures (Aspelund et al., 2005, p. 400).

Riediger et al. have investigated the longterm results of the pylorus-preserving pancreaticoduodenectomy, Frey procedure, and duode-

num-preserving pancreatic head resection methods in their study, and any differences have not been found regarding the pancreatic endocrine and exocrine deficiency (Riediger et al., 2007, p. 949).

Koninger et al. have compared the Berne modification and duodenum-preserving pancreatic head resection method in their study, and any differences have not been found in pain control (Koninger et al., 2008, p. 490).

In the prospective randomized study conducted by Andersen et al.; the local resection with or without the pancreatic head duct drainage and the duodenum-preserving pancreatic head resection method have provided more efficient results than the pancreaticoduodectomy, with low morbidity and mortality. Local resection of the pancreatic head provides the advantage of the lowest cost and early prevention of the morbidity and postoperative diabetes. Late incidence of recurrent pain, diabetes, and exocrine deficiency have been determined same for the each three surgical approaches. Local resection of the pancreatic head is seem to provide the best results, and the lowest risk for the treatment of chronic pancreatic pain (Andersen & Frey, 2010, p. 18).

Cahen et al. have conducted a randomized study, which compares the endoscopic and surgical drainage of the pancreatic duct in patients having advanced chronic pancreatitis. Additional drainage has been required in 68% of the patients, who had been treated with endoscopy, when compared with the surgical group, in which it was 5%. As a result, less procedures and less pain have occurred in the patients, which the surgery was applied, when compared with the patients, who had endoscopy. Furthermore, almost half of the patients, who have been treated with endoscopy, underwent surgery finally (Cahen et al., 2011, p. 1690).

Sutherland et al. have demonstrated that islet-cell autotransplantation may improve the pain and life quality in patients with total pancreatectomy, who have dependency to the narcotics. It has been found that significant islet functions were preserved in more than two thirds of the patients, who had received islet-cell autotransplantation, and independency to insulin has been seen in one fourth of the adults and in the half of the children (Sutherland et al., 2012, p. 409).

Biliary strictures.

Chronic scar and fibrosis of the pancreatic head is resulted with the compression of the intrapancreatic section of the common bile duct. Radiological evidences have been found for the bile duct expansion up to the one third of the patients having chronic pancreatitis, biliary obstruction is seen in 6% of the patients. Biliary strictures are typically appear as a

long symmetric stenosis, which includes the intrapancreatic section of the common bile duct in MRCP or ERCP. Temporary bile duct decompression with plastic stents is indicated with IV fluid and antibiotic treatment in the patients, who apply with cholangitis. The chronic pancreatitis may be treated with distal common bile duct stenosis stent placement in long term. Multiple, spontaneous stents are seem to be superior to the single stent placement, and may be useful in long term. Thus, it causes almost normalization of the bio-chemical tests of the liver functions and increase of distal common bile duct diameter. Placement of multiple stents has reduced the need for surgical procedures. Pancreastoduodectomy is indicated in the patients, which the malign diseases are not excluded before the surgery. A Roux-en-Y hepaticojejunostomy is an alternative treatment method for the patients, who do not have malign disease evidence or a significant indication, which prevents the resection of the pancreatic head (Catalano et al., 2004, p. 945).

Duodenal stenosis.

Duodenal stenosis is developed in patients up to 1.2% of having chronic pancreatitis. Stomach ache, nausea, vomiting, and significant weight loss may be listed among the clinical findings. The differential diagnosis include the causes of secondary gastric outlet obstruction to the upper gastrointestinal malign neoplasms and gastroparesis. Patients with severe malnutrition require IV hydration, nutritional support, and gastric decompression. The permanent treatment requires gastrojejunostomy (Vijungco et al., 2003, p. 1258).

Pancreatic pseudocyst.

Pancreatic pseudocysts are developed more frequently in patients with chronic pancreatitis, when compared with AP. Pseudocyst is developed in 30-40% of the patients. Spontaneous pancreatic pseudocyst is present only in 10% of the patients. The treatment indications include patients having secondary symptoms to stomach, duodenum, or biliary compression, and the complications associated with pancreaticopleural fistulas, rupture or spontaneous haemorrhage. Alternative treatments in treatment include endoscopic and surgical drainage. In a prospective randomized study, it has been demonstrated that the endoscopic approach for simple pancreatic pseudocysts have provided a more rapid improvement in life quality, and less hospital expenses (Varadarajulu et al., 2013, p. 583).

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Chapter 38

EFFECTS OF PROBIOTICS ON ORAL HEALTH

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Introduction

The oral cavity is like an entrance door of the body and is home to many microorganisms. It is stated in the literature studies that there are more than 700 species of microorganisms in the oral cavity. This variety of hosts, consisting of gram-negative and gram-positive microorganisms, is in equilibrium with the body defense system. This balance can turn in favor of opportunistic microorganisms as a result of nutritional habits, saliva pH changes, and changes in body health. If acidic pH increases, the incidence of tooth decay increases, and as saliva ph becomes basic, the incidence of dental calculus and periodontal ailments increase (1).

These microorganisms include prokaryotes and some eukaryotes (protozoa, yeasts, mycoplasma, candida, spirochetes), which are divided into two groups, mostly Gram-positive (*Streptococcus, Enterococcus, Micrococcus, Peptococcus, Peptosteptococcus, Lactobacillus, Corynebacterium, Actinomyces, Arachnia, Rothia, Eubacterium, Propioni bacterium, Bifidobacterium, Bacillus and Clostridium*) and Gram-negative (*Nisseria, Veillonela, Campylobacter, Eikenella, Actinobacillus, Capnocytophaga, Heamophilus, Simonsiella, Bactertoides, Fusobacterium, Porphyromonas, Provotella, Laptotrichia and Wolinella*). All of the microorganisms found in the oral cavity are often referred to as oral microbiota, oral microflora, or oral microbiome (2).

Oral diseases are a serious public health problem, as localized health problems such as dental caries and periodontal problems can cause an infection that may affect other organs of the body due to a hidden infection. According to NIH, the national institute for aging, the oral cavity needs planned individual care, such as brushing teeth twice a day, using regular mouthwash, and flossing to keep away from disease (3). Various oral probiotic strains are available for the prevention and treatment of caries, bad breath, and mouth diseases.

When probiotics are taken in sufficient numbers, they are defined as living microorganisms that have positive effects on host health. Probiotics play a powerful role in oral care as a preservative. Bacteriotherapy in the form of probiotic bacteria with inhibitors is a promising and low-cost agent, the effect of which has now been developed on oral pathogens.

Significant progress has been made in the field of functional and health food from probiotics to the present. Traditionally, probiotics have been associated with gut health, and most of the clinical interest has focused on the prevention or treatment of gastrointestinal infections and diseases. However, in recent years, it has been reported that the bioavailability of probiotic bacteria has increased, including increasing adaptation to the immune system, treating or preventing respiratory infections, and preventing or alleviating allergies and atopic diseases. (4)

It has been suggested that probiotics play an important role in the prevention and treatment of oral diseases such as tooth decay, periodontitis, bad breath, candida infections, and gingivitis (Figure 1). In recent years, the role of probiotics in maintaining ecological balance and its effectiveness in the normalization of oral microbiota has been an intensive research topic.

These studies are systematically summarized by many reviews in the past. (5)

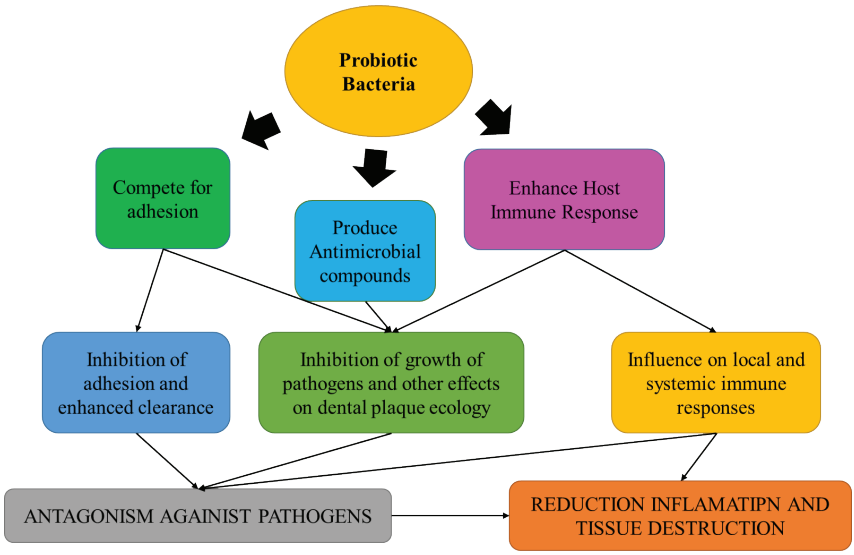

Figure 1. *Mechanism of action of probiotics in the oral cavity*

It offers the opportunity to affect the development of mucosal and systemic immunity by regulating the entire microflora composition. Probiotics naturally colonize the oral cavity, reducing the harmful effects of all pathogenic microorganisms. As a result, they play an antimicrobial role. It contributes to the transformation of the entire infected area in the oral cavity from an environment rich in inflammatory cytokines to a better environment by living in its beneficial organisms. Thus, they contribute completely to systemic human health.

There are common types used in oral probiotic preparations. These; *Lactobacillus bulgaricus, L. acidophilus, L. casei, L. helveticus, L. lactis, L. salivarius, L. plantarum, Streptococcus thermophilus, Enterococcus faecium, E. faecalis, Bifidobacterium and Saccharomyces boulardii.*

In the use of ready-made preparations of probiotic microorganisms, there has been a shift towards a more robust routine treatment / care strategy in the past few years, helping with dental care. this situation is considered as a promising condition for dental health (6).

It has been reported that probiotics increase the life of removable den-

tures by preventing the adhesion of unwanted microorganisms in the oral flora. Studies on the role of probiotics in preventing oral diseases should increase. The purpose of this review is to review research to highlight the use of probiotics in oral care and to support the effectiveness of probiotic therapy on oral health.

2. Diseases and probiotics

Any adverse change in the resident oral microflora, their metabolic activity and their local distribution leads to various disease. The use of probiotics reverses the imbalance in the oral microbial equilibrium and effectively normalize the microbiota. The ability of probiotics to adhere to mucus and epithelial cells, as well as to co-aggregate, is one of the most important selection criteria for potential probiotic strains. (8) This may be due to the increased salivary levels of matrix metalloproteinase (MMP-9) and tissue inhibitor of metalloproteinases (TIMP-1) following consumption of probiotics. Several studies have confirmed the antiviral activity of probiotics due to their immunomodulatory effect. A study by Khani et al. (9) suggests that L.rhamnosus enhances macrophage activation and viability for HSV-1 (Herpes Simplex Virus-1) elimination, when compared with non-probiotic *Escherichia coli*. A study by Cutler and Jotwani (10) suggests that probiotics modulate host immunity both systemically and locally.

Probiotics are administered to maintain or restore the natural micro-flora against a pathogen invasion, which is central to the development of the major oral diseases.

(1) Tooth decay

Tooth decay is generally defined as the localized chemical dissolution of the tooth surface. According to the Global Oral Health Database, caries prevalence ranges from 49% to 83% worldwide (11). *Streptococcus mutans* are the main causal organism in caries development (12).

The mutans family are very stable bacterial communities that can tolerate low pH. Low pH conditions, *Actinomyces spp., Atopobium spp., Bifidobacteria, non-mutated Streptococci, Propionibacterium spp*. It leads to an increase in such acidogenic species. Low pH conditions also leads to demineralization, which leads to erosion of tooth enamel (13).

A recent study (14) revealed that L. rhamnosus GG has the highest value of adhesion and inhibits *Streptococcus* by producing different antimicrobial components such as organic acid, hydrogen peroxide, carbon peroxide and diacetyl bacteriocins. (15) report a novel probiotic *Streptococcal species, Streptococcus dentisani,* isolated from dental plaque of caries-free individuals.

In a study by Lin, Chen, Tu, Wang, and Chen (2017) (16) four probiotic strains of *Lactobacilli* displayed strong inhibitory effects on Streptococcus mutans, isolated from children with active caries, with the inhibition rate reaching 70–90%.

In the study of Petersson et al (17)., pH value the combination of L. rhamnosus LB21 with fluoride has been shown to be very effective in reducing caries in schoolchildren and also in mineralization of tooth enamel in older adults.

Cagetti et al. (2013) (18) observed regular consumption of probiotic milk containing *L.rhamnosous* GG and L. reuteri reduced *S. mutans* associated caries risk and initial caries development.

Rodriguez et al. (2016) (19) observed that after consuming *L. rhamnosus* SP1 (10^7 cfu/mL) supplemented probiotic milk for ten months the caries prevalence in preschool children dropped from 65.8% to 54.4%.

(2) Plaque

Dental plaque is considered as the single most important factor that causes dental caries.

It is a whole where a wide variety of physical, metabolic, and molecular interactions take place. Plaque provides a favorable environment for the simultaneous protection of the growth of pathogenic microorganisms from antimicrobial agents and host defenses.

The plaque can be removed with a toothbrush in the most effective way, if the regular plaque is not removed from the tooth surface, it can cause tartar formation, which can lead to gum problems, tooth decay and even tooth loss (20).

Probiotics can modulate the systemic immune system by neutralizing the freely available electron charge, and also reduce mucosal permeability and reduce plaque initiation.

In an in vitro study of *Streptococcus thermophilus* and *Lactococcus lactis* strains, they were reported to adhere to the saliva-coated hydroxyapatite surface. (20).

(3) Gingivitis

Porphyromonas gingivalis is the most common organism causing gingivitis. It can colonize the lower gingival areas and lead to the progression of chronic inflammatory diseases. As a result, it surrounds the gingival epithelial cells and causes tissue damage. There are some parameters of gingivitis, these are inflammatory parameters such as GI (Gingival Index) and BOP (bleeding in probing) (21).

Probiotic strains help manage the gingivitis by helping to keep the oral cavity flora in balance. Acidogenic probiotic bacteria such as *Lactobacilli, Streptococci* and *Bifidobacterium* release antimicrobial substances that have inhibitory effect against pathogens through co-aggregation, production of toxic byproducts and competing for substrates. Nisin, a commercially available antimicrobial agent found effective in the reduction of plaque buildup and gingivitis in beagle dogs as compared to treatment with chlorhexidine and a placebo (22).

Schlagenhauf et al.(23) have been reported that regular consumption of lozenges containing *L. reuteri* is beneficial in controlling pregnancy-related gingivitis and plaque coverage in healthy pregnant women. In a study investigating the level of proinflammatory cytokines found in gingival crevicular fluid, it was reported that the use of gum containing *L. reuteri* contributed to the reduction of proinflammatory cytokines in gingival crevicular fluid (24).

(4) Periodontitis

Periodontal diseases are the most common inflammatory destructive conditions that include both soft and hard tissue. *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, A. actinomycetemcomitans, Bacteroides sp., Actinomyces sp., Staphylococcus intermedius*, and *Candida albicans* are the common pathogens associated with chronic periodontitis. In periodontal diseases, plaque accumulation is the main actor as in dental caries. Periodontal diseases can extend to deeper supporting structures that lead to destruction of the periodontal ligament and alveolar bone.

According to the World Health Organization, 15-20% of middle-aged (35-44 years) adults face tooth loss due to severe periodontal (gum) diseases. Periodontal pathogens less frequently can invade systemic circulation and cause end toxemia, complicate pregnancy outcomes and cause diabetes and nosocomial pneumonias (25).

Probiotics help in periodontitis by stabilizing the oral microbialflora. Most strains of Lactobacillus suppress growth of periodontal pathogens. Many latest studies showed application of beneficial bacteria, as an adjunct to scaling and root planing, can inhibit the recolonization of periodontopathogen and in overall pocket depth reduction and clinical attachment gain. Probiotics adhere to dental tissues as a part of the biofilm (or plaque) and compete with the growth of cariogenic bacteria or periodontal pathogens (26). Matsubara et al. (27) reported that probiotic bacteria can be used as an adjunct to traditional therapy and can be used as periodontitis with no side effects in the host. They can increase the antimicrobial effect of removal of the mechanical plate, reduce the need. It is stated that they can replace antibiotics for surgical intervention and treatment of human

periodontal infections in the future.

(5) Halitosis

Gram-negative bacteria (*Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia and Treponema denticola*) break down food proteins to produce volatile sulfur compounds (VSCs: hydrogen sulfide, methyl mercaptan and dimethyl sulfide). this happens under Anaerobic conditions, which contributes to the progression of periodontal diseases and the formation of bad breath (28). These volatile compounds originating from the oropharynx or expired alveolar air contain sulfur-containing gases such as hydrogen sulfide, methanethiol and dimethyl sulfide (29).

In addition, there are situations caused by gases such as indole, scatole, putresin, cadaver and acetone. These are among the most dominant causes of bad breath. The irritating commensal microflora balance present in bad breath is responsible for bad and unwanted odors. Besides the health problem of the mansion, the bad smell is an important psychological condition for social life (29, 30).

Conclusion

Oral health is important on individuals' quality of life and is in a direct relationship with welfare. Oral diseases can limit the individual's capacity to feed, communicate and smile. As a result, the individual decreases the quality of life in his personal and social life. In vitro and in vivo studies have revealed that probiotic strains are effective in preventing a mouth health problem in individuals and have an important role in the recovery of this process. In literature studies, probiotics are important in inhibition of oral pathogenic microorganisms. As a result, it has been found that when the probiotic preparations developed are used regularly and regularly (1-2 weeks), they prevent or reduce oral diseases significantly. If we associate the improvement of oral flora and indirectly with general health, it is thought that using oral probiotics to protect oral health will lead to important health problems.

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Chapter 39

DIAGNOSIS AND MANAGEMENT OF MIDFACIAL FRACTURES

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Introduction

The facial skeleton is usually defined by vertical and horizontal buttresses consisting of thin segments of bone encased and supported by thougher frames are called “buttresses.” These act like pillars toward forces (Morris & Tiwana, 2013).

In the midfacial complex vertical buttresses that provide protection from vertically directed forces are; zygomaticomaxillary, nasomaxillary, nasofrontal and pterygomaxillary (P. N. Manson, Hoopes, & Su, 1980). These are further supported by the horizontal buttresses which are; zygomatic arches, infraorbital rims, frontal and supraorbital bar (P. N. Manson, Shack, Leonard, Su, & Hoopes, 1983).

The midface is attached to the cranium with this frame. Forces applied to the facial skeleton are transmited and absorbed by the buttress system. In the presence of external forces, disruption of the facial skeleton is prevented by these components; however, if the critical level is reached, it results in fractures from weak anatomic sites which are patterned fairly predictable (Morris & Tiwana, 2013).

Le Fort Classification System

Le Fort fractures are specific facial bone fracture patterns that occur with the blunt facial trauma most commonly involving motor vehicle accidents, assault, or falls. Le Fort stated that, generally, in the presence of facial fractures, cranial bone fractures were not observed (LeFort, 1901). Fractures occur through three weak lines in the facial bony structure; those that protect the cranial cavity, those that circumscribe the midface, and those that cut across the face. According to these three lines, the Le Fort classification system was arised (P. N. Manson et al., 1983).

All Le Fort fracture types involve the pterygoid processes of the sphenoid bones and therefore, disrupt the intrinsic buttress system to the midface. However further differentiation among Le Fort fractures can be done according to involvement of the maxillary, nasal, and zygomatic bones (P. N. Manson et al., 1983).

Le Fort I

A Le Fort I fracture is a transverse fracture that extends through the maxillary bone at the level of the pyriform aperture and progresses posteriorly to the pterygoid plates. Fractures will include anterior, lateral, and medial maxillary walls, exposing the nasal cavity and sinuses in instances of open fractures.These fractures result in mobility of the tooth-bearing maxilla and hard palate from the midface and are associated with malocclusion and dental fractures.

The signs of Le Fort I fractures consist of: swelling, pain, paresthesia of infraorbital nerve, intraoral laceration, nasopharygeal hemorrhage, malocclusion (Cunningham, 2012; Morris & Tiwana, 2013).

Le Fort II

Le Fort II fracture is pyramidal and contains most of the maxillary, nasal and palatine bones with pterygoid plates and the dento-alveolar segment. The fracture extends from the nasofrontal suture through the nasal bones along the maxilla to the zygomatico-maxillary suture and includes the medial inferior third of the orbit. The fracture then continues through the zygomaticomaxillary suture and through the pterygoid plates. The segments may be intact under the line of fracture, but are often comminuted.

The signs of Le Fort II fractures other than Le Fort I consist of: Cerebrospinal fluid rhinorrea, epistaxis, diplopia, subconjunctival hemorrhage (Cunningham, 2012; Morris & Tiwana, 2013).

Le Fort III

Fractures at the Le Fort III level include; the maxilla, nasal bones, zygomas, palatine bone and the pterygoid plates. These fractures essentially separate the face along the base of the skull. The fracture line extends from the nasofrontal suture along the medial wall of the orbit through the superior orbital fissure. It then extends along the inferior orbital fissure and the lateral orbital wall to the zygomaticofrontal suture. The zygomaticotemporal suture is also separated. The fracture then extends along the sphenoid bone, separating the pterygoid plates (Cunningham, 2012; Morris & Tiwana, 2013).

Le Fort III fractures may have similar symptoms to those already mentioned in LeFort II injuries. Additionally in the anterior cranial base injury, bilateral periorbital ecchymosis and edema produce the “raccoon eyes” appearance. The surgeon should have a heightened suspicion for cranial injury and possible dural tears. These can be evaluated by assessing for cerebrospinal rhinorrhea or CSF otorrhea (Cunningham, 2012; Morris & Tiwana, 2013).

Clinical Examination and Imaging

For all trauma patients the protocols of advanced trauma life-support should be followed. Following the primary examination and if needed resuscitation, secondarily detailed maxillofacial examination is completed.

Clinically identified fractures are radiographically confirmed. In the past; facial lateral radiographs and the Waters’ view and were used; however, detailed visualization of the fracture area was difficult. Currently for more detailed visualizing in the presence of trauma, coronal and axial com-

puted tomography scans of the midfacial structures are prefered (Morris & Tiwana, 2013).

Management

First care should be taken to create an airway and control bleeding in all injuries. Mostly, the branches of the maxillary artery are the cause of hemorrhage (Harris, Rice, Watts, & Davies, 2010).

Isolated maxillary dentoalveolar process fractures should be manually reduced and fixation should be caried out with ligature wires and arch bars. In the presence of too large segmental fractures that can not be stabilized using only arch bars, with the costruction of occlusal splints or with the strengthened arch bars by adition of acrylics can be used to secure fractured segment. Complications include tooth loss, bone resorption, external root resorption and tooth ankylosis.

The treatment sequence depends on the presence of other facial fractures and preference of surgeon. Working from the "outside in" or "bottom up" sequences are both valid methods. Mandible being intact, creates the the easiest position to obtain projection of maxilla. For this reason before the stabilisation of maxilla strong consideration should be given to the repair of fractured mandible. Intermaxillary fixation (IMF) is the most reliable technique for establishing anterior projection of the maxilla. Intraoral access is often used for the internal fixation. For the allignment of fractured segments temporarily placed IMF may be performed. Reduction and fixation of the fractures are done under direct vision. Coronal flaps or periorbital incisions would be necessary for the complete fixation of Le Fort II and III fractures (Cunningham, 2012).

