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VOLUME 1

EDITOR: PROF. DR. CEM EVEREKLİOĞLU

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Theory and Research in Health Sciences II

Volume 1

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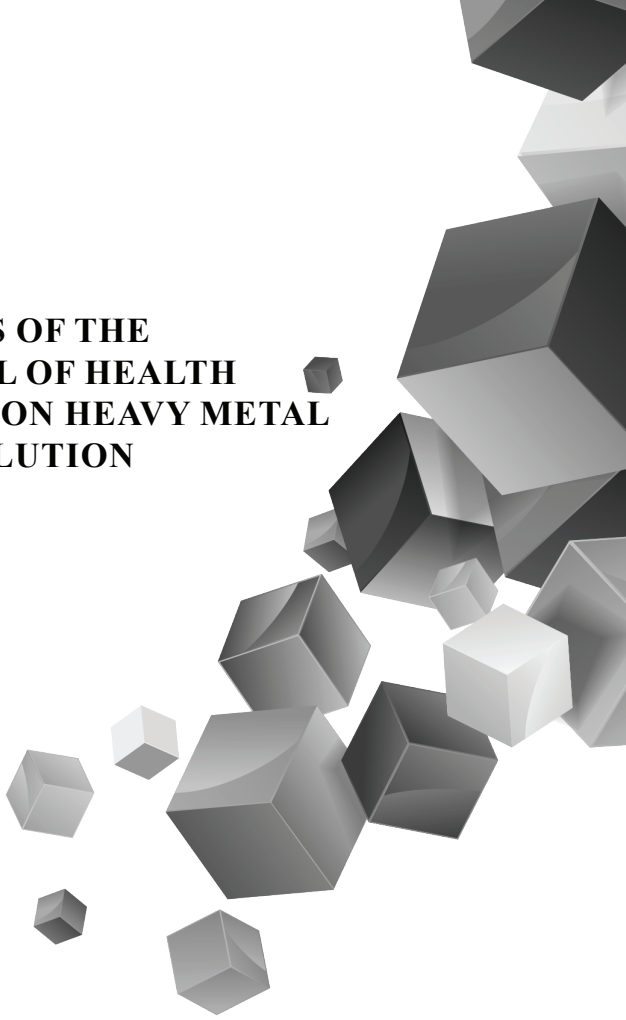
İÇİNDEKİLER

Chapter 1

KNOWLEDGE LEVELS OF THE VOCATIONAL SCHOOL OF HEALTH SERVICES STUDENTS ON HEAVY METAL AND RADIATION POLLUTION



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1. Introduction

Environmental pollution, which is one of the most important problems in today's world, threatens human health and the future of nature. Especially with the developing technology, questions are increasing on heavy metal and radiation pollution, which are known as types of environmental pollution. Humans are constantly exposed to natural radiation, radiated from the sun and space (in the earth's crust, structures, food and drinking water), naturally occurring radioactive materials. There are radioactive gases in the air we breathe. Our body itself is also radioactive. However, apart from natural radiation sources, people are also exposed to rays with their own radiation sources (1).

Although nuclear power plants, known as radiation sources, are one of the most discussed topics in the public, approximately 17% of the electricity production in the world is provided by nuclear power plants. This rate rises above 30% in the European Union and 78.2% in France. The most important drawback that can be attributed to nuclear energy in terms of environmental pollution is the problem of radioactive wastes (2).

Heavy metals are metals found in a certain dose in the human body. However, when their concentrations exceed a certain value, it has a toxic effect. Especially motor vehicles, inorganic fertilizers, factory wastes can cause an increase in the concentration of heavy metals in the air, soil and water and thus to enter the body (3). The most dangerous aspect of heavy metals that enter the soil structure is that they join the plant structure and pass on to other living things through the food chain. In addition, heavy metals that become free ions infiltrate the groundwater and mix into drinking water, and thus degrades the water quality. Apart from this, they affect their activities negatively by damaging soil creatures.

1.1. The Aim and Importance of The Research

Heavy metal pollution and radiation pollution are perhaps the most important pollution types today. Thermal power plants, which are quite high in our country, cause great damage to our environment both with solid heavy metals and gaseous heavy metals. Leaving the residues of the burned coal unconscious to the nature and not going through the necessary pre-processes leaves us confronted with an even more serious situation. At the same time, as a result of the burning of solid fuels, very large levels of gaseous heavy metal particles are released into the environment. Heavy metals taken above a certain dose in living beings result in negativities up to death. Cadmium, mercury, lead and chromium are important heavy metals. The wastes left by the plastic factory, which was established on the coast of Minimata Bay of Japan in 1951, and the mercury inside the food chain caused serious diseases such as partial paralysis and blindness

in many people in the region two years later. Many people were harmed in this event called “Minimata Tragedy”. The emergence of such serious public health and environmental problems in the world; It has led to the recognition of the environmental pollution incident and understanding of the danger dimensions (4).

Radiation pollution caused by nuclear power plants, which is very busy today, is also an important problem. Chernobyl reactor accident, which also affected our country closely, is the first major nuclear accident of the 20th century. It was learned by the whole world on April 30, 1986 that a large amount of fission products were released into the atmosphere after the nuclear accident that occurred in the early hours of April 26, 1986 in the 4th unit of the Nuclear Power Reactor in Chernobyl city of Kiev, Ukraine. Almost all European countries have been affected by this event at different levels, depending on various factors, especially meteorological conditions and distance to the accident site. Turkey is one of the countries affected by the accident (5, 6).

On March 11, 2011, Japan faced the biggest earthquake disaster in its history. After this earthquake measuring 8.9 on the Richter scale, the island nation faced a tsunami disaster this time. Unfortunately, it has been observed that giant waves have reached a height of 10-15 meters, displacing large vehicles, houses, ships, some buildings and dragging them from the shore for kilometers. The Fukushima Dai-ichi Nuclear Power Plant suffered the most from the earthquake and tsunami disaster. The accident was changed to what we know today, as backup power units (diesel generators) could not be used with the tsunami (7).

What is Environmental Pollution? Çepel defines environmental pollution as follows: It is called environmental pollution when foreign substances, which adversely affect the health of all living things, cause material damages on inanimate environmental assets and deteriorate their qualities, with air, water and soil. In other words, “Ecological damages caused by humans and disrupting the natural balance in ecosystems” can also be a definition of environmental pollution (1).

Heavy Metal Pollution:

Heavy metal pollution is a type of pollution that can be seen in all air, soil and water environments. Especially the wastes generated as a result of industrial activities developed with the industrial revolution cause heavy metal pollution. When some heavy metals found in nature are added to a living organism more than their dose, it results in negativities up to death. Cadmium, mercury, lead, zinc, copper and chromium come first among these. Environmental pollution caused by heavy metals can reach the soil with mining activities, fertilizers and pesticides, industrial wastes

and hydrocarbon combustion products that cause water, air and direct soil pollution (8). The causes of heavy metal pollution can be listed as natural resources, mining, industrial wastes, burning of fossil materials, urban wastes and waste water, fertilizers and drugs used in agriculture, detergents. As can be seen, it is the same with the factors that cause other contamination. Significant amounts of toxic elements from fertilizers and drugs used in agriculture are released to the soil (9). They can accumulate in the soil and be transported in the soil-plant-animal-human nutrition chain with increasing concentration. Heavy metal pollution has been seen since ancient times. With industrialization, pollution has reached enormous levels. After long periods of unconscious industrialization, pollution dimensions have increased to incredible levels. Nowadays, studies on the reduction or elimination of these harmful contents have gained momentum. Pollution causes changes in the structure of soil, water and air environments (10). Pollutants that spread to the air can be transported to distant places due to effects such as wind and air flow. In the aquatic environment, while pollutants increase their levels in stagnant environments, they are transported by formations such as rivers. Finally, it appears as the accumulation of heavy metals in the soil, which is a very important issue for humanity. Mainly, while trace elements are formed in the soil material, their amount is constantly increasing with the participation of human activities in the host soils. These contributions can be made with chemical fertilizers, industrial wastes, mine residues and automobile emission gases (11, 12).

Measures that can be taken to prevent heavy metal pollution can be listed as follows.

•Industrial technologies that cause high pollution should be avoided, clean industry technologies should be produced or preferred.

• The use of renewable energy sources should be expanded.

• Environmental protection and treatment processes should be handled and developed as regional projects.

• Measures should be taken for the establishment of treatment facilities, the disposal of solid wastes and also to prevent harmful wastes.

• A balanced distribution of industry between regions should be ensured and should be moved far enough from the city.

• Industrial wastes that cause toxic and permanent effects on ecosystems should be controlled and treated.

• More attention should be paid to the use of industrially sourced heavy metals that cause pollution.

• In order to reduce pollution, wastewater mixed with rivers should be discharged after treatment.

• A balanced distribution of industry between regions should be ensured and should be moved far enough from the city.

• Limit values should be determined for fertilizers, pesticides and wastes applied to agricultural lands that cause heavy metal entry into the soil and applications should be made accordingly.

• In order to prevent heavy metal pollution in irrigation water, industrial originated heavy metal-containing waste water should be used in irrigation after being treated with an appropriate method.

Radioactive Pollution

Radioactive contamination is the disruption of the electron-proton system, which is in equilibrium in the atoms of all beings, of certain substances that are self-emitting electrons and are qualified as radioactive (1). The fact that the number of neutrons in the nucleus of the atom is more than the number of protons makes the atom unstable. An unstable atom is in motion and the neutrons in its nucleus are broken down by emitting alpha, beta and gamma rays.

These substances that are broken down by radiating rays to their environment are called radioactive material and the emitted rays are called radiation (3). All living beings live with radiation, cosmic rays coming from outer space and the sun as a part of life are irradiated from natural sources such as soil and building materials, water and food as well as artificial sources due to radioisotopes in the earth's crust. People; They are exposed to an annual dose of approximately 2.5 mSv (milisievert), although their living standards vary depending on the physical characteristics of their living environment and geographical conditions. This dose; Approximately 87% is made up of natural resources, 12% from medical applications, and the rest from professional irradiation and other artificial sources (13). Natural resources are cosmic rays from the sun, with radioactive materials found in rocks in terrestrial environments and sediments in marine environments. Nuclear energy is gaining in importance day by day. Although nuclear energy is advantageous in terms of producing more energy in a shorter time, as a result of any accident, it may cause damages not only in the region where it was established but also in continental dimensions.

Measures that can be taken to prevent radioactive pollution:

• To establish power plants where renewable energy sources will be used instead of nuclear power plants

• Solving the waste problem in nuclear power plants

• Continuous radiation control

• Healthy and safe storage of radioactive materials

In this study; It is aimed to measure the knowledge levels of the Vocational School of Health Services students radiation pollution to the environment from sources such as heavy metals from traffic, batteries, industrial facilities, mines, and the Chernobyl Nuclear Power Plant accident near Kiev in Ukraine in 1986 and the Fukushima Daiçi nuclear power plant accident in Japan in 2011 and daily X-ray filming.

1.2. Problem and Sub-Problems

1.2.1. Problem Statement

What is the level of knowledge of the students on heavy metal and radiation pollution?

1.2.2. Sub problems

1) Is there a significant relationship between students' gender and their level of knowledge about heavy metals and radiation?

2) Is there a significant relationship between the students' departments and their level of knowledge about heavy metal and radiation?

3) Is there a significant relationship between students' classes and their knowledge level about heavy metals and radiation?

4) Is there a significant relationship between whether students follow environmental problems or not and their knowledge level about heavy metals and radiation?

5) Is there a significant relationship between the education levels of the mothers of the students and their knowledge on heavy metals and radiation?

6) Is there a significant relationship between the education level of the fathers of the students and their knowledge on heavy metal and radiation?

1.3. Limitations

This research;

a) With the fall semester of the 2019-2020 academic year,

b) With 277 students studying at Karadeniz Technical University, Vocational School of Health Services

c) The data obtained from the research is limited to the scope of the questionnaire and test.

2. Material and Method

2.1. Research Model

In the research, scanning model, one of the descriptive research methods, was used. Screening research is based on the opinions of the participants about a subject or event or their interests, skills, abilities, attitudes, etc. It is the type of research whose characteristics are determined on larger samples compared to other studies (14).

The reason for choosing this method is to determine the knowledge level of Vocational School Health Services students on radiation pollution. It is also planned to consider these levels of knowledge in terms of various variables.

2.2. Universe and Sample

The universe of the study consists of 1st and 2nd grade students studying at Karadeniz Technical University, Vocational School of Health Services, Department of Medical Services and Techniques in the 2019-2020 academic year. Distribution of students according to programs and classes is given in Table 1.

Table 1. *Distribution of students according to programs and classes*

Classes	Medical Imaging and Techniques (TGOR)	Medical Laboratory and techniques (TLAB)	Total
1	73	68	141
2	69	67	136
Total	142	135	277

The simple random sampling method, one of the random sampling methods, was used in the selection of the sample. According to the simple random sampling method, all units in the universe have an equal and independent chance to be selected for the sample (14).

The sample of the research; It consists of 142 Medical Imaging and Techniques program students, 135 Medical Laboratory Techniques program students studying at Karadeniz Technical University Vocational School of Health Services. Of the total 277 students included in the sample, 196 are female (70.76%) and 81 are male students (29.24%).

2.3. Data Collection Tools and Techniques

The data of the research were obtained from the Heavy Metal and Radiation Pollution Success Test. Personal Information Questionnaire was used for the demographic information collected from the sample. In

the questionnaire, the variables used in the study, which are department, class, gender, point of view on environmental problems, whether they are a member of an environmental organization, and the education level of the parents were asked. The collected data were evaluated and entered into the SPSS package program to analyze the data. In the study, the reliability (internal consistency) of the scale, which was created to measure the knowledge levels of the students, was examined by item analysis, and both item analysis (t-test) based on the difference between sub-upper group averages and item analysis based on correlation were performed. In order to determine the discrimination power of the items in the scale, the t-value of the difference between the upper and lower group achievement scores for each item was calculated. For this, achievement total scores are ranked from high to low. As a result of the analysis, the Cronbach Alpha Reliability Coefficient (α) of the final scale was determined as **0.72**. Data analysis was performed using the SPSS (Statistical Package for the Social Sciences) 16.0 program, after the data was encoded and transferred to the computer environment. In the research, among the descriptive statistical methods frequency, percentage, mean and standard deviation analysis, t-test was used to determine the differences between independent variables. First of all, the distributions of the personal characteristics of the students participating in the research were given.

3. Research findings

In this section, statistical analysis of the collected data and interpretation of the obtained results are included. The answers given by the students to the Heavy Metal and Radiation Pollution Success Test were analyzed.

Sub problem 1: Is there a significant relationship between the gender of the students and their level of knowledge on heavy metals and radiation?

The results of the analysis made to determine whether there is a significant relationship between the gender of the students and their knowledge on heavy metal and radiation are given in Table 3.1.

Table 3.1. *t-Test Results for Heavy Metal and Radiation Academic Achievement Scores of Students*

According to Gender

Cender	**N**	**X**	**S**	**Sd**	**t**	**p**
female	196	11.27	3.58	468	4.741	.000
male	81	12.93	3.86			

When Table 3.1 is examined, it is seen that the average scores of the heavy metal and radiation academic achievement test scores of female

students (x) are 11.27, while male students are (x) 12.93. It was seen that there was a significant difference in favor of male students between the heavy metal and radiation academic achievement test scores of the students according to their gender [t (1-468) = 4.741, p <.05].

Sub problem 2: Is there a significant relationship between the students' programs and their level of knowledge about heavy metal and radiation?

The results of the analysis conducted to determine whether there is a significant relationship between the students' programs and their knowledge on heavy metal and radiation are given in Table 3.2.

Table 3.2. *t-Test Results of the Heavy Metal and Radiation Academic Achievement Test Scores of the Students According to the Programs*

Programs	N	X	S	Sd	t	p
TGOR	142	12.89	3.98	412	3.182	0.07
TLAB	135	11.26	3.05			

When Table 3.2 is examined, the average of Achievement Test scores is 11.26 (x) for Medical Laboratory Techniques program and Medical Imaging Techniques Program (TGOR) students (x) is seen to be 12.89. According to the programs of the students, there was a significant difference between the Heavy Metal and Radiation Academic Achievement Test scores in favor of the Medical Imaging Techniques Program students [t (1-412) = 3.182, p <.05].

Sub problem 3: Is there a significant relationship between the students' classes and their knowledge level about heavy metal and radiation?

The results of the analysis conducted to determine whether there is a significant relationship between the students' classes and their knowledge on heavy metals and radiation are given in Table 3.3.

Table 3.3. *Average and Standard deviations of Heavy Metal and Radiation Academic Achievement*

Test Scores of Medical Imaging Techniques Program Students by Classes

Classes	N	X	S
1	73	10.63	3.12
2	69	13.41	2.96
Total	142	12.26	3.04

1. 1st class, 2. Second class

When Table 3.3 is examined, the average scores of the 1st grade students of the Medical Imaging Techniques Program is (x) 10.63, and the average of the scores of the 2nd grade students is (x) 13.41. When the table is examined, it is seen that there is an increase in the scores of the 2nd grade students. It is seen that there is a significant difference in favor

of the 2nd graders in terms of the distribution of heavy metal and radiation academic knowledge test scores according to classes.

Table 3.4. *ANOVA Results of Heavy Metal and Radiation Academic Achievement Test Scores of Medical Imaging Techniques Program Students by Classes*

Source of Variance	Sum of Sguares	sd	Average of Sguares	F	P	Significant Difference
Intergroup	695.583	5	378.145	18.564	.000	1-2
In-group	1241.451	157	12.658			1-2
Total		162				

1. 1st class, 2. Second class

When Table 3.4 is examined, it is seen that there is a significant difference in favor of the 2nd graders in terms of the distribution of heavy metal and radiation academic knowledge test scores of the Medical Imaging Techniques Program students [F (5-162) = 18,564, p <.05]. Distribution of success of Medical Laboratory Techniques Program students according to their classes is shown in Tables 3.5 and 3.6.

Table 3.5. *Average and Standard Deviation of Heavy Metal and Radiation Academic Achievement Test Scores of Medical Laboratory Techniques Program Students by Classes*

Classes	N	X	S
1	68	11.42	3.25
2	67	11.73	3.48
Total	135	11.26	3.67

When Table 3.5 is examined, the average score of Medical Laboratory Techniques Program 1st grade students is (x) 11.42, and the average score of 2nd grade students is (x) 11.73.

Table 3.6. *ANOVA Results of Medical Laboratory Program Students' Heavy Metal and Radiation Academic Achievement Test Scores by Program and Classes*

Source of Variance	Sum of Sguares	sd	Average of Sguares	F	P	Significant Difference
Intergroup	0.867	5	0.365	0.032	.840	-
In-group	1927.160	184	7348			
Total	1928.027	189				

When Table 3.6 is examined, no significant difference was found in terms of distribution of heavy metal and radiation academic achievement test scores of medical laboratory program students according to classes [F (5-189) = 0.032, p> .05].

Sub-problem 4: is there a significant relationship between whether students follow environmental problems or not and their level of knowledge about heavy metals and radiation?

The analysis results are given in Table 3.7 and Table 3.8.

Table 3.7. *Average and Standard deviations of Students' Heavy Metal and Radiation Academic Achievement Test Scores by Following Environmental Problems*

	N	X	S
follows	45	14.16	2.75
partially	219	12.07	3.42
does not follow	13	10.61	3.19
Total	277	12.93	3.18

When Table 3.7 is examined, the average of the test scores of the students following environmental problems is (x) 14.16, the average of the test scores of the students who follow partially (x) is 12.07, the average of the test scores of the students who do not follow (x) is 10.61. When the table is examined, it is seen that there is an increase in the scores of students who follow environmental problems.

Table 3.8. *ANOVA Results of the Heavy Metal and Radiation Academic Achievement Test Scores of the Students According to Their Environmental Problems Tracking Status*

Source of Variance	Sum of Sguares	sd	Average of Sguares	F	P	Significant Difference
Intergroup	146.521	4	139.002	17.246	.012	1-3, 1-2
In-group	6020.382	360	18.541			
Total	6166.903	364				

1.follows, 2.partially, 3.does not follow

When Table 3.8 is examined, it is seen that there is a significant difference in favor of the followers in terms of whether the students follow environmental problems or not, [F (4-364) = 17.246, p <.05]. It has been determined that this difference is between those who follow environmental problems and those who partially follow, those who do not follow and those who follow.

Sub-problem 5: Is there a significant relationship between the education levels of the mothers of the students and their knowledge about heavy metals and radiation?

The results of the analysis made to determine whether there is a significant relationship between the education status of the students' mothers and their knowledge about heavy metal and radiation are given in Table 3.9 and Table 3.10.

Table 3.9. *Average and Standard Deviation of the Heavy Metal and Radiation Academic Achievement Test Scores of the Students According to their Mothers' Educational Status*

Mother Education Status	N	X	S
Primary school graduate	109	10.43	3.24
Secondary school graduate	78	11.70	3.50
High school graduate	59	11.54	3.67
College graduate	31	12.93	2.89
Total	277	12.85	3.27

When Table 3.9 is examined, the average test scores of students whose mothers are primary school graduates (x) is 10.43, and the test scores of students whose mothers are secondary school graduates the average (x) is 11.70, the average of the test scores of the students whose mothers are high school graduates (x) is 11.54, the average of the test scores of the students whose mothers are college graduates is (x) 12.93.

Table 3.10. *ANOVA Results of the Heavy Metal and Radiation Academic Achievement Test Scores of the Students According to the Education Status of the Mothers*

Source of Variance	Sum of Sguares	sd	Average of Sguares	F	P	Significant Difference
Intergroup	65.840	2	26.247	7.126	.074	-
In-group	5286.002	386	12.840			
Total	5351.842	388				

1. Primary school graduate, 2.Secondary school graduate, 3.High school graduate, 4.College graduate

When Table 3.10 is examined, no significant difference was found between the education status of the mothers and their test scores [F (2-388) = 7.126, p> .05].

Sub problem 6: Is there a significant relationship between the education levels of the fathers of the students and their knowledge on heavy metals and radiation?

The results of the analysis performed to determine whether there is a significant relationship between the education status of the fathers of the students and their knowledge on heavy metal and radiation are given in Table 3.11 and Table 3.12.

Table 3.11. *Average and Standard Deviation of the Heavy Metal and Radiation Academic Achievement Scores of the Students According to Their Fathers' Education Status*

Father Education Status	N	X	S
Primary school graduate	101	10.58	3.12
Secondary school graduate	74	11.34	3.53
High school graduate	64	12.57	2.80
College graduate	38	11.70	3.07
Total	277	11.35	3.69

When Table 3.11 is examined, the average test scores of students whose fathers are primary school graduates (x) is 10.58, and the test scores of students whose fathers are secondary school graduates the average (x) is 11.34, the average of the test scores of the students whose fathers are high school graduates (x) 12.57, the average of the test scores of the students whose fathers are college graduates is (x) 11.70.

Table 3.12. *ANOVA Results of the Heavy Metal and Radiation Academic Achievement Test Scores of the Students According to the Education Status of the Fathers*

Source of Variance	Sum of Sguares	sd	Average of Sguares	F	P	Significant Difference
Intergroup	71.248	2	23.468	3.250	.286	-
In-group	5437.005	386	12.327			
Total	5508.253	388				

1.Primary school graduate, 2.Secondary school graduate, 3.High school graduate, 4.College graduate

When Table 3.12 is examined, no significant difference was found between the education status of the students' fathers and their test scores [F (2-388) = 3.250, p> .05].

4. Discussion and Conclusion

4.1. Discussion

The achievement scores of the students in the Heavy Metal and Radiation Academic Achievement Test were found to be medium. In a

study conducted at primary education level, Uluçınar et al. found that students' participation in environmental-related activities was quite low (15). In another study conducted at high school level, Kose (17) concluded that students do not know the environmental problems sufficiently.

It was observed that there was a significant difference in favor of male students between the heavy metal and radiation academic achievement test scores of the students according to their gender. This result is in parallel with the research result of Alp et al. and Aydın & Çepni (10, 17). While some studies conducted at primary and high school levels (15, 16, 18) did not find any significant difference regarding gender, Erol & Gezer (19), Çabuk & Karacaoğlu and Kaya et al. (6, 20) found a significant difference in favor of girls. Considering the studies in the literature, it is possible to say that gender is not an important variable.

The success scores of the Medical Imaging Techniques Program students are higher than the other program. According to the programs of the students, it was observed that there was a significant difference between the Heavy Metal and Radiation Academic Achievement Test scores in favor of the Medical Imaging Techniques Program students. The reason for this can be said that this program is more intertwined with radiation-related issues and students' interests are more focused on radiation issues during their education.

When the success scores according to the classes are examined, the average achievement scores of the Medical Imaging Techniques Program students are (x) 10.63 for the 1st grade students and 13.41 for the 2nd grade students. The higher the grade level, the higher the success average. The reason for this is the increase in practice lessons in the second year. The success score in the medical laboratory and techniques program was found to be lower than the medical imaging and techniques program. The reason for this may be due to the difference in the application course contents of these programs. The average of Heavy Metal and Radiation Academic Achievement Test of Medical Laboratories and Techniques program students is (x) 11.42 for first year students, (x) 11.73 for second year students,

Aydın & Çepni and Tortop et al. (17, 21) did not find a significant difference between the education level of the mother and success in her study, but found a significant difference between the educational status of the father and success. In their study, they found that as the father's education level increased, the knowledge level of the students also increased. In our study, it can be shown that the distribution in the sample is insufficient as the reason for the lack of a significant difference between the education status of the parents and test scores. The parents of most of the students participating in the study are primary school graduates.

4.2. Results

1. The success scores of the students in the Heavy Metal and Radiation Academic Achievement Test were found to be medium.