***Le Fort I*:** The standard approach to reduction and fixation is via an upper transoral vestibular incision.Arch bars should be applied to the dentiton when present. For isolated maxillary fractures at the Le Fort level, maxillomandibular fixation should be applied and maxilla manually reduced. The vertical buttresses of maxilla should be internally fixated with plates and screws . If it is difficult to manually reduce the maxilla because of impaction or because a significant amount of time has elapsed since the injury, Rowe disimpaction forceps can be applied to free the maxillary segment before fixation (Cunningham, 2012; Morris & Tiwana, 2013).

***Le Fort II and III*:** Wide surgical exposure is often necessary when approaching these fractures to ensure proper reduction and stabilization. Transcutenous and/or coronal incision can be used. Occasionally, a Le Fort II fracture can be reduced with the Rowe forceps and maxillomandibular fixation. Maxillomandibular fixation for 4 weeks may be enough for bony healing of stable fractures. More often, open reduction and internal fixati-

on is required to stabilize the fractures.

For edentulous patients establishment of intermaxillary fixation Gunning's splint is preferred. Bone grafting should be considered if there is significant gaps between fractured segments (Cunningham, 2012; Morris & Tiwana, 2013).

Pediatric Maxillary Fractures

The incidence of midfacial fractures in children is low. This is due to bone structure being softer, lack of pneumatization of sinuses compared to adults result in the protection of the midface because of the increased cranium to midface size ratio. Conservative use of rigid plating systems or resorbable plating systems are often used in children in cases of fixation requiring fractures (Morales, Skowronski, & Thaller, 2010).

Zygomaticomaxillary Fractures

Zygomaticomaxillary fractures are common in facial trauma and as with other facial fractures, most commonly occur among men in third decade of life as a result of interpersonal violence or motor vehicle collisions (Balle, Chrıstensen, Greısen, & Jørgensen, 1982). Because of its prominent position on the facial skeleton, the zygomatic bone is responsible for the anterior and lateral projection of the midface and thus is a very important part of facial aesthetics (Ellis, 2013).

The zygoma has four projections, creating the shape of quadrangle (the temporal, frontal, maxillary and the infraorbital rim) and articulates with the temporal, frontal, maxillary, and sphenoid bones, contributing significantly to the stability and strength of the midface. The zygoma may be separated from its four articulations. This is called a zygomatic complex fracture. The zygomatic arch may be fractured as part of a zygomatic complex fracture or independently. A zygomatic complex fracture includes disruption of the four articulating sutures: zygomaticotemporal, zygomaticofrontal, the zygomaticosphenoid and zygomaticomaxillary (Ellis, 2013).

Diagnosis and Examination

Zygomatic fractures are usually treated following the stabilization of more serious injuries after 4 to 5 days of injury with the decreased swelling. Signs of zygomatic fracture are; periorbital or maxillary vestibular ecchymosis, periorbital edema, flattened arch or malar prominence, deformity or displacement, trismus, displacement of palpebral fissure, enophthalmos, exophthalmos, chemosis, and conjunctival hemorrhage. Symptoms that indicate zygomatic injury are; pain, sensory deficit, crepitus from air emphysema, and diplopia (Foo, 1984; Larsen & Thomsen, 1978; Wiesenbaugh, 1970).

Once the examination of the patient has been completed, the anatomic dislocation of the zygoma can best be evaluated by digital exploration. The patient should be in the cephalad position during the examination; the surgeon should compare the right and left sides simultaneously, beginning at the zygomaticofrontal suture and palpating inferiorly and medially along the infraorbital rim. Then the zygomatic arch should be evaluated for dislocation, comminution, or pain. The buccal vestibule and antral wall should be palpated intraorally for detection of crepitus or bony disruption. Palpation is important because acute swelling after an injury can mask the displaced fracture (Bailey, 2012; Ellis, 2013). After the primary and secondary trauma survey, when lifethreatening injuries have been ruled out or managed, radiographic imaging should be considered. Currently, computed tomography (CT) is the gold standard for diagnosing bony injuries of the face (Bailey, 2012; Ellis, 2013).

Management

Once the decision has been made to treat zygomatic injuries surgically, the need for fixation depends on the stability of the structures. Typically, low-energy injuries can be treated with open reduction alone, whereas high-energy injuries will required open reduction and internal fixation. When the CT scan shows a displaced zygomatic fracture that is not comminuted and does not required orbital floor reconstruction, initial exposure should be obtained with a maxillary vestibular incision. If the fracture can be reduced and is stable, no fixation may be required; if the fracture is unstable after reduction, fixation should be performed. If the reduction remains unstable, exposure and fixation of the lateral orbital rim should be accomplished, followed by fixation of the infraorbital rim if necessary. When evidence of a comminuted fracture exists, or when orbital floor reconstruction is anticipated, exposure of all fracture sites should be accomplished before placement of any fixation. This exposure would typically include an approach to the zygomaticofrontal region and infraorbital rim and a maxillary vestibular incision. Fixation in each of these regions is usually necessary (Bailey, 2012; Ellis, 2013).

Blow-Out Fracture

The term “blow-out fracture” refers to an isolated defect in one of the orbital walls, most commonly the floor or medial Wall. Displacement of the bone can vary from minimal (not requiring repair), to significant, requiring reconstruction. The orbital rims and surrounding bones of the face remain intact (Messinger, Radkowski, Greenwald, & Pensler, 1989).

Most blow-out fractures are observed along orbits thin floor which is further weakened by the infraorbital canal presence. Orbital content herni-

ation, often fat tissue, to the maxillary sinüs occurs. Less commonly, blow-out fractures can occur along the medial wall. Isolated blow-out fractures of the orbital roof or lateral wall are considerably rarer. Fractures at these sites tend to be associated with other fractures in the surrounding bones (Antonyshyn, Gruss, & Kassel, 1989).

Diagnosis and Examination

Defect size and the presence of orbital content entrapment determines the clinical problems.

- *Diplopia* results from soft tissue entrapment. As the patient tries to move the globe (usually to look upwards) the tethered fat prevents the inferior rectus muscle from freely moving. Sometimes the muscle itself can become trapped in the fracture. This is a more serious problem, as direct injury to the muscle can result in scarring and persistent diplopia .

- *Enophthalmos* is the "sunken-in" appearance of the globe. Due to the compensatory effects of edema within the ortbit, this can not be detected at first examination. However, follow up of the patient should be carried out in order not to misdiagnose (NL & HC, 1968).

Management

Following the diagnosis, to determine the need for surgery to prevent further damage to the orbit, the initial survey is carried out. In the presence of concomitant facial fractures such as; nasoethmoid, zygoma or frontal bone these must be repaired first. The reason is, success of orbital defect repairs are based on key landmarks and for the support of implant infraorbital rims correct position is essential. In the presence of significantly displaced neighbouring bones it won't be possible (Morris & Tiwana, 2013).

Timing of surgery is dependent on a number of factors; however, immediate exploration and repair is rarely required. Indications for urgent repair include significant entrapment of the muscles. In most "blow-outs" it is the orbital fat that is trapped. However, muscle entrapment can potentially result in ischemic injury to the muscle and subsequent fibrosis. Inappropriately severe pain is considered by some to be a sign of this. If urgent repair is not indicated, most blow-out fractures can be left safely for up to 7–10 days if necessary. To enable further evaluation, resolution of the swelling should be allowed (Morris & Tiwana, 2013).

Naso-Orbito-Ethmoidal Fractures

The naso-orbito-ethmoid (NOE) region of the midface is defined by the orbits laterally, the glabella superiorly and the nose inferiorly. It is a particularly challenging region of the face to reconstruct due to its aesthetic prominence, its 3-dimensional contour and the delicate associated struc-

tures involved in the region (Leipziger & Manson, 1992). NOE fractures occur from direct blunt force trauma to the central face, such as striking the dashboard in a motor vehicle collision (J. Paskert, Manson, & Iliff, 1988).

Diagnosis

Palpation is an effective way to assess the location of fracture in physical examination (J. P. Paskert, Manson, & Manson, 1989). If intercanthal distance is more than 35mm, a displaced fracture may occur in medial wall and if the distance is more than 40 mm, the displaced fracture can be diagnosed. However, in acute phase, it will not be possible to examine physically due to intense pain and initial symptoms may cover up real existing clinical features resulting in misdiagnosis.

After physical examination if NOE fracture is suspected, a fine cut (0.5 mm) maxillofacial CT scan extending from vertex through menton should be obtained. Plain films are of no utility. Axial and coronal views are both required and additional sagittal and 3D views may be helpful if orbital involvement is extensive and/or the fractures are complicated (P. Manson, Markowitz, & Mirvis, 1990).

Classification

Currently the most frequently used classification system was detailed by Markowitz et al. in 1991 for grading injuries. Severity is graded into three types based on the degree of comminution of the NOE segment and the integrity of the medial canthal attachment (Markowitz et al., 1991).

Management

Once an NOE fracture is diagnosed, the surgeon must decide whether an operative intervention is warranted. Generally, this decision is based on amount of displacement and degree of comminution. A nondisplaced, nonmobile NOE fracture can be treated nonoperatively. A minimally impacted, large segment NOE fracture may be treated with closed reduction and splint stabilization, whereas a displaced, comminuted NOE fracture may require operative fixation via one or multiple surgical approaches (Cunningham, 2012; Morris & Tiwana, 2013).

NOE fractures are commonly associated with other facial fractures such as midface fractures, zygımaticomaxillary fractures, and especially frontal sinus fractures. It is paramount to identify and treat the NOE component of these multilevel fractures in order to attain an anatomic reduction. While most isolated NOE fractures do not cause compromise of frontal sinus drainage by blocking the nasofrontal outflow, obstruction of the nasofrontal outflow tracts by comminuted NOE fractures may increase the risk of mucocele formation with associated frontal sinus fractures. One

must first disimpact and fixate the NOE component before obliterating or cranializing the frontal sinus to avoid mucocele formation (Cunningham, 2012; Morris & Tiwana, 2013).

Proper restoration of form and fixation of NOE fractures usually require the wide access provided only by a coronal flap. Wide exposure of the nasal bones and medial orbital walls is enabled by vertical incision of the periosteum over the nasal bridge. An intraoral approach to the paranasal areas of the maxilla or a transconjunctival approach to the inferior orbital rim and infero-medial wall are also often needed. The central fragment must usually be repositioned ventrally by intranasal manipulation to re-establish projection of the nasal bridge and reconnect it to the frontal bone. Both nasal bones, if split and splayed, must be repositioned and held in the proper angle. The medial canthal ligaments must be restored to their correct position, preferably by exact reduction of their bone attachments. Various miniplates from different manufacturers are available to achieve these goals. The medial orbital walls are usually comminuted, and because the thinness of the lamina papyracea precludes use of any osteosynthesis devices, they must be reconstructed by autogenous bone grafts or implants. In cases where the medial canthal ligament is detached or attached to an unusable bone fragment, it must be engaged and fastened in its proper position (Cunningham, 2012; Morris & Tiwana, 2013).

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Chapter 40

LIVER DEVELOPMENT, LIVER HISTOLOGY AND THE ROLE OF THE LIVER IN SEPSIS

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1. Liver

1.1 Liver Development

The liver appears in the middle of the third week of development in the human, with the thickening of the endoderm in the anterior part of the region, which takes the name of the hepatopancreatic ring in the duodenum. It then deepens to form what is called the hepatic diverticulum or liver bud (Gürsoy & Koptagel, 1997). This protrusion, known as the liver bud, consists of cells that penetrate the mesodermal plate, the septum transversum, and show rapid proliferation between the pericardial cavity and the yolk stalk. As the liver cells continue to enter the septum, the connection between the hepatic diverticulum and the intestine (duodenum) narrows, forming the bile ducts. A small ventral protrusion from the sources of bile becomes the gallbladder and cystic duct. In later stages of development, epithelial cell cords mix with vitellin and umbilical veins to form hepatic sinusoids. The liver cords differentiate into the parenchyma and lay the inner surface of the bile ducts (Sadler, 2005). Fibrous tissue, hematopoietic tissue and Kupffer cells of the liver develop from mesenchyme in the septum transversum (Moore & Persaud, 2002), and hepatocytes from the endoderm of the anterior intestine (duodenum) (Gürsoy & Koptagel, 1997). When liver cells occupy the entire septum transversum, the organ grows towards the caudal in the abdominal cavity. At the same time, the septum transversum mesoderm, which is located between the liver and anterior intestine and between the liver and the abdominal wall, becomes membranous, forming a small omentum and falciform ligament, respectively. These two ligaments together form the peritoneal connection between the anterior intestine and the anterior abdominal wall and are called the ventral mesogastrium (Sadler, 2005). Mesoderm on the surface of the liver differentiates outside of a small area and forms the visceral peritoneum. In this small area, contact with the septum transversum is maintained. This part of the septum is in dense mesenchymal tissue and will form the tendinous part of the diaphragm in the future. This surface of the liver that comes into contact with the future diaphragm is not covered with the peritoneum and is known as the naked surface of the liver (Moore & Persaud, 2002; Sadler, 2005) (Figure 1. 1).

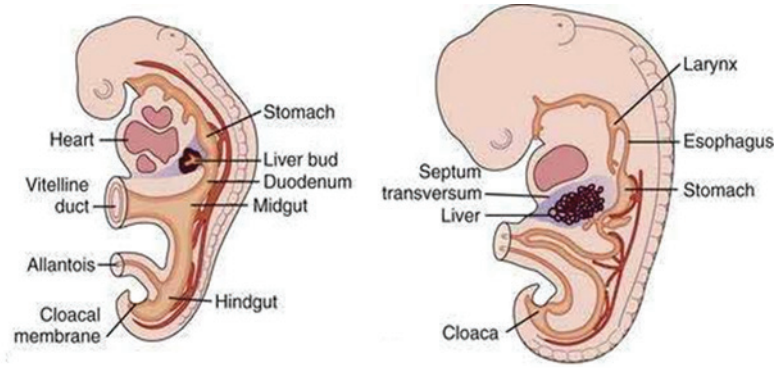

Figure 1.1. *Embryonic development of the liver (Sadler, 2005).*

The liver grows rapidly between the 5th and 10th weeks, filling most of the upper abdominal cavity. The amount of blood in the umbilical vein determines the development and segmentation of the liver. Initially, the right and left lobes are the same size, but soon the right lobe grows larger. Hematopoiesis, which starts in the sixth week, gives the liver a bright red color. This hematopoietic activity is also responsible for the size of the liver between the 7th and 9th weeks. In the ninth week, the liver is 10% of the total body weight (Moore & Persaud, 2002). The liver functions as a hematopoietic organ in the human embryo and fetus of 6-22 weeks of age (Gürsoy & Koptagel, 1997). Blood production in the liver gradually decreases over the last two months of intrauterine life and remains only small hematopoietic islets at birth. The weight of the liver at birth is about 5% of the body weight (Sadler, 2005). One of the important functions of the liver is bile production. Liver cells begin to produce bile from the 12th week. The produced bile flows through the choledochal duct, which is formed by the combination of the cystic duct and the hepatic duct, into the intestine. It gets dark green color due to bile content inside the digestive tract (Moore & Persaud, 2002; Sadler, 2005). As a result of changes occurring in the duodenum, the entry point of the choledochal canal from the initial anterior position of the choledocal canal changes from the initial anterior position to the posterior and the choledocal canal passes behind the duodenum (Arınc & Ehan, 2001; Moore & Persaud, 2002; Sadler, 2005).

1.2. Liver Histology

The liver, the body's largest organ after the skin, is located in the abdominal cavity under the diaphragm (Junqueira & Carneiro, 2006). The liver with an average weight of 1400 g constitutes 2% of body weight in adults (Sancak & Cumhur, 2004). The ratio of liver to body weight is higher in the fetus. The liver, which is reddish brown, has a structure with abundant veins and easily tears (Ekinci, Unur, & Ülger, 2005).

The liver, which consists of four weakly constrained lobes (Kierszenbaum, 2006), contains porta hepatis (liver hilus) in the form of the letter H between the lobes. Large vessels, lymph nodes, nerves and bile duct entering and exiting the organ pass through this area. The liver is exocrine due to the discharge of its secretion into the duodenum through the bile ducts, and it also has an endocrine gland feature as it gives the substances it synthesizes directly into the blood (Doğanyiğit, 2012). Blood is transported to the liver through two vessels, the portal vein and the hepatic artery. The portal vein, which provides 75-80% of the incoming blood volume, carries blood from the digestive tract, spleen and pancreas, while the hepatic artery, a branch of the celiac line, carries 20-25% of oxygenated blood. Blood from the branches of the portal vein and hepatic artery mixes in the sinusoids of the liver lobules (Kierszenbaum, 2006). Digestive products absorbed in the intestines and coming through the blood are metabolized in the liver, modified, then stored or given to the blood for use in other organs. The liver is externally covered with the visceral peritoneum. Under the peritoneum is a capsule called Glisson capsule, rich in elastic fibrils (Doğanyiğit, 2012). The Glisson capsule has a tight connective tissue structure. Its average thickness is about 70-100 μm. Serous mesothelium covers the peritoneum-facing side of the capsule. Mesothelium acts as a shield against pathogens and other harmful substances. There are collagen and elastic fibrils in the mesothelial structure. The Glisson capsule supports the liver from the outside, protects the shape of the organ, and also contributes to the support stroma by sending extensions to the liver parenchyma (Carnerio & Kelley, 1998; Eşrefoğlu, 2009). The thin connective tissue septum, which separates from the capsule in the porta hepatis region, enters the organ and separates the liver into lobes and lobules (Doğanyiğit, 2012). The liver lobule is a structural unit of the liver and is a polygonal structure with an average size of 0.7x2 mm (Junqueira & Carneiro, 2006). In the sections where the lobules concoin, the connective tissue increases and forms a triangular structure. The portal canal, the Glisson triangle, the Kiernan gap, or the Portal gap, contains a branch of the portal vein, a hepatic artery, a bile duct and lymphatic vessel. The triple structure consisting of the hepatic artery, portal vein and bile duct is called the 'portal triad' (Doğanyiğit, 2012). The venule is usually the largest in diameter. The venule wall is thin and lumen irregular. It carries blood from superior and inferior mesenteric and splenic veins. The artery, which has a smaller diameter than the venule, has a smoother lumen and a thick wall than the venule. It carries coming blood from the celiac branch of the abdominal aorta. The bile duct is covered with a single-layer cubic epithelium (Ross & Pawlina, 2017; Yağmurca, 2003) and ejaculates bile from hepatocytes into the hepatic canal (Junqueira & Carneiro, 2006). The portal canal surrounds hepatocytes located outside the lobule. The area between connec-

tive tissue stroma and hepatocytes is defined as the Mall region, and this area is thought to be liver lymph production areas (Doğanyiğit, 2012). In addition to this triple structure, there are lymph vessels and nerve fibrils in the portal areas. Sympathetic (postganlionic) nerve fibrils in the portal area originate from celiac ganglion and parasympathetic (preganglionic) nerve fibrils originate from N. Vagus. Both systems provide innervation of arterial muscles in the portal area (Ross & Pawlina, 2017; Yağmurca, 2003).

Three different liver lobules have been identified to explain the structural organization and functions of the liver (Figure 1. 2). These; the classical concept of the liver lobule based on structural parameters; the concept of portal lobule based on the approach of bile discharge from adjacent lobules to the same bile duct. The third approach is the concept of liver acinus (hepatic acinus, portal acinus) based on the distribution of oxygen throughout the venous sinusoids of adjacent lobules (Kierszenbaum, 2006). The classical liver lobule is polygonal, with vena centralis in the center and portal area in the corners. Blood from sinusoids is drains a vena sentralis which located in the center of the lobule. Hepatocytes are arranged radially and arranged like bricks of a wall. These cell plates front from the periphery of the lobul to its center. They are freely anastomosed to form a labyrinth and sponge-like structure. Sinusoid capillaries lie between these plates. In the classical liver lobule, blood flow is from periphery to center. Bile flows in the opposite direction of blood flow. Bile, to intralobular bile ducts from bile canalicules; then it passes to Herring canal and empties into bile ducts in the portal area (Kierszenbaum, 2006).

Portal lobule which another type of lobulation made by considering the secretion of bile, there is a portal canal in the center and a central vein at the corners of the triangle-shaped lobule. In the portal lobule, blood flow is from center to periphery and bile flow is from periphery to center (Kierszenbaum, 2006).

The concept of liver acini is better suited to describe the liver's regeneration conditions, metabolic activity and cirrhosis development. Liver acinus is an oval-shaped region located between two central veins and two portal triads in the axis of interlobular vessels. Here, the blood flows from the center of the acini towards the vena sentralis (Terzi, 2008).

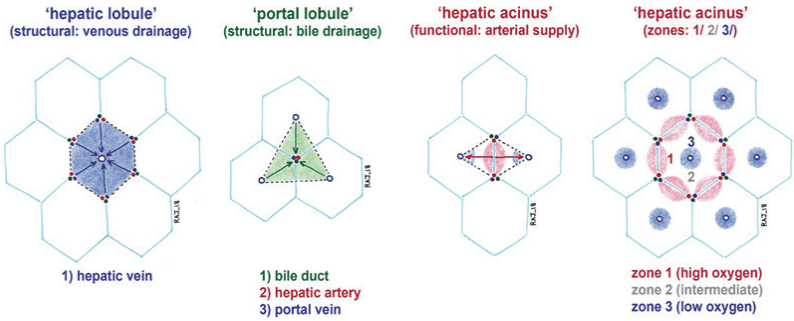

Figure 1. 2. *Liver lobule models (Browness & Taylor, 2019).*

Limits in liver acini can be determined by the last branch of a hepatic artery. The flow of throughout the venous sinusoids of coming blood from the artery creates a rating called zone I, zone II and zone III at the oxygenation and nutrition (Kierszenbaum, 2006). Zone I (peripheral zone); it is the richest region in terms of both food and oxygen. The cells in zone I are the closest to the vessels. In this region, since the blood moves from the center of the lobule to the periphery, zone 1 cells that encounter oxygen and nutrient-rich blood show continuous activity. Since this region is the first region to encounter blood, the first region to be affected in a possible toxic substance in the blood is again zone I (Arslan, 2008; Fawcett & Bloom, 1994). Zone III is the closest part to the central vein, and this area is the poorest part of oxygen. Therefore, the first region to be affected in case of hypoxia is zone III (Kierszenbaum, 2006). Zone III is the first region where physiological and pathological lubrication in the liver is first seen (Arslan, 2008). Zone II is in an intermediate state in terms of both nutrients and oxygen (Kierszenbaum, 2006).

There are sinusoid capillaries between the hepatocyte cords starting from the central vein and proceeding as anastomosing towards the periphery (Carnerio & Kelley, 1998; Eşrefoğlu, 2009; Ross & Pawlina, 2017). Sinusoids, one of the important structures of the liver, are special type capillaries with a wide lumen of 9-12 microns diameter and irregular shape (Doğanyiğit, 2012). Sinusoidal capillaries in the liver are irregularly enlarged vessels, consisting of intermittent windowed endothelium layer. The basal lamina underneath the endothelial cells is intermittent, and sinusoids are supported by a thin reticular fiber network (Junqueira & Carneiro, 2006). Sinusoids take blood from the vena porta and arteria hepatica and open to the vena centralis (Arslan, 2008). In zone I, sinusoids are curved, narrow and anastomotic and tend to be more parallel and wider than zone III (Bioulac-Sage, Le Bail, & Balaboud, 2007; Braet, Luo, & Spector, 2001; Enomoto et al., 2004). The blood flow is unidirectional in the sinusoids from zone I to zone III. Changes in microcirculation also occur in sinusoids (Bioulac-Sage et al., 2007). Sinusoids contain endothe-

lial cells, Kupffer cells and pericinusoidal fat storage cells (ito cells). Sinusoidal cells represent 6% of the lobular parenchyma (2.5% endothelial, 2% kupffer and 1.4% ito cells) and 26.5% of the liver plasma membrane (Bioulac-Sage et al., 2007; Ross & Pawlina, 2017). Endothelial cells are thin cytoplasm cells with heterochromatic nuclei located in the sinusoid wall (Arslan, 2008). It is of windowed type due to the large number of holes in its cytoplasm. The pinocytotic vesicles in the cytoplasm with a small number of organelles are indicative of having pinocytosis activity (Doğanyiğit, 2012). Both the intermittent placement of the endothelial cells and the intermittent basal laminate underneath the endothelial cells facilitate the passage of substance from the blood to the Disse interval. In addition to the endothelial cells, there are macrophages called Kupffer cells, which are mononuclear phagocyte system cells (Junqueira & Carneiro, 2006). First, it was demonstrated by Von Kupffer in 1876 in the liver pericinisoidal connective tissue by the gold chloroid method (Ovale & Nahirney, 2009; Ross & Pawlina, 2017). They are cells that resemble the star shape with cytoplasmic extensions towards the environment. It has cytoplasmic protrusions extending towards the lumen and the endothelium, which borders the sinusoid wall. They are located between the endothelial cells at certain intervals. They originate from blood monocytes and have phagocytosis abilities (Tekelioğlu, 2002). It is found in zone I more than zone III (Bioulac-Sage et al., 2007). Kupffer cells have a large oval-shaped nucleus. The granular endoplasmic reticulum and golgi apparatus are well developed. This shows that they actively synthesize protein. There is abundant lysosome in the cytoplasm (Kierszenbaum, 2006; Ross & Pawlina, 2017). The main functions of Kupffer cells are to eliminate aged erythrocytes, to phagocyte bacteria from the large intestine through portal blood and to secrete proteins related to immunological events. 15% of liver cells are Kupffer cells (Junqueira & Carneiro, 2006). Kupffer cells can be identified by monoclonal antibody such as KP1 (anti-CD68) (Bioulac-Sage et al., 2007). Another type of cell in the sinusoid wall is fat storage cells. These cells, often located in the inter hepatocytic recess, contain lipids and vitamin A. It has a thin and long cytoplasmic structure surrounding the endothelial cells (Wake, 1995). These cells have functions such as retrieval, storage and release of retinoids, synthesis and secretion of some extracellular matrix proteins and proteoglycans, and regulation of the sinusoid lumen diameter in response to various regulatory agents (e.g., prostaglandins and thromboxane A2) (Junqueira & Carneiro, 2006). In addition, they are thought to be involved in the production of reticular and collagen fiber and stem cells for hemopoiesis in the fetal period (Doğanyiğit, 2012).

The region between the hepatocytes and the sinusoidal endothelium is defined as the Disse interval, the pericinusoidal space or the subendothelial

space. It contains reticular fibrils and a small amount of collagen fibrils. Hepatocytes have abundant microvilli on their faces facing this range. This region, which does not contain any intermediates, does not contain shaped elements of blood. However, it is stated that the presence of blood plasma, the active exchange of metabolites between blood and hepatocytes, and the microvilli in the liver cell surface play an increasing role in this change (Doğanyiğit, 2012).

Hepatocytes are functional parenchymal cells in the lobule (Tekelioğlu, 2002). It is stated that the life span of these cells, which make up 80% of the liver cell totality, is 200-400 days. These cells with a diameter of 20-30 micrometers are polygonal and arranged as plates showing branching and anastomosis. Hepatocytes; it has three surfaces, sinusoidal, canalicular and intercellular, which play an important role in the transfer of substances between blood vessels and bile canaliculus. The sinusoidal surface, which constitutes 70% of the hepatocyte surface, is the surface of the hepatocytes that contains abundant microvillus overlooking the Disse range and are areas where material transfer is made between sinusoids and hepatocytes. The canalicular surface between the two adjacent hepatocytes is about 15% of the hepatocyte surface and the surface into which the bile is introduced into the canaliculus from the hepatocytes. The bile canaliculus is a tubular gap of 0.5- 2.5 μm in diameter between the smooth membranes of two adjacent liver cells. The hepatocyte membranes that limit this region form the microvilli extending into the lumen. The intercellular surfaces of hepatocytes are the surface between neighboring hepatocytes that are not in contact with sinusoids or canaliculi, and another 15% of the hepatocyte surface. Hepatocytes have nuclei with large rounded and smooth surfaces, centrally located, varying in size from cell to cell. Approximately 25% of liver cells that contain single nuclei can be double nuclei. The nucleus contains one or more vesicular nucleolus with several scattered chromatin groups (Doğanyiğit, 2012). Hepatocytes at different distances from portal areas show structural, biochemical and histochemical differences. A large number of mitochondria found in the cytoplasm synthesize ATP for use in a variety of cell functions. Hepatocyte cytoplasm contains abundant lysosomes, and these are important for the destruction and transformation of intracellular organelles (Ovale & Nahirney, 2009). The basophilic cytoplasm contains both Rough and Smooth Endoplasmic Reticulum (RER, SER). SER, which has a tubular network structure showing branching, functions in the production of glycogen. RER, which is monitored as cisterns parallel to each other, is the organelle responsible for protein synthesis. Hepatocytes contain about 200-300 peroxisomes with diameters ranging from 0.2-1 μm (Kierszenbaum, 2006; Yağmurca, 2003). The Golgi complex is typically located near the bile canalicula and around the nucleus in he-

patocytes. They function as the formation of the lysosome, the synthesis of plasma proteins, the secretion of glycoprotein and low-density lipoproteins. In hepatocytes, glucose is stored as glycogen. In electron microscopic examinations, glycogen appears as aggregated granules in SER clusters (Kierszenbaum, 2006; Ovale & Nahirney, 2009).

2. Sepsis

Despite advances in experimentally and clinically, sepsis is defined as an irregular systemic inflammatory response triggered by infection, trauma or toxins, morbidity and mortality rates are quite high. Inflammation observed in patients progresses to sepsis, severe sepsis, septic shock, and multiple organ failure syndrome. Especially; infants, children, and the elderly (> 65 years) with immune compromised individuals are susceptible to sepsis and sepsis-related deaths (Iwashyna, Cooke, Wunsch, & Kahn, 2012). Studies predict that by 2029 the number of cases of annual sepsis will be 50% more than that seen in 2001 (Gaieski, Edwards, Kallan, & Carr, 2013).

Classically, the acute phase of sepsis is initially characterized by a strong pro-inflammatory and natural immune state aimed at eliminating the pathogen, but in the later phases of sepsis, the immune system shifts towards an anti-inflammatory, immunosuppressive state, inflammation decreases and begins tissue repair (Ranieri et al., 2012). All of the typical features of sepsis, such as high fever, tachycardia, tachypnea, inflammation, immune activation, phagocytosis and acute phase reactant production, require physiological energy sources. However, despite increasing nutritional requirements, patients are often reluctant or unable to eat; this creates an energy problem. There is also a problem with mitochondrial respiration in sepsis (Protti et al., 2007).

Sepsis is a life-threatening disease triggered by germ infestation and disorder of the natural immune system (Wiersinga, Leopold, Cranendonk, & van der Poll, 2014). and uncontrolled cytokine production, reactive oxygen species (ROS) and nitric oxide (NO) cause systemic inflammatory response, redox imbalance and arterial hypotension. This condition, which contributes to the pathophysiology of sepsis, causes progressive and irreversible multi-organ dysfunction and high mortality (Angus & van der Poll, 2013).

3. The Role of the Liver in Sepsis

Inflammatory pathogenesis and organ damage leading to death from sepsis are not fully understood for vital organs, especially the liver. The liver works as a lymphoid organ in response to sepsis. The liver-mediated

immune response in sepsis is responsible for the removal of bacteria and toxins, as well as causing inflammation, immunosuppression and organ damage. To reduce morbidity and mortality rates from sepsis, alleviating liver damage and improving liver function may be an important option (Yan, Li, & Li, 2014). Sepsis is one of the most common causes of patient mortality, with an annual number of 18 million cases in intensive care units and a mortality rate of around 28-40% worldwide (Hotchkiss & Nicholson, 2006).

A centrally acting liver in metabolic and immunological homeostasis is the largest gland in the human body and is responsible for more than 200 functions such as detoxification, storage, energy production, nutrient conversion, hormonal balance and coagulation. These vital physiological functions make the liver a critical organ for sepsis. There are studies showing that liver damage and dysfunction can cause disease progression and death of the patient, especially in sepsis (Canabal & Kramer, 2008; Yan et al., 2014). Various pathogens, toxins, or inflammatory mediators cause damage to the liver, and if this injury cannot be cured, this progresses from hepatocellular dysfunction to liver damage and subsequent liver failure. Liver damage is defined as an irreversible injury that occurs in hepatocytes. Liver failure, on the other hand, is defined as continuous, serious damage to the liver and loss of function in 80-90% of liver cells (Jarrar & Chaudry, 2001). In the early stages of sepsis, liver functions have been shown to be impaired by many experimental and clinical studies (Kramer et al., 2007; Recknagel et al., 2012). Liver damage that occurs early and after sepsis has a critical effect on the severity and outcome of sepsis due to the multiple functions of the liver. The average incidence of liver dysfunction in patients with sepsis has a rate of 39.9% (Yan et al., 2014). 8.5% of patients with sepsis also have liver failure. Liver dysfunction and liver failure are associated with serious complications in sepsis. In patients with chronic liver disease such as liver failure, cirrhosis, trauma and drug-induced liver damage, risks such as sepsis, multiple organ failure (MOF) and death due to sepsis are quite high (Yan et al., 2014).

Liver damage in patients with sepsis is defined by histological changes in the liver. Hepatitis and liver steatosis were found in most autopsy reports of patients who died from sepsis. Liver lesions include portal inflammation, centrilobular necrosis, lobular inflammation, hepatocellular apoptosis, cholangitis / cholangiolitis and steatosis (Koskinas et al., 2008). When evaluating sepsis-related liver injuries in animal models, steatosis, cholestasis and hepatocellular damage associated with hepatocyte apoptosis, necrosis and neutrophil accumulation were observed after sepsis-related liver injuries were evaluated. The main histological changes observed in this process include necrosis, capsular inflammation, portal inflamma-

tion, balloon degeneration, steatosis and liver fibrosis (Akcan et al., 2006; Doganyigit, Okan, Kaymak, Pandir, & Silici, 2020; Gonnert et al., 2012; Muftuoglu, Aktekin, Ozdemir, & Saglam, 2006). Clinically, early signs of hepatic dysfunction include high serum bilirubin and alkaline phosphatase in patients, and in the future, elevation of transaminases after long-term hypotension is used as a marker. In addition, usable many biomarkers such as Glutathione S-transferase A1-1(Koo, Zhou, Chaudry, & Wang, 2000), carbamoyl phosphate synthetase 1 (Crouser, Julian, Huff, Struck, & Cook, 2006), and glucose 6-phosphatase (Maitra, Wang, Brathwaite, & El-Maghrabi, 2000) to demonstrate liver dysfunction in early sepsis.

The liver plays critical roles in clearing bacteria in sepsis, mediating inflammatory responses, and clotting that can regulate kidney failure, acute lung damage, acute respiratory distress syndrome, coagulopathy and hepatic encephalopathy (Yan et al., 2014). Bacterial clearance is the most important process in the survival of patients with sepsis (Protzer, Maini, & Knolle, 2012). Sepsis-induced liver dysfunction is induced by many pathogenic factors (Hattori, Hattori, Suzuki, & Matsuda, 2017), including lipopolysaccharide (LPS), inflammatory factors or pathogens. During sepsis and / or septic shock, the natural metabolism of hepatocytes is changed towards the inflammatory response. The main cytokine of the liver inflammatory response is interleukin-6 (IL-6), which is responsible for the synthesis of acute phase proteins such as C-reactive protein (CRP), α-1 antitrypsin, haptoglobin, prothrombin and fibrinogen (Aninat et al., 2008). IL-6 secretion is induced by endotoxin (lipopolysaccharide-LPS) and tumor necrosis factor-α (TNF-α). LPS also stimulates the secretion of TNF-α, interleukin-1β (IL-1β), interleukin-12 (IL-12) and interleukin-18 (IL-18) by kupffer cells (Kolios et al., 2008). IL-18 is the main factor responsible for LPS-induced liver damage. IL-18 causes interferon-γ (IFNγ) secretion, causing hepatocyte apoptosis, an increase in TNF-α concentration, and a monocyte / macrophage surface receptor responsible for binding of lipopolysaccharide-binding protein (LPS / LBP) complex (Woznica, Inglot, Woznica, & Lysenko, 2018).

The majority of lipopolysaccharide (LPS), the key molecule responsible for bacterial infection, is cleansed by the liver, and it has been shown in several studies that within 60 to 80% of bacteria administered by intravenous injection can be removed from the liver (Deng et al., 2013; Gonnert et al., 2012). Various cell types in the liver, especially Kupffer cells, function in this process (Dhainaut, Marin, Mignon, & Vinsonneau, 2001). The liver reticuloendothelial system quickly and effectively captures and eliminates bacteria. Kupffer cells, which show endocytic and phagocytic capacity to remove bacteria and soluble bacterial products, also cooperate with platelets and neutrophils in removing bacteria from the bloodstream.

By aggregation of platelets, bacteria are detected, and they are prevented from escaping from Kupffer cells. Neutrophils that migrate and accumulate to liver sinusoid through chemokines secreted by Kupffer cells during endotoxemia and sepsis then interact with platelets to capture and eliminate microbes (Protzer et al., 2012; Wong, Jenne, Petri, Chrobok, & Kubes, 2013; Yan et al., 2014). The role of Kupffer cells, which are macrophages of the liver, is to prevent bacteria and endotoxins from entering the systemic circulation by removing them from the portal vein blood. However, the inflammation cascade is strongly activated during sepsis. But this mechanism not only kills bacteria, but also damages the liver. In response to excessive LPS stimulation, kupffer cells secrete nitric oxide (NO), reactive oxygen species (ROS), TNF-α, IL-1β, IL-6, IL-12 and IL-18, causing endothelial cell and hepatocyte damage (Kolios et al., 2008).

Liver sinusoidal endothelial cell dysfunction contributes to the development of multiple organ failure syndrome (Aird, 2003). Liver sinusoidal endothelial cells (liver sinusoidal endothelial cells- LSEC) have the potential to produce immunoregulatory and pro-inflammatory cytokines such as NO, IL-1 and IL-6. It has been reported that this production increases when LPS is applied (Knolle et al., 1997). In particular, Endothelin-1, a potent vasoconstrictor released by LSECs, has been reported to have a strong correlation with inflammatory response. In addition to the expression of cytokines such as TNF-α, IL-1 and IL-6, nuclear factor enables transcription factors such as kappa B (NF-κB) to be activated (Yeager et al., 2012). In addition, endothelin-1 concentrations have proved to be an early and sensitive mortality marker in septic shock patients (Brauner, Rohde, & Clausell, 2000). In addition, LSECs act as antigen presenting cells for $CD4^+$ T cells, and LPS suppresses $CD4^+$ T cell activation through antigen presenting LSECs. In addition, LSECs have pores pierced with fenestrations that facilitate the transport of lipoproteins and macromolecules between blood and hepatocytes. In gram-negative bacterial sepsis, LSECs have been shown to be involved in the pathogenesis of hyperlipidemia by defenestration by bacterial toxins (Cheluvappa et al., 2010).

The liver is also the main site of inflammatory responses to bacterial endotoxins during sepsis. There are studies showing that the liver is the main source of production of cytokines and NO when exposed to host endotoxins (Siore et al., 2005). The injured liver can induce severe and systemic harmful inflammatory responses in other organs during sepsis. High levels of pro-inflammatory cytokines are associated with liver dysfunction and multiple organ dysfunction syndrome (Gustot, Durand, Lebrec, Vincent, & Moreau, 2009). Kupffer cells are responsible for the production of inflammatory cytokines in the early stages of sepsis. Following the attack of harmful bacteria and / or endotoxins, Kupffer cells increase the release

rate of several early pro-inflammatory mediators such as TNF-α, IL-1, IL-6, interferon gamma (IFN-y). Hepatic natural killer T (NKT) cells play a critical stimulating role in developing a local and / or systemic pro-inflammatory response to sepsis. NKT cells make up 10-20% of the total T cell population in the liver, which is much higher than other organs. Therefore, hepatic NKT cells play a critical role in regulating the systemic inflammatory response and promote survival in sepsis (Hu et al., 2009).

In addition to the production of pro-inflammatory cytokines, the liver's response to sepsis includes the induction of anti-inflammatory mediators, which play a critical role in preventing immune reactions such as IL-10, transforming growth factor β and glucocorticoids. Many liver-resident cell types can perform immune-regulating functions and work with circulating cells to induce liver tolerance of toxins, suppress systemic inflammatory responses, and protect organs from injuries (Yan et al., 2014). In the progression of sepsis, the roles of complex reactions and substances such as reactive oxygen species (ROS), nitrogen types, inflammation and apoptosis (Andrades et al., 2011; Andrades, Ritter, Moreira, & Dal-Pizzol, 2005), as well as changes in hepatocytes such as apoptosis and autophagy have been investigated (Chen et al., 2016; Cho, Kim, Choi, & Lee, 2016). Autophagy plays an important role in reducing sepsis-related dysfunction of multiple organs and may be an important target for the future management of sepsis (Jiang et al., 2015). In studies conducted, it has been reported that inhibition of autophagy causes organ dysfunction in patients with sepsis and increases the mortality by consuming immune cells (Lin et al., 2014; Ren, Zhang, Wu, & Yao, 2017). However, Takahashi et al. (Takahashi et al., 2013), in the sepsis model induced by cecal ligation and puncture, he suggested that hepatocyte apoptosis is inhibited, and liver damage is improved (Takahashi et al., 2013; Tang et al., 2013).

Conclusion

The liver plays an important regulatory role in sepsis and homeostasis. Therefore, in order to progress in sepsis, research on the pathophysiology of liver injury and mechanisms accompanied by liver dysfunction should be increased. This will contribute to the development of new strategies for the diagnosis and treatment of sepsis.

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Chapter 41

A RESEARCH STUDY CONDUCTED REGARDING THE IMPACTS OF UNIVERSITY STUDENTS' CONSUMPTION OF DIETARY SUPPLEMENTS ON THEIR NUTRITION

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1. INTRODUCTION

Good nutrition is described as the adequate and properly balanced intake of nutrients, which meet the requirements of the body and enable people to lead healthy lives. People need to get proper nourishment in every stage of their lives starting from the time they are in their mothers' wombs. However, in such periods as infancy and adolescence, an adequate and well-balanced diet becomes more important. Whether an individual is well or poorly nourished has a significant impact on their health throughout their lives (Baysal, 2009).

In recent years, due to the fact that people have become more knowledgeable about nutrition, their efforts to lead a healthier life have also increased, causing them to follow various dietary practices. While some of these promote good health, some, far from being suitable, present risks for health. Diets followed in ignorance especially to lose weight without consulting an expert, vitamin-mineral tablets taken in excessive doses, alternative medicine practices again applied without consulting an expert are the major risk factors in this issue.

Dietary supplements have various different forms, including vitamins, minerals, enzymes, extracts, herbal mixtures, herbal therapies and preparations sold without prescription (Stanner, 2005). Dietary Supplement Health and Education Act of the US Constitution defines dietary supplements as products that are intended to supplement the diet that bear or contain one or more of such dietary ingredients as a vitamin, a mineral, an herb or other botanical, an amino acid, metabolite or extract. As well as being composed of essential nutrients like essential vitamins, minerals, and amino acids, dietary supplements can also contain such substances as ginseng, gingko and other botanical products, which are not essential (Williams, 2005).

Dietary supplement industry is large and varied with its food substitutes, weight-loss foods, curative herbs, botanicals and mineral and vitamin tablets. In recent years, people have been introduced to dietary supplements at a rapid pace. Among these supplements, vitamin and mineral supplements are the ones which are sold the most (Briefel et al. 2005). Although the general image is that it is women who make use of alternative medicine and dietary supplements, men with prostate disorders also frequently turn to them in search for remedy (Andrew, Fleshner, Klotz, 2002). This study has been carried out in order to determine the dietary supplement intake status of young people, their purposes for supplement use, which supplement group they consume the most, whether their income status affects their supplement use and the relationship between the diet adequacy of the young people who take dietary supplements and those who do not.

MATERİALS AND METHODS

The study was carried out at Gazi University, the Faculty of Vocational Education. In order to facilitate the access to a greater number of young people, the university students were included in the study. A survey was conducted in order to collect data and information about the students' personal details, their dietary supplement intake status, how frequently they consume them, the types of dietary supplements they use and their attitudes towards healthy living. A food consumption chart was used to record the students' three-day food intake with the purpose of determining their food consumption status.

The survey and the food consumption scale were prepared by the researcher with the aid of several resources. The questionnaires were first distributed to the students and then they were collected after they had been filled out. The food consumption scales were given with the questionnaires and the students were asked to fill them in under the supervision and the guidance of the researcher.

For the questions in the first section regarding height and weight, taking into consideration the fact that the students might not know their exact weight and height or that they might show reluctance to provide correct information, the researcher measured and recorded their heights without their shoes on using a non-stretchable measuring tape, and similarly, she weighed them on a scales after having them take off their shoes and recorded their weights.

Before starting the evaluation of the data, the questionnaires were examined in detail in order to make sure that they had been properly filled in. Those questionnaires that were filled in incorrectly were considered invalid and were not included in the evaluation process. A computer based assessment of the data obtained from the questionnaires was made using a software package called SPSS (Statistical Package for the Social Sciences) 13 for Windows and then these data were evaluated and interpreted. The student version of Nutrition Database Program was utilized to assess food combinations and dietary contents.

In order to evaluate the intake of nutrients and energy, RDA values, which are recommended for daily intake according to gender and age, were utilized. According to these values, those who consume 67-133% of their recommended daily energy and nutrient intake are considered to receive sufficient nourishment, those who consume less than 67% are considered to receive insufficient nourishment and those who consume more than 133% are considered to receive excessive nourishment. When the adequacy level of the students' nutrient intake was assessed, it was determined based on the food intake of the students only; dietary supplements were

not included in it.

When the students were categorized according to their BMI (Body Mass Index), the BMI classification of WHO (World Health Organization) was taken as a basis. According to this, the individuals whose BMIs are lower than 18.5 kg/m² are considered underweight, those with a BMI of 18.5-24.9 kg/m² are considered normal, those with a BMI of 25-29.9 kg/m² are considered overweight, those with a BMI of 30-39.9 kg/m² are considered obese and those with a BMI of 40 kg/m² and above are considered extremely obese (WHO, 2020).

FINDINGS AND DISCUSSION

In this section, the demographic features of the students included in the study, their dietary supplement intake status, what types of dietary supplements they take and how frequently they take them, their purposes for taking dietary supplements, their nutritional education status and the results pertaining to the comparison of the influence of different variables on the intake of dietary supplements have been presented. A total of 340 university students, 73 of whom are male and 267 of whom are female, were included in the study. Due to the fact that the majority of the students included in the study were between the ages of 18-23, those under the age of 18 and those above 23 were not specifically categorized.