2. It was observed that there is a significant difference in favor of male students between the Heavy Metal and Radiation Academic Achievement Test scores according to the gender of the students.

3. According to the programs, it was seen that there was a significant difference between the Heavy Metal and Radiation Academic Achievement Test scores in favor of the Medical Imaging Techniques Program students.

4. There is a significant difference in favor of the 2nd grade students in terms of the distribution of Metal and Radiation Academic Achievement Test scores of the students of the Medical Imaging Techniques Program by classes.

5. It is seen that there is a significant difference in favor of the followers in terms of whether the students follow environmental problems or not. The average of test success scores of students who follow environmental problems was found to be higher.

6. There was no significant difference between the education status and success scores of the students' mothers and fathers.

4.3. Suggestions

First of all, the cultural structure of the society and the environmental attitudes of the individuals should be determined and efforts should be made to prevent environmental problems before they occur. It should be aimed to raise individuals who think, discuss, question, approach environmental problems sensitively, react to environmental problems, express their ideas and participate in the solution of these problems in universities. Trips should be organized to surrounding areas such as national parks, nature protection areas, natural monuments, nature parks, special environmental protection zones, botanical gardens, zoos, animal shelters, biogenetic reserve areas.Also, Turkey Atomic Energy Agency (TAEK) should be organized annual trips.

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Chapter 2

MOLECULAR DOCKING OF SMALL MOLECULES

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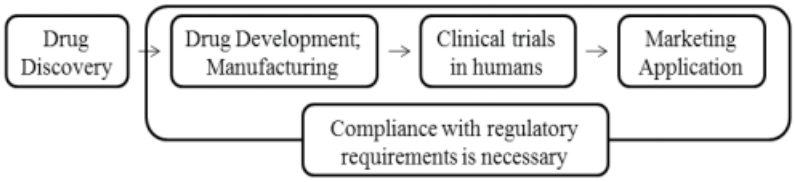
1. Computational Studies

1.1. Approval of the drug

Europe and USA are the two major regulatory agencies except Japan in the world. EU is a union of countries but USA isn't. This is why their regulatory agencies have been encapsulated for easy understanding (Kashyap *et al*, 2013).



Scheme 1. The basic regulation (Kashyap *et al*, 2013).

In USA, Food and Drug Administration (FDA) is responsible for confirming the safety and effectiveness of the drug. Within this responsibility, FDA monitors and controls approximately 1 trillion worth of drugs each year. FDA assesses the effectiveness, safety, good applicability, and whether it can be sold with or without a prescription, before approving the drugs that will land in the market. Over the past 10 years, FDA has approved more than 500 novel prescription drugs (Lipski & Shark, 2001). (Since the processes in both regulation offices are similar, only one of these processes was be explained.)

Table 1. Terms and Definitions of the Novel Drug Development Process.

Term	**Definition**
Phase 1	Determination of pharmacological activity and safe dose range
Phase 2	Evaluation of the activity with the volunteer control group
Phase 3	Determination of adverse effects by working with a high number of volunteers
Phase 4	Following the FDA approval, following the general use of the drug and identifying possible problems

Fast-track drug	Fast approval of the drug by the FDA to make it available to the patient in the treatment of serious and previously unavoidable vital diseases
Labeling	Writing all information about the drug on the package insert
Misbranding	Re-regulation of the drug in the FDA as a result of any misspellings or errors

1.2 Drug Development

The development of the drug is generally progressing in stages. In the first step, there is a preclinical phase usually completed in 3-4 years. If this stage is successful, the FDA's investigational new drug (IND) application is followed. After the IND approval is obtained, the processes specified in Table 1 are followed in order. Consequently, considering all these preclinical studies and approval periods, it takes 8 to 12 years on average for a single drug to reach the markets (Lipski & Shark, 2001).

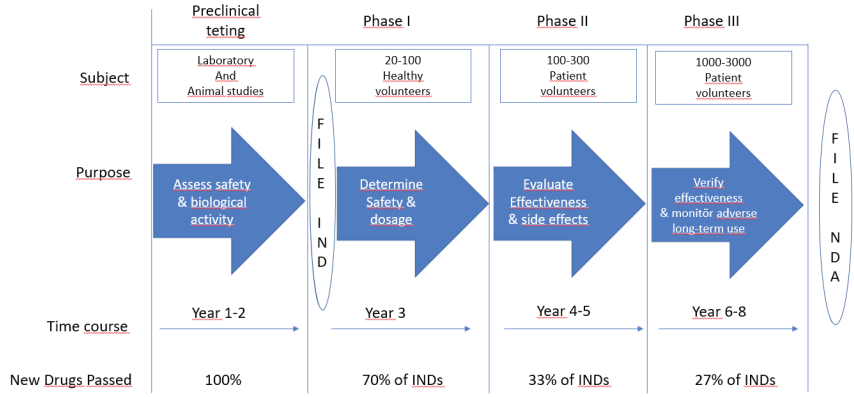


Figure 1. Development stages and process of a drug (Lipsky *et al*, 2001).

1.3. Design a New Drug Molecule

The first step to develop a new drug is to create a new drug molecule in the preclinical study. In order to create this molecule, it is necessary to understand the disease in the best way and benefit from the advantages of biology, chemistry and computer science.

The methods used can be listed as follows:

- Literature

- Academic environment
- High Troughput screening
- Research information
- Computer aided drug design (Sibley, 2017).

For over a quarter-century, Computer-aided drug discovery, and development have taken a great role in the improvement of novel drug molecules. These methods generally categorized as Ligand-based and structure-based procedures (for more information at part 1.5).

1.4. Molecular Docking

Research in the pharmaceutical industry is growing and progressing with modern medicinal chemistry. Medicinal chemistry; It also includes molecular modeling that reveals the structure-activity relationship (SAR) (Hughes *et al*., 2011). Furthermore to the SAR study, pharmacodynamic data, pharmacokinetic data (ADMET: Absorption, distribution, metabolism, elemination and toxicity) are also studied with applications included in molecular modeling (Lipinski *et al*., 2012). In this application, the contributions of advanced biomolecular spectroscopic methods such as X-ray crystallography and nuclear magnetic resonance (NMR) are great. With these techniques, more than 100,000 3D macromolecule structures have been exposed and thus, information has been obtained with the binding regions of drug molecules in macromolecules (Berman, 2000). Structure-based drug design (SBDD) modeling studies, which form a very important part of Medicinal Chemistry, are used as a biological target (3D structures) obtained by these spectroscopic methods (Salum *et al*., 2008). While defining molecular analysis (binding energies, intermolecular interactions, stimulated conformational changes, etc.), the SBDD strategy is highly related to structure-based virtual scanning (Structue-based virtual screening; SBVS) and molecular dynamic (MD) (Kalyaanamoorthy and Chen, 2011). The approach in drug design is to compare the specific target regions in the receptor in the ligand library using known ligands. This information is used in ligand-based drug design (LBDD) (Shim and Mackerell, 2011). Ligand-based virtual screening (LBVS), similarity research, quantitative-structure activity relationship (QSAR), and pharmacophore determination are the most common studies in the LBDD method (Bacilieri and Moro, 2006). SBDD and LBDD are techniques used in drug development in both academia and industry. In light of all these approaches, successful studies are conducted in structural, chemical and biological data research (Drwal & Griffith, 2013; Trossini *et al*., 2013; Valasani *et al*., 2014).

1.5. Structure-Based Drug Design (SBDD)

Identifying small-molecule ligands and understanding their interactions with proteins is of significant importance in drug research and development. SBDD refers to the systematic use of structural data (eg protein target- i.e. receptor) obtained by experimental or direct computational homologous modeling in general. The aim is to figure out the high-receptor binding affinities by designing ligands accordingly specific electrostatic and stereochemical behavior. The presence of three-dimensional macromolecular structures allows for rigorous examination of the binding site topology, including the presence of clefts, cavities, and lower pockets. Electrostatic properties such as charge distribution also need to be carefully defined. At present day, SBDD technique permit the create of ligands containing the properties necessary to effectively modulate the target receptor (Blaney, 2012; Mandal *et al.*, 2009).

SBDD is a cyclical process consisting of gradual acquisition of knowledge (Figure 2) (Ferreira *et al*., 2015). Starting from a known target structure, theoretical studies are carried out in a computer environment to identify potential ligands. Following molecular modeling studies, the process of determining the most promising molecules follows the synthesis of these molecules (Wilson & Lill, 2011). The next step is; the evaluation of biological features such as affinity and effectiveness is the transfer of all these results to various experimental platforms (Fang, 2012). If active compounds are identified, the 3D ligand-receptor complex can be resolved. The present structure permit the observation of several intra-molecular properties that support the molecular recognition processIt is useful for structural definitions of ligand-receptor complexes, an examination of binding conformations, characterization of basic intermolecular interactions, characterization of unknown binding sites, mechanical studies and elucidation of ligand-induced conformational changes (Kahsai *et al.*, 2011). When a ligand-receptor complex is identified, biological activity data is correlated with structural information (Shoichet & Kobilka, 2012). In this way, the SBDD process begins with new steps to include molecular modifications that have the potential to increase the affinity of new ligands for the binding site. The flexibility of the target receptor, keeping in mind that significant conformational changes may occur in ligand binding is an important feature to be considered in the modeling phase. Although it is not necessary in many cases, in some special cases, techniques such as flexible placement and MD are useful in solving the problem of flexibility (Chandrika *et al*., 2009).

Molecular docking is one of the most commonly used methods in SBDD. Because the active conformation of small molecule ligands within the appropriate target binding site is anticipated with a very high

rate (Figure 2). Molecular modeling became an important tool in drug development in the 1980s after the development of the first algorithm. For example, research involving important molecular events, including ligand binding modes and intermolecular interactions that stabilize the ligand-receptor complex, has become easily feasible. In addition, molecular clamping algorithms predict the quantitative values of binding energies (Ferreira et al., 2015).

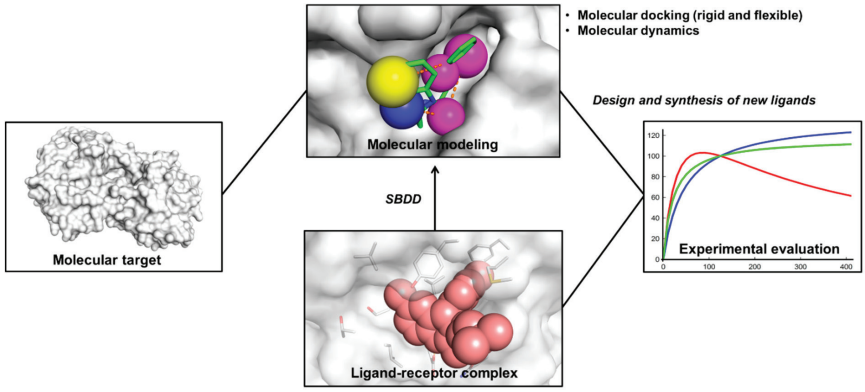


Figure 2. Data obtained from SBDD (Ferreira et al., 2015).

Identifying presumably binding conformations requires two steps:

- Investigation of a large conformational area representing various potential attachment modes

- Accurate estimation of the interaction energy related to every of the expected binding conformations (Kapetanovic, 2008).

Molecular clamping programs work by evaluating ligand conformations with specific function scores and this process is repeated until the ligands reach the lowest energy (Kapetanovic, 2008; Yuriev *et al.,* 2011).

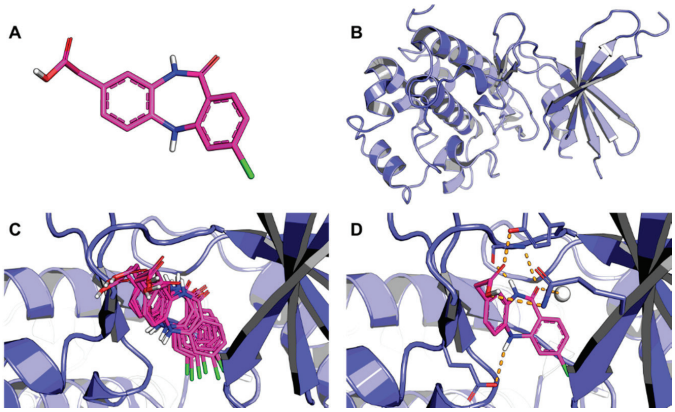


Figure 3. The result of the molecular docking method. A) 3D small molecule structure B) 3D macromolecule structure C) Ligand is placed in the binding well of the receptor and assumed conformations are investigated. D) the most probable binding confirmation and related intermolecular interactions as well are defined. The protein backbone is represented as a cartoon. Ligand and active site residues are indicated by stick depiction. The white sphere expresses water, and the dash lines represent hydrogen bonds. (Ferreira et al., 2015).

1.6. Structure of Protein

The protein that is targeted to be used in molecular docking is most commonly obtained from the Protein Data Bank (PDB) site. Where there is no experimental data, homology models (Sutclffe *et al*., 1987; Sander and Schneider, 1991) or pseudoreceptor models (Vedani *et al.*, 1995) are used as alternatives. However, it should be remembered that the quality of the protein structure is critical to the success of chelating experiments. Even a small change in structure can seriously affect the result of the computational docking experiment (Muegge & Rarey, 2001). Therefore, it is desirable that the solubility of the protein structure is 2.0 Å. However, this value can show flexibility up to 2.5 Å (Jones *et al.*, 1997).

2. Virtual screening (VS)

Virtual screening is one of the in silico methods that are rapidly increasing use, used to select promising compounds (Lyne, 2020; Ferreira *et al*, 2015). there are numerous tools to achieve those computational studies and generally, they can be classified as either receptor-based or ligand-based. the strategy of the ligand-based methods is to use knowledge provided by a compound that is known to bind to the target and these are used to determine other ligands that are tried to develop. This method might be done using many various methods such as 3D shape matching (Srinivasan *et al*, 2002), Pharmacophore matching (Mason *et al*. 2001), or similarity searching (Mestres and Knegtel, 2000). Receptor-based

computational based can be performed when the structure of the target is known. In this method, each ligand is explicitly docked into the binding site of the receptor, together with its docking score of the excellent of the fit of it in the target active site (Halperin *et al,* 2002).

A Typical virtual screening workflow exists of preparation phase of both the target and the data set and then following by molecular docking phase and concludes within ligand selection (Lyne, 2002). However, the docking programs achieve well in binding modes with the active site of the receptor and also pharmacologically active conformations of the small molecules. But current scoring functions are not successful to sort active and inactive molecules (Ece and Sevin 2013; Mascarenhasa and Ghoshal 2008).

A graphical definition of the workflow of virtual screening study against any selected receptor (the target) was given in figure 4 (Lyne, 2002).

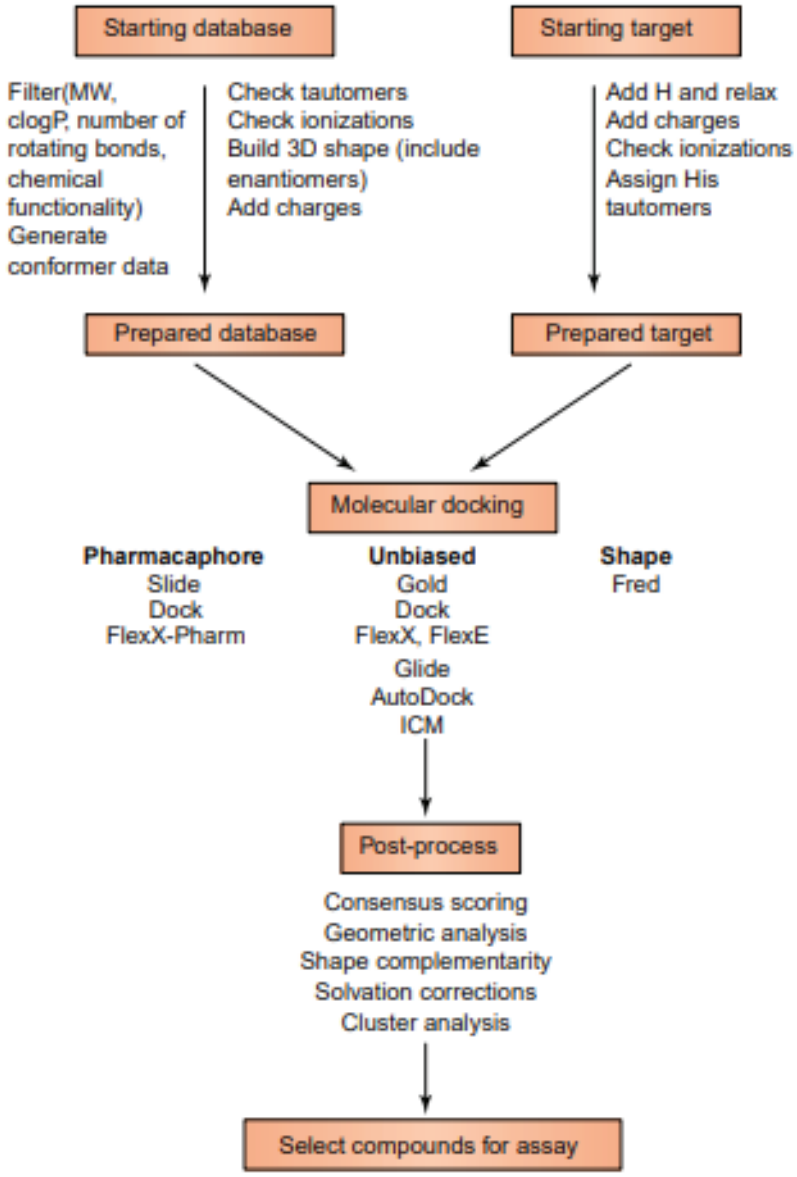


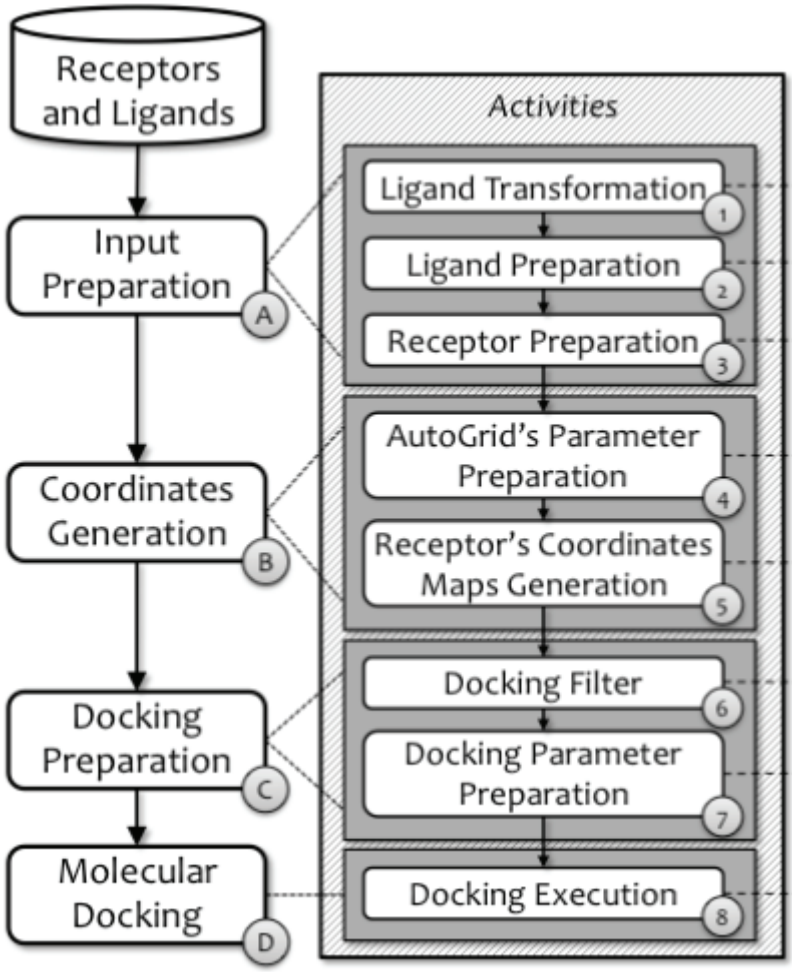
Figure 4. A workflow of virtual screening. This is not includes all list of molecular docking tools. But it gives some examples of common docking tools (Lyne, 2002).

3. Basic Molecular Docking Process and Scoring

In the light of the information given under the previous titles, how a simple modeling study should be done is as follows.

- Input preparation (Ligand transformation, Ligand preparation, Receptor preparation)
- Coordinates Generation (AutoGrid's parameter preparation or Receptors coordinates maps generation)
- Docking preparation (Docking filter, Docking parameter preparation)
- Molecular docking (Docking execution) (Ocana *et al*, 2014)

First, the target protein is retrieved from the Protein Data Bank, and before using it for calculating, missing amino acids were identified and added to the protein and then the macromolecule is both optimized and minimized, respectively (De vita *et al*., 2019). Afterward, both the native ligand and ligands (data set of small molecules) are prepared using the relevant module in the software. Before the docking process of the data set, The Grid cube is identified using native ligand as a center of the active site and RMSD (Root Mean Square Deviation) value calculated using these parameters and native ligand. RMSD is the measure of the similarity between two structures and must be less than 2 Å (kufareva and Abagyan, 2011). In the last step, the data set of ligands process is performed on the correctly prepared the receptor and its determined active site and possible chemical interactions of the molecules with the active site of the target protein are obtained. As a result of this study, ligand-receptor interactions are obtained in 2D and 3D-dimensions with virtual screening with together their the best docking score (Morris and Lim-Wilby, 2008; Neelarapu *et al,* 2011).



Scheme 2. A Basic molecular docking workflow

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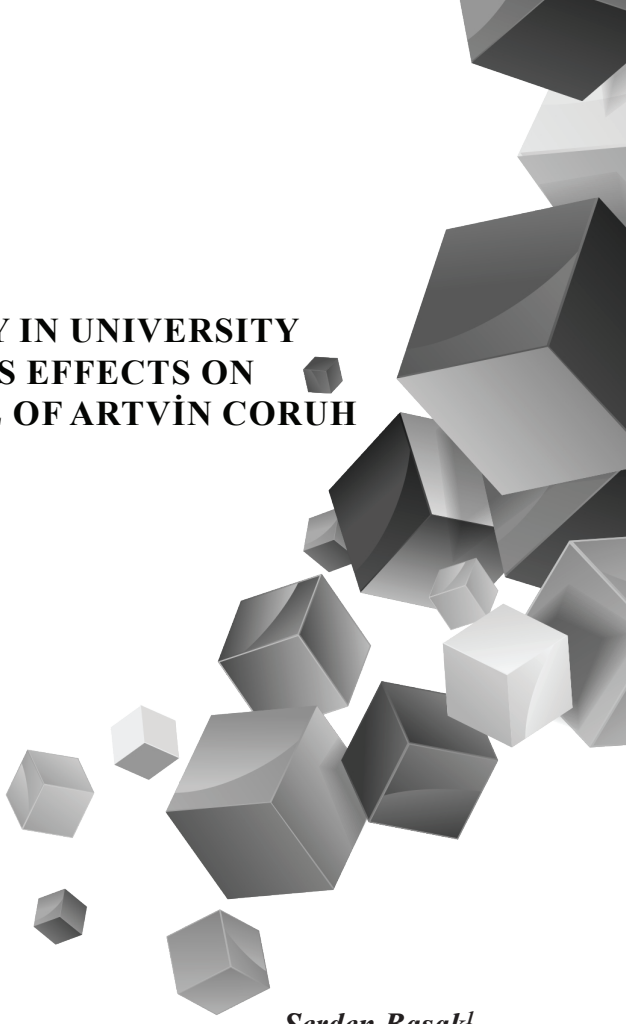
Chapter 3

INDOOR AIR QUALITY IN UNIVERSITY CLASSROOMS AND ITS EFFECTS ON STUDENTS: THE CASE OF ARTVİN CORUH UNIVERSITY



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INTRODUCTION

Pollutants that disrupt indoor air quality are sourced from indoor and outdoor environments. Human is the leading source of indoor pollution. Besides, indoor carpets, furniture, materials used for cleaning, cigarette smoke, stove smoke, and tools and devices used for various purposes are other internal pollutants. Also, building materials can be critical to internal pollutants and affect indoor air quality. Outdoor pollutants can be atmospheric dust, pollen, car exhausts, and industrially sourced pollutants. Pollutants in the outdoor air negatively affect indoor air quality with the outdoor air given in or outside air leaking in (Bulut, 2011, Basak et al., 2017).

People spend a significant part of their time in buildings, indoors. On the other hand, it cannot be said that the indoor air quality in buildings is generally high. Acceptable indoor air quality can be defined as delivering sufficient and sufficient clean air to the indoor environment. One of the critical points here is that the ventilation requirement can be calculated more precisely, such as the building's heating and cooling needs. In this context, indoor air quality needs to be defined to increase health, comfort, and operational performance (URL-1, 2020). Adhering to all these definitions, the occurrence of diseases in buildings can be examined in 2 main groups:

a) Building-Related Illness (BRI) are factors related to the building's interior environment. However, these are not factors that can be solved by ventilation and can be solved by removing the source.

b) Sick building syndrome (SBS): They differ from the building's diseases because the disturbances they cause are not easily noticed and cannot be easily eliminated. When the building is abandoned, disturbances may pass. It is not possible to diagnose these disorders (Çilingiroğlu, 2010).

Air conditioning and ventilation systems are also among the factors affecting indoor air quality. Air conditioning and ventilation play an essential role in providing optimum indoor air in buildings. Air movements inside the building gain importance in terms of thermal comfort and air quality. In Figure 1, the main factors affecting indoor air quality are shown (Parmaksız, 2017).

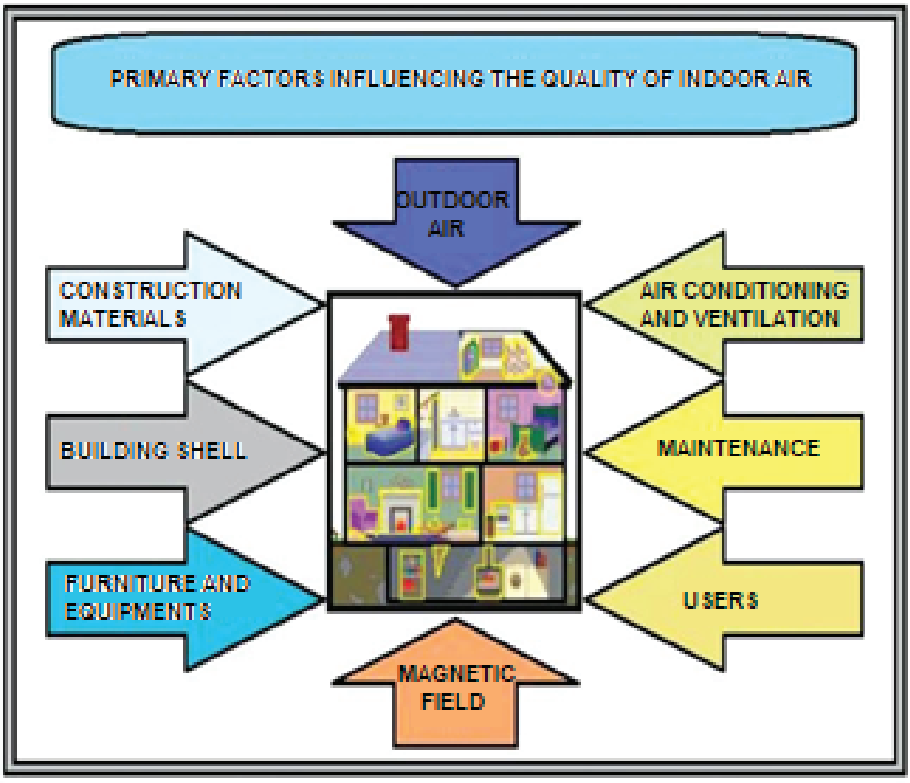

Figure 1. *Leading factors affecting indoor air quality (Kayhan 2005; Babaroğlu 2015)*

Among all parameters, the easiest and fastest parameter to measure is CO_2. CO_2 ratio is increasing in places where people are crowded and/or not ventilated. As a result of this, the disease called RLS and symptoms related to this disease can be seen in those who work with other pollutants or in those places. (Demirarslan and Başak, 2018). This study includes the responses of people working in a public building to the Indoor Air Quality questionnaire and the evaluation of these answers. (Parmaksız, 2017, Demirarslan et al., 2019).

MATERIALS AND METHODS

The "Indoor Air Quality Questionnaire" obtained from the Ethics Committee of Artvin Coruh University in 2017 was applied to the participants (Appendix 1). The questionnaire, which was applied in two different classes, was carried out before the pandemic by obtaining the verbal consent of 28 students from ACU Borcka Acarlar Vocational School and 6 students from ACU Engineering Faculty.

Classes volume was measured with a laser meter. On the day of the survey study, the classrooms' doors and windows were kept closed, and CO_2 measurement was also carried out against time. Measurements were made with PCE-AC 3000 brand CO_2 device. This device is used to control the carbon dioxide concentration in indoor spaces where people are concentrated. The device has an adjustable alarm that gives the acoustic and optical warning, a maximum and minimum value function, and a data logger that can be operated 24 hours. The measuring range is 0 to 3000 ppm CO_2, accurate to ± 5% or ± 50 ppm (URL-2, 2020). The survey results were evaluated with the SPSS 17 program**.**

RESULTS

Class sizes with 6 students and 28 students measured by laser meters are given in Table 1.

Table 1. *Physical dimensions of the classes*

Measurements	Class of 6 students	Class of 28 students	**Unit**
L	11.71	9,898	m
W	6.97	7,502	m
H	3.31	2,793	m
Area	81.65	74,25	m^2
Volume	270.57	207,39	m^3

The CO_2, temperature, and humidity measurements for 6 students (classroom 1) and 28 students (classroom 2) are given in Figure 2.

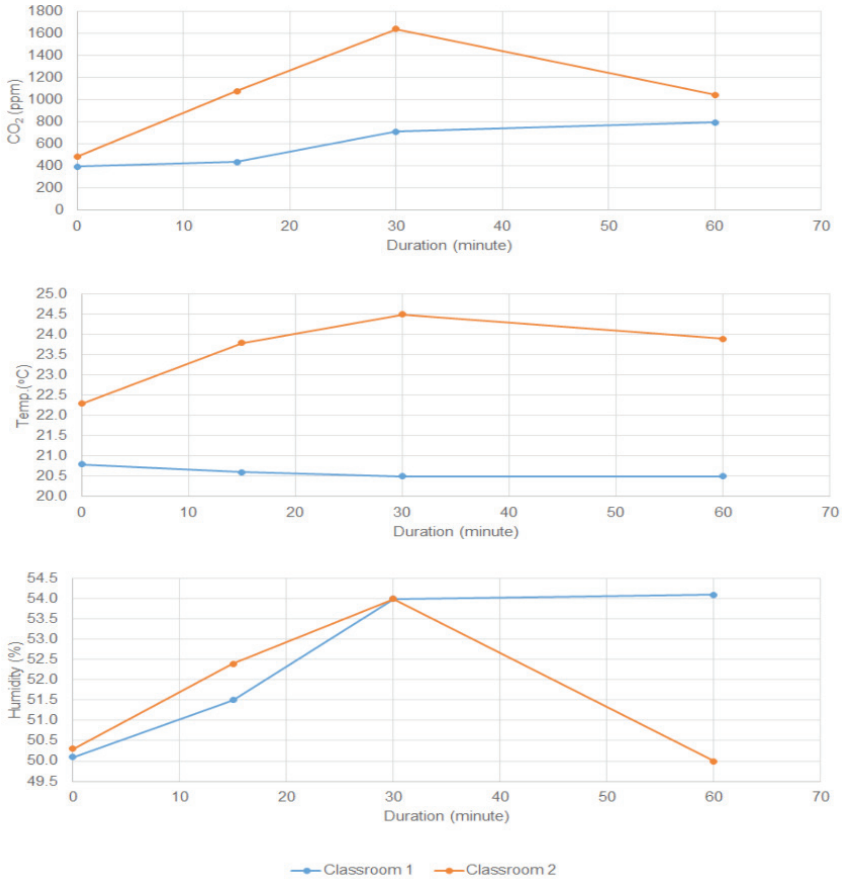

Figure 2. *CO_2, temperature, and humidity measurements for classes*

The obtained survey results are given in Table 1-17.

Table 2. *Means for some individual characteristics (n=34)*

	n	min	max	Mean	SD
Age	34	19	38	23	4.445
Working Year		2	6	2.27	0.898

Table 2 shows the averages of some individual characteristics. The study's average age in the study is 23 (± 4.445), and the average working year is 2.27 (± 0.898).

Table 3. *Data and frequencies of the participants (n=34)*

	Variable	n	%
Gender	Female	14	41.2
	Male	20	58.8
Education	Associate degree	28	82.4
	University	6	17.6
Job	Student	30	88.2
	Officer	3	8.8
	Other (import-export)	1	2.9
Working unit (School)	OHS	26	76.5
	Health	2	5.9
	Administrative	1	2.9
	Environment	5	14.7

In Table 3, the frequencies of some variables are included. A total of 36 people participated in the study. 41.2% of the participants in the study are women, and 58.8% are men. Since most of the study participants are male, a homogeneous gender distribution could not be made. Considering the educational status of the participants, it is seen that 82.4% are associate degree graduates and 17.6% are undergraduate. Besides, 88.3% of the respondents stated that they are students (not working), 11.7% are working students (civil servants and other professions). When evaluated in terms of the unit of study (the department studied), it is seen that 76.5% of the participants work in the field of occupational health and safety, 5.9% in the field of health, 2.9% in the administrative field, and 14.7% in the environment.

Table 4. *Participants' evaluations about the indoor environment and the frequencies of these data (n=34)*

Variable	n	%	SD
So hot	8	23.5	0.431
Very cold	12	35.3	0.485
Airless	10	29.4	0.463
Musty /scented	2	5.9	0.239
Other types of fragrances	1	2.9	0.172
Poor lighting	0	0	0
Other	0	0	0
Dusty	6	17.6	0.387
Noisy	12	35.3	0.485
Very dry	1	2.9	0.172
Very humid	1	2.9	0.172
Breezy	2	5.9	0.239
The environment is crowded	8	23.5	0.431
Vibratory	0	0	0
I have no complaints	7	20.6	0.410

Table 4 contains the frequencies of the 1st question of the survey. 23.5% of the respondents stated that the environment is sweltering, 35.3% is very cold, 29.4% is airless, 17.6% is dusty, 35.3% is noisy, and 23.5% stated that the environment was crowded. 20.6% of the participants do not have any complaints about their environment.

Table 5. *Personal characteristics of the participants and the frequencies of these data (n=34)*

Variable	n	%	SD
I wear contact lenses	0	0	0
I'm using glasses	6	17.6	0.387
I look at the screen for more than 1 hour a day in the building I am in.	19	55.9	0.504
I use chemicals (disinfectant, room fragrance, *etc.*) every day.	1	2.9	0.172
I am using paper without carbon copy	2	5.9	0.239
I use tobacco products	15	44.1	0.504
None of the above	8	23.5	0.431

Table 5 includes the frequencies of the second question of the survey. 17.6% of respondents wear glasses. 55.9% of the participants stated that they look at the screen for more than 1 hour a day in the building they are located in. According to the survey findings, the rate of those who use tobacco products is 44.1%. 23.5% of the participants chose none.

Table 6. *Chronic health information of the participants and the frequencies of this information (n=34)*

Variable	n	%	SD
I have an allergic rhinitis	-	-	-
I have asthma	-	-	-
I am allergic	-	-	-
I have eye cold / inflammation	3	0.288	8.8
I have sinusitis	5	0.359	14.7
I have emphysema (gas)	-	-	-
I have laryngitis (throat burn)	-	-	-
I have a bronchitis	1	0.172	2.9
I have other chest complaints	1	0.172	2.9
None	25	0.448	73.5

Table 6 contains the frequencies of the 3rd question of the survey. While 8.8% of the survey participants stated that they had eye fever/ inflammation, 14.7% had sinusitis, 2.9% had bronchitis and other chest complaints; 73.5% chose none.

Table 7. *Acute health information of the participants and the frequencies of this information (n=34)*

Variable	n	%	SD
Frequent cough	5	14.7	0.359
Wheezing (other than a cold)	2	5.9	0.239
Too much cold (more than 4)	9	26.5	0.448
Shortness of breath	2	5.9	0.239
Migraine	2	5.9	0.239
Burning or irritation in the eyes	7	20.6	0,410
None of the above	9	26.5	0.448
Nasal congestion	10	29.4	0.463
Sinus infection	1	2.9	0.172
Sore throat	7	20.6	0.410
Sound attenuation	2	5.9	0.239
Headache (at least 2 times a month)	12	35.3	0.485
Sneezing / sneezing attacks	4	11.8	0.327
Other (please specify)	1	2.9	0.172

Table 7 contains the frequencies of the 4th question of the survey. While 14.7% of the respondents marked the options for frequent cough, 25.6% for too much cold, and 20.6% for burning and irritation in the eyes, 26.5% marked none. Besides, 35.3% of the participants complain of headaches (at least 2 times a month) and 29.4% have nasal obstruction.

Table 8. *The drugs used by the participants and the frequencies of these data (n=34)*

Variable	n	%	SD
Pain killer	3	8.8	0.288
Nasal congestion medicine (decongestant)	2	5.9	0.239
I do not use any medication this often	27	79.4	0.410
Antidepressant	1	2.9	0.172
Allergy medicine (antihistamine)	1	2.9	0.172
Other (please specify)	3	8.8	0.288

Table 8 contains the frequencies of the 5th question of the survey. 79.4% of the respondents answered the medication you use daily and weekly as I do not use any medication at this frequency.

Table 9. *Participants' views on indoor air quality and the frequencies of these data (n=34)*

Variable (Indoor air quality)	n	%	SD
Good	7	18.9	-
Intermediate	22	59.5	-
Poor	5	13.5	-

The frequencies of indoor air quality (question 6) are listed in Table 9. Accordingly, 59.5% of the participants evaluated the indoor air quality of the building as a medium.

Table 10. *Data showing the relationship between indoor air quality and the seasons and the frequencies of these variables (n=34)*

Variable	n	%	SD
Yes	5	16.7	0.750
No	11	36.7	
I do not know	14	46.7	

Table 10 contains the frequencies of the 7th question of the survey. 36.7% of the participants answered this question as "no," and 46.7% as "I do not know." Participants left the 8th question of the questionnaire blank.

Table 11. *Data showing the relationship between indoor air quality and periods during the day and the frequencies of these variables (n=34)*

Variable	n	%	SD
Morning	1	20	1.342
Afternoon	2	40	
Night	-	-	
All-day	2	40	

Table 11 contains the frequencies of the 9th question of the survey. According to the findings of this question answered by those who answered "yes" to the questionnaire's 7th question, 40% of the participants stated that they found the lower indoor air quality in the afternoon and 40% of them all day.

Table 12. *Answers are given by the participants about the sick building syndrome and their frequencies (n = 34)*

Variable	n	%	SD
Headache	14	41.2	0.500
Irritation / burning in the eyes	3	8.8	0.288
Runny nose	3	8.8	0.288
Fever (> 38 °C)	0	0	0
Cough	4	11.8	0.327
Skin problems (redness)	1	2.9	0.172
Sinus congestion	1	2.9	0.172
Sore throat / burning	4	11.8	0.327
Dizziness	1	2.9	0.172
Fatigue / burnout	10	29.4	0.463
Wheezing	1	2.9	0.172
Muscle pain	7	20.6	0.410
Sinus infection / inflammation	0	0	0
Hoarseness	2	5.9	0, 239
Sneezing	3	8.8	0,288
Red / watery eyes	3	8.8	0,288
Shortness of breath	1	2.9	0,172
Other (please specify)	1	2.9	0,172

Table 12 contains the frequencies of the 10th question of the questionnaire. Accordingly, 41.2% of the respondents stated that they experienced a headache in the building where they were located, 11.8% had a cough and sore throat/burning, 29.4% had fatigue/burnout, and 20.6% had muscle pain. They stated that he lived. Answers to questions 11, 12, and 13 are given in Table 13.

Table 13. *Participants' responses to other questions about sick building syndrome and their frequencies (n = 34)*

	Variable	n	%	SD
Question 11	Yes	10	37	0.736
	No	12	44.4	
	I do not know	5	18.5	
Question 12	Yes	8	44.4	0.857
	No	5	27.8	
	I do not know	5	27.8	
Question 13	Yes	8	53.3	0.884
	No	3	20	
	I do not know	4	26.7	

In Table 13, while the rate of those who said no to the 11th question was 44%, the rate of those who said no to the 12th question was 27.8%, and the rate of those who said no to the 13th question was 20%. Answers to questions 14 are given in Table 14.

Table 14. *Responses of the participants regarding sick building syndrome in the last week and their frequencies (n = 34)*

	Variable	n	%	SD
Question 14	Headache	11	32.4	0.478
	Irritation / burning in the eyes	3	8.8	0.288
	Runny nose	4	11.8	0.327
	Fever (> 38 °C)	0	0	0
	Cough	6	17.6	0.387
	Skin problems (redness)	0	0	0
	Sinus congestion	1	2.9	0.172
	Sore throat / burning	2	5.9	0.239
	Dizziness	1	2.9	0.172
	Fatigue / burnout	9	26.5	0.448
	Wheezing	1	2.9	0.172
	Muscle pain	4	11.8	0.327
	Sinus infection / inflammation	1	2.9	0.172
	Hoarseness	1	2.9	0.172
	Sneezing	2	5.9	0.239
	Red / watery eyes	1	2.9	0.172
	Shortness of breath	1	2.9	0.172
	Other (please specify)	1	2.9	0.172

In Table 14, higher values were obtained in Headache, Cough, Fatigue/ burnout, Muscle pain responses in terms of a standard deviation compared to other answers. Answers to questions 15 and 16 are given in Table 15.

Table 15. *Participants' allergy/asthma rates and frequencies of these data (n=34)*

	Variable	n	%	SD
Question 15	Yes	6	17.6	0.436
	No	27	79.4	
	I do not know	1	2.9	
Question 16	Yes	2	6.1	0.459
	No	26	78.8	
	I do not know	5	15.2	

The vast majority (> 75%) of the 15th and 16th questions in Table 15 answered no. Answers to questions 17 and 18 are given in Table 16.

Table 16. *Time spent by participants in indoor spaces and their frequencies (n = 34)*

	Variable	n	%	SD
Question 17	0-25%	5	14.7	0.600
	26-50%	22	64.7	
	51-75%	7	20.6	
	76-100%	-	-	
Question 18	0-25%	5	14.7	0.774
	26-50%	13	38.2	
	51-75%	15	44.1	
	76-100%	1	2.9	

In Table 16, the majority answered 26-50% in the 17th question, while in the 18th question, the answer was 51-75%. Answers to questions 19 and 20 are given in Table 17.

Table 17. *The physical conditions of the participants' environment and the frequencies of these variables (n = 34)*

	Variable	n	%	SD
Question 19	Photocopy	10	29.4	0.463
	Laser printer	6	17.6	0.387
	Window	31	91.2	0.288
	Plant / flower	8	23.5	0.431
	Central ventilation	12	35.3	0.485
	Air conditioning	24	70.6	0.463
Question 20	Very bright	4	11.8	0.327
	Bright	9	26.5	0.448
	Lighting is suitable	20	58.8	0.500
	Darkly	1	2.9	0.172
	Dark	-	-	-

In Table 17, the window stands out as the answer to the 19th question, while the answer to the 20th question is that the lighting is suitable. Answers to questions 21, 22, and 23 are given in Table 18.

Table 18. *The physical and chemical conditions of the participants' environment and the frequencies of these variables (n = 34)*

	Variable	n	%	SD
Question 21	No	**30**	**90.9**	0.292
	Yes	3	9.1	
Question 22	No	**26**	**76.5**	0.431
	Yes	8	23.5	
Question 23	No	**27**	**79.4**	0.410
	Yes	7	20.6	

In Table 18 and questions 21-23, the answer "no" was preferred with a ratio of > 75%.

DISCUSSION

A total of 34 students from two different classes participated in this study. Although the classes' volumes are approximately the same, the volume of the 28-person class is smaller than the other. Simultaneously, because of the student density, the maximum CO_2 concentration was reached within 30 minutes. Although the lessons are specified as 40 minutes in the program, the duration can be extended to 60 minutes in the block lessons. However, if it is not possible to open windows due to the weather conditions in busy classrooms, it becomes challenging to concentrate students as time progresses. In studies conducted abroad, there are studies in which there is an inversely proportional relationship between the CO_2 concentration and the students' attention levels in the environment (Twardella & Matzen, 2012; Sendell et al.2004; Temprano et al.2020). When the survey study was evaluated statistically, the following results were obtained.

First of all, it was concluded that there was no statistically significant difference between those who found the environment very warm and the diagnoses made for them in the building where they were studied. A statistically significant difference was found between those who found the environment crowded and those who said they had an eye infection ($p = 0.009$), and those who said they had sinusitis ($p = 0.007$). Also, a statistically significant difference was found between those who said they did not have a complaint and those who said they had eye-catching / eye inflammation ($p = 0.006$) and those who said they had sinusitis ($p = 0.048$) ($p<0.05$).

There is no statistically significant difference between the diagnoses made for them in the working (read) building with the participants who find the environment very dry and those who find the environment inspired and the diagnoses made for them. Finally, there was no statistically significant difference between those who found the environment very humid, and the diagnoses made for them in the working building.

Borcka Acarlar Vocational School is located on the D010 roadside as a location and affects the 0-minute measurement of the classroom's outdoor CO_2 amount. Since the Faculty of Engineering is within the border of Seyitler village of Artvin Center, the external environment and therefore 0-minute measurements are much lower.

While there was no heater or air conditioning device in either class during the measurements, the intensive class's initial temperature increased 2 degrees in the measurements made on consecutive days and remained constant in the other class.

CONCLUSION AND RECOMMENDATIONS

Measurement of CO_2, temperature, and humidity is essential in indoor air quality. As it is known today, people spend most of their time in closed environments during the day. This situation mainly affects indoor air quality. The importance of indoor air quality increases even more in crowded environments such as schools. Students, teachers, and other staff spend most of their days in closed and crowded environments. This situation causes different effects on both instructors and learners. In environments where ventilation is not practical, CO_2, temperature, and humidity increase; thus, adverse effects such as fatigue, boredom, and carelessness are observed on both sides. In addition to all these, diseases such as sick building syndrome may occur. Providing the necessary ventilation in busy classrooms will increase the concentration of both the lecturer and the students. In periods when central ventilation is prohibited or not working due to the COVID-19 pandemic, it is essential to ventilate the environment by opening the windows. However, here, too, weather conditions and the amount of CO_2 in the outdoor environment gain importance. In classrooms that are busy and do not have ventilation facilities, it is more critical for lecturers and lecturers to adjust the lecture time properly.

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The original questionnaire approved by the Artvin Çoruh University Ethics Committee in 2017 is given in Appendix 1. It has been translated into English for publication in the text.

APPENDIX 1. INDOOR AIR QUALITY QUESTIONNAIRE

1. Age: ……..
2. Education Status: ……
3. Profession: …….
4. Gender: ……

7. How many years have you been working/studying in this building?
……

8. Which unit in the department you work/study in?
……

1. Mark one or as many of the following environmental issues, if any, about the world in which you are?

□ □ Too hot	□ □ Dusty
□ □ So cold	□ □ Loud
□ □ Airless	□ □ Too dry
□ □ Moldy/scented	□ □ Very humid
□ □ Other types of fragrances available (please specify?)	□ □ Breeze
	□ □ The environment is crowded
□ □ Poor lighting	□ □ Vibrating
□ □ Other	□ □ I have no complaints

2. Please mark one of the following options that suit you or as many as required?

□ □ I wear contact lenses
□ □ I am wearing glasses
□ □ I look at the screen for more than 1 hour a day in the building I am
□ □ I use chemicals (disinfectant, room fragrance, etc.) every day
□ □ I am using paper without carbon copy
□ □ I use tobacco products
□ □ None of the above

3. Have you been diagnosed with one or more of the following diagnoses since you were in the building where you worked/trained?

□ □ You have an allergic rhythm	□ □ I have emphysema (gas)
□ □ I have asthma	□ □ I have laryngitis (throat burn)
□ □ I have an allergy	□ □ I have a bronchitis
□ □ I have eye cold/inflammation	□ □ I have other chest complaints
□ □ I have sinus	□ □ None

4. Have you ever encountered one or more of the following complaints in your building during the last year?

□ □ Frequent cough	□ □ Nasal congestion
□ □ Wheezing (except for a cold)	□ □ Sinus infection
□ □ Too many colds (more than 4)	□ □ Sore throat
□ □ Shortness of breath	□ □ Sound attenuation
□ □ Migraine	□ □ Headache (at least two times a month)
□ □ Burning or irritation in the eyes	□ □ Sneezing/sneezing attacks
□ □ None of the above	□ □ Other (please specify)

5. Please tick below the drug categories you use daily or weekly, if any?

□ □ Pain relief	□ □ Antidepressant
□ □ Nasal congestion medicine (decongestant)	□ □ Allergy medicine (antihistamine)
□ □ I do not use any medication this often	□ □ Other (please specify)

6. How would you evaluate the indoor air quality of your building?

□ □ Good	□ □ Medium	□ □ Poor

7. If you think there is a problem in indoor air quality, do you encounter this problem mostly in a specific year?

□ □ Yes	□ □ No	□ □ I do not know

8. If you answered "Yes" to Question 7. Please list the seasons you encountered this problem? ("1 is least, two is medium, three is more, four is most")

□ □ Winter (December-February)	□ □ Spring (March-May)	□ □ Summer (June-August)	□ □ Autumn (September-November)

9. Suppose you answered "Yes" to Question 7. At what time of the day does indoor air quality feel lower?

□ □ Morning	□ □ Afternoon	□ □ Evening	□ □ All-day

10. Which of the following symptoms did you experience in your building?

□ □ Headache	□ □ Sinus congestion	□ □ Sinus infection / inflammation
□ □ Eye irritation/burning	□ □ Sore throat/burning	□ □ Hoarseness
□ □ Runny nose	□ □ Dizziness	□ □ Sneezing/sneezing
□ □ Fever (> 38 °C)	□ □ Fatigue / burnout	□ □ Redness / watery eyes
□ □ Cough	□ □ Wheezing	□ □ Shortness of breath
□ □ Skin problems (redness)	□ □ Muscle pain	□ □ Other (please specify)

11. Do the symptoms mentioned above or symptoms disappear within 1 hour after leaving the building?

□ □ Yes	□ □ No	□ □ I do not know

12. If "No," do the symptoms disappear the next morning?

□ □ Yes	□ □ No	□ □ I do not know

13. If "No," do the symptoms disappear on vacation?

□ □ Yes	□ □ No	□ □ I do not know

14. Which of the following symptoms have you had in your building in the past week?

□ □ Headache	□ □ Sinus congestion	□ □ Sinus infection / inflammation
□ □ Eye irritation/burning	□ □ Sore throat/ burning	□ □ Hoarseness
□ □ Runny nose	□ □ Dizziness	□ □ Sneezing/sneezing
□ □ Fever (> 38 °C)	□ □ Fatigue / burnout	□ □ Redness / watery eyes
□ □ Cough	□ □ Wheezing	□ □ Shortness of breath
□ □ Skin problems (redness)	□ □ Muscle pain	□ □ Other (please specify)

15. Have you ever had an allergy and/or asthma test?

□ □ Yes	□ □ No	□ □ I do not know

16. Do you have allergies and/or asthma?

□ □ Yes	□ □ No	□ □ I do not know

17. What percentage of the day do you spend in your building?

□ %0 - %25	□ %26 - %50	□ %51- %75	□ %76-%100

18. What percentage of the day do you spend in your room or classroom?

□ %0 - %25	□ %26 - %50	□ %51- %75	□ %76-%100

19. Please tick the ones below in your environment?

□ □ Copier	□ □ Laser printer	□ □ Window	□ □ Plant / flower	□ □ Central ventilation	□ □ Air conditioning

20. Do you think the lighting of your environment is sufficient?

□ □ Very bright	□ □ Bright	□ □ Lighting is appropriate	□ □ Darkly	□ □ Dark

21. Has renovation work been done in your environment? (New carpet, whitewash/paint, new items, *etc.*)

□ □ No	□ □ Yes (please specify)

22. Is there visible moisture or mold on the walls, ceiling, and/or floor of your environment?

□ □ No	□ □ Yes (please specify)

23. Do you enter the chemical laboratory or cleaning room?

□ □ No	□ □ Yes (please specify)

24. If your answer is "Yes," please write down the chemical you remember the last time you used?

Chapter 4

TRADITIONAL USES OF *POTENTILLA* L. (ROSACEAE) SPECIES

Selen İLGÜN[1]

Gökçe ŞEKER KARATOPRAK[2]

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1. General features of *Potentilla* genus (Botany, chemistry and pharmacological effect)

Medicinal herbs play a vital role in the prevention and treatment of diseases. Various parts of medicinal plants, which are very popular in recent years, can be used for medicinal purposes. Moreover, its use as a raw material in the pharmaceutical industry is also very important (Sofowora, Ogunbodede et al. 2013). According to the data; more than 50% of the drugs developed and approved are directly derived either from modified products of medicinal plants or from the active ingredients of these herbs. Because, with the advancement of modern medicine and pharmaceutical research, it has become important to use herbs as the main source instead of medicinal products obtained by chemical synthesis (Wang, Xu et al. 2020)

Throughout the history, people have passed down their knowledge and experience to new generation about the medicinal use of herbs and the traditional use of medicinal plants still maintains its popularity. According to World Health Organization (WHO) data, 80% of the world's population prefer traditional medicines to meet their basic health care needs so in developing countries, the majority of the world's population cannot take pharmaceutical medicines and have to use their own herbal medicines (Wang, Xu et al. 2020).