Table 1 displays the distribution of the students regarding their demographic features. According to the table, of the students who took part in the study, 23.5% were 21, 20.4% were 20, 18.5% were 19 years old and 78.5% were female students. As for the individual sources of income of these students, the table shows that 81.8% of the students receive money from their parents and 74.1% of them benefit from student loans or scholarships as sources of income. When the students' places of accommodation are considered, it can be seen that more than half of them (60.9%) stay in dormitories, 25.3% stay in rented flats with their friends and 10.3% stay with their families. Finally, when students make a self-assessment of their health conditions, 53.5% of them consider themselves to be in good health, 30.9% of them consider themselves to be in not poor health and 12.4% of them consider themselves to be in very good health. Those who consider themselves to be in poor health are 3.2%.

Table 1. Demographic Distribution of the Students Included in the Study (n=340)

Age	Number	%
18 and under	26	7.6
19 years	63	18.5
20 years	69	20.4
21 years	80	23.5
22 years	51	15.0
23 years and above	51	15.0
Gender		
Male	73	21.5
Female	267	78.5
***Individual Source of Income**		
Loans and Scholarships	252	74.1
Family	278	81.8
Allowances From Relatives	27	7.9
Money Earned Through	19	5.6
Work	2	0.6
Other(Pension)		
Place of Accommodation Throughout School Life		
With Family	35	10.3
With Relatives	12	3.5
Student House	86	25.3
Dormitory	207	60.9
Self-Assessment of Health		
Poor	11	3.2
Not poor	105	30.9
Good	182	53.5
Very good	42	12.4
Body Mass Index(BMI)		
18.5 and Under	49	14.4
18.6–24.9	266	78.2
25–29.9	22	6.5
30–39.9	3	0.9

*As more than one choice has been stated, the ratios have not been calculated over the sum.

In terms of their BIMs, the majority of the students (78.2%) appear to be normal, 14.4% of them appear to be underweight and 6.5% appear to be overweight. It has been found out that 0.9% of the students are obese. Also, in another study where the participants are again university students, it was discovered that in relation to their weights, 80.5% of the students were normal, 13.8% were underweight and 5.7% were overweight (Garibağaoğlu, Mergen, Öner, 2005). In another study covering obese individuals, it was detected that as the individuals became older, their BMIs

also became higher (İçen, 2006). In a study covering university students, it was observed that there is a positive correlation between the individual incomes of the students and their BMIs (Vançelik and Ark, 2007).

Table 2. The Distribution of Students' State and Type of Vegetarianism on the basis of their Statements

Vegetarianism	**Number**	**%**
Yes	20	5.9
No	320	94.1
TOTAL	340	100.0
Vegetarian Types Seen Frequently		
Vegan	1	5.0
Lacto-ovo	12	60.0
Vegetarian	2	10.0
Lacto-vegetarian	4	20.0
Ovo-vegetarian	1	5.0
Fruto-vegetarian		
TOTAL	20	100.0

Of the students participated in the study, the number of those who are vegetarians is quite small (5.9%). 60.0% of the vegetarian students are lacto-vegetarians, that is, they follow a diet free of animal meat, but they consume milk and eggs.

Table 3. The Impact of the Students' State of Vegetarianism upon their Intake of Dietary Supplements

Existence of Impact	**Number**	**%**
Influential	5	25.0
Not influential	15	75.0
TOTAL	20	100.0

When it is examined whether the dietary supplement intake of students is influenced by the fact that they are vegetarians, 25.0% have stated that they are influenced while 75.0% have stated that they are not.

It has been discovered that 52.1% of the participants have received nutrition education whereas 47.9% have not received any nutrition education.

Table 4. The Distribution of Nutrition Education of the Students According to Gender

Nutrition Education	Gender				TOTAL	
	Male		Female			
	No.	%	No.	%	No.	%
Received	50	28.2	127	71.8	177	100.0
Not Received	23	14.1	140	85.9	163	100.0
TOTAL	73	21.5	267	78.5	340	100.0

As can be seen in Table 4, when the nutrition education of the participants are considered according to their sexes, 28.2% of those who received nutrition education are male students and 71.8% are female students. 85.9% of the students who did not receive nutrition education are female students.

Table 5. The Distribution of the Sources the Students Received Nutrition Education From

(n=177)*

Nutrition Education Source	Number	%
School(as a course)	140	41.2
Teacher	107	31.5
Newspapers-Journals etc.	46	13.5
Family	41	12.1
TV Programs	38	11.2
Dietician	14	4.1
Doctor	4	1.5

*More than one choice has been marked.

According to Table 5, when the sources of the nutrition education that were given to students are taken into consideration, 41.2 % of the students have stated that they have received their education from their schools, 31.5% from their teachers, 13.5% from newspapers and journals and 12.1% have stated that they have received their nutrition education from their families.

78.5% of the participants have said that they have breakfast and 21.5% of them have said that they do not have breakfast.

Table 6. The Distribution of Students According to Their Nutrition Education and Breakfast Consumption

Nutrition Education	Breakfast Consumption				TOTAL	
	Yes		No			
	No.	%	No.	%	No.	%
Received	142	80.2	35	19.8	177	100.0
Not Received	125	76.7	38	23.3	163	100.0
TOTAL	267	78.5	73	21.5	340	100.0

$p>0.05$ SD=2 $X^2=0.241$

As presented in Table 6, 80.2% of the students who have received nutrition education have stated that they have breakfast while 19.8% of them have stated that they do not have breakfast. It can also be seen that 78.5% of the students who have not received nutrition education have breakfast whereas 23.3% of them do not. In terms of not having breakfast, those with nutrition education rate lower as compared to those without nutrition education.

Table 7. The Distribution of Students According to Their Nutrition Education and Self-Assessment of their Health Conditions

Assessment of Health	Nutrition Education				TOTAL	
	Received		Not Received			
	No.	%	No.	%	No.	%
Poor	6	54.5	5	45.5	11	100.0
Not Poor	51	48.6	54	51.4	105	100.0
Good	102	56.0	80	44.0	182	100.0
Very Good	18	42.9	24	57.1	42	100.0
TOTAL	177	52.1	163	47.9	340	100.0

$p>0.05$

It has been found out that, of the participants who consider their health to be in poor condition, 54.5% have received nutrition education whereas 45.5% have not. Of those who consider their health to be in very good condition, 42.9% have received nutrition education while 57.1% have not. Of those who consider their health to be in good condition, 56.0% have received nutrition education, on the other hand, 44.0% have not.

It has been analyzed whether there are any discrepancies between the way the participants assess their health conditions and regular breakfast habits. The results have been presented in Table 8.

Table 8. The Distribution of Students According to Self-Assessment of their Health Conditions and Regular Breakfast Consumption

Health Assessment	Regular Breakfast Consumption				TOTAL	
	Yes		No			
	No.	%	No.	%	No.	%
Poor	4	36.4	7	63.6	11	100.0
Not Poor	84	80.0	21	20.0	105	100.0
Good	145	79.7	37	20.3	182	100.0
Very Good	34	81.0	8	19.0	42	100.0
TOTAL	267	76.1	73	21.5	340	100.0

p>0.05

As presented in the table, of the students who consider their health to be in very good condition, 81.0% have breakfast regularly. It has been determined that 63.6% of the students who consider their health to be in poor condition do not have breakfast. It has also been discovered that while 80.0% of the students who consider their health not to be in poor condition have breakfast, 20.0% of them do not and that 79.7% of the students who consider their health to be in good condition have breakfast regularly.

Table 9. The Distribution of Students According to Their BMI and Nutrition Education

BMI	Nutrition Education Status				TOTAL	
	Received		Not Received			
	No.	%	No.	%	No.	%
18.5 and less	22	44.9	27	55.1	49	100.0
18.6- 24.9	138	51.9	128	48.1	266	100.0
25-29.9	15	68.2	7	31.8	22	100.0
30-30.9	2	66.7	1	33.3	3	100.0
TOTAL	177	52.1	163	47.9	340	100.0

p>0.05

It has been found out that, of the participants who have normal weights, 51.9% have received nutrition education. It has also been found that 68.2% of the students who are overweight have received nutrition education. 55.1% of the students who haven't received nutrition education are underweight and 48.1% are normal in terms of their weight.

Table 10. The Distribution of Students According to Their BMI and Gender

BMI	Gender				TOTAL	
	Male		Female			
	No.	%	No.	%	No.	%
18.5 and less	1	2.0	48	98.0	49	100.0
18.6- 24.9	59	22.2	207	77.8	266	100.0
25-29.9	13	59.1	9	40.9	22	100.0
30-30.9	-	-	3	100	3	100.0
TOTAL	73	21.5	267	78.5	340	100.0

$p<0.05$

As shown in Table 10, 2.0% of the participants with BMI 18.5 and less are male and 98.0% are female. 77.8% of the female students and 22.2% of the male students are normal in terms of their BMI. 59.1% of the male students and 40.9% of the female students appear to be overweight. It has been observed that there is a significant relationship between gender and BMI ($p<0.05$).

68.2% of the participants have stated that they do not use dietary supplements. The percentage of those who have stated that they use dietary supplements is 13.3% while that of the participants who have stated that they sometimes use dietary supplements is 18.5%.

Table 11. The Distribution of Students According to the Duration of Dietary Supplement Intake

Duration of Supplement Intake	No.	%
1-3 Months	42	38.9
3-6 Months	25	23.1
6-12 Months	13	12.0
1-3 Years	14	13.0
More than 3 years	14	13.0
TOTAL	108	100.0

When the durations of dietary supplement intake stated by the students are evaluated, it is seen that they generally use them for short periods of time. 38.9% of the students have used them for a period of 1-3 months, 23.1% have used them for 3-6 months while 13.0% have used them for 1-3 years or more than 3 years. In a research study conducted, 58.0% of the female students stated that they used dietary supplements regularly. It was found out in the same study that 88.0% of the students using dietary supplements consumed a multivitamin at least twice a week. Moreover, 27.0% of this group stated that they consumed multivitamins 6-7 times a week (Brech, 1998).

Table 12. The distribution of the Students According to the Purpose and Frequency of Their Dietary Supplement Intake (n=108)

Frequency of Use / Purpose of Use	Every Day		A Few Times a Day		A Few Times a Week		Once a Week		Once a Month		TOTAL	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
To reduce the risk of chronic diseases	2	1.8	-	-	4	3.7	3	2.7	7	6.4	16	14.8
To prevent possible future illnesses	8	7.4	2	1.8	8	7.4	4	3.7	5	4.6	27	25.0
To meet the need for nutrients that cannot be fully provided by food	8	7.4	5	4.6	9	8.3	4	3.7	2	1.8	28	25.9
To take precautions against illnesses such as the flu and common cold	11	10.1	10	9.2	18	16.6	9	8.3	10	9.2	58	53.7
To provide energy(To boost)	6	5.5	7	6.4	15	13.8	5	4.6	3	2.7	36	33.3
To have healthy hair, nails, skin, etc.	5	4.6	2	1.8	12	11.1	4	3.7	4	3.7	27	25.0
To facilitate digestion	7	6.4	4	3.7	13	12.0	7	6.4	3	2.7	34	31.4
To gain weight	2	1.8	4	3.7	5	4.6	3	2.7	2	1.8	16	14.8
To lose weight	1	0.9	2	1.8	12	11.1	1	0.9	6	5.5	22	20.3
To reduce or prevent stress	2	1.8	2	1.8	6	5.5	1	0.9	6	5.5	17	15.7

As can be seen in Table 12, 53.7% of the students participating in the study have stated that they take dietary supplements in order to take pre-

ventive measures against ailments such as the flu and the common cold. When the frequency of their use is examined, it can be seen that they consume dietary supplements at most a few times a week (16.6%). As for the other purposes of use, 33.3% of them use dietary supplements in order to provide themselves with energy, 31.4% use them to facilitate digestion, 14.8% use them to gain weight and reduce the risk of chronic diseases. In a study carried out by Aytekin (2000) similar results were obtained. In another study that was conducted it was discovered that participants consumed dietary supplements in order to prevent illnesses, to get adequate nourishment, to increase energy intake, to feel better and to provide athletic body performance (Driskell, Graud, 1996). In a study conducted to determine the attitude of health care specialists towards botanicals and the extent of students' consumption of botanicals, it was found out that more than half of the students used botanicals primarily to cure physiological symptoms, to prevent illnesses and to maintain their general well-being.

It has been identified that 57.4% of the students taking part in the study took advice when they started using supplements and 42.6% started using them without getting any advice.

Table 13. The Distribution of Sources of Advice When Students Start Taking Supplements (n=62)

Sources of Advice	**Number**	**%**
Doctor	36	58.0
Friends	25	40.3
TV- radio programs	17	27.4
Family	13	20.9
Newspapers and magazines	11	17.7
Natural therapists (herbalists, spice dealers, etc.)	8	12.9
Pharmacist	7	11.2
Nurse	5	8.0
Dietician	4	6.4

According to Table 13, with 58.0%, doctors rank the first as a source of advice for students who start taking supplements. Friends come second with a percentage of 40.3%. While TV and radio programs rate 27.4%, with a percentage of 20.9%, families rate almost half as much as the advice of their friends. This situation indicates that a circle of friends are more influential on young people's lives than are their families. As sources of advice for students, the ones which rate the lowest are dieticians, nurses, pharmacists and natural therapists (6.4%, 8.0%, 11.2%, 12.9%), respectively. In a research study that was carried out, it was found out that in order to prevent illnesses, to get adequate nutrition, to boost energy intake, to feel better and to obtain athletic body performance, participants consumed

supplements that were either recommended or prescribed by individuals -- be it health care specialists or not -- and the media (Driskell, Graud, 1996). In another study, it was discovered that dieticians and nutritionists were less influential as regards the use of dietary supplements, whereas it was seen that doctors were the most influential group on women and the elderly individuals as a source of advice regarding the use of supplements (Creighton, Seaborn, 1997). This study established the fact that doctors are the most significant group with regard to advice on supplement use. In a study conducted concerning the supplement consumption of adolescents, it was reported that it is their families, friends and doctors who first introduce adolescents to dietary supplements (Herbold et al. 2004). Furthermore, it was revealed in another study that family and friends constituted the most important source of both advice on the use of botanicals and information regarding related products (Ambrose, Samuels, 2004).

The majority of the students who participated in the study (97.6%) have stated that they have no such health conditions as those which require a regular intake of supplements. Those who have stated that they have health problems are 2.4% (5 students are anemic and 3 students have digestion problems).

Table 14. The Distribution of Students as Regards the Period They Use Supplements the Most (n=108)

Period	**Number**	**%**
I use it regularly	9	8.3
When I lose weight	9	8.3
In winter months	39	36.1
Throughout illnesses	45	41.6
When I do not feel well	35	32.4
When I have exams	22	20.3
Any time	21	19.4

As Table 14 illustrates, supplements are most frequently consumed during periods of illnesses (41.6). This percentage is followed by winter months (36.1%) and then comes the period during which students do not feel good (32.4%). It has also been found out that some students use dietary supplements during the period when they have exams (20.3%). Although the percentage of the students who regularly use supplements and that of the students who use them when they lose weight are the same, it has been detected that these are the periods during which supplements are consumed the least (8.3%).

Table 15. The Distribution of the Supplement Types the Students Use and Their Frequency of Use (n=108)

Supplement Type	Every Day		More Than Once a Day		Once a Week		More Than Once a Week		Once a Month		TOTAL	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Vitamin C	16	14.8	11	10.1	13	12.0	10	9.2	7	6.4	57	52.7
Vitamin E	1	0.9	4	3.7	6	5.5	2	1.8	2	1.8	15	13.8
B vitamins(Single or as a complex)	1	0.9	2	1.8	3	2.7	2	1.8	6	5.5	14	13.0
Vitamin D	2	1.8	2	1.8	1	0.9	3	2.7	2	1.8	10	9.2
Vitamin K	-	-	1	0.9	-	-	2	1.8	2	1.8	5	4.6
Folic acid	-	-	2	1.8	-	-	1	0.9	1	0.9	4	3.7
Iron	10	9.2	3	2.7	-	-	3	2.7	3	2.7	10	9.2
Calcium	2	1.8	2	1.8	2	1.8	5	4.6	2	1.8	13	12.0
Phosphorus	1	0.9	1	0.9	1	0.9	1	0.9	1	0.9	5	4.6
Zinc	2	1.8	2	1.8	1	0.9	2	1.8	2	1.8	9	8.3
Iodine	2	1.8	2	1.8	-	-	2	1.8	1	0.9	7	6.4
Multivitamins/ Multiminerals	7	6.4	5	4.6	6	5.5	8	7.4	4	3.7	30	27.7
Sodium	1	0.9	1	0.9	1	0.9	1	0.9	2	1.8	6	5.5
Fiber/Roughage	1	0.9	-	-	1	0.9	1	0.9	3	2.7	6	5.5
Fish oil	3	2.7	-	-	3	2.7	3	2.7	5	4.6	14	13.0
Prebiotic yoghurt	3	2.7	-	-	5	4.6	5	4.6	2	1.8	15	13.8
Kephir	-	-	-	-	2	1.8	3	2.7	5	4.6	10	9.2
Herbal Teas	17	15.7	12	11.1	15	13.8	18	16.6	10	9.2	72	66.6
Herbal Mixtures	3	2.7	2	1.8	4	3.7	5	4.6	5	4.6	19	17.5
Gingko	-		-	-	-	-	1	0.9	1	0.9	2	1.8
Energy Drinks	3	2.7	-	-	7	6.4	5	4.6	5	4.6	20	18.5
Pollen	-	-	1	0.9	1	0.9	2	1.8	2	1.8	6	5.5
Royal Jelly	1	0.9	-	-	1	0.9	-	-	2	1.8	4	3.7
Senna	1	0.9	2	1.8	2	1.8	8	7.4	6	5.5	19	17.5
Garlic	3	2.7	2	1.8	8	7.4	-	-	4	3.7	17	15.7

As indicated in Table 15, the highest percentage of supplements that the participants consume is that of herbal teas with 66.6% and when the frequency of consumption is taken into consideration it can be seen that the number of those who consume herbal teas more than once a week is higher. The ratio of the participants who consume herbal teas every day (15.7%) is close to the ratio of those who consume them more than once a week (16.6%). Among the supplements that the students consume the most, vitamin C ranks the second with 52.7% and of the students who take vitamin C, 14.8% have stated that they take it every day. The third

most frequently consumed supplements are multivitamins-multiminerals (27.7%) and when the frequency of consumption is examined it can be seen that the ratio of those who consume them more than once a week is higher (7.4%). The percentages of the students who use herbal mixtures and senna are equal and these supplements are also among those that are consumed frequently (17.5%). In a study conducted among military personnel, it was discovered that the most frequently consumed supplements were multivitamins. In the same study it was found out that while male soldiers generally preferred to use amino acids, protein powders and carbohydrate-based supplements, female soldiers more frequently used multivitamins. Moreover, it was detected that female soldiers used significant amounts of weight-loss supplements (Warber, Cline, Mc Graw, 1997). In another study that was carried out, the consumption frequency order of the primary supplements were recorded as multivitamins, calcium, vitamin C, vitamin E and B-complex vitamins, respectively. In a study carried out by Stang et al. (2000), it was concluded that 47.6% of the adolescents who participated in the study used only multivitamins, 17.9% used them with minerals and 17.2% used vitamin C when taking iron as a supplement. In another study, it was reported that 68.8% of the participants who used supplements consumed multivitamins or multiminerals, 35.6% consumed a single nutrient and 16.0% used mixtures of these (Dwyer et al. 2001).

Table 16. The Distribution of the Students According to Their Demographic Features and Supplement Intake

AGE	State of Dietary Supplement Intake						TOTAL		SD	X^2	P
	Yes		No		Sometimes						
	No.	%	No.	%	No.	%	No.	%			
18 and under	3	11.5	15	57.7	8	30.3	26	100.0	10	,779	p>0.05
19 years old	8	12.7	44	69.8	11	17.5	63	100.0			
20 years old	10	14.5	43	62.3	16	23.2	69	100.0			
21 years old	11	13.8	58	72.5	11	13.8	80	100.0			
22 years old	8	15.7	35	68.6	8	15.7	51	100.0			
23 years old and above	5	9.8	37	72.5	9	17.6	51	100.0			
TOTAL	45	13.2	232	68.2	63	18.5	340	100.0			
GENDER											
Male	5	6.8	55	75.3	13	17.8	73	100.0	2	,167	p>0.05
Female	40	15.0	177	66.3	50	18.7	267	100.0			
TOTAL	45	13.2	232	68.2	63	18.5	340	100.0			
PLACE WHERE THE LONGEST PART OF LIFE HAS BEEN SPENT											
Village or a small town	5	13.2	24	63.2	9	23.7	38	100.0	4	,783	p>0.05
District	13	14.8	57	64.8	18	20.5	88	100.0			
Province	27	12.6	151	64.8	36	16.8	214	100.0			
TOTAL	45	13.2	232	68.2	63	18.5	340	100.0			

As can be seen in Table 16, when the supplement intake of students according to their ages are compared 57.7% of those who are 18 years old and under have states that they do not use dietary supplements. Similarly, in other age groups, the percentages of those who do not use dietary supplements are higher than those who use them. When the age groups where the density of non-users is the highest are examined, it is observed that two groups have the same ratios: the 21 year-old group and the group in which the students are 23 years old and above (72.5%) and it has been noted that statistically there is no significant discrepancy between the results($p>0.05$). In a study carried out between the years 1987 and 2000 to find out the trend in dietary supplement intake, a continuous rise was observed in the US, from 1992 until 2000 (Millen, Dodd and Subar, 2004). In another study, the supplement intake of young consumers was examined and it was found out that 81.9% of consumers used dietary supplements regularly and the intake of Vitamin E increased with age (Horwitz,1998)..

In the analysis to find out whether there existed a correlation between the genders of the students who took part in the study and their use of dietary supplements, no statistically significant discrepancy was detected ($p>0.05$). As can be seen in the table, the proportion of the male students

who do not use supplements (%75.3) is higher than that of the female students (%66.3). 6.8% of the male students have stated that they use supplements, whereas this ratio is 15.0% for the female students. Similar results have been obtained in another study that was conducted. The dietary supplement intake of college students was observed and it was discovered that a higher percentage of female students used supplements when compared to male students (Kim et al. 1997). Moreover, in another study, the dietary supplement intake of graduate students was observed and it was concluded that 32.0% of the female students and 17.0% of the male students took dietary supplements regularly, and that 35.0% of both genders used them rarely (Driskell, Graud, 1996). Furthermore, in the study carried out by Stang and Ark (2000), the dietary supplement intake of adolescents was analyzed and similar results were obtained. It was reported that 59.1% of the female adolescents and 40.9% of the male adolescents consumed dietary supplements.

Furthermore, in a study that was conducted, it was reported that individuals with high socio-economic status consume dietary supplements more frequently compared to those with average or low socio-economic status (Kim, Han and Keen, 2001).

The analysis of the differences between the students' places of residence and their supplement intake has revealed that the percentages of the students who use and who do not use supplements are similar in all three groups, i.e., villages, districts and provinces. As a result, no significant relationship has been observed between the two ($p>0.05$).

The distribution of the students' genders and their opinions on the effectiveness of supplements has been examined and in all three groups, female students rated higher. Of the participants who think dietary supplements are effective and beneficial, 17.7% are male students and 82.3% are female students. As regards those who believe only some supplements are useful, again the percentage of female students are higher in this group (%79.5).