The *Potentilla* genus belongs to the Rosaceae family and represented by approximately 110 genera and more than 3000 species worldwide (Shulaev, Korban et al. 2008). Many of them are important fruits, nuts, ornamental plants and wood products. Members of this family provide very valuable nutrients, aesthetic or highly appreciated products for industry (Tanker, Koyuncu et al. 1998).

Taxonomically; *Potentilla* belongs to the sub-tribe Potentillinae, one of the 10 tribes of the Rosaceae family (Augustynowicz, Latté et al. 2020). According to its latest monograph and current floristic research around the World; the genus includes about 300 to 430 species; moreover, the genus is among those with a rich plant diversity and spreaded in the temperate, arctic and alpine areas, chiefly in the northern hemisphere (Dobeš and Paule 2010, Dobeš, Lückl et al. 2013). Plants generally perennial, rarely annual herbs and shrubs. Flowers usually terminals, in cymes or solitary 5- or rarely 4- merous. Hypantium weakly concave with a central hemispherical receptacle. Epicalyx present. Petals yellow or white rarely reddish. Stamens are about 20, but sometimes more or fewer. Fruits are achenes. Style subterminal, lateral or basal, longfiliform or short, usually thickened and sometimes papillose at the base, shorter than or longer than achene, generally decidious in fruit (Pesmen ,1972).

Potentilla is taxonomically very complicated and difficult genus. The phylogenetic relationships of the species belonging to the genus *Potentilla* are quite complex, since the speciation of the species is a continuous process. In addition to crossings with various species from other genera of the Rosaceae family (eg *Fragaria, Sibbaldia*), interspecific hybridization events lead to the emergence of polyploids and/or new morphobiotypes with chromosome number polymorphisms (Eriksson, Hibbs et al. 2003)

In addition, the different breeding ability of *Potentilla* species make difficult the taxonomy of the genus. At present, it is necessary to clarify the phylogenetic relationships within the genus and to better understand the mechanisms of plant conformation to different environmental situations. For this reason, *Potentilla* genus is being studied intensively at morphological, chemical and molecular grades (Samatadze, Zoshchuk et al. 2018)

Table 1. *Scientific clasifications of Potentilla*

Divisio	: Spermatophyta
Subdivisio	: Angiosperma
Classis	: Dicotyledones
Ordo	: Rosales
Family	: Rosaceae
Subfamily	: Rosoideae
Tribe	: Potentilleae
Subtribe	: Potentillinae
Genus	: *Potentilla* L.

Data on the traditional medical use of *Potentilla* species are available in almost every part of the world. For this reason, detailed studies have been carried out on the pharmacological efficiency and plant-drug safety profiles of the genus (Melzig and Böttger 2020).

Phytochemical investigations of the genus *Potentilla,* historically began with the identification of the components of the tannin fraction. The aerial and underground parts of the *Potentilla* species, also known as “Cinquefoils”, that show a series of biological activity, are especially rich in polyphenols and triterpenes (Tomczyk and Latté 2009), (Zhao, Cai et al. 2008). In the literature; there are several studies showing that many compounds have been detected from *Potentilla* species. Examples are; flavonoid aglycones, flavonoid *O*-glycosides and *O*-glucuronides, flavonoids *C*- glycosides, diflavonol ester Potentilin A and isoflavons

aglycone *C* and *O*- glycosides. On the other hand, hydrolysable and condensed tannins and derivatives, organic acids and phenol carboxylic acids, coumarins, chromones, sterols, triterpenoids and lignans has been defined. Chemical contents of some *Potentilla* species subject to recent bioactivity studies are given in Table 2.

Table 2. Chemical constituent of some Potentilla species

***Potentilla* species**	**Compounds**	**References**
P. glabra	Hyperoside, Isoquercitrin, Miquelianin, Quercitrin, Rutin, Reynoutrin, Rhamnetin 3-*O*-glucoside, Rhamnetin 3-*O*-rhamnoside, Rhamnetin 3-*O*-rutinoside, Ellagic acid, (+)-Catechin, Caffeic acid, Ferulic acid	(Wang, Wang et al. 2013), (Han, Bai et al. 2016)
P. discolor	Apigenin, Luteolin, Tricetin, Tenaxin I, Comosiin, Apigenin 7-*O*-β-D-glucuronide, Astragalin, Cynaroside, Kaempferol 3-*O*-β-D-glucuronide, Kaempferol 7-*O*-α-L-rhamnoside, Vincetoxicoside B, isovitexin, Vicenin 1, 2 and 3, Schaftoside, Isoschaftoside, Brevifolin, Ellagic acid, Ellagic acid 3-*O*-methyl ether, Chlorogenic acid, Cryptochlorogenic Acid, Neochlorogenic acid, Citric acid, *p*-Coumaric acid, Gallic acid, Maleic acid, Xspolyphenol B, Oxalic acid, Protocatechuic acid, Pyruvic acid, Salicylic acid, Succinic acid, Tartaric acid, β-Sitosterol, Chaenomiside F, Potentillalignan A and B, Aceriphyllic acid A, Aceriphyllic acid A methyl ester, α-Amyrin, β-Amyrin, Asiatic acid, Arjunic acid, Betulinic acid, Gypsogenic acid, Maslinic acid, Myrianthic acid	(Cheng, Wang et al. 2020), (Zhang, Huang et al. 2018), (Wang, Zhu et al. 2019), (Yang, Chen et al. 2010), (Jang, Kim et al. 2006, Yang, Chen et al. 2010), (Weisheng, Xiaoke et al. 1996)
P. chinensis	Kaempferol 4′,5,7-trimethyl ether, Kaempferol 3-O-α-L-arabinofuranoside, Tiliroside, Cis-tiliroside, Avicularin, Quercitrin, β-Sitosterol, Blumenol A, Ellagic acid 2,3,8-tri-*O*-methyl ether	(Gao, Shen et al. 2007), (Shen, Wang et al. 2006), (Wang, Li et al. 2006)
P. anserina	Potenserin B, Resveratrol, Resveratrol 3-*O*-β-D-(6-*O*-galloyl)-Glucopyranoside, (Z)-Resveratrol 3,5-*O*-β-diglucoside, Phloridzin, 3-Hydroxy-phloridzin, Daidzin, Puerarin, 3-Methoxydaidzein 8-*C*-glucoside, Daidzein 8-*C*-apiosyl-(1→6)-glucoside, Potentilin A, Puerarin 3′-methyl ether, Ellagic acid, Ducheside B, Ellagic acid 4-*O*-α-L-arabinofuranoside, Potentillanoside G, Potentillanoside H, Ellagic acid 3-O-α-L-rhamnopyranoside, (+)-Catechin 7-*O*-β-glucopyranoside , (+)-8-carboxymethylcatechin methyl ester , (-)-Epicatechin, (-)-Epicatechin 7-*O*-β-glucoside, (-)-Epigallocatechin-7-*O*-β-D-glucoside, Potenserin A, 8,8′-Methylenebiscatechin, 6,8′-Methylenebiscatechin, Procyanidin B3, (+)-Catechin-(4_→8)-(-)-epicatechin, Potenserin C, Prodelphinidin B3, Prodelphinidin C Arjunic acid, Potenserin A, C ,Cecropiacic acid ,Maslinic acid, Pomolic acid, 28-*O*-β-D-glucoside 2-oxopomolic acid 3-epi-2-oxopomolic acid, Potentillanoside A,B,C and D	(Wang, Li et al. 2006), (Morikawa, Ninomiya et al. 2014), (Yang, Wang et al. 2020), (Kombal and Glasl 1995), (Schimmer and Lindenbaum 1995), (Morikawa, Imura et al. 2018)

P. fulgens	Apigenin 7-O-β-D-glucuronide, Astragalin, Tiliroside, Cynaroside, Hyperoside, Rutin, Scutellarin, Potentene A and B, (-)-Epiafzelechin, (+)-Afzelechin-(4α→8″)-(+)-catechin,	(Jaitak, Kaul et al. 2010), (Choudhary, Radhika et al. 2015), (Kaul, Jaitak et al. 2011)
P. fruticosa	Apigenin, Luteolin, Ampelopsin, Rhamnetin, Miquelianin, Quercetin 7-*O*-β-D-glucuronide, Sorhamnetin 3-*O*-β-D-glucuronide, Digalloylglucose, Trigalloylglucose, Tetragalloylglucose, Pentagalloylglucose, Epigallocatechin 3-*O*-p-coumarate, Pigallocatechin-dimethylgallate, (-)-Afzelechin-(4α→8)-(-)-afzelechin, 6,8′-Methylenebiscatechin, 8,8′-Methylenebiscatechin 6,8′-Methylene(7-*O*-glucosyl)-biscatechin, Chlorogenic acid, *p*-Coumaric acid, Gallic acid, Quinic acid, Protocatechuic acid, 5-Hydroxysalicylic acid, Bergapten, Betulinic acid, Oleanolic acid	(Yu, Pu et al. 2016), (Luo, Wang et al. 2016), (Malyutina, Pravlotskaya et al. 2018), (Khramova and Vysochina 2010)
P. fragarioides	β-sitosterol, β-daucosterol, ursolic acid, pomolic acid, swinhoeic acid, (1-p-hydroxy-cis-cinnamoyl)cinnamic acid, trans-caffeoylisocitric acid, trans-caffeic acid, quercetin, quercetin-3-O-β-D-glucuronide, (+)-catechin and 3-O-methylellagic acid-4′-O-α-L-rhamnopyranosid	(Li, Li et al. 2020)
P. longifolia	Pernambucone, Orobanone, Rosamultin, Oleanolic acid, ent-16β,19-dihydroxykaurane, Carthamidin, 1,5-dihydroxy-2-(2'-methylbutanoyl)-3-methoxy-6-methylbenzene, bis (2-ethylheptyl) phthalate, Ganyearmcaoosides A and B and Ganyearmcaoic acid A	(Piao and Yuan 2020), (Lin, Zhao et al. 2020)

Scientific studies have confirmed the effects of *Potentilla* species whose traditional use is known, and also revealed their pharmacological features through various experimental tests. Especially antimicrobial, antihyperglisemic, antitumor, antioxidative, antiimflamatory, and impairments effect on triodes glands are the important pharmacological effects. (Cheng, Wang et al. 2020, Kowalik, Paduch et al. 2020, Lin, Zhao et al. 2020, Wang, Zhang et al. 2020)

2. Traditional Uses of *Potentilla* Species

Potentilla species are plants whose effects have been known since ancient times, and the oldest argument for the use of *Potentilla* species is probably the Papyrus Ebers. This ancient Egyptian papyrus is considered the most extensive medicinal papyrus dating to 1550 BC. According to a data obtained from papyrus; It is stated that *Mandragora* and *Potentilla* species make teeth healthy. The philosophers of the Roman Empire mentioned this plant in their works and suggested "Quinquefolium" as a remedy for skin ailment. *Potentilla* species are called "Heptaphyllon" or "Pentaphyllon", "Septifolium" and "Quinquefolium" in Greek and Latin

languages. The famous Greek physician Dioscorides suggested the root of "Pentaphyllon" in his "De Materia Medica" against toothache and, throat problems, as well as for the treatment of stomatitis and skin ailments. He also recommended this herb to be used internally against dysentery, diarrhea, gout and sciatica, and recommended the use of its leaves against epilepsy and jaundice (Augustynowicz, Latté et al. 2020).

Avicenna, also known as Ibn-Sina, used the roots of *P. reptans* for the treatment of liver diseases, sore throat and kidney stones and also mentioned that it is used in the treatment of diabetes in his book "Al-Qānūm fī Tibb".(Augustynowicz, Latté et al. 2020).

Especially *P. erecta* was defined as "Tormentil" in medieval herbal books and has been used in many European countries hitherto. Recently, monographs for "Tormentillae rhizome" are available in the European Pharmacopoeia, Committee on Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA) and the European Scientific Cooperative on Phytotherapy (ESCOP). The German Commission E stated in a monograph that *P. erecta* rhizomes have found use in acute diarrhea as well as inflammations of mild mucous membrane (Melzig and Böttger 2020)

It has been stated that in Ayurveda, Unani, Siddha, Chinese and Tibet medical systems, the aerial and underground parts of more than three hundred *Potentilla* species are used in the treatment of various diseases due to their rich content of polyphenolic compounds (Kaul, Jaitak et al. 2011).

Rhizome extracts of *P.erecta* (L.) Raeusch., known as "Tormentil," have been commonly used in traditional medicine for a long time in Central Europe and Russia. The traditional usage were for the treatment of toothache, inflammations of the throat, ulcers of the mouth, jaundice, and dysentery and as a homeostatic and for wound healing. A bitter liqueur is made from the chopped tormentil rhizomes in Germany, called ''Blutwurz'', used for improving the digestion (Zhao, Cai et al. 2008). Also in the Ukraine, tormentil rhizomes are used to flavor the''Kalhanivka'' vodka which is considered as a remedy for many disease and as a general tonic (Shushunov, Balashov et al. 2009).

P. erecta which is used for the treatment of purulent facial eczema and buccal ulcerations traditionally, is formally registered in pharmacopoeias of various European countries and also registered in homeopathic pharmacopoeias for preparation of homeopathic remedies (Tomovic, Cupara et al. 2015)

In the Prokletije Mountains (Montenegro) *P. erecta* used as a remedy for the treatment of diarrhea, external inflammation of the mouth and

pharynx and for poorly healing wounds (Menković, Šavikin et al. 2011)

P. fulgens Wall. Ex Hook known as the Himalayan Cinquefoil in English, is an significant medicinal plant used in the Himalayan high regions. North East India's different ethnic groups used different parts of the plant as medical resources, although their mechanism of action has not yet been determined. The inhabitants of this region masticate the main root of *P. fulgens*, betel nut (*Areca catechu*) and betel leaves (*Piper betel*) for various disorders (Tripathy, Choudhary et al. 2015)

P. fruticosa mostly accepted as "Jinlaomei drug" and "Gesanghua" in China usually used as a garden plant but apart from this common application, it also has various medicinal properties. These medicinal properties can be exemplified for the conventional use in the treatment of diarrhea, hepatitis, rheumatoid and scabies in China. In addition, leaves of *P. fruticosa*, which are sweet and cool, are used as additives in foods and as a component in cosmetic products (Liu, Yin et al. 2015). Also, *P. fruticosa* L. as a tea widely used for strengthening the stomach and the spleen and promoting metabolism (Roy, Swargiary et al. 2010)

In Tibet, *P. anserina* root extracts, a traditional medicinal herb, are used for the treat some viral infections, as well as to treat malnutrition, anemia, diarrhea, and hemorrhage (Wang, Wang et al. 2013, Morikawa, Ninomiya et al. 2014).

In the North Serbia; *P. recta (*sulfur cinquefoil) has traditionally quite different uses. For example; the herb has been used as an astringent, styptic, stomachic, anti-inflammatory, cleansing, and antipyretic and tonic agent. It also finds use in bleeding, diarrhea, internal and external inflammations, gonorrhea, throat cleansing, wounds and ulcers (Bazylko, Piwowarski et al. 2013), (Popović, Smiljanić et al. 2014).

A popular traditional herb in folk medicine, *P. chinensis* Ser. is used to treat Type-2 diabetes, immune disorders and liver diseases in China (Huang, Zhang et al. 2015), (Wei, Huang et al. 2013). Different parts of *P. chinensi* have been used in eastern medicine for diseases, such as dysentery and carbunculosis (Wan, Tao et al. 2016).

Aerial parts decoction of *P. reptans* used as anti-diarheic in Italy (De Natale and Pollio 2007). Dry leaves of *P. reptans* were used traditionally in the treatment of cardiovascular disorders. In Northern Navarra region of Spain, as a magical or religious ritual, the fresh plant *P. reptans* is kept in a bowl and is believed to heal hemorrhoids when the plant is dry (Cavero, Akerreta et al. 2011).

P. discolor is one of the most important raw materials among all herbal medicines used in Type-2 diabetes, in Traditional Chinese Medicine

for the therapy of hyperglycaemia and hyperlipidemia (Li, Li et al. 2014)

P. fulgens L. is a folk remedy and commonly found in the high altitudes of India. It used for a variety of ailments, including diabetes. In addition, the aqueous root bark extract of this local medicinal plant is prepared and consumed to treat intestinal parasite infections in India (Roy, Swargiary et al. 2010, Syiem and Warjri 2011).

In the literature, it is recorded that *P. evestita* plant has antimicrobial, anti-inflammatory, analgesic, anti-diarrheal, anti-diabetic, hepatoprotective, anticancer and antispasmodic effects (Rauf, Khan et al. 2014).

Buds and leaves of *P. peduncularis* used for healing fever, influenza, cough and aerial parts of *P. multifida* used for hepatitis, enterobiasis, functional uterine hemorrhage, type 2 diabetes, in addition roots of *P.* atrosanguinea has wound healing effects and used this purpose in China, Korea, Japan, India. (Tomczyk and Latté 2009)

In some European countaries like Sweden, Serbia and Montenegro, Russia, Bulgaria and, Turkey, *P. aurea* is another species that has used for various purposes and there are data that it also used in diabetes (Buchholz and Melzig 2016)

Aerial parts of *P. kleiniana* used for stomach problems, some types of cancer, diabetes and, helminthiasis in Asian countries (Roy, Swargiary et al. 2010).

Instead of *P. discolor* Bunge, which is widely used in the treatment of diabetes in China, *P. multicaulis* Bunge has also been used as a folk remedy (Jia, Wang et al. 2013).

In Pakistan; whole plants and roots of *P.* mooniana used for colic, spasmodic pain, gastric problems, intraoral ulcer, and infections caused by bacteria and fungi (Laloo, Prasad et al. 2014).

The stems and leaves of *P. simplex* are used in the treatment of fungal infections in the USA, especially in Canada, while the roots of *P. arguta* are used for viral infections (Tomczyk and Latté 2009).

In Asian countries such as China, Korea, Japan, India, and in North Africa and North America, it is registered that the aerial parts of *P. supina* are used for arthritis, asthma, bloody discharge, bleeding, some types of cancer, dysentery, helminthiasis and high fever (Nam, Kim et al. 2017), (Lee, Shin et al. 2017). Similarly in Korea, *P. rugulosa* is a different type of *Potentilla* used for fever and bleeding (Choi, Lee et al. 2020).

In India, it is stated that taproots of *P. polyphylla* are chewed daily for treatment of gastric problem, pyorrhea, colic and spasmodic pain (Borborah, Baruah et al. 2014).

In Mongolia aerial parts of *P. parvifolia* are used for skin ailments, mastitis, edema, some types of cancer (Yuan, Suo et al. 2018).

In Turkey; especially *P. recta* and *P. reptans* used as antipyretic, tonic and astringent. Also; Table 1 provides detailed information on the traditional use of species belonging to the *Potentilla* species in Turkey.

Table 1. *Common popular ethnobotanical uses of the Potentilla species in Turkey (Dogan, Bulut et al. 2016) (Tuzlacı,2016)*

Common popular uses	Plant
Dental and oral healthcare, Respiratory diseases Various skin diseases Wound healing effects Gastrointestinal disorders Neurological disorders	P. recta
Pain relief	P. caucasicus
Urinary system disorders and diseases Cardiovascular system disorders and diseases *Diabetes mellitus* Astrenjan	P. inclinata
Cardiovascular system disorders and diseases Various skin diseases Muscular and skeletal system diseases Gastrointestinal disorders Hemoroid	*P. reptans*
Various skin diseases	P. speciosa var. speciosa
Respiratory diseases	*P. anserina* subsp. *anserina*
Cancer	P. speciosa

Conclusions

Although the medical and pharmaceutical industry has made great progress around the world, people still have difficulties in accessing modern treatment methods, especially for economic contitions. Consequently, many people, especially those living in rural areas, have to use plants for therapeutic purposes which belonging to the local flora. As a result, the rich chemical content of *Potentilla* species and their widespread traditional use make the plant important for its use in treatment.

The pharmacological evaluation of *Potentilla* species and their extracts (*in vitro* and *in vivo*) has been made especially since the 1980s. Studies have proven that *Potentilla* species have anti-diabetic, anti-inflammatory, and anti-microbial properties. In particular, clinical studies confirm that *Potentilla alba* exerts positive effects on thyroid function. Also clinical trials have been carried out on the use of *P. erecta* root extracts in the treatment of ulcerative colitis and in viral diarrhea in children. These results show that *Potentilla* species need clinical studies as well as proving their effect son phytochemistry and pharmacology. At the same time, due to the abundance of ethno-pharmacological data on the use of the genus *Potentilla*, it is important to make the toxicological evaluation of these preparations used by the public

As a result, many studies have been conducted showing the pharmacological activities of *Potentilla* species and it has been observed that the obtained data are compatible with the results obtained with traditional knowledge.

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Chapter 5

PHYTOCOSMETICS AND NANO CARRIERS USED

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Introduction

Plants have been one of the important sources of medicine since the beginning of human civilization. Herbal-based medicines, healthcare products, pharmaceutical products, food supplements, cosmetics, etc. There is an increasing demand for. Researching the chemical components of plants and conducting pharmacological screenings can provide us with the basis for finding ways to develop new agents In addition, plants have provided us with vital life-saving drugs used in the effectiveness of modern medicine (GOYAL et al. 2007). Phytocosmetics are rich in ingredients and natural cosmetic products in terms of plant content. Each community has its own traditional cosmetics that contain herbs and herbs, minerals, and oils to maintain the radiance and beauty of the skin (TOLULOPE 2014). Phytocosmetics are products of natural origin, and also contain natural ingredients from plants and algae. Active ingredients in phytocosmetics include natural ingredients such as extracts, essential or fixed oils, resins, waxes. Natural ingredients are obtained from natural or organic sources that do not contain synthetic compounds (HETTA 2016). Active ingredients can be obtained from plants as one of the important sources in the cosmetics industry. It has oils or extracts of plants whose biological benefits are often related to their functions of preventing or treating skin, hair or teeth problems. For example, antioxidants are responsible for preventing oxidative damage by chain breaking free radicals (NASIR et al. 2018). In recent years, the inclusion of phytochemicals as purified compounds or extracts has become very important, which has led to a significant increase in popularity and demands for cosmetics containing phytochemicals (phytocosmetics or natural cosmetics) (SARKER et al. 2018). Cosmetic products used in traditional cosmetics show little efficiency. The new technology has shown great potential to increase the effectiveness and efficiency of the yield of nutraceuticals and bioactive compounds. Recent advances in nanotechnology provide its use as potential cosmetics for poorly soluble, poorly absorbed and unstable herbal extracts and phytochemicals (SARAF et al. 2008). Recently, interest in the use of traditional cosmetics and anti-aging skin care products has increased due to less toxic effects in the treatment of skin problems and for reasons such as skin toning, softening and increased shine, hydration (TOLULOPE 2014). It is aimed to show the results of the use of phytochemicals in combination with nanocarriers and how natural compounds can be used more effectively. In addition, the common plant types used in cosmetics, the history of phytocosmetic application, the use of nano-carriers that will benefit from the plants will be explained.

Phytocosmetics

According to the Turkish Cosmetic Law published on the official newspaper on February 26, 1994; It is prepared to apply to different parts of the human body such as epiderma, nails, hairs, hair, lips and genital

organs, mouth and teeth and mucous membranes, its purpose and side purpose is to clean and maintain these parts by maintaining them in a good condition and changing appearance and body. All preparations and / or substances, including hair dyes and hair color lighteners, which are to correct their smell, are called cosmetics (GERÇEK 2017).

Phytocosmetics is defined as products containing only cosmetics produced from plants or herbal ingredients. plants, plant extracts / extracts, essential oils, distillation products, aromatic waters, meats, aqueous extracts, tinctures, resins, gums and the like, vegetable oils-lipids, waxes, mucilage, plant carbohydrates or purified plant components (KAPOOR 2005; STRASSBOURG 2001).

Phytocosmetic is a common practice in many cultures used for skin, hair and body. Most traditional cosmetics are used to increase beauty, eliminate bad odors of the body, clean and treat certain skin disease conditions in both children and adults. Each community has its own unique traditional cosmetics that contain herbs, minerals and oils to maintain the radiance and beauty of the skin (Tolulope 2014).

In the cosmetics industry, there is a trend towards increasing use of plant extracts as bioactive additives, which are essential for the creation of new formulations with medicinal and prophylactic properties. It should be noted that the same demands are made for extracts used in cosmetic products as well as those for phytotherapy purposes. The variety of extracts included in cosmetics is constantly expanding, using new wild species and vegetables, orchard and technical products and waste from their processing (GUSAKOVA et al. 1998).

Natural products have often been the only source of choice for most people, from food to medical preparations and beauty items. However, many products of science and technology, natural products are still very important for every area of the economy. Considering modern drug discovery, natural products are the first choice to look for new chemical structures for a long time. Natural products are ultimately biochemical metabolites (COSTA 2015).

The term phytochemical comes from a wide variety of compounds produced by vegetable foods. Phytochemicals are usually secondary metabolites produced by plants and often have several biological and pharmacological properties that have long been used to find new drug molecules for the treatment of human diseases. However, properties such as antioxidants, emollients and antimicrobials, as well as color and fragrance, have been used in some cosmetic products available to date (SARKER et al. 2018).

Use of Phytocosmetic Products in History

The history of the use of cosmetics and perfumery began with human history and developed together. Its first applications are found in religious ceremonies, hunting and war. In the 16th century, essential oils were isolated by distillation of wine and beer, fragrant essential oils were used in my perfume production. People who can survive with their hunting and fighting abilities have tried to protect their bodies by using their skin for camouflage purposes or to scare their enemies. Aboriginals in Australia embellish themselves with flower garlands, lubricate their bodies, stick feathers and dye their wool with white clay.

The natives of North America decorated their bodies with bright war paints. Shamans adorn their skins and carried a wild animal head such as deer, bear, wolf, and panther on their heads. Cosmetics, aramotic trees and oils were used in religious ceremonies in the early eastern medina. Even the ruins in the cemetery belonging to the old dynasty times present the ruins of make-up from that period(GERÇEK 2017).

The use of plants is as old as the human race. For example Aloe vera, MÖ. It is one of the oldest known African plants dating back to the great Greek philosopher Aristotle, who enumerated the medicinal benefits of this extraordinary plant, dating back to an Egyptian papyrus dating back to the 3500s. Numerous biological properties such as bleach, anti-acne, anti-wrinkle and anti-pigmentation are found in various herbal cosmetics. Egyptians were very proud of their regularity, care and cosmetics were an integral part of daily hygiene routines. The hot and dry climatic conditions of Egypt were the main reasons for the use of creams and oils for protection. The main elements of the plants of the majority of perfumes used in religious practices and the protection of the dead were the types such as cedar, peppermint, almond oil, rosemary, rose, Aloe vera, sesame oil, chamomile and lavender.

It was the birthplace of ancient Egyptian aromatherapy; women used exquisite, sugary perfumes and fragrance oils from pine trees and flowers, which shows the vital role of cosmetics for this civilization (HETTA 2016).

Ancient Egyptians used natural resources to produce cosmetics, up to 4000 MÖ. They used what was available in their environment. Natural resources used by the ancient Egyptians:

• Henna • Kohl• Red Ocher • Minerals • Chalk and fats are used for cleansing the skin by a cream combination preparing of (HETTA 2016).

In the old literature of Ayurvedic and Unani medicine, the knowledge and benefits of herbal medicines are many. Charaka Samhita (1000 MÖ), one of the first applications of Indian medicine, mentions that more than 2000 herbs are used for medicinal purposes (GOYAL et al. 2007).

It is known that the Romans dye their hair with henna. Even in Eastern countries, nails, palms and feet are painted red with henna leaves. Chinese nobles used arabic resin, egg whites, gelatin and wax to make their nails shiny. In Japan, geishas painted their faces with false saffron (Carthamustinctorius), colored with powder they obtained from rice powder, rubbed softened wax on their hair (Real 2017). The purpose of cosmetics is not only to enhance the appearance, but also for medical benefits. In addition, ancient Egyptians used hair colors and oils containing heavy metals (lead, copper, etc.) to protect them from bacterial infections (HETTA 2016).

By the 20th century, the first synthetic hair dye was produced in 1907 by Eugéne Schueller, the founder of L'oreal. In 1936 Eugéne Schueller launched the first sunscreen cream. The new generation red lipstick, red nail polish, dark eye makeup and bronze skin look of the 1920s were produced by Coco chanel. After 1990s, anti-aging, dermocosmetic products based on scientific data, products containing natural agents, organic hair dyes without ammonia, natural hair reducers, and special shampoos have found increasing market share. Cosmetics today also include compounds such as antioxidants, vitamins, plants, plant originated substances, biological substances, alpha-hydroxy acids. Today, natural products and isolates are included in the formulations of cosmetics and skin care products (GERÇEK 2017).

Why Phytocosmetic Products?

Natural ingredients have been used for skin care for centuries and are now becoming more common in formulations because of consumers' concerns about synthetic ingredients and chemicals. The main benefits reported for herbal extracts used in skin care include antioxidant and antimicrobial activities and tyrosinase inhibition effect. The use of bioactive extracts or phytochemicals from various botanicals in cosmetics has been used mostly to care for the body and to affect the biological functions of the skin and provide nutrients for healthy skin as components. In general, botanical products are a rich source of vitamins, antioxidants, essential oils, hydrocolloids, proteins, terpenoids and other bioactive substances. The compounds may provide different properties than these extracts, according to their composition (RİBEİRO 2015).

More than 40% of the drugs used in medicine are based on preparations of plant origin. Most biologically active substances and individual drug preparations isolated from plants are less xenogenic than synthetic substances and have no pronounced side effects on the organism for prolonged use. (GUSAKOVA et al. 1998).

Given the natural economic potential in using natural resources in ecosystems, plant extracts can be used in cosmetic science to beautify physiological balance and protect human skin. Also, compared to synthetic cosmetic ingredients, herbal products are mild and biodegradable and show low toxicity. (RİBEİRO 2015).

Consumers tend to return to the use of plant / herbal products for a variety of uses in order to practice a more natural lifestyle based on the belief that it is safer. Since the early ages, numerous civilizations in the past have used cosmetics as a cosmetic application of plants. The demand and use of herbal cosmetics, also called phytocosmetics, has increased significantly in the personal care system. This demand arises from the excessive use of synthesized chemicals, unnatural and artificial products, chemical colorants and their non-herbal cosmetics for 150 years. As a result, their production and application causes a large number of negative effects that cause many diseases in the human body.

Recently, many famous cosmetics groups have announced that a deceased female talcum powder has been sued by the user's family, claiming that ovarian cancer has been linked directly to the application of chemical powder for more than 10 years. The belief that phytocosmetics are naturally safe has led to increased consumer demand for cosmetics containing natural or organic ingredients. The search for phytocosmetics is constantly increasing globally, with the popularity of the use of natural ingredients, which is currently largely rising. Therefore, the plants used in many cultures, which are an integral part of cosmetic applications, must be recognized and properly documented, their use according to their active ingredients must be investigated (MAHOMOODALLY 2017).

Many herbal originated substances have been used in medicine and cosmetics because of their pharmacological effects. Compared to synthetic cosmetic products, herbal products are light, biodegradable and have a low toxicity profile (SAWARNLATA et al. 2008).

Phytocosmetic Fields and General Plant Samples

Skin care

They have many biological activities such as photo protection, antiaging, moisturizing, antioxidant, astringent, antiirritan and antimicrobial plants and extracts. Exposure of the skin to sunlight and other substances reacts with proteins and fatty acids in atmospheric conditions, causing the production of reactive oxygen species, which can cause oxidative damage and disruption of the antioxidant system. This type of injury damages the regulation ways of the skin and leads to the development of photoaging and skin cancer. Effects of aging include wrinkles, roughness, the appearance of fine lines, lack of elasticity and traces of hyperpigmentation. Herbal

extracts act in these areas and show healing, softening, rejuvenating and sunscreen effects (SARAF et al. 2008).

Coconut oil is excellent as a skin moisturizer and emollient. A 2004 study by Agero et al shows that extra virgin coconut oil is effective and safe when used as a moisturizer (Agero et al. 2004). Another study by another group reported that using coconut oil for hair prevents and strengthens protein loss (AARTİ 2003). ***Sunflower oil*** contains lecithin, tocopherols, carotenoids and candles. It has softening properties in cosmetics ***Aloe vera*** soothing gel is a component used in cosmetics for skin lotion, minor burns and sunburn. (GEDİYA et al. 2011).

Anti-aging treatment

The skin is a vital organ of the body. Skin aging is mainly influenced by external factors such as pollution, chemicals and radiation to internal factors. Exposing the skin to these external factors can damage the epidermis, which causes accelerated skin aging. Among the popular phytochemicals, cosmetic industries that produce various anti-aging products include a variety of natural antioxidants such as curcumin, resveratrol, epicatechin, ellagic acid and flavone, apigenin. It can reduce the physiological symptoms of aging by resisting skin reactive oxygen species, protecting and stimulating matrix-related proteins, absorbing their radiation, and maintaining a water balance, however, one of the most common side effects that can be caused by these components may occur itching, rash and inflammation. Due to their antimicrobial properties, many phytochemicals such as phenolic compounds and mono- and sesqui-terpenes or phyto-extracts, Castanea sativa, are also added to skin care cosmetics (SARKER et al. 2018).

Golden Root has long been used in traditional medical systems in Europe and Asia to increase an organism's resistance to physical stress. It is thought to have antioxidant properties.***Carrot*** is a valuable plant because it is a natural source of vitamin A and other essential vitamins. Carrot seed oil is indicated as anti-aging and invigorating. Because it promotes the formation of new cells and helps reduce wrinkles. It acts as a natural rejuvenator for the skin. ***Ginkgo*** is best known as the circulating tonic for strengthening small organs to all organs, especially the brain. Capillaries become more flexible and as a result, more oxygen is given to the brain and eyes it is important in old age. Ginkgo also protects the nervous system and fights oxidation (GEDİYA et al. 2011).

Dandruff treatment

Henna is has a natural affinity with the proteins in our hair, which allows it to "stain" the color, hair shaft. ***Neem*** is used for a number of medical purposes. It is a skin cleanser in some areas where it can be used in the treatment of common cosmetic problems (GEDİYA et al. 2011).

Skin protection

Green tea protects against direct damage to the cell and relieves inflammation, according to the research of the Department of Dermatology at Columbia University, New York. Studies show that catechins in green tea are 20 times stronger than vitamin E in their antioxidant power. Men, women and children should frequently use this super shield against the devastating effects of the sun. ***Turmeric***, is used in many celebrations of Hindus. At Indian weddings, their bride rubs their body with turmeric for a radiant look. Newborn babies also rub with turmeric for good luck. Traditionally, women rub turmeric on their cheeks to produce a natural golden glow. **Calendula** is used topically to treat acne, reduces inflammation, controls bleeding, and soothes irritated tissue (GEDİYA et al. 2011). There are several studies showing that calendula cream or ointment is effective in the treatment of radiation dermatitis (MCQUESTİON 2006; BOLDERSTON 2006).

H*air care*

Amlan is rich in vitamin C, tannins and minerals such as phosphorus, iron and calcium that provide nutrition to the hair and also cause the hair to darken. ***Almond oil*** Almond oil contains a small amount of super unsaturated Omega-3 essential fatty acids. It proves to be very nourishing, softens and strengthens hair. Almond oil also proves to be a very good cleaning agent. (GEDİYA et al. 2011).

Essential Oils Used in Phytocosmetics

Essential oils are natural fragrances extracted from almost every part of a plant. Essential oils are essential and liquid aroma compounds from natural plants, usually plants. Essential oils often contain volatiles such as terpenoids, benzenoids, fatty acid derivatives and alcohols. Although essential oils are widely used in cosmetics, their actual effect is not fully understood. The use of essential oils is determined by their chemical, physical and sensory properties, which differ greatly from oil to oil. Each of the individual chemical compounds that can be found in the oil contribute to the overall character. Essential oils Inhalation, Baths, Massage, Compressors, Steam treatments, Room Odor etc. It can be used in various ways for cosmetic purposes (GEDİYA et al. 2011).

Often essential oils are used as follows:

• As auxiliary protector• To smell• For hair care• Anti-dandruff• For skin care (HETTA 2016).

Some fixed oils are used as excipients in cosmetic preparations: Rose oil, Eucalyptus oil, avocado oil, Citronella oil, almond oil, rapeseed oil, apricot kernel oil, sesame oil, sunflower oil, flaxseed oil, and palm oil (HETTA 2016; GEDİYA et al. 2011)

Antioxidants Used in Phytocosmetics

Whether synthetic or natural, exogenous or endogenous antioxidants can be effective in preventing free radical formation by clearing them or promoting their degradation and suppressing such disorders (GEDİYA et al. 2011). Epidemiological and in vitro studies on medicinal plants and vegetables strongly supported the idea that plant components with antioxidant activity may have protective effects against oxidative stress in biological systems (BLOCK 1992). It is naturally controlled by various useful compounds known as antioxidants. In addition to fruits and vegetables, herbs without a certain nutritional value can also be an important source of antioxidants. Leaves from black and green tea, which have long been used between the western and Asian populations, represent potentially health-preserving antioxidants and the most popular daily plant consumption worldwide (HİGDON 2003).

Tamarind seeds have radical scavenging activity, lipid peroxidation and anti-microbilativity activity. Its antioxidant activity is suitable for anti-wrinkle cosmetics (KRİSHNA, 1999). Vitamin C prevents free radical damage. It is beneficial in strengthening the immune system. The main source of vitamin C is carrot, orange, peach, sweet potato, broccoli etc. Vitamin E It is known to be beneficial against some cancer and heart problems. It is known as the 'scavenger of free radicals'. Vitamin E nuts, whole grains, almonds, vegetable oils, etc. (GEDİYA et al. 2011).

Undesirable Effects of Phytocosmetics

Within the scope of the increase in the use of herbal health products and the side effects of these products, the concept of phytopharmacovigilance has been developed and focused on the correct diagnosis of the plant, raw material supply, process of processing, percentage of active ingredient, microbial load, heavy metal limits, finished product and trained manpower. Phytovigilance concept for herbal products is similarly encountered (ŞARDAŞ 2010; ERNST 2004). Cosmetovigilance is the activities of collecting, evaluating and monitoring spontaneous notifications of undesirable effects observed with the use of cosmetic products, including phytocosmetics, under normal or predictable conditions.

Undesirable effect The adverse effects it creates on human health as a result of the use of a cosmetic product.

Serious adverse effects are defined as disability, functional insufficiency in hospital treatment, congenital anomalies, or adverse effects resulting in sudden life-threatening or death (YAPAR 2016).

Undesirable effects in cosmetic products are locally; allergy, irritation, phototoxicity, photoallergy, systemically; Respiratory system, blood

and organ systems are grouped as damages, teratogenic, embryotoxic, estrogenic effects, mutagenic / photomutagenic and carcinogenic / photocarcinogenic effects. . Some of the most common allergic reactions are seen as originating from fragrance / essential oil and some of them are reported as originating from vegetable raw materials (NİGAM 2009; GOOSSENS 2015).

Some effects of herbal ingredients that may cause undesirable or toxic effects with the use of phytocosmetics or cosmetic products; hypersensitivity reaction, liver toxicity, estrogenic activity, allergic reaction, skin burning, photosensitivity, dysfunction neuromotor, conjunctival inflammation, convulsion, mutagenic effect or carcinogenic effect (YAPAR 2016).

Manufacturing Standards and Stability of Phytocosmetics

Phytocosmetics should determine the intended / expected effects / properties of their herbal ingredients during the preparation / production phase and after use, after they become a finished product. Producers must ensure the quality of vegetable raw materials and finished products with appropriate quality control assessments and tests such as ISO 16128 and ISO 22716 of the International Standardization Organization (ISO) to the relevant international standards (YAPAR 2016).

Stability is the ability to maintain the quality, physical, chemical and microbiological properties of a component or finished product for the specified shelf life (Shumen 2009). Stability has a wide scope and the stability of phytocosmetics can be examined in the following four main groups.

Chemical stability: It includes the stability of the active substances in the formulation.

Physical stability: It covers the properties of the active substances in the formulation and the preparation in general (organoleptic properties-color, odor and taste, suspensibility etc.).

Microbial stability: It is important both for the level of microbial contamination and the effectiveness of the antimicrobial agent, as well as for degradation due to microbial contamination.

Toxicological stability: Interactions, incompatibility, and unexpected effects are evaluated.

The concept of expiration date in phytocosmetics is stated as the time that the product remains durable within the limits set in the packaging to be marketed. When there is no contrary record, the time required for a 10% change can be taken as shelf life. This period must be written on the inner and outer packaging label. If the period is given as month and year, it is understood that the preparation is durable until the last day of that month. If

the stability test results show that undesirable results may occur as a result of keeping the product at low or high temperatures, storage information should be included on the package. If there is no special directive or limit for storage, care should be taken to protect the products from moisture, freezing and excessive heat. Stability tests should be carried out under conditions where thermal stability and, if possible, humidity sensitivity can be examined. The test period must bear all the time and conditions from the production process to its transportation and use by the consumer (SHUMEN 2009).

Controls and analyzes can be made on plant raw materials and additives;

1. organoleptic controls, botanical controls, crop packaging, batch number, labeling (harvest date)

2. Physical controls

3. Chemical controls

4. Biological controls

5. Chromatographic and spectroscopic analysis

The components used in phytocosmetics should be evaluated in terms of irritation and sensitization reactions in body use and necessary precautions should be taken. For this purpose, sensitivity test and irritation test can be done. Accordingly, long-term stability and dermatological tests are important in evaluating the availability of herbal raw materials or phytocosmetics. Obtaining the expected effect in usability is related to the activity of phytocosmetic. The activity of phytocosmetics; The activity, storage conditions and packaging process of the chemical composition of the vegetable source depend on the microorganism and pesticide residue associated with the vegetable source. The evaluation of phytocosmetics is the same as other cosmetics in terms of legal requirements (YAPAR 2016; ZHANG 2012).

It is important in terms of chemical, physical, microbiological and toxicological stability to ensure the stability of the active substance / substances in the formulation and manufacture of a phytocosmetic within the scope of certain standards. Effectiveness and / or safety may vary partially or completely due to phytochemistry and / or product degradation. These changes can also cause undesirable effects caused by phytocosmetics. Therefore, it is necessary to determine and ensure the conditions that will ensure that the quality, effectiveness and safety of the product will remain within certain limits during the storage, distribution and use process.

Phytocosmetic and Nanotechnological Applications

An innovative approach can increase both the aesthetics and performance of a cosmetic product. The application of new approaches can also increase the effectiveness of plants in relation to the continuous effect on the human body. Today, there are many products in the market with its improved effectiveness. The formulation and selection of the approach to be used for herbal cosmetics will depend on the purpose of preparation (for topical or systemic effect; properties of the drug or plant extract, such as natural hydrophilic or hydrophobic; surface properties of a system such as permeability and charges; biodegradability, biocompatibility, toxicity; release profile Examples are the size of the required product and the antigenicity of the final product. (Newman et al. 2009). The use of nanotechnology products is increasing day by day thanks to its superiorities. Skin, which is the largest organ of our body, has a favorable feature for local and systemic drug applications. The stratum corneum layer, which is the outermost layer of the epidermis, acts as a good barrier against the external environment. Topically applied preparations from the lipid layers of the stratum corneum penetrate by diffusion. The physicochemical properties and particle size of the compound in the prepared preparation significantly affect the degree of penetration during the transition. There are passages along the hair follicles, especially hydrophobic compounds, supromolecular proteins and an important passage for carrier systems. Nanoparticles penetrate into the hair follicle ducts and are stored there. Dermotological applications of nanoparticles are for protection, diagnosis and treatment purposes. For example, there are three different types of protection methods in anti-aging product formulations developed against the harmful effects of UV rays:

• Antioxidant use

Stimulation of repair mechanisms

• Use of physical photon blocking agents

Studies have shown that products containing TiO2 and ZnO nanoparticles in Group 3 remain in the living epidermis layer and do not penetrate into the lower layers. This result is important for the reliability of nanosystems (NEWMAN et al. 2009).

Purposes of nanoparticles in cosmetic products

• Increasing the encapsulation and stability of sensitive substances such as unsaturated fatty acids, vitamins, antioxidants in the nano carrier.

• Increasing penetration of cosmeceuticals from the epidermis

• Transport of targeted active ingredients to the skin layers

• Preparation of more effective formulations of expensive substances to reduce costs

• Controlled release of cosmetic active ingredients

• Ensuring encapsulation in the carrier to minimize irritation of irritant substances on the skin.

• It can be used for purposes such as increasing the effectiveness and tolerance of UV filters such as TiO2 and ZnO on the skin surface.

Cosmetic nano-carrier systems and application examples

Herbs and spices have been used in maintaining and improving human beauty because herbs have many beneficial properties such as sunscreen, antiaging, moisturizing, antioxidant and antimicrobial effects. Chemicals have a higher toxicity. In order to improve the properties of phytocosmetics, researches are carried out to develop new approaches that can increase both the aesthetic appeal and performance of the products. In this context, approaches studied and discussed include liposomes, phytosomes, transferosomes, nanoemulsions, nanoparticles, microemulsions, nanocrystals and cubosomes (SARAF et al. 2008).

Microemulsions

Microemulsions are capable of encapsulating non-polar molecules such as lipids, flavors, antimicrobials, antioxidants and vitamins (CHEN 2006). An oil-in-water microemulsion is formulated using lecithin and an alkyl glucoside as a cosmetic tool for arbutin, and kojic acid, naturally occurring whitening agents. The stability of these compounds is higher in microemulsions than in aqueous solutions. Microemulsion of lipids with surfactant mixtures and a polar phase for sunscreen agents and octylmethoxysinamate as soy lecithin produces a water-in-water microemulsion, water-in-oil and oil-in-oil emulsions and a multiple cosmetic emulsion, nonionic, non-ethoxylated, skin compatible emulsifiers. In this system, the property of the cosmetic material is preserved (for example, ascorbic acid, an antioxidant material) (SARAF et al. 2008).

Multiple emulsions

Multiple emulsions are complex polydisperse systems that are stabilized by lipophilic and hydrophilic surfactants, respectively, with both oil-in-water and water-in-oil emulsions simultaneously. Among multiple emulsion oil-in-water and oil-in-water types, the first has wider application areas and has therefore been studied in great detail. Multiple emulsions are formulated cosmetically. As a skin moisturizer. Long-term release of chemicals was obtained with multiple structures (Okochi et al. 2000). These systems have some advantages, such as preservation. The incorporated substances and the inclusion of several substances are active

in different departments. These properties can be successfully adopted for cosmetic formulations that take different herbal extracts (RAYNAL et al. 1993).

Liposomes

Liposomes are spherical vesicular with a membrane consisting of phospholipid and cholesterol double layer. Topically applied liposomes; Since they are similar to the composition of the epidermis, they provide advantages such as increasing the accumulation of substances in the skin, encapsulating water and oil-soluble substances, being non-toxic and reducing the side effects by preventing systemic absorption (RAHİMPOUR et al. 2012).

Liposomes include water and lipid soluble pharmacologically and cosmetically active ingredients. Amphiphilic and lipophilic substances (Oil-soluble UV filters) can be included in two layers of lipids. Empty liposomes are also used in cosmetics, as they increase skin moisture. They can be single or multilayered, and their size can range from 20 nm to several hundred micrometers. While liposomes support the excretion of the encapsulated active ingredients in the epidermis and dermis, the permeability rate decreases. This helps fix the active ingredients to the outermost skin layers as desired for cosmetic products. Simultaneously, for example, sun care products containing liposome encapsulated UV filters can be delayed to be water resistant (CEVC 1997).

They have shown that liposomallinoleic acid is more effective in reducing hypermigmentation than non-liposomal formulation. Topical application of coenzyme Q10, an important antioxidant that protects cells against skin aging, protects the skin from wrinkle formation and premature aging. Lee and Tsai, solvent injection method prepared liposomformulations loaded with coenzyme Q10 of less than 200 nm. They monitored topically applied formulations in vivo. They stated that liposomal alkoenzyme Q10 formulations increased the stratum corneum penetration of the substance compared to suspension formulations (LEE et al. 2010).

Phytosomes

Phytosomes are standard extracts or purified fractions that are complexed with phospholipids for better bioavailability and enhanced activities. They are lipophilic in nature and improve the topical absorption of complex molecules, hydration, collagen structure, enzyme balance, etc. activity in skin functions such as. Topical absorption of biologically active phyto-components provides local application where needed (SARAF et al. 2008).