Table 17. The Distribution of the Students According to Their Genders and Regular Breakfast Consumption

Regular Breakfast Consumption	**Gender**				**TOTAL**	
	Male		**Female**			
	No.	**%**	**No.**	**%**	**No.**	**%**
Yes	54	73.9	213	79.7	267	78.5
No	19	26.1	54	20.3	73	21.5
TOTAL	73	100.0	267	100.0	340	100.0

P>0.05 SD=2 X^2=0.105

As can be seen in the Table, 73.9% of the male students have stated that they have regular breakfasts, while 26.1% have stated that they do not. As for the female students, 79.7% of them have breakfast regularly, whereas 20.3% do not. No statistically significant discrepancy has been observed between female and male students.

Table 18. The Distribution of the Students Who Have and Who Do Not Have Breakfast According to Their Supplement Use

Breakfast Consumption	**Dietary Supplement Intake**						**TOTAL**	
	Yes		**No**		**Sometimes**			
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Yes	35	13.1	179	67.0	53	18.9	267	100.0
No	10	13.7	53	72.6	10	13.7	73	100.0
TOTAL	45	13.2	232	68.2	63	18.5	340	100.0

P>0.05 SD=4 X^2=0.197

When the differences between students' breakfast consumption and supplement intake are examined it can be seen that there is no statistically significant relationship between the two. The percentage of those students who have regular breakfast and that of the students who do not seem to be similar to each other (%13.1 and %13.7 respectively). Regarding non-users, the highest percentage belongs to the students who have stated that they do not have breakfast (%72.6), which is followed by those who have breakfast (%67.0). In another study, it was found out that 19.0% of the students did not have breakfast and the percentage of the female students who did not have breakfast (%23.0) was higher than that of the male students who did not have breakfast (%14.0). In the same study, it was reported that 70.0% of the students had breakfast without using vitamin-mineral supplements and 11.0% of them used supplements along with their breakfasts (O'Neil, Nicklas, Reger, Myers, 1998).

Table 19. The Distribution of Students According to Their Nutrition Education and Dietary Supplement Intake

Nutrition Education	**Dietary Supplement Intake**						**TOTAL**	
	Yes		**No**		**Sometimes**			
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Received	22	12.4	126	71.2	29	16.4	177	100.0
Not Received	23	14.1	106	65.0	34	29.9	163	100.0
TOTAL	45	13.2	232	68.2	63	18.5	340	100.0

p>0.05 SD=2 X^2=0.026

According to Table 19, 71.2% of the students who have received nutrition education have stated that they do not use dietary supplements. In the group that has not received nutrition education, the percentage of the students who use supplements regularly (%14.1) and that of the students who sometimes use them (%29.9) are higher when compared to the percentage of the students who have received nutrition education. Similar results were also obtained in a previous study. It was reported that the rate of supplement intake among the students who received nutrition education was lower compared to those who did not. However, although the ratio of those who use supplements is higher in both groups, it was discovered that 47.0% of the students who received nutrition education and 53.9% of the students who did not receive nutrition education used dietary supplements (Aytekin, 2000).

Table 20. The Distribution of Students According to Self-Assessment of Their Health and Dietary Supplement Intake

Self-Assessment of Health Condition	Dietary Supplement Intake						TOTAL	
	Yes		No		Sometimes			
	No.	%	No.	%	No.	%	No.	%
Poor	1	9.1	7	63.6	3	27.3	11	100.0
Not Poor	18	17.1	67	63.8	20	19.0	105	100.0
Good	21	11.5	127	69.8	34	18.7	182	100.0
Very good	5	11.9	31	73.8	6	14.3	42	100.0
TOTAL	45	13.2	232	68.2	63	18.5	340	100.0

As can be seen in Table 20, most of the students who have been included in the study to classify the assessment of health condition do not use dietary supplements. Although the number of students who do not use supplements is similar in all four groups, of the students who assess their health conditions as being very good, the ones who do not use supplements are higher in percentage compared to others (%73.8). Furthermore, as the students' health conditions deteriorate, the number of non-users declines. Conversely, it has been discovered that the rate of the students who sometimes use supplements drops in direct proportion to improvement in health condition. Similar results were obtained in a study examining the supplement intake in different ethnic groups. It was reported that 54.0% of the participants used vitamin and mineral supplements and that most of them assessed their own health conditions as being good or very good (Harwell, Driskell, 1996).

Table 21. The Distribution of Students According to Their Dietary Supplement Intake and BMI

BMI	Dietary Supplement Intake						TOTAL	
	Yes		No		Sometimes			
	No.	%	No.	%	No.	%	No.	%
18.5 and under	4	8.2	32	65.3	13	26.5	49	100.0
18.6- 24.9	39	14.7	180	67.7	47	17.7	266	100.0
25–29.9	2	9.1	17	77.3	3	13.6	22	100.0
30–30.9	-	-	3	100	-	-	3	100.0
TOTAL	45	13.2	232	68.2	63	18.5	340	100.0

14.7% of the participants within the normal limits of body weight have stated that they use supplements, 17.7% have stated that they sometimes use supplements and 67.7% have stated that they do not use supplements. Of the participants who are underweight, 65.3% have stated that they do not use supplements while 26.5% have stated that they do. Of those who have been classified as overweight, the majority (%77.3) have stated that they do not use dietary supplements. In a study that was conducted at the University of Rush, it was reported that of the students who used supplements, more than half were obese (Glew et al. 2004).

Table 22. The Distribution of Students According to Their Dietary Supplement Intake and Practices for a Healthy Life

Practices for a Healthy Life	Dietary Supplement Intake						TOTAL	
	Yes		No		Sometimes			
	No.	%	No.	%	No.	%	No.	%
I try to learn about healthy eating and integrate this new information into my life	33	14.5	153	67.4	41	18.1	227	100.0
I have regular check-ups	6	15.8	22	57.9	10	26.3	38	100.0
I consume food that is completely grown or prepared in a natural environment	10	18.2	36	65.5	9	16.4	55	100.0
I try to remain healthy by using dietary supplements	4	22.2	3	16.7	11	61.1	18	100.0

I do sports regularly	6	8.3	47	65.3	19	26.4	72	100.0
I have breakfast regularly	22	15.5	95	67.4	24	17.0	141	100.0
Rather than using pharmaceuticals I try to consume products of alternative medicine	6	15.4	20	51.3	13	33.3	39	100.0

As can be seen in Table 22, 22.2% of the students who use dietary supplements have stated that they try to maintain their health by using supplements whereas 67.4% of the students who do not use supplements have stated that they try to learn about healthy eating habits and integrate the new information into their lives and a same number of students have stated that they have breakfast regularly. Of the students who do not use dietary supplements, 57.9% have stated that they have regular check-up in order to remain healthy. When the students who sometimes take supplements are considered, 61.1% of them have stated that they try to remain healthy by using dietary supplements/

Table 23. The Distribution of Supplement Types According to Gender

Supplement Type	**Gender**				**TOTAL n=340**	
	Male n=73		**Female n=267**			
	No.	**%**	**No.**	**%**	**No.**	**%**
Vitamin C	15	20.5	42	15.7	57	16.7
Vitamin E	4	5.4	11	4.1	15	4.4
B vitamins(Single or as a complex)	4	5.4	10	3.7	14	4.1
Vitamin D	3	4.1	7	2.6	10	2.9
Vitamin K	3	4.1	2	0.7	5	1.4
Folic acid	1	1.3	3	1.1	4	1.1
Iron	3	4.1	16	5.9	19	5.6
Calcium	3	4.1	10	3.7	13	3.8
Phosphorus	1	1.3	4	1.4	5	1.4
Zinc	3	4.1	6	2.2	9	2.6
Iodine	1	1.3	6	2.2	7	2.0
Multivitamins/Multiminerals	10	13.6	20	7.4	30	8.8
Sodium	1	1.3	5	1.8	6	1.7
Fiber/Roughage	1	1.3	5	1.8	6	1.7
Fish oil	5	6.8	9	3.3	14	4.1
Prebiotic yoghurt	4	5.4	11	4.1	15	4.4
Kephir	2	2.7	8	2.9	10	2.9
Herbal Teas	14	19.1	58	21.7	72	21.1
Herbal Mixtures	3	4.1	16	5.9	19	5.6

Gingko	-	-	2	0.7	2	0.5
Energy Drinks	8	10.9	12	4.4	20	5.9
Pollen	-	-	6	2.2	6	1.7
Royal Jelly	-	-	4	1.4	4	1.1
Senna	1	1.3	18	6.7	19	5.6
Garlic	2	2.7	16	5.9	18	5.3

As regards the distribution of the supplement intake of the participants according to their gender, it is clearly understood from the table that the most commonly used supplement among males is Vitamin C (%20.5), whereas it is herbal teas among female students (%21.7). It has been found out that 15.7% of the female students take Vitamin C. 19.1% of the male students have stated that they use herbal teas as supplements. 5.9% of the female students and 4.1% of the male students have stated that they take iron as a supplement. As for the rates of multivitamin and multimineral use, 13.6% of the male students and 7.4% of the female students take them as supplements. It has also been discovered that 10.9% of the male students and 4.4% of the female students consume energy drinks as supplements. It has been noted that while supplements such as gingko, pollen and royal jelly are never consumed by male students, they are consumed by female students, though in small amounts.

Table 24. The Distribution of the Purposes of Supplement Use According to Gender (n=108)

Purpose of Use	**Gender**			
	Male n=18		**Female n=90**	
	No.	**%**	**No.**	**%**
To reduce the risk of chronic diseases	6	33.3	10	11.1
To prevent possible future illnesses	6	33.3	21	23.3
To meet the need for nutrients that cannot be fully provided by food	5	27.7	23	25.5
To take precautions against illnesses such as the flu and common cold	14	77.7	44	48.8
To provide energy (To boost)	12	66.6	24	26.6
To have healthy hair, nails, skin, etc.	7	38.8	20	22.2
To facilitate digestion	4	22.2	30	33.3
To gain weight	3	16.6	13	14.4
To lose weight	-	-	22	24.4
To reduce or prevent stress	3	16.6	14	15.5

As can be seen in Table 24, the male students who use dietary supplements use them in order to take precautions against illnesses such as the flu and common cold. Furthermore, of the male students who take supplements, 66.6% have stated that they use supplements in order to as a source of energy and 38.8% have stated that they use them in order to have healthy hair, nails, skin, etc. It has been detected that the percentage of the male students who take supplements to reduce the risks of chronic diseases and that of those who take supplements to prevent possible future illnesses are equal to each other (%33.3). When the purposes of the female students who take supplements are examined, just as the male students, most of them (%48.8) have stated that they use supplements in order to take precautions against illnesses such as the flu and common cold. The percentage of those who use supplements to facilitate digestion is 33.3% among the female students. 24.4% of the female students have stated that they take dietary supplements in order to lose weight whereas none of the male students has stated such a purpose.

Table 25. The Averages of the Points Assigned to the Opinions Regarding the Effects of Vitamins and Minerals on Health

OPINIONS	**Nutrition Education Received(n=177) Average ± SS**	**Nutrition Education Not Received (n=163) Average ± SS**	**t**	**P**
Extra intake of vitamins and minerals provides energy and power.	2.54±0.858	2.67±0.744	1.44	0.148
People need to take supplementary vitamins and minerals for adequate nutrition.	2.02±0.828	2.28±0.724	2.99	0.003*
Dietary supplements may help reduce stress.	2.12±0.679	2.17±0.662	0.65	0.515
People need extra vitamins and minerals when they are exhausted	2.56±0.824	2.65±0.834	1.01	0.310
Vitamin C may prevent common cold	3.11±0.841	3.28±0.671	1.97	0.050
Health may be protected by taking vitamins and minerals in amounts more than required	1.56±0.817	1.87±0.771	3.54	0.000*
Several psychological disorders are caused by vitamin deficiency	1.89±0.691	1.93±0.619	0.47	0.632

Diseases like cancer may be caused by mineral and vitamin deficiencies	2.23±0.715	2.20±0.670	0.48	0.704
Consuming a variety of foods is sufficient for the intake of all the vitamins and minerals	2.40±0.906	2.47±0.772	0.38	0.475
TOTAL	2.27±0.393	2.39±0.340	0.71	0.004*

*p≤0.05

When the averages of the points assigned by students to the opinions regarding the effects of vitamins and minerals on health are examined, the belief that Vitamin C prevents common cold has a high average both in the group who received nutrition education and the group who did not (3.11±0.841, 3.28±0.671, respectively). The point averages of the opinion that people need to take supplementary vitamins and minerals for adequate nutrition are 2.02±0.828 in the group who received nutrition education and 2.28±0.724 in the group who did not receive nutrition education, which indicates a significant relationship when statistically evaluated.

In the groups who received nutrition education and who did not the opinion that health may be protected by taking vitamins and minerals in amounts which are more than required has averages of 1.56±0.817 and 1.87±0.771, respectively. When statistically evaluated, these figures indicate a significant relationship (p≤0.05). In a study carried out by Vançelik et al. (2007), dietary knowledge and habits of university students were examined. It was found out that the average points for dietary knowledge were higher for female students than for male students. In the same study, it was discovered that the students who lived in cities prior to their university education were more knowledgeable about nutrition compared to the students who lived in rural areas.

Table 26. The Distribution of the Students' Intake of Nutrients According to Their Nutrition Education Status

NUTRIENT	NUTRITION EDUCATION											
	RECEIVED (n=177)						NOT RECEIVED (n=163)					
	INSUFFI-CIENT		SUFFI-CIENT		HIGH		INSUFFI-CIENT		SUFFI-CIENT		HIGH	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Energy	98	55.4	77	43.5	2	1.1	102	62.6	61	37.4	-	-
Carbohydrate	12	6.8	98	55.4	67	37.9	12	7.4	88	54.0	63	38.7
Fat	50	28.2	111	62.7	16	62.7	42	25.8	114	69.9	7	4.3
Protein	35	19.8	117	66.1	25	14.1	25	15.3	112	68.7	26	16.0
Vitamin A	37	20.9	103	58.2	37	20.9	43	26.4	87	53.4	33	20.2

Vitamin D	168	94.9	4	2.3	5	2.8	154	94.5	3	1.8	6	3.7
Vitamin E	6	3.4	45	25.4	126	71.2	10	6.1	54	33.1	99	60.7
Vitamin K	2	1.1	21	11.9	154	87.0	1	0.6	22	13.5	140	85.9
Thiamin	140	79.0	37	20.9	-	-	139	85.3	24	14.7	-	-
Riboflavin	65	36.7	99	55.9	13	7.3	67	41.1	88	54.0	8	4.9
Niacin	16	9.0	132	74.6	29	16.4	16	9.8	132	81.0	15	9.2
Panthotenic acid	126	71.2	50	28.2	1	0.6	130	79.8	33	20.2	-	-
Vitamin B_6	103	58.2	71	40.1	3	1.7	108	66.3	55	33.7	-	-
Biotin	82	46.3	83	46.9	12	6.8	84	51.5	74	45.4	5	3.1
Vitamin B_{12}	60	33.9	79	44.6	38	21.5	65	39.9	71	43.6	27	16.6
Vitamin C	67	37.9	79	44.6	31	17.5	76	46.6	60	36.8	27	16.6
Folic acid	146	82.5	29	16.4	2	1.1	144	88.3	19	11.7	-	-
Calcium	145	81.9	32	18.1	-	-	149	91.4	14	8.6	-	-
Phosphorus	23	13.0	96	54.2	58	32.8	19	11.7	110	67.5	34	20.9
Iron	122	68.9	36	20.3	19	10.7	135	82.8	16	9.8	12	7.4
Zinc	56	31.6	112	63.3	9	5.1	51	31.3	104	63.8	8	4.9
Iodine	87	49.2	87	49.2	3	1.7	66	40.5	85	52.1	12	7.4
Copper	8	4.5	101	57.1	68	38.4	9	5.5	71	43.6	83	50.9
Sodium	2	1.1	39	22.0	136	76.8	-	-	25	15.3	138	84.7
Potassium	160	90.4	17	9.6	-	-	157	66.3	6	3.7	-	-
Fiber	132	74.6	44	24.9	1	0.6	125	76.7	38	23.3	-	-

As can be seen in Table 26, 55.4% of the students who have received nutrition education and 62.6% of those who have not take energy less than the required daily amount.

It has been noted in both groups that, in terms of vitamin intake, the students who take insufficient vitamin D are close in percentage and also very high in number. The Vitamin D intake of 94.9% of the students who have received nutrition education and 94.5% of the students who have not is lower than the required daily amount. Vitamin D functions importantly in increasing the absorption of calcium in the intestines and mineralization in the bone tissue (Erden, Tanyeri, 2004).

In terms of other vitamins, 82.5% of the students who have received nutrition education and 88.3% of those who have not received nutrition education take insufficient amounts of folic acid; 79.0% of the students who have received nutrition education and 85.3% of those who have not take insufficient amounts of thiamin; 71.2% of the students who have received nutrition education and 79.8% of those who have not take insufficient amounts of panthotenic acid; 58.2% of the students who have received nutrition education and 66.3% of those who have not take insufficient amounts of Vitamin B_6 and 46.3% of the students who have received nutrition education and 51.5% of those who have not take insufficient amounts of biotin. Moreover, it has been determined that 58.2% of the students who have received nutrition education and 53.4% of those who have not take sufficient amounts Vitamin A; 55.9% of the students who have received nu-

trition education and 54.0% of those who have not take sufficient amounts of riboflavin; 74.6% of the students who have received nutrition education and 81.0% of those who have not take sufficient amounts of niacin.

As for the daily mineral intake, 81.9% of the students who have received nutrition education and 91.4% of those who have not take insufficient amounts of calcium; 90.4% of the students who have received nutrition education and 66.3% of those who have not take insufficient amounts of potassium; 68.9% of the students who have received nutrition education and 82.8% of those who have not take insufficient amounts of iron minerals to meet their daily needs. Furthermore, it has been detected that 63.3% of the students who have received nutrition education and 63.8% of those who have not take sufficient amounts of zinc.

The daily fiber intake of 74.6% of the students who have received nutrition education and 76.7% of those who have not is lower than the sufficient amount. 87.0% of the students who have received nutrition education and 85.9% of those who have not take Vitamin K which is usually more than the required daily amount; similarly, 71.2% of the students who have received nutrition education and 60.7% of those who have not take Vitamin E which is usually more than the required daily amount. As for fat, 62.7% of the students who have received nutrition education and 4.3% of those who have not display a higher intake level than the required daily amount. When the minerals taken more than required daily amounts are examined, it can be seen that 38.4% of the students who have received nutrition education and 50.9% of those who have not exceed the daily required amount of copper in their intake, and 76.8% of the students who have received nutrition education and 84.7% of those who have not exceed the required daily amount of copper in their intake.

In a study where the subjects were adolescents of ages 11-14, the fiber intake of these adolescents was examined and it was found out that 27/.5% of young people take insufficient amounts of fiber (Yabancı, Pekcan, 2005). In another study, children suffering from chronic constipation were compared with the control group, and as a result, it was identified that children with chronic constipation hardly had the habit of eating regular meals and, compared to the control group, their consumption of carbohydrates was higher, but their consumption of fibers and liquids was lower (Kasırga et al. 2004).

Table 27. The Distribution of the Students' Daily Nutrient Intake According to Their BMI

NUTRIENT	UNDERWEIGHT(n=49)						NORMAL (n=266)						OVERWEIGHT(n=22)					
	INSUFFI-CIENT		SUFFI-CIENT		HIGH		INSUFFI-CIENT		SUFFI-CIENT		HIGH		INSUFFI-CIENT		SUFFI-CIENT		HIGH	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No	%
Energy	26	53.1	23	46.9	-	-	158	59.4	107	40.2	1	0.4	14	63.6	7	31.8	1	4.5
Carbohydrate	5	10.2	25	51.0	19	38.8	15	5.6	151	56.8	100	37.6	4	18.2	8	36.4	10	45.5
Fat	13	26.5	33	67.3	3	6.1	72	27.1	178	66.9	16	6.0	5	22.7	13	59.1	4	18.2
Protein	10	20.4	32	65.3	7	14.3	50	18.8	174	65.4	42	15.8	-	-	20	90.9	2	9.1
Vitamin A	13	26.5	23	46.9	13	26.5	60	22.6	155	58.3	51	19.2	6	27.3	10	45.5	6	27.3
Vitamin D	44	89.8	3	6.1	2	4.1	254	95.5	3	1.1.	9	3.4	21	95.5	1	4.5	-	-
Vitamin E	1	2.0	13	26.5	35	71.4	15	5.6	79	29.7	172	64.7	-	-	6	27.3	16	72.7
Vitamin K	1	2.0	4	8.2	44	89.8	2	0.8	35	13.2	229	86.1	-	-	4	18.2	18	81.8
Thiamin	36	73.5	13	26.5	-	-	224	84.2	42	15.8	-	-	16	72.7	6	27.3	-	-
Riboflavin	19	38.8	26	53.1	8	8.2	100	37.6	153	57.5	13	4.9	12	54.5	6	27.3	4	18.2
Niacin	6	12.2	39	79.6	4	8.2	24	9.0	207	77.8	35	13.2	2	9.1	15	68.2	5	22.7
Panthotenic acid	35	71.4	14	28.6	-	-	203	76.3	63	23.7	-	-	15	68.2	6	27.3	1	4.5
Vitamin B_6	29	59.2	20	40.8	-	-	166	62.4	99	37.2	1	0.4	13	59.1	7	31.8	2	9.1
Biotin	27	55.1	20	40.8	2	4.1	128	48.1	127	47.7	11	4.1	10	45.5	8	36.4	4	18.2
Vitamin B_{12}	21	42.9	23	46.9	5	10.2	94	35.3	119	44.7	53	19.9	9	40.9	7	31.8	6	27.3
Vitamin C	17	34.7	23	46.9	9	18.4	112	42.1	112	42.1	42	15.8	13	59.1	4	18.2	5	22.7
Folic acid	39	79.6	10	20.4	-	-	233	87.6	32	12.0	1	0.4	15	68.2	6	27.3	1	4.5
Calcium	41	83.7	8	16.3	-	-	232	83.7	34	12.8	-	-	19	86.4	3	13.6	-	-
Phosphorus	7	14.3	30	61.2	12	24.5	29	10.9	166	62.4	71	26.7	5	22.7	9	40.9	8	36.4
Iron	46	93.9	2	4.1	1	2.0	197	74.1	43	16.2	26	9.8	11	50.0	7	31.8	4	18.2
Zinc	11	22.4	35	71.4	3	6.1	83	31.2	171	64.3	12	4.5	12	54.5	8	36.4	2	9.1
Iodine	19	38.8	28	57.1	2	4.1	125	47.0	130	48.9	11	4.1	9	40.8	11	50.0	2	9.1
Copper	-	-	29	59.2	20	40.8	17	6.4	130	48.9	119	44.7	-	-	11	50.0	11	50.0
Sodium	-	-	7	14.3	42	85.7	2	0.8	55	20.7	209	78.6	-	-	2	9.1	20	90.9
Potassium	46	93.9	3	6.1	-	-	249	93.6	17	6.4	-	-	19	86.4	3	13.6	-	-
Fiber	34	69.4	15	30.6	-	-	203	76.3	62	23.3	1	0.4	2	66.7	1	33.3	-	-

As can be seen in Table 27, 53.1% of the students who are underweight, 59.4% of the students who are within normal weight limits and 63.6% of the students who are overweight take less energy than required per day. As regards the daily vitamin intake, 89.9% of the students who are underweight, and 95.5% of the normal-weight students and overweight students take less amounts of Vitamin D than required per day. As for thiamin, 73.5% of the underweight students, 84/2% of the normal-weight students and 72.7% of the overweight students take lower amounts of thiamin than the required daily intake. When the sufficiency of panthotenic acid is examined, it can be seen that 71.4% of the students who are underweight, 76.3% of the normal-weight students and 68.2% of the overweight students take insufficient amounts of panthotenic acid. Similarly, 59.2% of the students who are underweight, 62.4% of the normal-weight students and 59.1% of the overweight students take insufficient amounts of Vitamin B_6; 55.1% of the students who are underweight, 48.1% of the normal-weight students and 45.5% of the overweight students take insufficient amounts of biotin; 79.6% of the students who are underweight, 87.6% of the normal-weight students and 68.2% of the overweight students take insufficient amounts of folic acid. Moreover, the riboflavin intake of 54.5% of the overweight students and again the Vitamin C intake of 59.1% of these students are lower than the required amounts per day.