The phytosis process intensifies the vegetable compounds by increasing absorption, increasing bioavailability and increasing conduction

to tissues. By combining the emulsifying effect of phospholipid with standardized botanical extracts, the phytosome form significantly increases bioavailability and provides faster and improved absorption on the skin. Many phytosomes have been prepared for many popular herbal extracts, such as Ginkgo biloba, grape seed, hawthorn, milk thistle, green tea and ginseng, as they have better effects than liposomes. Ginkgo biloba terpene phytosis has been shown to be effective in relieving individual contact reactions to other substances in topical formulations. The sedative activity of silymarin has been shown to increase more than six times in the phytosome of the silymarin in experimental models. It is due to the higher affinity of the complex for skin phospholipids compared to free active principles in the activity of the phytosome form. Phosphatidylcholine, phytosis process; In addition to acting as a carrier, it nourishes the skin because it is an important part of the cell membrane. It shows better stability profile because chemical bonds are formed between phosphatidylcholine molecules and phyto-component (YANYU et al. 2006).

Transferosomes

Transferosomes are administered by a skinless method that penetrates the stratum corneum lipid lamellar regions as a result of hydration or osmotic force on the skin. It can be applied as a drug carrier for a number of small molecules, peptides, proteins and nutraceuticals. (Benson 2006) Transfersomes can penetrate the stratum corneum and provide nutrients locally, resulting in skin care to maintain their function. Both small and large hydrophobic and hydrophilic molecules are transmitted through the layer after conjugation with transferosomes (CEVC 1997).

Transferzomes are called highly deformable, elastic or ultra-flexible liposomes. The rapid penetration of the staratum corneum through the intercellular way is the reason that they are highly deformed.

Nanoparticles

Nanoparticles are very stable and have a high affinity to the stratum corneum, so it produces high bioavailability of the encapsulated material to the skin. Application of a gel containing nanoparticles loaded with vitamin A and vitamin E derivatives increases skin moisture compared to controls. Increased skin moisture is due to the high water binding capacity of phospholipids, which make up nanoparticles. It is clear that nanoparticles penetrate the upper layers of the stratum corneum. There they fuse with skin lipids and active agents are released. Ultra fine particles form a sticky film that causes an occlusive effect on the skin, which supports the penetration of active ingredients. Active ingredients such as vitamins, sunscreen, fragrance and essential oils are widely used as nanoparticles. They provide improved skin hydration and protection through improved

stability of chemically unstable active ingredients, controlled release of active ingredients, pigment effect and film formation on the skin. The preparations have low viscosity, are oil-free and show high bioavailability. Compared to liposomes, lipophilic substances are much higher burdened by nanoparticles. Effects on skin hydration and viscoelasticity are important criteria during the development of new cosmetic formulations. Solid lipid nanoparticles are a promising compound for moisturizing new cosmetic formulations. Due to their good physical stability and compatibility with other ingredients, nanoparticles can be added seamlessly to existing cosmetic formulations. Alpha lipoic acid, a new antiaging agent, is chemically unstable and produces an unpleasant odor from decomposition. Therefore, the active is encapsulated into the solid lipid nanoparticle to overcome this problem. Similarly, the amount of molecular sunscreen can be reduced by 50% while maintaining the level of protection compared to traditional emulsion (SARAF et al. 2008).

Nanoemulsions

Nanoemulsions are emulsions with a small droplet size (20-300 nm). They can be used for lipophilic and hydrophilic substances with increased biophilicity. Nanoemulsions with droplets above 100 nm appear white, while dispersions around 70 appear opaque to 100 nm and transparent below. Coenzyme Q10, also known as ubiquinone, is a unique cosmetic substance that protects the skin from premature aging, wrinkle formation and loss of cell activity. ; it is extremely lipophilic and its topical bioavailability is very low. Encapsulation of ubiquinone in nanoemulsion increases its concentration in the dermis compared to traditional formulations. Similarly, the bioavailability of herbal cosmetic extracts may also increase (ZULLİ 2006). Kim and colleagues prepared nonoemulsions of astaxanthin, a carotenoid with high antioxidant activity but limited use due to its low solubility and poor photostability, and conducted stability studies. They stated that the nanoemulsion foromulation they prepared can be considered as a stable skin care product with anti-wrinkle and moisturizing properties (KİM et al. 2011).

Multiple nanoemulsions

They are in the nano range and allow the application of several incompatible substances simultaneously. Vitamin E and coenzyme Q10, when mixed together, form a dark complex; double nanoemulsion can be successfully prepared for cosmetic purposes (MERİSKO et al. 2003).

Nanocrystals

Nanocrystal technology is an attrition where large micron-sized crystals are ground in a water-based stabilizing solution. The process produces physically stable dispersions of nanometer-sized drug crystals.

Such systems are used for flavonoids, sunscreens, nutrients, bioactive agents, etc. It can be used to improve the cosmetic formulation (YANG et al. 2002). Nanocrystals has been developed to increase the solubility and dissolution rate of low-solubility active ingredients and cosmetic active compounds, and the particle size ranges from 200-600 nm. Mitri et al. Produced the high pressure homeginasation method ilelutein nanocrystals. They allowed the substance to dissolve 24 times more in solubility. They showed that, due to the increase in surface area and solubility, the penetration of lutein into the skin and its localization in the skin increase (MİTRİ et al. 2011).

Cubosomes

Cubosomes are two-continuous cubic phases, consisting of two separate, continuous but non-intersecting hydrophilic regions, consisting of a lipid layer that is converted into a periodic minimum surface with zero mean curvature. Continuous and periodic structure, high viscosity of the bulk cubic phase. However, cubomoms prepared in dispersion maintain a nanometer structure that is the same as that of the bulk cubic phase, but give a much lower, water-like viscosity. Compared to liposomes, cubosomes have a much higher volume of particles from the two-layer area. The cubic phase is strongly bioadhesive; so it can find applications in aroma release. They have the ability to include lipophilic, amphiphilic and water-soluble cosmetic molecules. This system can be used as a challenging system for cosmetic formulations (EMBİL et al. 1996).

Transdermal delivery system

Cosmetics can be applied in many ways with a variety of approaches. However, maintaining constant in vivo concentrations over an extended period of time can be problematic. Hills and grooves are often seen when cosmetic actives are applied through the skin. In addition, high concentrations can be irritated, whereas low active concentration can be subameliorative. To alleviate such a problem, manufacturers have developed cosmetic patches, an idea adopted from the pharmaceutical industry (CHADAWAR et al. 2007).

Microsponges

Microsponges are unique for controlled release of topical agents and consist of macroporous beads typically 10 to 25 microns in diameter loaded with the active agent. When applied to the skin, its active ingredient is in a time mode and also to other stimuli (rubbing, temperature, pH, etc.) Currently used in cosmetics, over-the-counter skin care, sunscreens and prescription products. By gradually distributing the active ingredient to the skin, for example, peroxide formulations have excellent effectiveness, with minimal irritation. Macro sponges can provide more efficacy for topically

active substances with improved safety, extended product stability and improved aesthetic properties in an effective new form (SARAF et al. 2008).

Ethosomes

Lipid carrier systems, whose average dimensions can vary from 30 nm to microns, consisting mainly of phospholipids, ethanol and water, can be singular or multilayered. It is named as an ethosome because it carries a high concentration of ethanol. It has been reported that the enzymes can effectively lock molecules with various lipofolic properties and increase the permeation of substances from the skin (TOUİTOU et al. 2000).

Lipid Nanoparticles

The most important advantages of solid lipid nanoparticular (KLN) s; They are prepared with lipids, which are biodegradable, physiological compounds, they can provide controlled release of substances, and they can remain stable for a long time as their water dispersions (Numanoğlu et al. 2006). Nanostructured lipid carriers (NLT), which are second generation lipid nanoparticles prepared with solid and liquid lipid mixtures, offer advantages such as higher loading efficiency and reducing the risk of leakage of the substance from the lipid matrix during storage. When applied to the skin, KLN and NLT form a film layer and increase the moisture content of the skin by providing occlusive effect and decreasing the loss of transepidermal water. They are used as carrier for molecular sunscreen agents. Thus, the UV blocking effect increases, penetration into the skin and possible side effects decrease (WİSSİNG 2003).

Conclusion

Phytochemicals are useful chemicals found in plants. The natural compounds that we will obtain from plant sources have less side effects and higher activities. The conscious selection and evaluation of these herbal resources will contribute to benefit from them at the highest level and to lead a healthy and fit life. We can achieve good results using these compounds in phytocosmetic. By making use of the advances in technology, we will benefit from the phytocosmetic products that we will use with nanocarriers.

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Chapter 6

THORACIC TRAUMAS

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1. INTRODUCTION

Trauma is the third leading cause of death among all age groups after cancer and cardiovascular diseases (Soybir, 2005). Thoracic injuries that include chest wall, pulmonary, esophagus and cardiovascular injuries account for about 10-15% of all the traumas. It is the leading cause of deaths in the first decades around the world and also responsible for about 35% of preventable deaths after trauma (Yaldız, 2018). Morbidity and mortality associated with thoracic trauma are due to the disruption of respiration and/or circulation. Respiratory failure can occur due to direct injury to the airway or lungs, as is the case with pulmonary contusions, or indirectly after rib fractures. The common consequence is the development of ventilation-perfusion mismatch and thus the reduction of pulmonary compliance.

Thoracic traumas can be simple so that the patient can be discharged after analgesic administration, or they can be severe so as to require urgent surgery, even fatal (Yıldız & Kılıç, 2018). The main life-threatening thoracic injuries include tension pneumothorax, massive hemothorax, open pneumothorax, cardiac injuries or major vascular injuries (Schellenberg & Inaba, 2018). Trauma imaging, which has been dominated by direct radiography, has been previously used for diagnosis, while full-body tomography scans are now used especially in patients with multiple traumas. Using bedside ultrasound has stood out for unstable patients. This is mainly because very severe injuries cannot be seen in evaluating radiographs taken while the patient with thoracic trauma is laid down (Uz et al., 2013). The gold standard method is considered as computed tomography (CT).

Thoracic traumas often cause pneumonia, pleural sepsis and respiratory failure that require intensive care. They are responsible for 25% of cases that require ventilatory support (Sirmali et al., 2003).

2. ANATOMY

The chest wall consists of the rib cage (sternum, 12 pairs of ribs, 12 vertebra and intervertebral discs) and intercostal muscles. There are neurovascular bundles containing an intercostal artery, vein, and nerve, which run along the inferior boundary of each rib. Intercostal nerves contain pain fibers since they receive somatic innervation. A visceral pleura layer covers intrathoracic structures. The parietal pleura covers the internal surface of the thoracic wall. The potential space between the visceral and parietal layers is called the pleural cavity.

The main function of the thoracic wall is to facilitate breathing. During inspiration, the contraction of the diaphragm and intercostal muscles increases intrathoracic volume and decreases intrathoracic pressure. Thus,

air is passively supplied to the lungs. After the diaphragm and intercostal muscles return to their normal positions, the intrathoracic pressure is increased, causing air to be forced out of the lungs. The thoracic wall also protects the intrathoracic structures from external injuries. The sternum and clavicles serve as the connection point for the pectoralis minor and major muscles. The posterior scapula covered by muscles provides additional protection for the superior thorax. It is bounded by anteriorly the sternum, superiorly by the clavicles, inferiorly by the xiphoid, posteriorly by the vertebral column and bilaterally parietal pleura and lungs and extends towards the diaphragm. The organs in the thoracic cavity: pharynx, larynx, trachea, esophagus, bronchi, lungs, pleura, diaphragm, heart and heart veins.

3. ETIOLOGY

It is usually seen after blunt or penetrant injuries, according to the mechanism of occurrence. It often occurs after blunt traumas due to motor vehicle accidents (Liman, Kuzucu, Taştepe, Ulaşan, & Topçu, 2003). The thorax is the fourth most injured area in unrestrained motor vehicle passengers but is the most commonly injured area in individuals who are restrained by a seat belt (Mayberry, 2000). Only motor vehicle accidents account for about 70-80% of blunt chest trauma cases (Morely, Johnson, Leibner, & Shahid, 2016). Blunt traumas may occur as compression,

direct trauma (fractures) and vascular laceration. Other causes of blunt traumas may include falls, non-vehicle accidents, high-energy assault, getting stuck and explosions. Trauma does not cause any complication in the pediatric age group due to the elasticity of the thoracic wall, while it may cause a rib fracture in the elderly population.

Penetrating injuries are continuously increasing and may occur due to gunshot injuries or sharp object injuries (knife, wedge, sword, etc.). The mortality rate is higher in penetrating injuries (Edgecombe, Sigmon, Galuska, & Angus, 2020). <10% of patients with blunt trauma require surgical intervention, while 15-30% of patients with penetrating chest injury require surgical intervention.

4. PHYSICAL EXAMINATION

During the inspection of thorax, abrasions, open rib or sternum fractures, paradoxical movement of the thoracic wall (flail chest), open pneumothorax, entrance and exit wounds in perforating injuries, abdominal breathing and whether both hemithorax are ventilated equally are evaluated. Assessment of breathing is necessary to recognize major thoracic injuries such as tension pneumothorax, open pneumothorax, pulmonary contusion and massive hemothorax. Palpation, percussion and especially auscultation to be performed during the examination will provide information about whether

a pneumothorax is present (Waydhas, & Sauerland, 2007). Palpation should be started from the clavicles, and whether there is tenderness to palpation in both hemithorax, sternum and rib should be checked. Crepitations due to fractured rib or subcutaneous emphysema can be felt by palpation. Chest tenderness, presence of crepitation and the depth of wound in penetrating injuries are also evaluated by palpation. If patients with pain and tenderness in the inferior ribs have also abdominal pain, they are at increased risk of intraabdominal organ injuries (Holmes et al., 2005).

In traumatic asphyxia, subconjunctival hemorrhage and cyanosis of face and neck can be seen. Subcutaneous emphysema, which may occur in pneumomediastinum, pneumothorax or tracheal injuries, is very prominent and can progress to the neck and eyelids. In tension pneumothorax, the face and neck veins may be swollen due to the acute torsion of both vena cava. Percussion is not often used in practice, however; it is known that tympanic sound is heard in pneumothorax, and opaque sound is heard in hemothorax. In auscultation, whether the lungs are ventilated equally is checked. In pneumothorax or hemothorax, breathing sounds are decreased or absent in the affected side. In tension pneumothorax, the cardiac apex beat is displaced. Tachycardia, hypotension, tracheal displacement, and swollen neck veins are diagnostic findings, which especially should suggest tension pneumothorax (Döner, & Sivriköz, 2018).

5. DIAGNOSIS

Chest radiography (CXR) is considered as the standard initial imaging method for all stable or unstable trauma patients. One-dimensional chest radiography (CXR), which can be also used as portable, has many advantages since it is quick and cheap, and has low radiation load as well as allow for scanning the whole thorax without the need for removing the patient from the trauma unit. CXR allows for evaluation of not only the lungs and the pleural area but also the bony structures including mediastinum, aorta, diaphragm and ribs, thoracic vertebrae, clavicle, rib cage, and scapula. ATLS emphasized that CXR should be used as a part of any evaluation following a traumatic injury to make a decision on CT scanning. In a study by Exadaktylos et al. CT revealed more than one pathological finding in about 50% of patients with blunt chest trauma with whom the first CXR appeared normal (2001).

Pneumothorax, hemothorax, pericardial tamponade, rib fractures and sternum fractures can be detected by bedside USG. It has the same specificity and higher sensitivity compared to CXR especially in the detection of c. USG has a sensitivity of 81-98% and a specificity of nearly 100% in the detection of pneumothorax (Uz et al., 2013). In emergency departments, occult pneumothorax can be detected by USG as accurately as by CT (Blaivas, Lyon, & Duggal, 2005).

CRX scan is not required in patients with a penetrating trauma away from the thorax. However, physicians should note that unknown bullet trajectories may cause damage in remote areas. For patients with multisystem blunt trauma, CRX should be taken with the patient in lying position. Patients with abnormal CRX findings should be scanned by CT (Rodriguez, Hendey, & Mower, 2017).

Since FAST is a rapid and practical method, it can be applied quickly in the trauma region in stable and unstable patients. There are four windows in FAST: right upper quadrant, left upper quadrant, cardiac and pelvic window. Recently, EFAST has been introduced by adding thoracic window to these 4 windows to evaluate pneumothorax and hemothorax. FAST has a specificity of nearly 100% and a sensitivity of 97% in the determination of penetrating cardiac injuries. In the case of cardiac injuries, the laceration of the pericardium may lead to the decompression of blood to the left hemithorax, causing the pericardial sac to appear falsely normal in USG. In EFAST, the USG image of the thorax to evaluate pneumothorax is obtained by scanning the lung areas in the midclavicular line in the 2^{nd}-4^{th} intercostal spaces and in the midaxillary line in the 6^{th}-8^{th} intercostal spaces. EFAST has a sensitivity of 86-100% and a specificity of 97-100% in the determination of pneumothorax. This makes EFAST superior than CXR in the investigation of pneumothorax because CXR has a sensitivity of 27-83% and a specificity of nearly 100%. Thorax CT scan has a sensitivity of 74-82% and a specificity of 100%. CT scanning has important limitations such as being expensive and radiation exposure. Since each patient cannot be scanned by CT, the patients should be selected carefully. A balance should be established between the risks arising from the number of patients to be skipped when a thorax CT scan cannot be performed and discharging the patients, and the cost and radiation exposure if a thorax CT scan is performed (Kea et al., 2013). Unstable patients with penetrating thorax trauma should be evaluated firstly by bedside USG and portable CXR for cardiac tamponade, tension pneumothorax, massive hemothorax.

6. THORAX INJURIES

6.1. Pneumothorax

It is defined as the presence of air between parietal and visceral pleural cavity. It may occur due to blunt or penetrating traumas. The size of pneumothorax was classified into three categories: mild (if it covers 15% of the total lung tissue), moderate (15-60%) (**Picture 1**) and massive (more than 60%). There are 4 types of pneumothorax including simple, open, tension and occult.

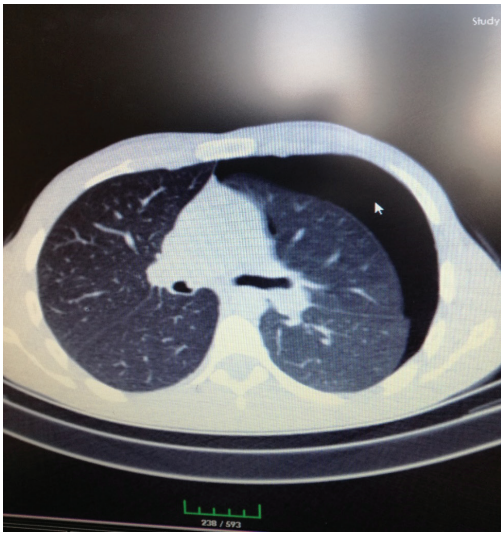

Figure 1. *Moderate pneumothorax in the left hemithorax*

6.1.1. Simple Pneumothorax

It often occurs due to the penetration of rib fractures to the lung. It may sometimes occur without a rib fracture due to the rupture of bullae in the parenchyma with the effect of barotrauma following blunt trauma. Physical examination shows that breathing sounds are decreased or absent in the affected side. Hypersonority to percussion is detected, while subcutaneous emphysema is felt by palpation. It can be diagnosed by seeing the lung border on CXR. In suspicious cases where any decision cannot be made with CXR, CT or USG is preferred. The size of pneumothorax is calculated by the method "the percentage of pneumothorax = 100-collapsed volume/ hemothorax volume" (Uz et al., 2013).

In an occult or low-percentage pneumothorax, it is sufficient to observe the patient. In a moderate or massive pneumothorax, tube thoracostomy (TT) is applied. In patients who need mechanical ventilation after trauma, TT should be applied even in the presence of minimal pneumothorax. In cases where there is no expansion and there is a persistent air leak, tracheobronchial tree rupture should be considered and bronchoscopy should be performed immediately. If it is due to a rupture in the tracheobronchial tree or esophagus, urgent surgery should be performed.

6.1.2. Open Pneumothorax

It mostly occurs due to penetrating traumas. The presence of a wide wound in the pleural cavity in the thoracic wall causes the penetration of air into the pleural cavity, and thus open pneumothorax occurs. If the cavity in the thoracic wall is more than 2/3 of the diameter of the trachea,

air passes preferentially through a less-resistant pathway, thus the pleural cavity is filled with air with each inhaled breath. Air passes through the thorax so that it causes severe respiratory distress and hypoxia. Therefore, as air enters and exits from the pleural cavity, the mediastinum shifts to the healthy side during inspiration and to the injured side during expiration. This is called mediastinal flutter. This may lead to mediastinal fluctuation, bending of the vena cava, decreased venous return, hypotension and tachycardia and eventually the death of the patient. Hence, after the defect in the thoracic wall causing open pneumothorax is closed immediately, air in the pleural cavity should be discharged by TT. The defect should be closed with a wide and impermeable sponge by plastering three edges. Thus, air will exit from the wound hole with each expiration, but it will not enter inside during inspiration. If the diameter of the defect is 7 times higher than the diameter of the trachea, then sudden death may occur (Çevik et al., 2006).

6.1.3. Tension Pneumothorax

It is a serious condition that requires immediate intervention. Tension pneumothorax occurs due to a one-way air leak from the tracheobronchial system to the pleural cavity and/or lung parenchyma. It creates a one-way valve system in pulmonary injury; it allows for air intake during inspiration, while intrapleural air is significantly increased due to blocked air discharge during expiration. The increased air in the pleural cavity causes increased pressure and thus tension pneumothorax. Thus, each time air enters inside, the affected lung deflates and thus collapses rapidly. Pressure occurs in the other side, which pushes vascular structures in the mediastinum and the heart, thus preventing venous return to the heart. Clinically, respiratory failure and cardiovascular collapse are observed.

Pneumothorax is seen in the majority of penetrating traumas, while it is seen in about 15-50% of blunt traumas (Tuncozgur et al., 2001). Tension pneumothorax may result in death if it is diagnosed and treated immediately. Tension pneumothorax should be identified clinically and is not necessarily verified radiologically (Cangir et al., 2005). During the examination of patients, chest pain, respiratory distress, tachycardia, dyspnea, hypotension, unilateral respiratory sound loss are observed. In the late period, tracheal deviation, venous distension in the neck and cyanosis may be seen. The similarity of symptoms and clinical presentation can sometimes be confused with cardiac tamponade. It is distinguished from cardiac tamponade due to hypersonarity at percussion and absence of respiratory sounds.

The leading causes of tension pneumothorax include barotrauma caused by mechanical ventilation as a result of PEEP (positive expiration end pressure) application, and blunt thorax traumas leading to serious

parenchyma laceration and major rupture of emphysematous bullae. In the treatment, decompression should be applied immediately. A wide-diameter branule is advanced into the pleural cavity, grazing the superior of the rib at the intersection point of the second intercostal space and the mid-clavicular line. Thus, we convert it to open pneumothorax, and then TT is applied. In patients with cardiac arrest, the most common reversible cause of death is tension pneumothorax (Mistry, Bleetman, & Roberts, 2009).

6.1.4. Occult Pneumothorax

It is diagnosed by chest radiography or thorax tomography. If a pneumothorax is detected by tomography (**Picture 2**), but it cannot be seen in chest radiographs, then it is called as occult pneumothorax (Barthwal et al., 2016). Occult pneumothorax is seen in 1.8-6% of all trauma registries. About 40% of patients with traumatic pneumothorax have an occult pneumothorax (Hills, Edmisten, Holtzman, & Wright, 1999). In occult pneumothorax cases, if clinical observation cannot be made, then TT should be applied.

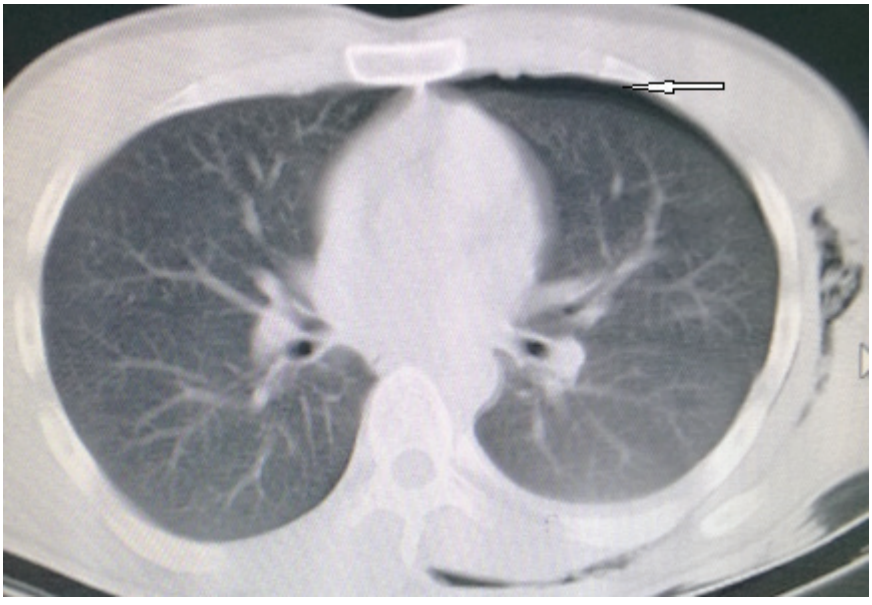

Figure 2. *Subcutaneous emphysema in the left side and minimal pneumothorax in the left hemithorax following penetrating injury*

6.2. Hemothorax

It is the accumulation of blood between the pleura and the lung, mostly after a trauma. Traumas can be classified as sharp object injuries, blunt injuries and blast injuries. Its etiology includes traffic accidents, firegun and sharp object injuries, falling from height and compression injuries

(crushing under heavy objects). Traffic accidents have a particular role in the development of hemothorax (Battistella & Benfield, 2000).