When the distribution of mineral intake according to BMI is examined, it can be seen that 83.7% of the underweight and normal-weight students, and 86.4% of the overweight students take insufficient amounts of calcium on a daily basis; 93.9% of the students who are underweight, 74.1% of the normal-weight students and 50.0% of the overweight students take insufficient amounts of iron mineral. While the sodium intake of 85.7% of the students who are underweight, 78.6% of the normal-weight students and 90.9% of the overweight students is higher than the required amount per day, the potassium intake of 93.9% of the students who are underweight, 93.6% of the normal-weight students and 86.4% of the overweight students is lower than the required amount per day. Diets high in sodium, but low in magnesium, calcium and potassium are among the risk factors for hypertension, osteoporosis and depression (Aydın, 2003).

As for the daily fiber intake of the students, 69.5% of the students who are underweight, 76.3% of the normal-weight students and 86.4% of the overweight students take insufficient amounts of fiber.

Table 28. The Distribution of the Students' Daily Nutrient Intake According to Their Dietary Supplement Use Status

NUTRIENT	STUDENTS USING DIETARY SUPPLEMENTS						STUDENTS NOT USING DIETARY SUPPLEMENTS						STUDENTS SOMETIMES USING DIETARY SUPPLEMENTS					
	INSUFFICIENT		SUFFICIENT		HIGH		INSUFFICIENT		SUFFICIENT		HIGHLY SUFFICIENT		INSUFFICIENT		SUFFICIENT		HIGH	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Energy	24	53.3	21	46.7	-	-	133	57.3	97	41.8	2	0.9	43	68.3	20	31.7	-	-
Carbohydrate	5	1.1	24	53.4	16	35.6	16	6.9	124	53.4	92	39.7	3	4.8	38	60.3	22	34.9
Fat	11	24.4	31	68.9	3	6.7	61	26.3	154	66.4	17	7.3	20	31.7	40	63.5	3	4.8
Protein	11	24.4	27	60.0	7	15.6	38	16.4	157	67.7	37	15.9	11	17.5	45	71.4	7	11.1
Vitamin A	6	13.3	26	57.8	13	28.9	55	30.2	129	55.6	48	20.7	19	30.2	35	55.6	9	14.3
Vitamin D	38	84.4	4	8.9	3	6.7	224	96.6	3	1.3	5	2.2	60	95.2	-	-	3	4.8
Vitamin E	1	2.2	9	20.0	35	77.8	10	4.3	71	30.6	151	65.1	5	7.9	19	30.2	39	61.9
Vitamin K	-	-	4	8.9	41	91.1	3	1.3	30	12.9	199	85.8	-	-	9	14.3	54	85.7
Thiamin	35	77.8	10	22.2	-	-	190	81.9	42	18.1	-	-	54	85.7	9	14.3	-	-
Riboflavin	14	31.1	28	62.2	3	6.7	87	37.5	129	55.6	16	6.9	31	49.2	30	47.6	2	3.2
Niacin	5	11.1	34	75.6	6	13.3	20	8.6	183	78.9	29	12.5	7	11.1	47	74.6	9	14.3
Panthotenic acid	34	75.6	11	24.4	-	-	171	73.7	60	25.9	1	0.4	51	81.0	12	19.0	-	-
Vitamin B_6	27	60.0	18	40.0	-	-	143	61.6	86	37.1	3	1.3	41	65.1	22	34.8	-	-
Biotin	19	42.2	24	53.3	2	4.4	115	49.6	103	44.4	14	6.0	32	50.8	30	47.6	1	1.6
Vitamin B_{12}	13	28.6	23	51.1	0	20.0	87	37.5	102	44.0	43	18.5	25	39.7	25	39.7	13	20.6
Vitamin C	17	37.8	17	37.8	11	24.4	98	42.2	99	42.7	35	15.1	28	44.4	23	36.5	12	19.1
Folic acid	41	91.1	4	8.9	-	-	195	84.1	35	15.1	2	0.9	54	85.7	9	14.3	-	-
Calcium	38	84.4	7	15.6	-	-	198	85.3	34	14.7	-	-	58	92.1	5	7.9	-	-
Phosphorus	6	13.3	27	60.0	12	26.7	26	11.2	138	59.5	68	29.3	10	15.9	41	65.1	12	19.0
Iron	39	86.7	4	8.9	2	4.4	172	74.1	35	15.1	25	10.8	46	73.1	13	20.6	4	6.3
Zinc	13	28.9	31	68.9	1	2.2	71	30.6	149	64.2	12	5.2	23	36.5	54	57.2	4	6.3
Iodine	21	46.7	20	44.4	4	8.9	109	47.0	114	49.1	9	3.9	23	36.5	38	60.3	2	3.2
Copper	4	8.9	22	48.9	19	42.2	10	4.3	120	51.7	102	44.0	3	4.8	30	47.6	30	47.6
Sodium	1	2.2	9	20.1	35	77.7	-	-	46	19.8	186	80.2	1	1.6	9	14.3	53	84.1
Potassium	43	95.6	2	4.4	-	-	215	92.7	17	7.3	-	-	59	93.7	4	6.3	-	-
Fiber	32	71.1	13	28.9	-	-	179	77.2	53	22.8	-	-	46	73.0	16	25.4	1	1.6

As can be seen in Table 28, the energy intake of 53.3% of the students who use dietary supplements, 57.3% of those who do not use dietary supplements and 68.3% of those who sometimes use dietary supplements is lower than the required daily amount.

When the vitamin intake of the students are taken into consideration, the vitamin D intake of 84.4% of the students who use dietary supplements, 96.6% of those who do not use dietary supplements and 95.2% of those who sometimes use dietary supplements is lower than the required daily amount. The thiamin intake of 77.8% of the students who use dietary supplements, 81.9% of those who do not use dietary supplements and 85.7% of those who sometimes use dietary supplements is lower than the required daily amount. It has been noted that 75.6% of the students who use dietary supplements take insufficient amounts of Vitamin B_6 and 91.1% of them take insufficient amounts of folic acid. Of the students who sometimes use dietary supplements, 81.0% follow a diet which is low in panthotenic acid, 65.1% follow a diet low in Vitamin B_6 and 85.7% follow a diet low in folic acid. 73.3% of the students who do not use dietary supplements have an insufficient intake of panthotenic acid, 61.6% of them have an insufficient intake of Vitamin B_6 and 84.1% have an insufficient intake of folic acid.

When the mineral intakes of the students are taken into consideration, of the students who use dietary supplements, 84.4% take insufficient amounts of calcium, 86.7% take insufficient amounts of iron and 95.6% take insufficient amounts of potassium. Of the students who sometimes use dietary supplements, 85.7% take insufficient amounts of calcium, 73.1% take insufficient amounts of iron and 93.7% take insufficient amounts of potassium. Of the students who do not use dietary supplements, 85.3% take insufficient amounts of calcium, 74.1% take insufficient amounts of iron and 92.7% take insufficient amounts of potassium.

As for fibers, 71.1% of the students who use supplements, 77.2% of those who do not use supplements and 73.0% of those who sometimes use supplements take insufficient amounts of fiber.

When the students are evaluated according to their consumption of the nutrients which they take more than the required daily amounts, it can be seen that the Vitamin E intake of 77.8% of the students who use supplements, 65.1% of those who do not use supplements and 61.9% of those who sometimes use supplements surpasses the required daily amount. The Vitamin K intake of 91.1% of the students who use supplements, 85.5% of those who do not use supplements and 85.7% of those who sometimes use supplements surpasses the required daily amount. Finally, the sodium intake of 77.7% of the students who use supplements, 80.2% of those who do not use supplements and 84.1% of those who sometimes use supplements surpasses the required daily amount.

6. CONCLUSION AND IMPLICATIONS

In this study, which was carried out in order to determine the dietary supplement intake status of young people, their purposes for supplement use, which supplement group they consume the most, whether their income status affects their supplement use and the relationship between the diet adequacy of the young people who take dietary supplements and those who do not, 21.5% of the participants consisted of male students and 78.5% consisted of female students. While the source of income was the family for 81.8% of the students, it was student loans or scholarships for 74.1% of them. Throughout their education %60.9 of the students stayed in dormitories, %25.3 in student houses as rented flats and %10.3 with their families. 78.2% of the students were normal-weight, 14.4% were underweight and 6.5% were overweight. 53.5% of them considered their health as being good, 30.9% considered it as being not poor and 3.2% considered it being poor.

5.9% of the students who took part in the study were vegetarians, and of these 60.0% were lacto-ovo vegetarians. 47.1% of the students had three meals a day and 40.6% had one or two meals a day. 52.1% of the participants had previously received nutrition education while 47.9% had not. Of those with nutrition education, 41.2% received it at school, 31.5% from teachers, 13.5% from newspapers and magazines and 11.2% received it from TV programs.

68.8% of the participants did not use dietary supplements, 13.2% did and 18.5% sometimes used them. Of the supplement users, 38.9% used them for 1-3 months and 23.1% used them for 3-6 months. 66.3% of the female students and 75.3% of the male students did not use dietary supplements. In terms of BMI, 65.3% of the underweight students, 67.7% of the normal-weight students and 77.3% of the overweight students were non-users. It was found out that 12.4% of the students who had received nutrition education and 14.1% of those who had not used dietary supplements regularly. 16.4% of the students who had received nutrition education and 29.9% of those who had not sometimes used supplements. Of the dietary supplement users, 57.4% started using them upon advice and of these, 58.0% took advice from doctors, 40.3% took advice from friends and 6.4% took advice from dieticians. Of the users, 41.6% took supplements most frequently when they were ill, 36.1% took them most frequently in winter months, 32.4% took them most frequently when they did not feel well and 20.3% took them most frequently during the period they had their exams. Moreover, 53.7% of the supplement users took them to reduce the risks of such illnesses as the flu and common cold, 33.3% took them to provide energy, 31.4% took them to facilitate digestion and 25.9% took them to meet the need of the nutrients which cannot be fully provided by food

intake. For 71.2% of the students, the form of supplement intake was tea, for 62.0% it was capsules and 21.3% used alternative medicine products for their supplement intake. Herbal teas (%66.6), Vitamin C (%52.7) and mutivitamins/multiminerals (%27.7) rated the highest as the most- used supplement types.

The results of the study also showed that 65.9% of the participants do not believe that dietary supplements are useful.

It was found out that the participants followed diets in which several of the nutrients were consumed in insufficient amounts. 58.8% of the students took less energy than the required daily intake. 94.7% of them followed a diet deficient in Vitamin D, 85.3% followed a diet deficient in folic acid, 82.1% followed a diet deficient in thiamin, 75.3% followed a diet deficient in panthotenic acid, 62.1% followed a diet deficient in B_6 vitamins and 93.2% took insufficient potassium, 86.5% took insufficient calcium, 75.6% took insufficient iron minerals.

On the other hand, Vitamin K intake of 86.5% of the students, sodium intake of 80.6% of the students and Vitamin E intake of 66.2% of the students were higher than the required daily amounts.

53.3% of the dietary supplement users, 57.3% of the non-users and 68.3% of the students who sometimes used them obtained less energy than the required daily intake. Vitamin D intake of 84.4% of the supplement users, 96.6% of the non-users and 95.2% of the students who sometimes used them was found to be insufficient. The thiamin intake of 77.8% of the students who used dietary supplements, 81.9% of those who did not use dietary supplements and 85.7% of those who sometimes used dietary supplements was also lower than the required daily amount. Moreover, panthotenic acid intake of 75.6% of the dietary supplement users, Vitamin B_6 intake of 60.0% of the supplement users and folic acid intake of 91.1% of the dietary supplement users were found to be insufficient. As for the students who sometimes used supplements, panthotenic acid intake of 81.0% of these students, Vitamin B_6 intake of 65.1% of them and folic acid intake of 85.7% of the students who sometimes used dietary supplements were found to be insufficient. Of the non-users, 73.3% took insufficient amounts of panthotenic acid, 61.6% took insufficient amounts of Vitamin B_6 and 84.1% took insufficient amounts of folic acid. Furthermore, calcium intake of 84.4% of the supplement users, iron intake of 86.7% of the supplement users and potassium intake of 95.6 % of the supplement users were discovered to be insufficient. Of the students who sometimes used dietary supplements, 85.7% took insufficient amounts of calcium, 73.1% took insufficient amounts of iron and 93.7% took insufficient amounts of potassium. Of the students who do not use dietary supplements, 85.3%

took insufficient amounts of calcium, 74.1% took insufficient amounts of iron and 92.7% took insufficient amounts of potassium.

Individuals who cannot obtain the nutrients that are required on a daily basis can meet their needs through dietary supplements. However, when, in particular, herbal mixtures and other botanical supplements are to be used, one has to make sure that they choose the ones that have been thoroughly studied and concluded to be beneficial by experts. The reason for this is that, while dietary supplements can be useful for health, they can also have harmful effects such as being toxic or causing unwanted drug interactions. It is essential that individuals who are experts in their fields be consulted in order to obtain information on the effectiveness of dietary supplements and their interactions with drugs and natural products. In addition, instead of using dietary supplements every time a person feels unwell, they should first have a check-up and then start taking dietary supplements upon the doctor's advice and prescription. In order to provide the necessary nutrients, the primary approach should always be increasing the quality of nutrition and consuming sufficient sources of vitamins and minerals.

Although a healthy diet satisfies hunger, it can also be improved so as to protect and promote better and it should consist not only of delicious but also of healthy and nutritious food. As alternatives to drinks containing caffeine and cola, the consumption of fruit juice and *ayran -yoghurt drink-* can be promoted. Vegetables and fruit are quite rich in fiber, vitamin and mineral content. Furthermore, dried leguminous seeds, lentil and whole meal foods such as corn, wheat, oat and bread, all rich in fiber, should frequently be included in the diet. In the daily diet, the consumption of salt should be reduced whereas the consumption of fiber should be increased. Intake of fiber has an important role in protecting and maintaining health. For example, it is useful in excretion, in the prevention of colon cancer, in the regulation of blood cholesterol or glucose and in the reduction of the heart attack risks.

For vegetarians, vitamin and mineral supplements play an important part in their diets. Dietary supplements may be used in order to cover the need for animal-based vitamins and minerals.

The study points out to the fact that the number of students who follow diets deficient in Vitamin D is very high. A national policy might be drawn up and developed in order to supply nutrients like Vitamin D whose deficiency is widespread. It could be compulsory especially in elementary school canteens to sell food products determined by specialists. Moreover, the most frequently consumed food products may be enriched by nutrients which are usually low in quantity in people's diets.

The results of the study indicate that the majority of university students are inadequately nourished. While menus are being planned at university cafeterias, being keen on the fact that the meals contain a balanced amount of all the essential nutrients would have positive effects on the diets of the students who eat their meals in the cafeteria.

Individuals spend a significant amount of time in front of the TV. Therefore, broadcasting programs that would positively affect these individuals' educational knowledge on nutrition would contribute to nutrition education in the country.

In the articles about dieting and nutrition which frequently appear in the written media, including expert opinions that would provide information on the areas of use for dietary supplements and on the health problems that could arise due to wrong use of supplements could also be beneficial to the public.

This study indicates that the percentages of insufficient intake of similar nutrients in both groups are high although the number of students with nutrition education is lower than that of the ones without nutrition education. Nutrition education should cover simple explanations and introduction of food groups, nutrients and their functions starting from nursery and pre-school level. This education should be continued and developed at elementary school level through reinforcement and various applied methods. Receiving such education would help students put their knowledge into practice in the later stages of their lives. Starting to educate individuals about nutrition from early ages on, would be more effective in enabling them to put theory into practice. While giving nutrition education, educators could also cover some information about the use of dietary supplements. Moreover, in Public Education Centers, concentrating on nutrients and the types and functions of dietary supplements, educators could help establish a new culture among all parts of society to follow a healthy, regular and balanced diet.

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Chapter 42

GENDER ASSESSMENT STUDIES FROM SKELETAL BONES IN TURKISH POPULATION

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INTRODUCTION

Studies aiming to identify people's gender, height and age are important for identification [1-3]. Because of the importance of the effect of population differences, gender, height and age estimation studies were conducted by considering these factors [4,5].

Biological gender estimation is one of the main parameters that are needed in the construction of biological profiles of a deceased person with unknown identity. When the corpse is decomposed at further levels, or it is skeletonized or mutilated severely, the analysis of bones might be the only way to determine the gender [6]. The measurements on body and body parts and their relations with gender vary at significant levels because of the genetic and environmental factors [7]. For this reason, there are studies conducted in different populations on the same bone types [8-10].

The same types of bones were assessed for gender assessment and different anatomical structure of these bones were examined, yielding different accuracy rates in gender estimation [11-13]. The chance of achieving high accuracy levels in gender estimation is related with analyzed skeletal components and the degree of reliability of techniques used in define the differences in the shape and size between the genders [14].

Computerized Tomography (CT), which is one of the methods employed in gender assessment, is considered to be a successful method in identification studies, and has been increasingly used in the forming population-based reference databases in recent years [14]. X-rays, Magnetic Resonance Imaging and caliper were used in the measurements in evaluation of anatomical structures in these studies [15-17].

Gender, height and age estimation studies conducted for each bone in human skeleton are important because they contribute to the identification process, and must be identified for each population. In this article, the purpose was to examine the studies conducted on gender assessment in skeletal bones in Turkish population, and also to investigate the accuracy rates obtained from the bones and the parameters used in evaluation.

GENDER ASSESMENT

1. APPENDICULAR SKELETON BONE

1.1. Upper Extremity Bones

1.1.1. Scapula

Özer I et al.'s study [8] was conducted on gender determination. They used the scapula in Medieval Skeletons from East Anatolia. Their aim was

to determine metric standards for gender determination for medieval Anatolian populations by using scapular measurements. The database for their study consisted of 93 adult skeletal remains (47 males and 46 females) that were obtained from Dilkaya Medieval Collection. A total of 4 measurements were made; maximum scapular height and breadth, glenoid cavity height and breadth. They also made use of Discriminant Function Analysis. Their measurements on maximum scapular breadth revealed some degree of sexual dimorphism with the highest accuracy of gender determination (94.8%). The accuracy rates of the functions ranged between 82.9% and 95.0%. They reported higher accuracy rates for female skeletons compared to males. They developed population-specific discriminant formulas with measurement combinations to be used in ancient Anatolian populations.

1.1.2. Humerus

In their study, Atamtürk D et al. [18] aimed to examine the applicability of the measurements made in the humerus for gender assessment. They also aimed to contribute to the creation of Discriminant Function Equations for Anatolian populations for forensic use. Their material consisted of the archived X-ray images of the cases. They analyzed a total of 84 X-ray films of 46 females and 38 males. The ages of the cases varied between 20 and 79 years (mean age was 48.96 years). They measured 5 dimensions, which included maximum length, vertical head diameter, head + greater tubercle diameter, right-left diameter at midshaft, and epicondylar breadth for direct and stepwise discriminant function analysis. According to their results, the proximal part of the humerus yielded greater diagnostic accuracy compared to distal and middle parts. The accuracy rates of correct classification varied between 73.2% (right-left diameter at midshaft) and 93.2% (vertical head diameter) for univariate analyses. In multivariate analyses, they produced 3 functions with an accuracy ranging between 90.0% and 92.7%. According to their results, the dimensions of the humerus (particularly when the measurements are taken from the proximal parts) could be used for gender determination.

Metin Tellioglu A [19] conducted a study on gender determination, and used 104 (52 females and 52 males) left dry adult humerus bones. They made a total of 22 measurements on the humerus bones; 8 in proximal, 3 in the body, and 11 in distal end. Their results yielded that there were significant differences between the genders in almost all of the measurements except for the fossa radialis depth and trochlea of humerus width. According to ROC Analysis results, it was determined that humerus head transvers diameter (92.31%), epicondylar width (92.31%), minimum body diameter (90.38%) and humerus maximum length (90.38%) measurements were important in gender determination. As a result of their study, they reported that their study would be a reference for further studies to be con-

ducted on gender determination by using the humerus since their study represented the entire Turkish population and was the first one in this field.

1.1.3. Forearm Bones

In their study in which Üzün I et al. [20] examined gender variation, they used forearm bones in Turkish population. The sampling consisted of 42 males and 38 females that had a mean age of 40 and 36, respectively. The remains used in the study were obtained from Department of Morgue, Council of Forensic Medicine, Istanbul, Turkey. Various measurements were made in the radius (length, head diameter, midshaft anteroposterior, midshaft transverse, distal breadth) and ulna (length, midshaft anteroposterior, midshaft transverse, notch height, distal minimum head diameter, distal maximum head diameter) with lengths, midshaft diameters, and epiphyseal breadths. The measurements were made with a digital sliding caliper that had a 0.01-mm sensitivity with the exception of radial and ulnar lengths, which were made with an osteometric board. The classification results were 92% for the radius, and 91% for the ulna. The accuracy rates were about 92% and 83%, respectively for incomplete bones. They reported that further studies were needed regarding the entire skeleton to understand whether the forearm is more dimorphic compared to the other bones in the Turkish population.

Celbis O and Agritmis H [21] examined the radius and ulna bones in their study in which they evaluated gender estimation from radial and ulna bone lengths in a Turkish corpse sampling in Forensics Medicine Institution. The cases consisted of 80 males and 47 females between the ages of 18 and 63 (mean age of males was 36, and 30 of females). The measurements made in radius and length in the ulna were obtained by revealing the epiphysis ends of the long bones, similar to dry long bones. They used a caliper with moving and static scales to make the measurements. The measurements in the right radial and ulnar lengths were made by dissecting the soft tissue and joints at both ends in the autopsy. The distances between radius head and processus styloideus radii and, olecranon and processus styloideus ulnae were recorded. The caliper was kept parallel to the bone shaft in measurements. Their results showed that discriminant function analysis statistics could reveal accuracy rates at 91.6% in radius, 95.7% in the ulna, and 93.6% in both bones for gender estimation.

1.1.4. Hand bones

In their study conducted at Department of Radiology, Alıcıoğlu B et al. [22] aimed to obtain a differential equation for metacarpal bones and phalanges in a live Turkish population. They included 65 adult cases, including 22 male and 43 female, to evaluate the use of the interarticular distance of the metacarpal bones and phalanges of the left hands obtained

with digital X-rays in gender estimation. The cases that were not mature in terms of skeletons, those with congenital and developmental dysplasia, metabolic bone diseases, those with recent pathologic lesions like trauma or surgery, and those that had tumors, osteoarthritis and arthritis were excluded from their study. The distances between the midpoint of the basis and distal tip point of all the metacarpal bones and phalanges (proximal, middle and distal) were measured with electronic calipers, and were then used as the interarticular distance. All finger bones (distal phalange, middle phalange, proximal phalange) and metacarpal bones were examined, and a multi-variable logistic regression model was created for gender estimation. They concluded that gender estimation could be achieved by using all the metacarpal bones and the interarticular distances of the phalanges the left hand, and by taking the cut-off value as 0.5 at a rate of 72.7% in male, 90.7% in female, and 84.6% in total individuals. As a conclusion, they reported the anthropometric data of the interarticular distance of metacarpal bones and phalanges in modern Turkish individulals, and provided a mathematical model for gender determination. The speculated that their discriminant function could be used as an alternative in forensic cases especially when the skull and pelvis were not present. They also argued that when all metacarpal bones and phalanges were present, the gender determination was accurate.