Bleeding usually depends on the damage to the lung and intercostal veins by fractured rib tips due to a rib fracture. Venous bleeding usually stops spontaneously. In complicated thoracic wall injuries, there are multiple fractures in at least 3 consecutive ribs, which is often accompanied by hemothorax. Late hemothorax may occur following intercostal vein injury with movements of fractured tips during breathing or coughing.

The severity of hemothorax due to penetrating trauma depends on the type of sharp object and the pathway in the thorax that it follows. The amount of bleeding and the severity of clinical presentation are higher in firegun injuries than sharp object injuries. In penetrating thorax injuries, lung parenchyma is also mostly injured (Eser & Günay, 2016).

If the amount of bleeding in the pleura is ≤400 ml, then the hemothorax is called minimal; if it is between 400-1000 ml, then the hemothorax is called moderate; and if it is >1500 ml, then the hemothorax is called massive. The reason why the threshold is taken as 400 ml is the fact that a liquid is identifiable in CXR when its amount reaches about 400 ml. In patients with massive hemothorax, bleeding may also occur outside the lung; there may be intraabdominal injuries leading to severe organ injuries such as liver, splenic, etc. In case of severe blood losses, hypovolemia as well as hypoxia due to alveolar hypoventilation may occur. CT should be the first choice in patients with multiple traumas since blood is diffuse in the pleural cavity while the patient is in lying position. CT also helps to identify pathologies accompanying hemothorax such as rib fracture, pneumothorax and lung contusion (Goodman, Traill, Philips, Berger, & Gleeson, 1999). If TT is applied early, the patient may die due to excess blood loss since the pressure on the region where bleeding stops will be eliminated. Liquid replacement should be made to prevent this.

6.3. Lung Contusion: It is characterized by bleeding and edema due to compression-decompression injuries of the lung parenchyma or secondary to rib fractures. It is the most common lung injury after blunt traumas. Decreased breathing sounds may be noted in the affected hemithorax. Contusions are often located near bony structures. Visible opacity areas in scanning are diagnostic for lung contusion. It should be noted that the first radiograph taken may be normal, and a radiological finding may be obtained after 6 hours. Ground glass opacities are seen on radiographs, and diffuse consolidation areas are seen in the presence of severe contusion. The presence of lung contusion promotes pneumonia predisposes to pneumonia. In patients with whom the contusion area is more than 20% of the lung volume, the probability of occurrence of acute lung injury is 80% (Miller e al., 2001). Lung contusion is diagnosed by CT with 100%

accuracy, and the complications of acute lung injury may be estimated according to the severity of the contusion. CT findings: faint ground-glass density in the parenchyma and consolidation (Kaewlai, Avery, Asrani, & Novelline, 2008). The primary treatment involves the administration of analgesics and ventilation support. In patients whose less than a fourth of the total lung volume is affected, ventilation support is not required, otherwise ventilation support is required.

6.4. Pneumomediastinum

It occurs when air leaks into the mediastinum after alveolar rupture due to blunt trauma. There may be no clinical symptoms. Mild chest pain, voice change, cough and stridor may be seen. Regression is noted in repetitive scans. The larynx, pharynx, trachea, bronchus and esophagus should be checked for severe injuries.

6.5. Traumatic Chylothorax

It is a rare condition that mostly occurs due to trauma. The thoracic duct is ruptured due to sudden hyperextension while passing through the thorax. In such injury that occurs just above the diaphragm, cheilosis liquid accumulates in the thorax cavity. Chylothorax may occur due to firegun or sharp object injury as well as iatrogenic left subclavian catheterization (Çobanoğlu, Ekin, & Kemik, 2017). Upper injuries are mostly located in the left hemithorax, while lower injuries are mostly located in the right side. The primary treatment involves inserting a thoracic tube.

7. Thoracic Wall Injuries

7.1. Rib Fractures

These are the most common bone fractures in thorax trauma, and they constitute the main reason for hospitalizations (Henry et al., 2014). 50% of rib fractures, especially in the first five ribs, are not prominent in conventional radiographs taken in the first days of the injury. In patients with severe trauma, lung scans should be taken in the presence of severely displaced rib fractures (Chung et al., 2014). The 4^{th} - 9^{th} ribs are affected mostly. If there is a fracture in the 9^{th} -12^{nd} ribs, abdominal organ injury should be investigated. A fracture of the first 2 ribs shows that the trauma is severe.

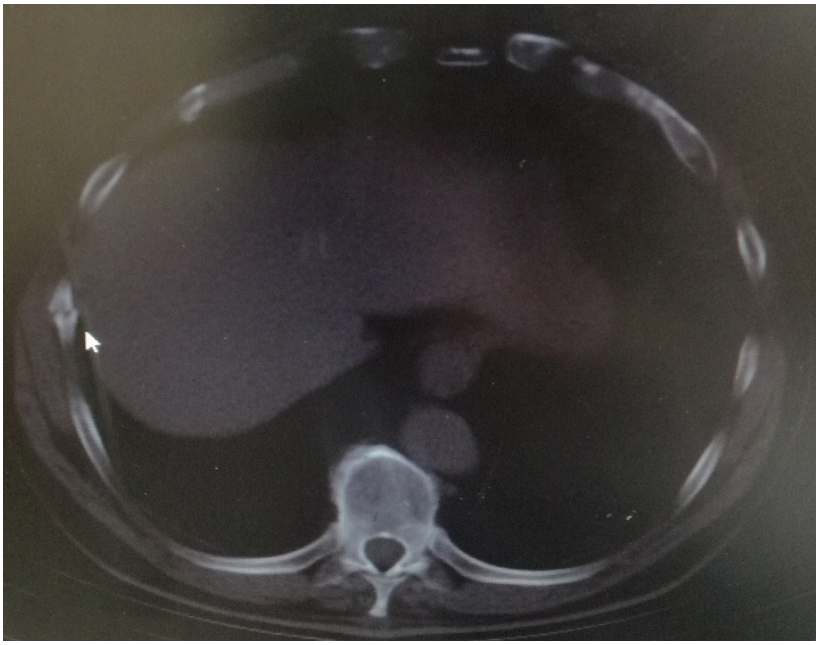

Figure 3. *View of the fracture in the 7th rib in the right side*

In the case of rib fractures, hospitalization is usually not required (**Picture 3**); however, older and obese patients with 2 ≥ fractures should be hospitalized to prevent complications such as pain-related immobilization, having difficulty coughing up, having difficulty discharging secretion, atelectasis and pneumonia. If the patient can do breathing exercises and has regressed clinical symptoms, then he/she can be discharged. Surgical intervention is preferred in those who do not sufficiently benefit from mechanical ventilation treatment, those whose thoracic wall integrity has been severely impaired, and those whose pain has not been relieved despite of intercostal block, narcotic and nonsteroidal anti-inflammatory analgesics due to improper union (A. Sarıtaş, Güneren, P. U. Sarıtaş, Kızılkaya, & Ugış, 2014).

7.2. Sternal Fracture

Sternal fracture following thorax traumas is less common, however, it has been increased due to decreased seat belt usage rate. A sternal fracture occurs due to high-energy trauma. It is mostly seen in women and elders (Odell et al., 2013).

Pain and localized tenderness on the sternum, and respiratory distress symptoms may be seen. Sternal fractures mostly occur in the body or the manubrium (Çobanoğlu, Hız, Sayır, Ediz, & Şehitoğulları, 2013). The best diagnosis methods are lateral chest radiography and thorax CT. USG is a useful diagnosis method, even some researchers think that it is more

effective than conventional radiographs (You, Y E. Chung, Kim, Park, & S. P. Chung, 2010). The mortality rate is low (<1%). ECG is required in these patients due to the risk of myocardial contusion. The presence of pericardial fluid should be checked by ECHO. In the follow-up of the patients, control ECG should be performed and cardiac enzymes should be checked. If there are no excessively displaced fracture tips and the fracture is not open, bed rest for about 1 month is sufficient. Open reduction and fixation may be required in those with excessively displaced and/or open fractures (Dubus, 2018).

7.3. Clavicle Fractures

The clavicle is mostly fractured from its middle. In severe segmental or angled fractures, subclavian artery vein and brachial plexus injuries should be investigated. There may be pain and deformity in the fracture localization. It can be easily diagnosed by direct radiography. Sternoclavicular dislocations may be anterior or posterior. In anterior sternoclavicular dislocations, analgesic administration and immobilization in a sling are sufficient. In posterior sternoclavicular dislocations, the immediate reduction is required. The proximal clavicle is pulled up using towel clamp (Morell & Thyagarajan, 2016).

7.4. Scapula Fracture

It is a rare condition that occurs due to severe thorax trauma. Because the scapula is a thick as well as well-protected bone. The fracture line may be detected by CXR and thorax CT. Such fractures may be accompanied by brachial plexus injury, rib fractures, lung contusion, axillary or brachial artery injuries. Neck-arm sling or posterior eight-bandage are sufficient in the treatment. Surgical treatment is required in fractures that cause rarely severely displaced and neurovascular injury (especially glenohumeral joint fractures) (Dubus, 2018).

7.5. Traumatic Asphyxia (Perthes syndrome)

It occurs as a result of sudden compression of the abdomen or chest when the glottis is closed during a deep inspiration. In the meantime, the pressure is increased in the superior vena cava and the venous flow is reversed. Thus, capillary vessels in the face and neck are ruptured. Therefore, craniofacial cyanosis, petechiae and edema, subconjunctival bleeding and periorbital bruises occur. In the treatment, neurological follow-ups should be performed with the patient's in 30^{o} head-up position (Dubus, 2018).

7.6. Flail Chest

Flail chest, which causes paradoxical breathing of the thoracic wall, occurs as a result of the fracture of at least three consecutive ribs in two

or more places (Uz, 2017a). A flail chest may also occur as a result of unilateral or bilateral impairment of thoracic wall stability in costochondral junction separation (Çubuk & Yücel, 2012). The free movement of a part of the thoracic wall causes paradoxical breathing and respiratory failure. Mostly, there is an underlying lung contusion. At first, it may not be noticed as the patient breathes superficially due to the pain. The patient has severe chest pain and dyspnea. The fractured region collapses during inspiration, while it moves out during expiration, causing paradoxical movement. Thus, lung expansion is prevented. The fractured ribs can be seen by CRX; however, costochondral junction separation cannot be seen (**Picture 4**). Complications such as rib fractures, lung contusion and hemopneumothorax can be evaluated more easily with the thorax and upper abdominal CT (Dubus, 2018).

In the treatment of mild and moderate traumas, analgesics and oxygen support will be sufficient. Narcotics, epidural analgesia and local anesthetics as a pain reliever, and intercostal nerve block can be used. Ventilator support may be needed in severe traumas. The injured lung is susceptible to fluid overload, and care should be taken against overload. In the choice of intubation and mechanical ventilation, the decision should be made based on respiratory rate, arterial oxygenation and fatigue of respiratory muscles. If the respiratory rate is >30, the O_2 saturation is <90%, PaO_2 is <60mmHg, $PaCO_2$ is >55 mmHg, then the patient should be intubated (Jones, 2008). Surgical intervention is performed to reduce the need for mechanical ventilation support.

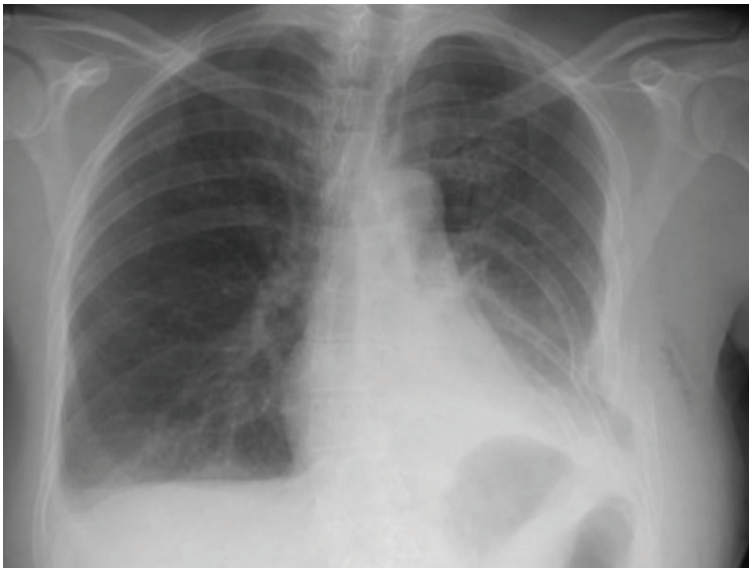

Figure 4. *CRX image of flail chest with a diffuse rib fracture and bilateral hemothorax in the left side*

7.7. Subcutaneous Emphysema

It is a condition when air passes into subcutaneous tissues. Emphysema may occur as a result of the piercing of the fractured ribs into the lung due to thorax trauma, traumatic tracheal rupture and/or esophageal perforation. Since it usually has a mild course, it does not require special treatment; however, if there is an excessive air leak so that it causes respiratory distress and prevents lung expansion, then additional treatment methods such as transcutaneous cannulas, skin incision, pre-tracheal fasciotomy may be required (Toktur & Kurkcuoglu, 2012).

Post-traumatic subcutaneous emphysema and pneumomediastinum mostly indicate underlying lung parenchyma or air leak from certain organs such as the trachea or esophagus (Molnar, 2010). Subcutaneous emphysema and pneumomediastinum are the most common findings in central airway injuries. Especially in the presence of severe pneumomediastinum increasing with mechanical ventilation, persistent pneumothorax, atelectasis, and subcutaneous emphysema, bronchoscopy should be performed with suspicion of tracheobronchial injury (Jr et al., 2017).

8. Cardiovascular Injuries

The heart is protected within the rib cage against external impacts. However, blunt heart trauma is responsible for about 15% of deaths associated with traffic accidents (Karakuş, Kuvandık, & Fansa, 2014). Both blunt and penetrating traumas affect mostly the right ventricle of the heart.

8.1. Cardiac Tamponade: It mostly occurs as a result of penetrating injuries. Blood accumulates rapidly between the pericardial leaves, which restricts the movement of the heart. In the physical examination, conventional Beck's Triad (hypotension, muffled heart tones and increased venous pressure), Kussmaul's sign (paradoxical venous pressure anomaly) and pulsus paradoxus signs are seen. However, these physical examination findings are not too specific; the Beck's Triad is seen only in 40% of those who are operated due to cardiac tamponade (Kapadia & Topol, 2007). In the diagnosis, USG is an important examination to evaluate the pericardium. USG shows the pericardial fluid with an accuracy of 90% (Uz, 2017b). In patients with tamponade, urgent pericardiocentesis will correct the hemodynamics of the patient.

8.2. Myocardial Contusion: It is often seen in the presence of sternum and rib fractures. Following contusion, arrhythmia, myocardial rupture, ventricular rupture, hypotension, and left ventricular failure occur. ECG shows sinus tachycardia, ST changes, atrial fibrillation, right bundle branch block, and ventricular premature beats. ECHO shows pericardial

fluid and wall motion anomaly. Cardiac marker levels in the blood are elevated (Rajan & Zellweger, 2004).

8.3. Myocardial Laceration and Rupture It is a rare condition that occurs as a result of blunt thorax traumas and is mostly seen in the right ventricle. However, it is the leading cause of death associated with blunt thorax traumas. The cause of death is severe bleeding and/or cardiac tamponade.

9. Other Special Injuries

9.1. Esophageal Rupture: It occurs especially as a result of penetrating injuries of the neck region. The morbidity and mortality rates are very high. The clinical symptoms may include chest pain, dysphagia, subcutaneous/mediastinal air, pneumothorax, pleural effusion. Thorax CT with contrast is performed for diagnosis. CT may show impaired esophageal wall integrity, extramural air around the esophagus, pneumomediastinum, mediastinitis, esophageal wall thickening and cervical emphysema (N. B. Topal & U. Topal, 2009). In the treatment, thorax tube should be inserted immediately.

9.2. Diaphragm Rupture: It is more commonly seen on the left side, while it is less commonly seen on the right side due to the buffer effect of the liver. Ruptures are mostly seen in the posterolateral section which is the weakest part of the diaphragm. CXR may show irregularity or disappearance in the diaphragm contour, and herniation of the abdominal organs into the thorax in the affected side (**Picture 5**) (N. B. Topal & U. Topal, 2009). It causes respiratory distress and poses a risk of strangulation. In the diagnosis, CXR shows unilateral diaphragmatic elevation and an increase in basal infiltration. Thorax CT is used for the final diagnosis. A nasogastric catheter is inserted to the patient for decompression before surgery.

9.3. Tracheal and Bronchial Injuries: Bronchial injuries are more common than tracheal injuries, and mostly occurs from the carina level. On CXR, posterolateral displacement of the lung due to disconnection of the injured portion is called ‘fallen lung sign’. The image of intramural air on the affected side is called ‘double-wall sign’. However, both findings are rarely seen in practice. CT more clearly shows the wall discontinuity in the affected area of the airway (Traub et al., 2007).

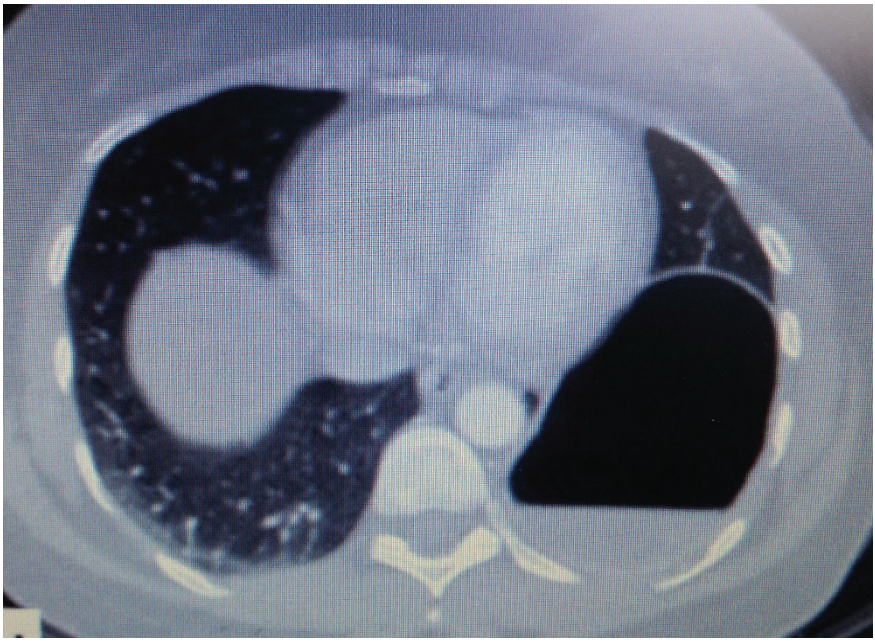

Figure 5. *Gastric herniation in the left hemithorax due to diaphragm rupture on CT*

10. TREATMENT

The most effective treatment method is intermittent positive pressure mechanical ventilation. However, mechanical ventilation is not indicated only in the presence of paradoxical movement. If the patient has tachypnea, the PaO_2 pressure in blood gas is less than 60 mmHg and the $PaCO_2$ pressure is higher than 45 mmHg, mechanical ventilation treatment should be applied. Continuous positive airway pressure may be also ensured by CPAP (Continuous Positive Airway Pressure) method. It prevents atelectasis and deterioration of blood gas in patients with spontaneous ventilation.

In patients with flail chest, the presence of lung contusion increases the risk of pneumonia. Rigid bronchoscopy may be required to clear secretion. In such patients, the mortality rate may range from 10% to 15%. The cause of early mortality is mostly massive hemothorax or lung contusion. The cause of late mortality is ARDS (Dubus, 2018). In patients with pneumothorax, radiologic scans should be repeated to follow the clinical course.

10.1. Tube Thoracostomy Procedure

It is the evacuation of air and blood using the underwater drainage system after a catheter is inserted into the chest through the incision into the thorax via a scalpel.

Firstly, the skin is cleaned with povidone-iodine. The incision is preferably started from the 5th intercostal space in the middle axillary line. A local anesthetic is administered to subcutaneous deep tissues, muscles and parietal pleura. Then, a skin incision of about 2 cm is made to establish a tunnel over the rib via a clamp. The intercostal muscle is incised over the rib to reach the pleura via a clamp. When the tube is inserted into the pleural space, it should be pulled anteriorly and superiorly towards the apex as much as possible. If there is hemothorax, the tube should be placed posteriorly and laterally. After the procedure is completed, the tube is fixed by suturing to the skin. Then, the position of the tube is checked by CXR. 24F-28F tube should be preferred for pneumothorax, and 32F-40F tube should be preferred for hemothorax. The underwater drainage tube should be located at 1 m below the patient.

The aspiration tube should be left in place for at least 24 hours after the air leaks from chest tubes completely stop (if they are inserted for simple pneumothorax) or the rate of serous drainage fluid is <200 mL/24 hour (if hemothorax tube is inserted) (Younes, Gross, Aguiar, Haddad, & Deheinzelin, 2002). It was found that the administration of prophylactic antibiotics to patients with whom the chest tube will remain for a long time has reduced the occurrence of empyema by about 6% (Wilson & Nichols, 2000). If the patient will be transferred to another health center, then the chest tube should be clamped.

If there is a leakage in the underwater drainage system or outer part of the tube, the tube is not positioned properly, the bronchi or bronchioles are obstructed by secretions, and there is a wide rupture in one of the main bronchi or lung parenchyma, then the lung cannot be fully expanded or pneumothorax cannot be evacuated.

10.2. When chest surgery is required?

In the majority of patients with intrathoracic bleeding, evacuation of hemothorax by tube provides sufficient treatment. However, <5% of cases need surgical treatment (thoracotomy). The ATLS guidelines define this as follows:

I. In case of blood loss > 1,500 mL at first or more than 200 mL/hour during the first 2–4 hours from chest tube drainage

II. Hemoptysis

III. Massive subcutaneous emphysema

IV. Severe air leakage in the underwater drainage tube

V. Uncertain CXR or thorax CT images

VI. Penetrating chest trauma.

10.3. When urgent thoracic surgical intervention is indicated?

I. Blood loss 150 mL - 200 mL/hour within 2-4 hours

II. Endobronchial blood loss

III. Impaired mechanical ventilation

IV. Stubborn pneumothorax

V. Presence of large air leakage despite chest tube

VI. Massive contusion

VII. Tracheobronchial tree injury

VIII. Injury of the heart or large vessels

(ATLS Subcommittee, American College of Surgeons' Committee on Trauma, International ATLS working group, 2013).

If the injury involves the diaphragm, then laparotomy or laparoscopy may be required due to an increased risk of intraabdominal injury. Urgent bronchoscopy should be performed to detect and repair the injury in a part of the tracheobronchial tree or the patency of the bronchi. Early thoracotomy is indicated if the air leakage continues or the lung is not sufficiently expanded despite these applications.

11. CONCLUSION

Patients with thorax trauma should be evaluated rapidly and systematically. In patients with blunt trauma, mostly thoracic wall injuries, rib fractures, sternum fractures, lung contusion, pneumothorax, hemothorax, myocardial contusion are seen. However, in patients with penetrating trauma, mostly pneumothorax, hemothorax, cardiac tamponade and large vessel injuries are often seen (Başoğlu, Akdağ, Çelik, & Demircan, 2004). The thoracic injury should be scanned according to the Advanced Trauma Life Support (ATLS) protocol. The main purpose is to determine life-threatening injuries in an emergency and to treat and stabilize the patient. Rapid and correct interventions by physicians will reduce the morbidity and mortality rates.

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Chapter 7

SHOULDER REGION TRAUMAS

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Introduction

The shoulder joint is the joint with the greatest range of motion in the body. We very often benefit from the 360° range of motion of the joint to meet our daily needs. Therefore, it is at higher risk for instability than other joints. Shoulder region traumas are very often seen in emergency departments, and shoulder dislocations are the most commonly seen shoulder region injuries. Shoulder region injury occurs as a result of a trauma or overuse (Matsen and Arntz, 1990). Since complications in traumas of this region will make our daily lives difficult, the shoulder region should be very well known by physicians and appropriate treatments should be applied.

Anatomy of Shoulder Region

The shoulder joint is the most complex joint in the body, connecting the upper limb to the trunk and allowing it to take numerous positions. The shoulder joint is not a simple joint, which consists of glenohumeral joint (GHJ), acromioclavicular joint, sternoclavicular joint and scapulothoracic joint. Normal shoulder movements involve the common movement of these four joints which are called as the shoulder junction (Hawkins and Abrams, 1987) (Figure 1).

The clavicle, scapula and humerus compose the bone skeleton of the shoulder junction. The shoulder is connected to the axial skeleton with a large amount of muscular structures as well as by the articulation of the clavicle with the thoracic cage in the sternoclavicular joint (Dalton,1998) (Figure1).