1.2. Lower Extremity Bones

1.2.1 Pelvis

In Gülhan Ö's study [14], they aimed to develop gender determination standards with the pelvic bone specific for the Turkish population. They used 3 Dimensional (3D) pelvic reconstructions obtained from CT images of 50 (25 males and 25 females) individuals who had pathological findings in their evaluations. The age distribution of cases ranged between 28 and 65 (45.28 ± 9.71) in female, and 20 and 63 (40.4 ± 11.95) in male. They made 5 measurements in each case as transverse pelvic outlet, midpelvic width, transverse pelvic inlet, anterior superior iliac spine, and the height of anteroinferior point of ischial tuberosity and pelvic outlet width. The accuracy rate of 5 measurements was evaluated with discriminant function analysis in gender determination. As a result of the single discriminant analysis they used in the study, the highest accuracy rates for female were 88% with midpelvic width as 84%, transverse pelvic stroke values as 84%. For male, these rates were determined at transverse pelvic outlet with 92% and at midpelvic width with 88%. The accuracy rates of other parameters ranged between 84% (transverse pelvic inlet) and 74% (anterior superior iliac spine and anteroinferior margin of ischial tuberosity). They reported that the accuracy rate was increased to 96% when the 5 variables were taken into account in multivariate discriminant analysis.

In the study of Karakas HM et al. [23], the subpubic angle in the pelvis, which was the most extensively studied bone, was examined for gender determination. They suggested several qualitative classifications, dimensional measurements, and indices for investigative anthropometry and forensics. An accuracy rate of 70-75% was reported with the independent use of these parameters. They also investigated the accuracy rate of the subpubic angle in gender determination living Anatolian Caucasians. They used subpubic angle as the study material, and identified and measured 3D CT images of pelvis. Their data were obtained by using 64-detector CT (MDCT) at a 500-mm isotropic resolution. A total of 66 males (41.6 ± 14.9 years of age) and 43 females (41.1 ± 14.2 years of age) were included in their sampling. The measurements of the subpubic angle were taken on a 3D image analysis workstation. They reported that there were statistically significant differences between males and females about the subpubic angle, and that the accuracy of the subpubic angle was 90.8% in gender determination. The sensitivity of the subpubic angle in detecting the female phenotype was 88% with a specificity of 95% and a cut-off value of 74°. As a result, they concluded that the subpubic angle is an accurate parameter in gender determination with high sensitivity and specificity.

1.2.2. Femur

Harma A and Karakas HM [13] provided data on *in vivo* femoral dimensions of Anatolian Caucasians. A total of 50 males and 54 females with ages ranging from 18 to 68 were investigated with CT. They examined the maximum length, vertical head diameter, midshaft transverse diameter and anterior bowing femur parameters. The femoral dimensions of Anatolian Caucasians were not similar to the single racial group as reported. Significant differences were reported in their study between males and females about maximum length and vertical head diameter. There was no sexual dimorphism in midshaft transverse diameter and anterior bowing. In the Discriminant Analysis for gender, 83.3% accuracy was achieved when maximum length was used, and 76.9% accuracy when vertical head diameter was employed. When these parameters were used in a combined way, the overall accuracy rate increased to 84.6%. In vertical head diameter, a 44.9 mm cut-off value yielded 94% sensitivity and 83% specificity. For maximum length, a 428.6 mm cut-off value yielded 80% sensitivity and 67% specificity.

Özer I et al. [8] attempted to create metric standards for gender determination with femur measurements for ancient Anatolian populations. The osteometric data were obtained from skeletal remains of 130 adults (67 males and 63 females) from Dilkaya medieval collection. A total of 8 femur measurements were made, and the data were analyzed with t-test and Discriminant Analysis. Basic statistics showed that all measurements

(maximum femur length, trochanter length, transverse diameter, maximal anteroposterior diameter, perimeter, subtrochanteric transverse diameter, subtrochanteric anteroposterior diameter, condyle breadth) were sexually dimorphic. Precision of gender determination was 86.5% with the condyle breadth for univariate discriminant function derived. Their prediction values demonstrated that gender differentiation could be performed with femur measurements in a reliable way between 76% and 88.5%, with values for female slightly higher than for males. It was speculated in their study that discriminant formulas developed with the combinations of femur measurements could be used for gender determination accurately on fragmentary skeletal remains in ancient Anatolian populations.

1.2.3. Leg bones

Ekizoglu O et al. [24] conducted their study on gender assessment, and examine the left tibia morphometry of a modern Turkish population by using CT images. They made measurements in seven parameters (maximum length, proximal epiphyseal breadth, distal epiphyseal breadth, maximum diameter at the nutrient foramen, medial-lateral diameter at the nutrient foramen, circumference at the nutrient foramen, intercondylar breadth) from the left tibia in 19-92 age group of 203 individuals (124 males and 79 females). The single-variable distinctive functions resulted in classification accuracy that ranged between 66% and 86%. They found that the best rate in gender dimorphism was firstly in proximal epiphyseal breadth (86%), followed by distal epiphyseal breadth (85%). It was also found that the medial-lateral diameter at the nutrient foramen and the intercondylar breadth values were 74% and 66%, which meant the lowest reliability in gender determination. They created 8 multi-variable discriminant functions by using different variable combinations. The classification accuracy for functions varied between 79% and 86%. The maximum length, proximal epiphyseal breadth and distal epiphyseal breadth were used in the best discriminant function, which resulted in 86.2% accuracy rate. They concluded that the tibia is a very useful bone for gender prediction in modern Turkish population.

Özer BK et al. [25] conducted a study to provide gender determination by using discriminant analysis from tibia measurements in an ancient Anatolian population. In their study, 7 tibia measurements were made in 123 adults with known gender (62 males and 61 females) in Medieval Dilkaya population. The osteometric measurements were the length, circumference of midshaft and minimum, transverse and sagittal diameters of midshaft and nutrient foramen levels. The data were analyzed with Student t-test and Discriminant Analysis. They concluded that grouping based on gender differentiation was accurate with tibia metric values between 73.5% and 90.2% in Dilkaya population. They concluded that the midshaft circumfer-

ence was the best single discriminating variable. They speculated that discriminant formulas developed with tibia measurements could be used for gender determination accurately in fragmentary skeletal remains in ancient Anatolian populations.

1.2.4. Foot bones

In their retrospective study, Ekizoglu O et al. [26] they worked on CT angiography images of the cases. Only the left calcaneus was evaluated in 408 cases between the ages of 18 and 99 (218 males, mean age: 38.21 ± 15.9 and 210 females, mean age: 37.02 ± 16.6 and a mean age for both genders 34.44 ± 16.4). They performed 3D reconstructions, and made 9 measurements on each left calcaneus, which were maximum length, load arm length, minimum breadth, middle breadth, body height, maximum height, dorsal articular facet length, dorsal articular facet breadth, cuboidal facet height. The data were evaluated after dividing them into two groups, the original group consisting of 348 individulas (179 males and 169 females), and the validation group consisting of 80 individuals (39 males and 41 female). The original group was used to create formulas to distinguish genders, and the validation group was used for cross validation. The statistical analysis of the original group was made with Discriminant Function Analysis Method. All the calcaneus measurements were found to be significantly larger in males than in females. The only variable that had the highest classification accuracy was the minimum breadth parameter that showed the accuracy rate of 100% in the original group and 90% accuracy rate in the validation group. It was found that the dorsal articular facet breadth was the best single variable combining high overall classification accuracy and low gender deviation for the validation sample. Multi-variable Discriminant Functions (DF1-DF5) and Logistic Regressions (LOGR1-LOGR5) were developed for different variable combinations in direct and gradual analyses. All variables were used in DF1. For the original group, 100% accuracy was obtained; and for the verification group, 94% accuracy rate was obtained. Four variables were selected in DF2 gradual analysis. DF2 yielded an accuracy rate of 100% for the original group, and 93.78% accuracy for the verification group. Length, height and width variables were used in DF3, DF4, and DF5, and in general, it was found that the accuracy rates were between 84.87% and 93.84% for the verification group. LOGR1 yielded the highest classification accuracy (97.5%) for the verification group. In general, it was found that the formula with the highest accuracy rate was LOGR1 for the verification group.

Ayşe A [27] made metric examinations on adult calcaneus (N=56) and talus (N=64) for gender prediction. The measurements in calcaneus and talus bones were made by using a digital caliper. Maximum calcaneus length and maximum talus height were selected for measurements. A total of 30

individuals with mean maximum calcaneus length larger than 72.85 mm were estimated to be male, and 26 individuals with smaller values than the abovementioned value were estimated to be female. The same application was calculated for the talus as well. A total of 30 individuals with a mean maximum talus height value bigger than 53.44 mm were estimated to be male, and 34 individuals with smaller values were estimated to be female.

2. AXIAL SKELETON BONE

2.1. Cranium Bones

Türk E et al. [28] retrospectively examined the frontal sinus dimensions of 178 individuals (105 females, 73 males) ranging in age between 20 and 85 on Conic-Ray Computed Tomography (CRCT) images. The width, height and also the total width of the frontal sinus were measured, separately for the right and left coronal CRCT sections. The frontal sinus anteroposterior depths were measured in axial CRCT sections, to the right and left separately. The data were evaluated with the Discriminant Function Analysis. As a result of the analyses, the rate of predicting the male gender accurately was 61.6%, the rate of predicting the female gender accurately was 78.1%, and the accurate gender estimation rate for the entire population was 71.3%. The most dimorphic parameters were identified as left anteroposterior depth, right anteroposterior depth, depth and maximum total width of the frontal sinus. As a result, it was determined that the frontal sinus was dimorphic in gender determination, but it was not enough alone to determine gender.

Buran F et al. [29] conducted a study and aimed to examine the possibility determination the age and gender by measuring the bimastoid width in cases whose skulls were available. They examined the cranial CT images of 600 individulals, including 300 males and 300 females between the ages of 20 and 50, from the Radiodiagnostic Department. The sampling was divided into 6 subgroups with 5 years difference in terms of age as 50 females and 50 males in each group. In the study, the mean age of the population was 35.49 ± 8.64 for the entire population; 35.43 ± 8.61 in male cases and 35.56 ± 8.68 in female cases. The images of males and females of all ages in equal numbers were examined. In 3D images, which were obtained with reconstruction, the width was measured between the ends of the “mastoid process” parts of both mastoid bones on the back of the skull on coronal plane, and the resulting values were recorded in millimeter. The results were evaluated with Discriminant Function Analysis Method. The accuracy rates for the measured values were 82.7% in females and 80% in males. As a conclusion, the morphometric measurements of the bimastoid width provided high dimorphism rate for gender determination in modern Turkish population; however, it did not have an important dimorphism rate

for age determination.

Kaya A et al. [30] examined the 3D Computerized Tomography Angiography (3D-CTA) screening of 52 females and 60 males individulals between the ages of 13 and 86 in terms of orbital width and height. The mean age of female cases was 58.37±2.86 (min: 13; max: 78) and the mean age of male cases was 63.12±1.72 (min: 25; max: 86). The orbital parameters and bookmarks that were used for orbital measurements were evaluated with sexual dimorphism discriminant function analysis method. Males had left and right orbital width and orbital height at significantly larger levels than females. When single variables were analyzed, it was found that the most reliable variable was the left orbital width (71.7%) for males and left orbital height (69.2%) for females. It was determined that the least reliable variable was the right orbital height for males and females (61.7% and 57.7%, respectively). It was found that the left orbital width and orbital height model had the best gender estimation rates (80.0% - 69.2% for males and females, respectively) for both genders when more than one variables were used in discriminant function analysis.

In their study, Ekizoglu O et al. [31] performed morphometric analysis of the maxillary sinuses to determine gender. They used 1-mm thick coronal and axial paranasal sinuses CT scan was used for morphometric analyses. They included 140 individuals (70 females and 70 males) ranging in age between 18 and 63 (mean 31 ± 11.2). Right anteroposterior diameter, right transverse diameter, right cephalocaudal diameter, right maxillary sinus volume, left anteroposterior diameter, left transverse diameter, left cephalocaudal diameter and left transverse diameter were measured for all cases. The size of the maxillary sinus was found to be significantly small in females in all measurements. The measurement results were evaluated with the Discriminant Function Analysis Method. As a result of the study, the accuracy rate according to gender was found to be approximately 80% in females, 74.3% in males, and 77% in the entire study population.

In their retrospective study, Ekizoglu O et al**.** [32] examined the images of 400 cases (200 males, mean age: 30.1 ± 15.9 and 200 females, mean age: 31.6 ± 16.6 and overall mean age for both genders: 30.7 ± 11.2) in cranial CT images. In this study, which was conducted at a training and research hospital, 14 cranial parameters were measured: maximum cranial length, maximum cranial breadth, bizygomatic diameter, basion-bregma height, cranial base length, basion-prosthion length, upper facial breadth, bimastoid diameter, orbital breadth, orbital height, biorbital breadth, interorbital breath, foramen magnum length and foramen magnum breadth. When the measurements were made, the proper images were selected from sagittal, coronal and axial techniques for the images in which measurement points could be determined best. The average values of all parameters were

found to be significantly higher in males. From the logistical regression analysis results made by using morphometric measurements, the most interesting measurements were identified as maximum cranial length, bizygomatic diameter, basion-bregma height, and cranial base length in terms of dimorphism. It was found that the most dimorphic structure was the bizygomatic diameter, which has an accuracy rate of 83% in females and 77% in males. When the results were analyzed in multiple terms, the best results were reported to be the four parameters (maximum cranial length, bizygomatic diameter, basion-bregma height, and cranial base length), which were obtained at the 4th stage, and 87.5% of females and 87.0% of males were reported to be classified accurately.

Tatlisumak E et al. [33] conducted a study and examined the place of frontal sinus in gender determination on 180 males and 180 females paranasal CT scans obtained from Radiology Department with a mean age of above 20. They made a total of 8 measurements (width of the right frontal sinus, width of the left frontal sinus, height of the right frontal sinus, height of the left frontal sinus, anteroposterior length of the right frontal sinus, anteroposterior length of the left frontal sinus, volume of the right frontal sinus, volume of the left frontal sinus). The widths and heights of the sinuses were measured on coronal plain, and their anteroposterior lengths were measured on axial plane. It was found that all measurements in frontal sinus were higher in males than in females. These differences were statistically significant in the width of the left frontal sinus, height of the left frontal sinus, anteroposterior length of the right frontal sinus, anteroposterior length of the left frontal sinus, volume of the right frontal sinus, and volume of the left frontal sinus, but were statistically insignificant in width of the right frontal sinus and height of the right frontal sinus. It was found that all measurements on the frontal sinus were higher on the left side in females, males and the total population.

Teke HY et al. [34] aimed to investigate whether the width, length and height of maxillary sinuses could be used to determine gender. The width, length and height of the right and left maxillary sinuses were measured in a total of 127 adult cases [62 (48.8%) females and 65 (51.2%) male], who applied to Department of Radiology for CT scans of their sinuses. The width and length measurements were made where the maxillary sinus was in the widest position. The height of the maxillary sinus was determined by marking the first and last scans of the sinus in the CT scan, and the number of sections between them was determined. Eventually, the number of the sections was multiplied by 3 to find the height of the sinus. The data obtained in CT scans were evaluated with Discriminant Function Analysis Method to determine whether the measurements of maxillary sinuses could be used for gender determination. In their study, it was found that the size

of maxillary sinuses of females was smaller than male. The accuracy rate of right maxillary sinus measurements was 59.7% in females, and 67.7% in males, and 63.8% on average. It was determined that the accuracy rate of left maxillary sinus measurements was 67.7% in both males and females. The accuracy rates of right and left maxillary sinus measurements were 69.4% in females, 69.3% in males, and 69.3% on average. As a result, they reported that CT measurements of maxillary sinuses were useful with a lower accuracy rate in supporting gender determination in forensics.

In their study, Polat YD et al. [35] aimed to determine the usability of maxillary and frontal sinus measurement parameters in the skull for gender determination and superiority of each other. They evaluated the paranasal CT images of 128 cases (56 females and 72 males) retrospectively. The parameters that examined in frontal and maxillary sinuses were the height and width of sinus, the distance between the anteroposterior diameter and the sinuses on the right and left sides. They reported significant differences between the genders in terms of all frontal sinus measurements; and males had the mean value as higher than in females in all measured variables. Among the maxillary sinus measurements, only the right maxillary sinus height and anteroposterior diameter did not yield significant differences in terms of left maxillary sinus width measurements. When multivariate discriminant function test was made, all measurements classified 60.4% of females and 80.9% of males accurately. When all parameters were evaluated together, the mean accuracy rate was found to be 71.9%. The best differential measurements were detected in the right frontal sinus. The right frontal sinus height could differentiate 51.9% of females, and 73.5% of males. As a result of their study, they found that especially frontal sinus size was more distinctive in gender determination. Their study also confirmed that paranasal CT could be used in anthropological studies.

Günay Y and Altinkök M [36] aimed to determine if the foramen magnum area was a useful criterion for gender determination in fragmented skulls. Measurements were made in 219 skeletons (170 males and 39 females) of Turkish adults in terms of the length of foramen magnum and foramen magnum width by using simple calipers with a sensitivity of 0.01 m. Statistically significant differences were detected between the averages of foramen magnum areas in both genders. However, the correlation coefficient was found to be 0.27. Because of this very low correlation coefficient, discriminant analysis was not made. The measurements confirmed that the average foramen magnum area was lower in females than in males. As a conclusion, it was found that the foramen magnum area was not a very useful indicator for gender determination, and could only be used in some cases as a supportive finding.

Apaydın B et al. [37] conducted a study to analyze the effects of men-

tal foramen, gonial angle, and antegonial angle measurements on gender determination by using digital panoramic radiography. They planned a retrospective study with 150 digital panoramic radiography images (75 males and 75 females, aged between 20 and 49). The study was conducted in Department of Maxillofacial Radiology. The radiography images were analyzed by dividing them equally to two gender groups (male and females). Various parameters were compared to determine gender. The distances from the upper and lower borders of the foramen mentale to the base of the mandibula on the right were measured. The gonial and antegonial angles were evaluated bilaterally. Statistically significant differences were detected between males and females in terms of all parameters they evaluated. Although the parameters for foramen mentale were higher in males, it was determined that the gonial and antegonial angles were higher in females. As a conclusion, the measurements in gonial angle, antegonial angle and foramen mentale were found to be statistically significant between males and females for the study population, and it was reported that these parameters could be used for gender determination in the Turkish population.

Gungor K et al. [38] conducted a study to determine any possible changes in gonial angle in time in ancient Anatolian populations. They also tried to show the symmetry of the gonial angle in jaws and sexual dimorphism. Gonial angle values (right and left) were measured in 267 Turkish adults who had no craniomandibular disorders, orthodontic history or treatment with panoramic radiographs. The data of the past populations were collected from previous studies. The comparison between right and left and sexual differences were tested with the Paired Student t-test. They also made discriminant analyses. According to their results, it was concluded that there were no significant differences between the right and left gonial angles of the individuals; however, a significant difference was detected at the left gonial angle between genders. Also, they found no statistically significant differences for the gonial angle between the selected past populations with their current sampling.

In Akansel G et al.'s study [39] lateral angle of the internal acoustic meatus of 92 cases (47 females and 45 males) was measured on axial slices of high resolution computerized temporal bone tomography scans. The measurement technique was modified from a cadaveric study where a significantly higher mean lateral angle measurement was detected in females compared to males. However, 35^{o} and lower measurements were 93.6% specific for male gender and 60^{o} and bigger were 97.7% specific for female gender. They concluded that CT of the temporal bone is capable of replicating the results from the cadaveric measurements for lateral angle determination.

Güleç E and Duyar I [40] used the foramen magnum of 83 females and

77 males from the Ottoman Period skulls. In their study, they conducted a discriminant analysis based on 3 measures of foramen magnum (length and width index) and 26 measurements of the skull. As a result of their discriminant analysis, it was found that the accuracy rate of the 3 foramen magnum measurements used in gender determination was 80%.

Kasar H et al. [41] aimed to develop a formula in their study in which they examined the mandibula of 83 males and 17 females cases in gender estimation. They conducted discriminant analyses with biomathematical methods on the mandibula parameters they examined. They found that gender determination could be possible with the formula they developed at a rate of 83.1% in males, and 76.47% in females.

Bayrak S and Göller Bulut D [42] evaluated foramen magnum (area, sagittal dimension, transverse dimension) and clivus (length, width) for gender determination, and examined Cone-Beam CT images of 412 individuals (248 females and 164 males). As a result, according to their results, significant relations were detected between the gender of the individuals and the sagittal and transverse dimension and areas of the foramen magnum and the length and width of the clivus. Also, a significant relation was detected between the length of the clivus and the ages of the individuals.

2.2. Hyoid Bone

In their study, Balseven-Odabasi A et al. [43] used the bodies as cadavers after autopsy in the Morgue Office of the Forensic Science Institution. The hyoid bones of 85 (53 males and 32 females) was excised to obtain metric measurements and to evaluate non-metric variations of the hyoid bone. It was reported that the age of the cadavers varied between 21 and 80. The excised hyoid bones were inserted on a black background and photographed in the front and superior-inferior at a distance of 55° with a digital camera. A total of 33 measurements (21 lengths, 7 widths and 5 angles) were made in each bone. The measurements were evaluated with Discriminant Function Analysis Method. It was found that males had significantly greater values than females in 18 (12 lengths, 6 widths) of these 33 measurements. As a conclusion, the accuracy rate for gender determination was 77.4% for males and 81.3% for females. When all of these 33 measurements were considered, it was determined that 92.5% of males and 78.1% of females were classified correctly by using the discriminatory functions.

2.3. Vertebral Column Bones

In the study of Sertel Meyvaci et al. [44], 8 parameters were examined in CT images of the first cervical vertebra (Atlas), and it was found that gender determination could be made with an accuracy ranging between

73% and 80%. They also found that the distance between lateral (FTL) or medial (FTM) edges of foramen transversarium in Atlas was sexually dimorphic and conducive to first cervical vertebra's gender determination. They used 6 parameters with the help of CT and the cut-off values of the FTM_{AVG} and FTL_{AVG} parameters (4.64 cm, 5.92 cm respectively). These cut-off values were compared with FTM_{AVG}, which was derived from dry Atlases whose genders were not known, and 76% were compared with FTL_{AVG} with 80% accuracy. According to their findings on 8 parameters obtained from CT images, when the 84 dry Atlases whose genders were not known were evaluated, it was estimated that 49 could be females, 22 could be males, and 11 could be children. The accuracy rate obtained was 78.8%.

Etli Y et al. [45] conducted a study for gender estimation with 21 sacral and coccygeal metric parameters obtained with CT images of a Turkish population consisting of 480 cases that were equalized in terms of gender and age. They used univariate discriminant analysis, linear discriminant function analysis, stepwise discriminant function analysis, and multilayer perceptron neural networks. They reported maximum 67.1% accuracy in univariate discriminant analysis, 82.5% in linear discriminant function analysis, 78.8% in stepwise discriminant function analysis, and 86.3% in multilayer perceptron neural networks. Although they did not obtain an acceptable accuracy rate of 95% or more for the sacrum and coccyx, gender estimation with neural networks was reported as a promising study area in corpses in which identification is not possible otherwise. They also recommended to conduct further studies on other bones with new techniques to obtain useful information.

2.4. Costae and Sternum

In the study of Koçak A et al. [46], they investigated the right sternal end of the 4^{th} costa of from a total of 251 individuals (78 females and 173 male) from different age groups. They obtained the bones from Forensic Science Institution. They made 3 measurements on superior-inferior height, anterior-posterior breadth and medial fossa depth with the help of a 0.1-precision caliper. These measurements were the maximum distance between the most superior and inferior points at the end of the rib, between the most anterior and posterior points at the end of the bone, maximum depth of the rib's concavity at the medial articular surface, where the distance between the bottom of the pit and top of the adjacent wall is greatest. The sampling was divided into 3 groups to determine the effect of age and to improve the gender determination functions: 1. Young Group (15-32 years) with stages 1-4; 2. Old Group (33-89 years) including stages 5-8; and 3. Total Group (15-89 years) including stages 1-8.

The data were analyzed with discriminant function analysis. Gradual Discriminant Function Analysis showed that gender determination could be possible at a rate of 86.1% in young males in the group, 91.7% in young males in the group, and 88.6% in total. It was seen that the most effective parameter for gender determination was maximum distance between the most superior and inferior points at the end of the rib. In the elder group, it was determined that the most effective gender parameters were maximum distance between the most superior and inferior points at the end of the rib and between the most anterior and posterior points at the end of the bone. In this group, the accuracy rate was 85.1% in males, 90.5% in females, and 86.5% in total. In the total group analysis results, the accuracy rate for gender determination was found to be 85.5% for males, 87.2% for females, and 86.1% for total.

Çölolu AS et al. [47] conducted a study on 294 the 4th costa's sternal ends after autopsy for the purpose of determining gender over the costa for the Turkish population. They used a caliper to measure the dimensions from the superior edge to the inferior edge and the anterior edge to posterior edge. The sampling was separated into 3 age groups as "young", "old" and "total". They developed 3 formulas one for each group by using Discriminant Function Analysis. It was concluded in their study that the superior edge to the inferior height was the most dimorphic dimension and that both dimensions could yield an accuracy of 86% to 90%.

In the study conducted by Ekizoglu O et al. [48], the purpose was to examine the benefits and reliability of sternal morphometric analysis for gender estimation. In this study, 443 cases (202 females and 241 male) between the ages of 33 and 60 (mean: 44±8.1) were evaluated by using Multidetector CT. The measurements used were manubrium length, mesosternum length, sternebra 1 width, sternebra 3 width and sternal index. The manubrium length and mesosternum length were measured from sagittal images, and the sternebra 1 width and sternebra 3 width were measured from coronal reshaped images. The mean manubrium length, mesosternum length, sternebra 1 width and sternebra 3 width were significantly higher in males, and the sternal index was higher in females. It was found in the discriminant analysis that the mesosternum length had a high accuracy rate of 80.2% in females and 80.9% in males. Mesosternum length also showed the best sensitivity (75.9%) and specificity values (87.6%). It was also found that the accuracy rates were over 80% in 3-Stage discrimination analysis for both genders. Similar to previous studies, it was determined that females had smaller sternum sizes, and the reliability of the sternal index was low. Step-wise 1 (manubrium length, mesosternum length, sternebra 1 width, sternebra 3 width) yielded the highest accuracy rate in Gradual Discrimination Analysis at a rate of 86.1% in females and 83.8% in males.

To assess gender in the Turkish population, Gülhan Ö et al. [49] examined CT images of 200 (100 male and 100 female) adult cases with known ages taken from the Department of Radiology. They reported that the mean age of adult males and females was 51.74 and 54.95 (20-80). A total of 200 three-dimensional femur models were created from CT scans. Thirteen parameters were identified from the measurements made in each femur: maximum length of femur, femur bicondylar length, femur trochanteric length, medial-lateral subtrochanteric diameter, vertical caput diameter, medial-lateral medium shaft diameter, femur neck vertical diameter, femoral neck axis length, femoral proximal width, femoral bicondylar width, epicondylar width, lateral condyle antero-posterior diameter, medial condyle antero-posterior diameter. A total of 13 anthropometric parameters were measured and analyzed by basic descriptive statistics and discriminant analysis methods. The accuracy rate of gender estimation was found to be between 63.5% and 88% in single variables. The most dimorphic single measurement was found to be the femur neck vertical diameter with a general accuracy rate of 88% when used alone. In the gradual analysis, the epicondylar width, femur neck vertical diameter, and medial lateral subtrochanteric diameter were found to be the most distinctive parameters with an accuracy rate of 91%.

In their prospective study, Ramadan SU et al. [50] randomly selected their cases from those who applied to the radiology department for thoracic CT examination with various complaints. The Turkish cases' thorax CT imaging was performed by using Multi-Section Computed Tomography (MSCT) in 340 (143 females, 197 males; mean age: 57.6 ± 15.2). The accuracy of the gender determination by using costa measurement data was investigated on the sternum obtained from chest MSCT imaging and the 4th costa. They used 6 (length of the manubrium, length of the sternal body, combined length of the manubrium and body, fourth rib width, manubrium width, corpus sterni width, Hyrtl's law, sternal index, sternal area) measurements and 3 indices identified in the literature in their study. The left fourth rib width was used in gender determination because the it was found to be a more significant difference than the right fourth rib width. An accuracy rate of more than 80% was achieved in gender determination for only combined length of the manubrium and body. Hyrtl's Law and sternal index did not provide adequate accuracy for gender determination in cases. When the sternal area and fourth rib width were used together, the highest accuracy rates (88.2%) were reached for gender determination. As a result, it was found that radiological methods like MSCT were useful in making reliable measurements and determining accuracy in various anthropological and forensic studies.

In Coltu A et al.'s [51] study, they evaluated the sternum measure-

ments for gender assessment. As a result of the metric measurements obtained from 141 chest front wall X-ray graphics of 111 male and 30 female autopsies (manubrium length, manubrium width, corpus length, sternum length, sternal index, 1, 2, 3rd segment width) were evaluated, it was found that the sternum length was 80% in females, and 89.2% in males. When corpus length, 1st segment width, 2nd segment width, 3rd segment width were examined together, the best accuracy rate was determined in male sternum at 100% (above 181 mm), and in females at a rate of 93.5% (less than 181 mm).

CONCLUSIONS

We examined the studies conducted for gender assessment by evaluating bones in the Turkish population. Although there were many studies conducted on cranium bones, we found that there were no studies conducted on other cranium bones and anatomical structures. When we examined other bones in the body, we could not detect studies conducted on some bones and especially on clavicula, fibula, and patella. In the metric evaluation of bone parameters, Computerized Tomography, Radiography images and direct measurements made with the help of caliper were used. We observed that researchers preferred Discriminant Function Analysis, which is a method commonly used by researchers to demonstrate gender estimations in the analysis of the values of parameters. Although the equations that were obtained as a result of these analyses were repetitive and reliable, they require experience to use in practice. For this reason, practical, simple and rapid applications are needed to contribute to gender assessment. As a result, gender assessment on each bone in the human skeleton will provide contribution for identification process. Studies should be conducted in different populations to contribute to morphological assessment of different bones in human skeleton with various evaluations for each bone in the skeleton, and practical and applicable methods should be selected and important contributions should be made to the identification process.

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Chapter 43

PLATELET-RICH FIBRIN IN ORAL SURGERY

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Evolution of Platelet Concentrate

Blood-derived products have been proposed for many years to stimulate the healing process and seal wounds with the use of fibrin glues, which are constituted of concentrated fibrinogen. Platelet concentrates were originally used for the prevention and treatment of hemorrhage. The origin of the platelets is megakaryocytes in the bone marrow. Platelets not only prevent hemorrhage by developing blood clot, but they also constitute the main source of cytokines and key growth factors that are capable of matrix remodeling, stimulation of angiogenesis, and cell proliferation during wound healing(SEZGİN & TANER, 2012). Platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), transforming growth factor beta-1 (TGFβ-1), and epidermal growth factor (EGF) are the key elements of tissue repair and released from platelets(Dohan Ehrenfest, Rasmusson, & Albrektsson, 2009). Whitman et al. first described the use of platelet concentrates to improve healing with the replacement of fibrin glues(Whitman, Berry, & Green, 1997). Platelet-rich plasma (PRP) is an autologous modification of fibrin glue and it was the first substance introduced to promote the healing process by using growth factors(Kanno, Takahashi, Tsujisawa, Ariyoshi, & Nishihara, 2005). A natural blood clot contains 5% platelets, 1% white blood corpuscles (WBCs) and 94% red blood corpuscles (RBCs) while PRP contains 95% of platelets. Protocols for harvesting PRP involve the use of anticoagulants and high g-forces to selectively separate blood cells based on density(Fujioka-Kobayashi et al., 2020).

General Classification

This classification has separated the preparations in terms of the presence of a cell content (especially leukocytes) and fibrin architecture.

1. **Pure Platelet-Rich Plasma (P-PRP):** Without leukocytes and with a low-density fibrin network after activation. Liquid solution or activated gel form.

2. **Leukocyte-and Platelet-Rich Plasma (L-PRP):** With leukocytes and with a low-density fibrin network after activation. Liquid solution or activated gel form.

3. **Pure Platelet-Rich Fibrin (P-PRF):** Without leukocytes and with a high-density fibrin network. Strongly activated gel form.

4. **Leukocyte-and Platelet-Rich Fibrin (L-PRF):** With leukocytes and with a high-density fibrin network. Strongly activated gel form(Dohan Ehrenfest et al., 2009).

Fibrin

Fibrin is the activated form of fibrinogen and commonly presents in plasma and platelet *alpha*-granules. It plays a potential role in platelet aggregation. Fibrin matrix is a natural mediator for angiogenesis, immunity, and epithelial cover that are major keys to healing and soft tissue healing. Fibrin matrix leads directly to angiogenesis induction by binding of numerous different growth factors. Fibrin and fibrinogen degradation products stimulate neutrophil migration and permit adhesion of the neutrophil to endothelium to promote the immune response. Fibrin matrix guides the coverage of injured tissue, affecting the metabolism of fibroblasts and epithelial cells(Joseph Choukroun et al., 2006a).

Platelet-Rich Fibrin

PRF is a second generation of concentrated platelet that was first described by Choukroun et al. in 2001 and has gained tremendous momentum, having been utilized or a variety of medical and dental procedures(J Choukroun, Adda, Schoeffler, & Vervelle, 2001; Fujioka-Kobayashi et al., 2020). This new concept is an autologous cicatricial matrix that is neither like fibrin glue nor like a PRP concentrate. It is a centrifuged blood derivation without any anticoagulant or other artificial biochemical modifications. PRF composed of a fibrin matrix with the incorporation of leucocytes, circulating stem cells, cytokines, and platelets. The interaction between these cells and the fibrin matrix stimulates a slow release of growth factors that may result in a better wound healing.

Advantages of PRF over PRP

- The lifespan of PRF growth factors is longer than that observed in PRP
- Does not require biochemical handling of blood
- Eliminates the use of bovine thrombin (promotes conversion of fibrinogen to fibrin) which is crucial for PRP and avoids the use of anticoagulants
- Cost-effective and simplified procedure over PRP
- The production time is reduced
- The more efficient proliferation and cell migration
- Supportive effect on the immune system

Disadvantages of PRF

- Low amount of derivation due to autologous nature
- Quick handling is needed to achieve maximum effect

- Tissue banks are unfeasible

Preparation Protocol

A venous blood sample is collected around 5 ml in each of the two sterile vacutainer tubes of 6 ml capacity without anticoagulant. The tubes should be centrifuging within 60 seconds after the start of the venepuncture. For this reason, it is recommended to load tubes into the centrifuge two by two. The coagulation of the blood sample starts almost in contact with the tubes. Rapid manipulation is an essential way to obtain a clinically convenient PRF clot. The tubes are centrifuged at 2700-3000 revolutions per minute (rpm) for 10-12 minutes. This process settles the blood into 3 distinct layers:

1. **Top Layer:** Straw colored, amorphous plasma layer
2. **Middle Layer:** A mesh network that contains the majority of the fibrin and platelets (buffy coat)
3. **Bottom Layer:** RBC layer / RBC corpuscle

The top layer is then removed and the middle PRF layer is collected 2 mm below the lower dividing border. The bottom layer is also unused. The PRF layer can be shaped into a plug or prepared as a membrane depending on the aim of use. The PRF clot by itself contains a great amount of exudate that is rich in growth factors, and this exudate can be expressed by gentle compression of the clot with specially engineered boxes to obtain stronger PRF membrane (Figures 1-8).

Current Terminology for PRF Derivations

L-PRF: Conventional Choukroun‘ s PRF system

A-PRF™: Advanced-PRF. Modified platelet fibrin concentrate presented with a “low-speed centrifugation concept” (LSCC) theory. Samples are centrifuged at 1500 rpm for 14 minutes. Morphologically different from L-PRF with a more porous structure.

i-PRF: Injectable PRF. High relative centrifugal forces (RCF) provides a fibrin clot with a dense structure including leukocytes and platelets, which were mostly distributed unequally throughout the scaffold accumulated at the proximal portion of the PRF clot. By reducing the RCF and modifying the centrifugation time, a more porous fibrin structure can be achieved with more growth factor release and leukocytes compared to conventional PRF preparation techniques. This process ensures not only the improvement of the structure and also the enhancement of the regenerative potential. The protocol is based on the LSCC. The centrifugation process can be reduced down to 600 rpm and 4 minutes.

C-PRF: Concentrated PRF. following the L-PRF protocols, the majority of cells were detected within the buffy coat layer. Directly above the RBC layer approximately tenfold increase in baseline concentrations especially in this 0.3 – 0.5 ml buffy coat layer given the working name concentrated PRF. This part of PRF can exhibit higher growth factor release.

Use of PRF in Oral Surgery Protocols

Considering the clinical advantages of PRF, it can be used in a wide range throughout all areas that require regeneration and healing. Sinus lift (crestal/lateral approaches), guided bone regeneration with or without implant, socket preservation, and periodontal therapy are the main fields of usage.

PRF and Sinus Lift

Sinus-lift is an effective and remarkable surgery for implant placement. The protocol has evolved either technical aspect or the launch of new biomaterials. Various materials are proposed for this procedure: autogenous bone grafts, xenografts, allografts, and synthetic materials. PRF can be combined with these materials to accelerate the healing process. Recently, studies suggested that PRF would be used in a maxillary sinus lift with the lateral approach without any graft material(He, Lin, Hu, Zhang, & Wu, 2009; Stumbras, Kuliesius, Januzis, & Juodzbalys, 2019). Previous studies reported that graft materials combined with PRF revealed accelerated histologic maturation and reduced healing period before implant placement by about 4 months compared with the group without PRF combination(Joseph Choukroun et al., 2006b). However, the use of this combination does not provide any additive value for implant survival and the volume stability of the graft. Therefore, a new method was suggested that PRF as sole graft material would be used in simultaneous maxillary sinus lifting and implant placement(Simonpieri, Choukroun, Corso, Sammartino, & Ehrenfest, 2011). Following the concepts of guided bone regeneration, PRF not only prevents damage to the maxillary sinus membrane, but it also prevents the leakage of the bone materials into the maxillary sinus when there is a perforation in the Schneiderian membrane. The major complications of the crestal sinus augmentation technique are perforation of the membrane and difficulties to verify these perforations. Studies also reported that perforation in the membrane is observed about 30% of surgeries(Kim, Jeong, & Oh, 2012). PRF can reduce or repair the damage of the Schneiderian membrane in this technique. Limited studies have shown an increase in the peri-implant bone density with the combination of PRF with xenograft(Inchingolo et al., 2010; Tatullo et al., 2012). Bölükbaşı et al. showed significantly lower resorption for this combination at areas of implant placement(Bolukbasi, Ersanli, Keklikoglu, Basegmez, & Ozdemir, 2015).

PRF, Guided Bone Regeneration and Peri-implant Bone Defects

Platelet derivations may not be relevant to improve the osseointegration process, but they may help the regeneration of peri-implant defects. The combination of platelet derivations and bone graft materials can increase the ratio of bone to the implant contact area. Limited in vitro studies have been carried out on the effects of PRF on the regeneration of peri-implant bone defects. Lee et al. showed that peri-implant bone defects were successfully repaired with PRF alone in an animal model. However, clinical studies are needed to the extent of this issue. In augmentation procedures, PRF is mostly preferred as a barrier membrane apart from combination of bone graft materials. It accelerates the regeneration of the gingiva and also prevents the leakage of the bone graft material. PRF membrane can promote healing by increasing the thickness of gum around the implants. Marginal bone loss in the peri-implant area is decreased in this manner(Lee et al., 2012).

The results concluded that PRF cannot replace bone graft materials, but it can play a significant role as a barrier membrane. Besides, PRF can promote soft tissue healing as gingiva and periosteum(Joseph Choukroun et al., 2006a).

PRF and Socket Preservation

PRF membrane was demonstrated to enhance the local healing of gingiva and reduce postoperative pain response in the extraction sockets. New blood vessels are generated and epithelialization is promoted with the use of PRF. Hence, more rapid wound coverage is facilitated. PRF expresses a substantial effect on the proliferation and differentiation periods of healing and releases autologous growth factors. Consequently, rapid wound coverage can be achieved. Socket preservation with PRF increases the novel bone formation by stimulating bone regeneration. It decreases the risk of complications or exposure since a barrier membrane is not used. Furthermore, there is an associated decrease in the chance of a foreign body reaction. Nonetheless, clinical research on the approximate height and width of alveolar bone PRF can maintain is insufficient regarding socket preservation(Kawase et al., 2015).

PRF and Periodontal Regeneration

The main objective of periodontal therapy is to block the spread of infection and to regenerate the periodontal tissues. There is a wide range of treatment modalities that can be used for regenerative periodontal therapy as well as the use of growth factors, guided tissue regeneration, bone grafting, or a combination of listed approaches. Autologous blood derivations offer a safe and functional approach to deliver high concentrations

of growth factors to surgical wounds following periodontal therapy. The adjunctive use of PRF in combination with the barrier membrane is giving more effective treatment results than the barrier membrane alone. The mentioned combination treatment model has given the significant improvement of clinical parameters in intrabony defects when compared with open flap debridement alone. Probing depth reduction, gaining clinical attachment level and defect filling are the clinical benefits providing by PRF(Sharma & Pradeep, 2011).

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Figures

Figure 1. *Venous blood collection*

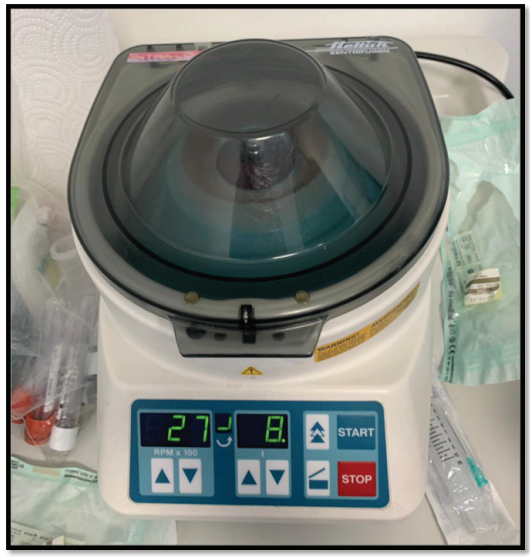
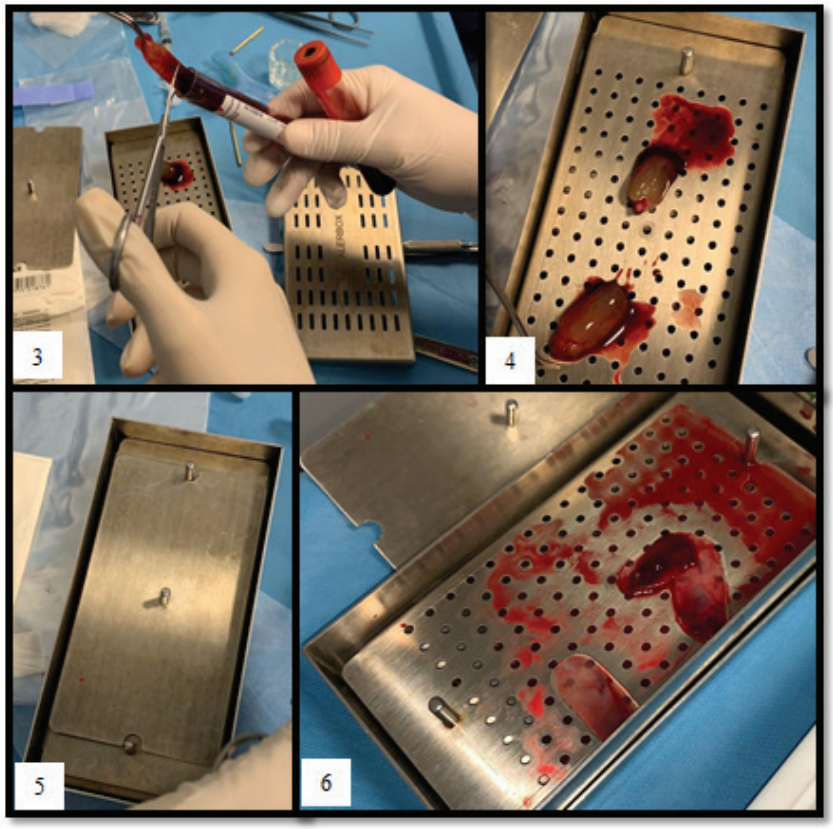


Figure 2. *Centrifuge process*



Figures 3-6. *Separating the fibrin layer and compressing procedure to obtain PRF membrane*

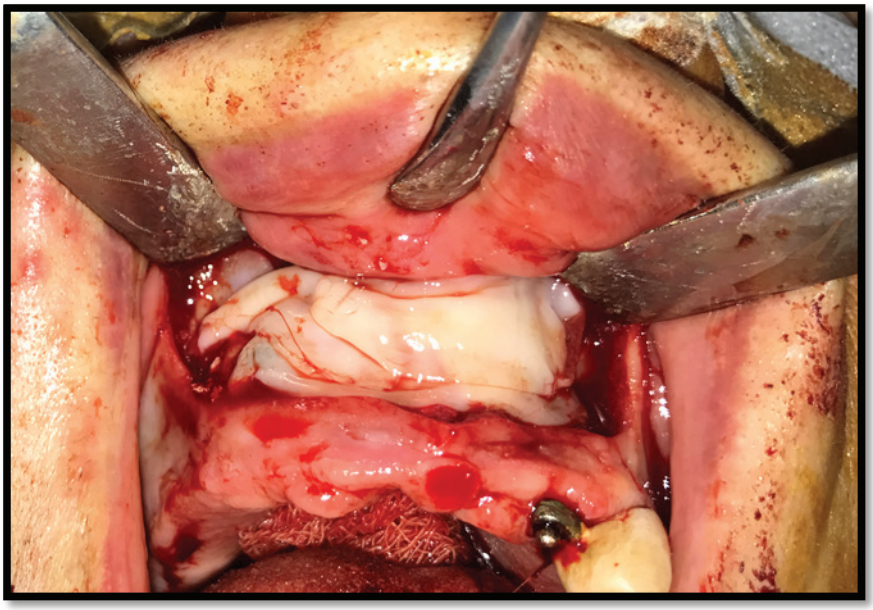


Figure 7. *PRF membrane cover in bone augmentation*