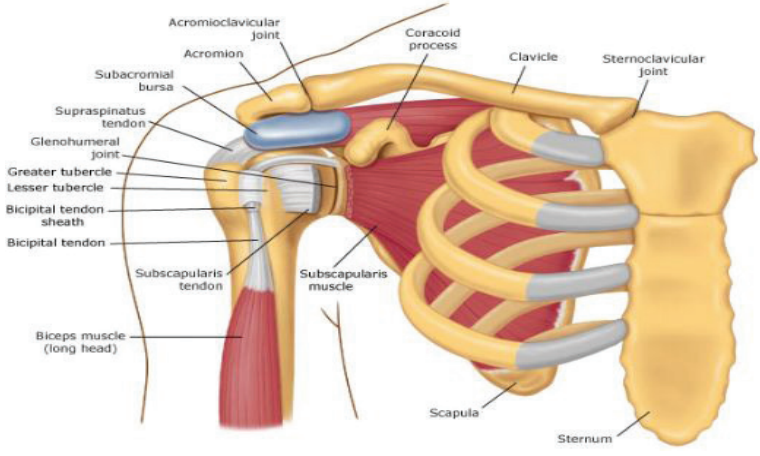

Figure 1: Anatomy of Shoulder Region

Bone Structure of the Shoulder Junction:

Clavicle: The clavicle connects the upper extremity to the axial skeleton. The clavicle is a flat bone when viewed from front, while it looks

like the italic S letter in the transverse plane. It articulates with the sternum and 1st rib interiorly and with the acromion exteriorly. The clavicle, that holds the arm separate from the trunk and serves as a support, plays a role in transmission of the force applied on the upper extremity to the axial skeleton (Snell, 1995).

Scapula: The scapula is a flat and triangular bone that lies between the 2nd and 7th ribs in the posterior thoracic wall. The free outer end of the spina scapula, called the acromion, articulates with the clavicle. There is pear-shaped glenoid cavity in the upper outer corner of the scapula, which articulates with the humeral head. The coracoid process originates from the base of the neck of the scapula glenoid and has a hook-like structure, pointing laterally forward (Jobe, 1998).

Humerus: The humerus articulates with the scapula to form the shoulder joint and with the radius and ulna to form the elbow joint. The proximal humerus consists of head, neck, lesser tubercle and greater lesser tubercle. The tendon of the long head of the biceps muscle passes between the two tubercles. The humeral head forms an angle of 130-150° with the shaft (Netter,2003).

Shoulder Junction Joints and Ligaments:

Sternoclavicular Joint: It is the only joint between the upper extremity and the axial skeleton. It connects the shoulder joint and the upper extremity to the thorax. It is the joint between the manubrium of the sternum and the proximal of clavicle (Gürsel, 2002). This disk helps to absorb shock from the arm and shoulder, and to stabilize the shoulder together with the ligaments (Bogumill, 2002). The greatest ligaments of the joint are anterior and posterior sternoclavicular ligaments (Jobe, 1998).

Acromioclavicular Joint: The acromioclavicular joint is a plane synovial joint within the glenoid, which helps increase the range of motion of the humerus. The joint is divided into two by a fibrocartilaginous disc in between (Magee, 1996). Upward and downward movement of the acromioclavicular joint allows for 20° rotation between the acromion and the clavicle in the first 20° and last 40° of shoulder abduction (Jobe, 1998).

Glenohumeral Joint: The glenohumeral joint is a ball-socket joint between the glenoid fossa and the humeral head (Peat, 1986). Static stability of the joint is provided by the joint capsule and ligaments, while its dynamic stability is provided by rotator cuff muscles. The joint capsule surrounds around the humeral head in a wide area, while it tightly attaches to the bone around the glenoid (Peat, 1986). The joint capsule is located between the neck of the humerus and the wall of the glenoid (Akgün, 1997). When the capsule loses its laxity and is hardened, the movements of the joint are significantly limited (Gürsel, 2002). The static

(passive) stabilizers of the glenohumeral joint are joint capsule, glenoid labrum, coracohumeral ligament, glenohumeral ligament, coracoacromial ligament and articular surface of the glenoid pit (O'Brien, 1990). The dynamic stabilizers of the glenohumeral joint are rotator cuff muscles. The subscapularis is located anteriorly, the supraspinatus is located superiorly, and the infraspinatus and teres minor muscles are located posteriorly. The activity of these muscles is to ensure that the humeral head is located in the center of the glenoid cavity (Dalton,1998).

Artery and Nerves of the Shoulder Joint:

The shoulder joint is supplied with blood by 6 arteries. These are anterior and posterior circumflex humeral, suprascapular, thoracoacromial, suprahumeral, subscapular arteries (Peat, 1986).

Neural innervation of the shoulder joint is provided by nervus axillaris, nervus musculocutaneus, nervus subscapularis and suprascapularis nerves (Peat, 1986).

General Approach

Medical History

The complaints of most patients with trauma are pain, stiffness, instability or weakness. Pain arises from shoulder-specific factors or many other factors. Extrinsic causes of shoulder pain include disorders of the cervical spine, thoracic outlet myocardium and diaphragmatic irritation.

Depending on the shoulder trauma, the patient should be asked questions about the time and mechanism of the injury, the precise location of the pain and associated motor complaints. Shoulder pain can manifest in an insidious manner without any precipitating factor. In this case, the duration, location, character, aggravating and alleviating factors of the pain should be also investigated in detail.

Stiffness may result from a restricted range of motion due to a previously existing painful condition of the shoulder. It may cause instability or a sensation of the shoulder almost "going out". A rotator cuff tear or an underlying nerve lesion usually causes a severe shoulder weakness.

Physical Examination

In addition to the axilla, the anterior, posterior and lateral sections of the shoulder should be also examined. The presence of any obvious deformity, ecchymosis, laceration, swelling or hematoma should be investigated.

Palpation of the shoulder should be performed starting from the sternoclavicular joint and moving laterally to the acromioclavicular joint. Then, the scapula, glenohumeral joint, and humerus should be

palpated. The presence of any tenderness point, crepitation, swelling, or deformity should be noted.

Active and passive ranges of motion are tested. However, such acute pain may be sometimes limited with the cause. Active range of motion is well evaluated with the patient in the sitting position. However, passive range of motion is best evaluated with the patient in the supine position. For the rotator cuff, resisted internal rotation, resisted external rotation and empty can test are performed.

The examination is completed by neurovascular function evaluation. The complete motor and sensory examination of the brachial plexus is performed. The radial pulse should also be checked, even if there is an underlying vascular injury. The presence of pallor, paresthesia, or severe hematoma should suggest a vascular injury.

Radiologic Imaging

Physical examination is usually sufficient to determine the majority of shoulder injuries. However, if required, radiographs are the most important element of radiologic examination in emergency department. In certain cases, computed tomography scan (CT) and Magnetic Resonance Imaging (MRI) are used to obtain detailed information for additional bone and soft tissue injuries. In certain cases, bedside ultrasonography (USG) may be used as a reliable screening method for some clavicle fractures, and soft tissue injuries such as rotator cuff tears and biceps tendon ruptures (Abbasi et al, 2013).

The initial assessment of traumatic injuries is performed by a three-view trauma radiography consisting of full anteroposterior, transscapular lateral, and axillary lateral views. The full anteroposterior view should be preferred over standard anteroposterior view.

Special Injuries and their Treatments

Fractures

Clavicle Fractures

Clavicle fractures account for 3-5% of all fractures, and its incidence is 2 times higher in men than women. It is the most commonly fractured bone in children. Clavicle fractures are classified anatomically and mechanistically into 3 groups. Fractures of the medial third are rare (5%) and occur as a result of a direct impact to the anterior chest. Fractures of the middle third are the most common, accounting for 80% of all injuries. The mechanism of injury involves a force applied to the lateral side of the shoulder as a result of sport injuries, falling or motor vehicle collision. Fractures of the lateral third occur due to a force applied to the top of the shoulder, accounting for approximately 15% of fractures (Van der Meijden et al, 2012).

A patient with clavicle fracture has pain over the fracture site. The affected extremity is held close to the body. The patient's head is often slightly tilted downward toward the injury site. Ecchymosis, crepitation, and deformity may be seen on the fracture site. The presence of a tent-like bump on the skin should be noted (Bahk et al, 2010).

The majority of fractures are clinically determined by chest or shoulder radiography. Clavicle-specific plain radiography may be required to confirm the presence of fracture (Figure 2). In children, radiation exposure may be reduced or eliminated by bedside ultrasonography (Croos et al, 2010).

For simple fractures, the first step is to control the pain, to provide immobilization for patient comfort and to determine appropriate follow-up management principles. The superficial cervical plexus block may be performed in fracture-related pains (Herring et al, 2012). The clavicle fractures may be appropriately immobilized by a simple arm sling. The figure-of-eight splint is no longer recommended (Rasmussen et al, 2011). In case of open fractures or accompanying neurovascular injury, orthopedics consultation should be requested (Van der Meijden et al, 2012).

Most clavicle cracks heal smoothly. Arm sling should be recommended until the patient feels comfortable. Early passive movement exercises are recommended. Adolescents and adults require immobilization for about 4-6 weeks. The most common complications are delayed union, nonunion, and symptomatic malunion (Van der Meijden et al, 2012).

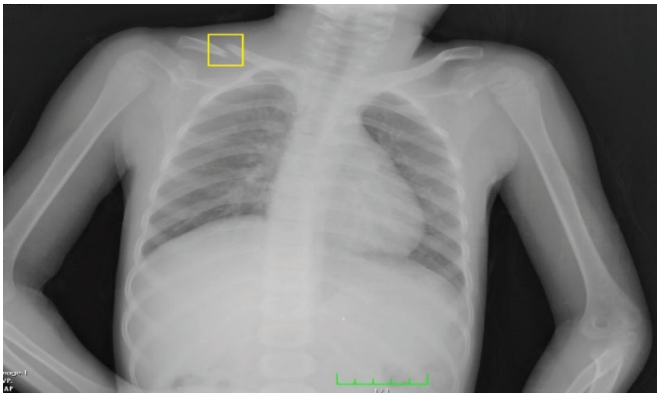

Figure 2: Clavicle Fracture

Scapula Fractures

Scapula fractures are rare, accounting for about 1% of all shoulder fractures. They usually occur as a result of high-energy falls or accidents (Cole, Gauger & Schroder, 2012). Scapula fractures rarely require management, but are associated with the complications of the same side lung, thoracic cage or shoulder girdle (Cole,2013). The most common

associated orthopedic injuries are fractures of the ribs, proximal humerus, and clavicle (Cole,2013).

Scapula fractures are traditionally divided into two main classes; extra-articular (trunk, acromiaon process, coracoid process and spine) and intra-articular (partial or full glenoid involvement) (Südkamp, 2011).

A conscious patient holds the shoulder in a comfortable position. The arm is held close to the body and any movement results in intense pain. Tenderness, crepitation or hematoma may be noted over the fracture site.

The three-view shoulder graphs are useful to show scapula fractures. These fractures can be also determined by careful examination of the chest radiograph (Cole,2013) (Figure 3). Comparative radiography should by preferred considering anomalies. The presence of scapula fractures can be determined by radiography, but the best determination method is CT (Cole, Gauger & Schroder, 2012).

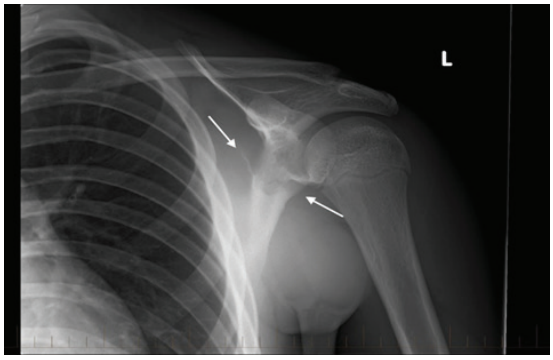

Figure 3: Scapula fracture

In scapula fractures, thoracic, intracranial, orthopedic and neurovascular injuries should be also evaluated together. Severely fractured scapula heals rapidly with treatment without the need for surgical operation (Südkamp, 2011). As initial treatment, immobilization by a sling to support the ipsilateral upper extremity and passive movement exercises are recommended (Cole, Gauger & Schroder, 2012).

Non-displaced fractures of the trunk, spine and acromion process require no further treatment. Displaced acromial fractures that impinge on the glenohumeral joint should be treated surgically. Severely displaced coracoid fractures with ruptured coracoclavicular ligaments require open reduction and internal fixation (Cole, Gauger & Schroder, 2012).

Most complications are associated with the ipsilateral lung, thoracic wall and shoulder girdle. In case of neurovascular injuries, fractures of carocoid process, scapular neck, trunk or spine may be seen together (Cole,2013).

Proximal Humerus Fractures

Proximal humerus fractures occur primarily in the older population as a result of low-energy falls due to structural changes that weaken the proximal humerus. Although most of these injuries involve displacement and are appropriately managed with conservative treatment, displaced fractures usually require surgical intervention (Murray et al, 2013).

Fractures of the proximal humerus separate along old epiphyseal lines, producing four distinct segments consisting of the joint surface, greater tuberosity, lesser tuberosity, and humeral shaft. There are four major fracture patterns. These are minimal displacement, two-part displacement, three-part displacement and four-part displacement (Murray et al, 2013).

The injury usually occurs as a result of fall on an outstretched and abducted arm. It limits concurrent pronation and abduction, and the humerus resists against the acromial process. This causes a fracture or dislocation, depending on the tensile strengths of the bone and surrounding ligaments. Fracture may also occur due to an impact to the lateral side of the arm or an axial load transmitted through the elbow (Vachtsevanos et al, 2014).

The affected arm is held close to the body, and movements are restricted by pain. Tenderness, hematoma, ecchymosis, deformity or crepitation may be noted over the fracture site. To identify injuries of the axillary nerve, brachial plexus or axillary artery, a thorough neurovascular examination should be performed. The number of fracture fragments, the presence of displacement and the degree of angulation should be evaluated by three-view radiographs (Vachtsevanos et al, 2014) (Figure 4).

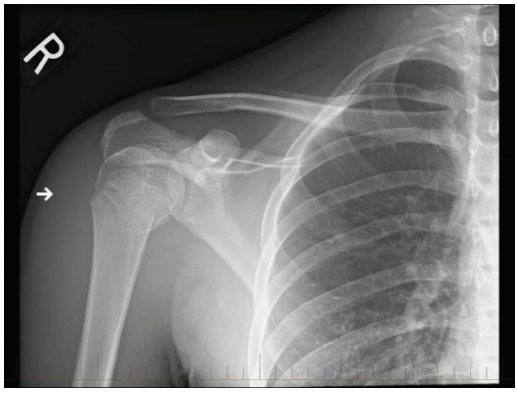

Figure 4: Proximal Humerus Fracture

Minimally displaced fractures account for 80% of all cases (Vachtsevanos et al, 2014). Initial treatment consists of administration of adequate analgesia and immobilization with an arm sling. It is recommended to start with passive exercises and gradually replace them

by more active exercises. Most nondisplaced fractures heal over 4-6 weeks (Handoll, Ollivere & Rollins, 2012). Two-part, three-part and four-part displaced fractures usually require fixation, and orthopedics consultation should be requested for such patients (Murray et al, 2013).

Orthopedics should be consulted before reduction of fractures and dislocations. The most common complication of proximal humeral fractures is adhesive capsulitis. The most devastating complications is avascular necrosis, fractures and dislocations, which are more commonly seen in segmental fractures (Murray et al, 2013).

Proximal humeral epiphysis fractures

Proximal humeral epiphysis fractures are rare and account for a small portion of all childhood fractures (Popkin, Levine & Ahmad, 2015). The injury can occur at any age while the epiphysis is open, however it is most common in adolescent boys. It usually occurs as a result of fall onto the outstretched hand. The fracture typically occurs along the hypertrophy zone in the epiphyseal plate (Popkin, 2015).

The patient holds the injured arm against the body by tightly holding it with the opposite hand. The area over the proximal humerus is swollen and painful with palpation. The diagnosis is confirmed by radiographs taken at 90 degrees to each other. Comparative radiographs are helpful for minimal displacements. 90% of such injuries can be determined by bedside USG (Croos et al, 2010).

The growth plate is responsible for 80% of the longitudinal growth of the bone, thus fractures of the proximal humerus epiphysis may cause permanent injuries and disabilities. In case of such injuries, urgent orthopedic consultation should be requested. The complications are rare and include malunion, growth plate dysfunctions and neurovascular injuries (Popkin, 2015).

Dislocations

Sternoclavicular Joint Dislocation

Sternoclavicular joint (SCJ) dislocations are rare and account for less than 1% of all dislocations (Martetschläger, Warth & Millett, 2014). The most common causes are prolonged injuries in high-impact contact sports and motor vehicle collisions. The majority of dislocations are in anterior direction. Posterior dislocations are rare; however, they may cause life-threatening injuries within the superior mediastinum (Glass et al, 2011).

SCJ injuries can be graded into three types (Martetschläger, 2014). Grade I injuries are a mild sprain of the sternoclavicular and costoclavicular ligaments. Grade II injuries are associated with subluxation of the joint secondary to disruption of the sternoclavicular ligament and the capsule.

Grade III injuries are the injuries in the form of complete rupture of the sternoclavicular and costoclavicular ligaments (Martetschläger, 2014).

Clinical suspicion is the only factor in diagnosing these injuries. The injured extremity is flexed at the elbow and supported transversely by the opposite arm. Any movement of the upper extremity results in pain. The SCJ may be mildly swollen and tender. In case of an anterior dislocation, the displaced medial end of the clavicle may be palpable. In posterior dislocations, pain is more severe. The patient's head is flexed toward the painful side in resting position (Sewell et al, 2013).

Although the diagnosis is made clinically, the SCJ dislocations should be confirmed radiologically. Standard anteroposterior, oblique, and 40-degree cephalic views are taken but it is very difficult to interpret the SCJ findings with these views. Thus, the best method for such dislocations is the BT imaging (Blakeley et al, 2011) (Figure 5).

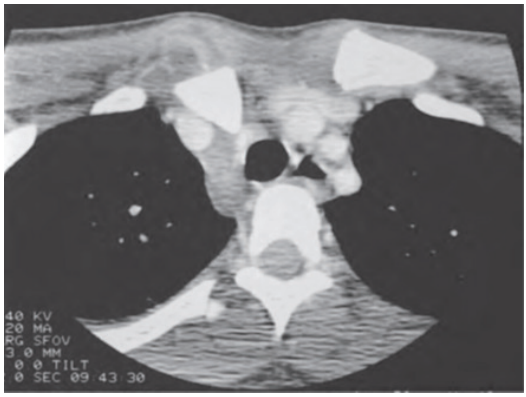

Figure 5: CT scan of the posterior dislocation of the right SCJ (Donald Sauser).

Grade I injuries are treated by immobilization in an arm sling. Immobilization should be recommended until the symptoms improve and the full movement is made without pain. Grade II injuries require longer immobilization period (Martetschläger, 2014). Grade III injuries are treated by close reduction and rarely open reduction.

Posterior dislocations are orthopedic emergencies and should be reduced immediately (Blakeley et al, 2011). Ideally, the reduction should be performed under general anesthesia or sedation. The dislocated SCJ is reduced by placing a rolled sheet posteriorly between the shoulder blades to elevate both shoulders approximately 5 cm above the table. Traction is applied to the arm in an extended and abducted position. If reduction does not occur, an assistant apply inward pressure on the medial end of the clavicle (Malik, Chiampas & Leonard, 2010). Complications of anterior dislocations mostly have cosmetic consequences (Martetschläger, 2014).

Acromioclavicular Joint Dislocations

Acromioclavicular Joint (ACJ) Injuries occur mostly in young men as a result of motor vehicle collisions, bicycle accidents or participation in contact or blow sports. The most common mechanism of injury involves a fall or an impact to the point of the shoulder with the arm adducted. Classification is made by the degree of damage to the acromioclavicular and coracoclavicular ligaments based on the Rockwood 6-class classification system (Simon,2015).

Patients should be examined while they are in the standing position because the supine position can mask ACJ instability, and both shoulders should be viewed simultaneously to assess for symmetry. Type I and type II injuries are associated with minimal deformity and full range of motion (even if it is painful) as well as mild tenderness and swelling over the ACJ margin. Patients with type III, IV, V, and VI injuries are characterized by severe pain and hold the arm tightly in adduction to reduce traction stress across the joint (Simon,2015).

The recommended projections include routine anteroposterior and axillary lateral view, vertical migration of the clavicle and anteroposterior displacement. Anteroposterior views are ideally obtained with a view of both joints on a single wide radiograph (Simon,2015). Radiography taken with the patient in standing position can help unmask a higher-level injury. The normal coracoclavicular distance ranges from 11 to 13 mm. A difference of more than 5 mm between the injured and uninjured sides indicates a complete coracoclavicular disruption. In type I injuries, the radiographic appearance is actually normal. In type II injuries, radiograph shows upward or posterior displacement of the clavicle. In type III, IV and V injuries, radiographic features include a widened joint, an increased coracoclavicular distance, and superior or posterior displacement of the clavicle where the lower part of the clavicle is in front of the top of the acromion (Simon,2015).

Type I and II injuries should be immobilized in an arm sling. When pain subsides (1 to 2 weeks), the range-of-motion and strengthening exercises can begin. The management of type III injuries varies; however, mostly non-surgical method is preferred initially (Simon,2015). Selected patients who are young, engage in athletics professionally, have severe displacement (more than 2 cm), and perform repetitive overhead activities may be candidates for surgical intervention. The treatment of type III injuries in emergency department should include immobilization in an arm sling and early orthopedic referral. Type IV, V and VI injuries require early surgical treatment (Simon,2015). The most common complications of ACJ injuries are residual symptomatic instability and joint tenderness (Simon,2015).

Glenohumeral Joint Dislocations

The GHJ is the most commonly dislocated major joint in the body. There are two distinct age peaks, the first in men aged 20 to 30 years and the second in women aged 60s years. The GHJ can dislocate anteriorly, posteriorly, inferiorly or superiorly. Anterior dislocations account for 96-98% of glenohumeral dislocations (Khiami, Gérometta & Loriaut, 2015). Posterior dislocations account for the majority of the remainder. However, inferior (luxatio erecta) and superior dislocations are rare.

Anterior Dislocations

Anterior dislocations can result from indirect or direct forces. In younger persons, the injury usually occurs during rapid movements with the arm in elevated, abduction and external rotated position during the athletic activities, or rarely due to a direct force applied posterolaterally (Khiami, 2015). Anterior dislocations can be classified according to their causes, frequency, and the anatomic position of the dislocated humeral head (Streubel et al, 2014).

The patient presents with severe pain and by supporting the dislocated shoulder with the opposite extremity. The lateral edge of the acromion process is prominent, and the normally rounded shoulder takes a “squared” appearance. The patient leans away from the injured side and suffers severe pain when he/she adduct the shoulder even slightly. Dislocations may be associated with injuries of brachial plexus, axillary nerve or artery. Nerve injury, even to a certain extent, may be seen in nearly half of the patients (Khiami, 2015).

Radiographs will confirm the clinical diagnosis and determine the position of the humeral head (Figure 6). For patients who are normally healthy, but have repetitive dislocation and concurrent major injury without diagnosis uncertainty, radiography is not required before relocation. Bedside USG may be helpful in diagnosis (Abbasi et al, 2013). Some patients with dislocation have an associated fracture. The most common is posterolateral compression fractures of the humeral head.

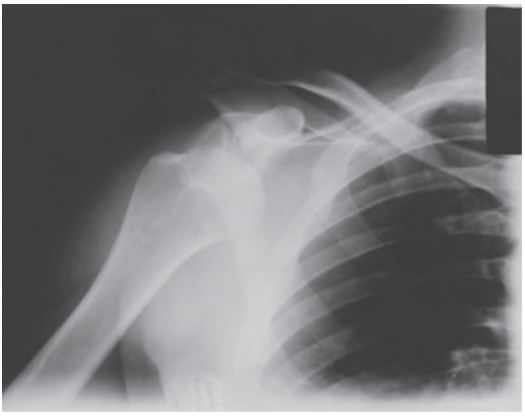

Figure 6: Anterior Humerus Dislocation

The reduction of the dislocation should be performed rapidly because it becomes difficult to reduce the dislocation over time due to incidence of the neurovascular complication and muscle spasm. For most patients, reduction may be attempted after interviewing with the patient before taking a scan. Radiography is recommended after the reduction (Abbasi et al, 2013).

Procedural sedation is used to facilitate the reduction in emergency department. Adequate analgesia may be provided by intraarticular injection of local anesthesia agent. This technique is useful especially when procedural sedation is contraindicated. Local anesthesia agents with prolonged effect may provide longer post-reduction analgesia (Jiang et al, 2014). If examiner is ready to stop the procedure when the patient feels pain, the reduction can be initiated without analgesic or sedation and completed successfully, depending on the duration of the dislocation and the technique (Shin et al, 2012).

Reduction can be performed using various techniques including mostly traction, leverage and scapular manipulation principles (Bonz and Tinloy, 2015). The ideal method should be simple, quick and effective, and require little help and not cause any additional injury to the shoulder. Being familiar with several reduction techniques is important because none of them is completely successful. The indicators of a successful reduction include sensation of the shoulder “back into its place”, disappearance of pain, restoration of the anatomy and improved range of motion. The neurovascular examination should be repeated and findings recorded after every reduction attempt (Bonz and Tinloy, 2015). After the reduction, the affected extremity is immobilized with an arm sling and shoulder bandage. The patients are discharged with adequate analgesic and follow-up. Primary dislocations and complex cases should be followed by an orthopedist. In emergency department, the patients should be immobilized with an arm

sling for 1 to 2 weeks (Gül et al, 2014).

The complications include fractures and neurovascular injuries (Khiami, 2015).

Posterior Dislocations

Posterior dislocations are rare and account for less than 5% of all glenohumeral dislocations. The glenoid fossa serves as a partial support against posterior dislocations. Posterior dislocations are easily missed if x-rays cannot be obtained on both planes and carefully reviewed. Then, they can remain unrecognized for weeks or months (Paterson et al, 2010).

The posterior dislocation may result from several distinct mechanisms of injury. A fall onto the outstretched hand with the arm in flexion, adduction and internal rotation or a direct impact to the anterior aspect of the shoulder may cause a posterior dislocation. Acute posterior dislocations are anatomically classified into three types; subacromial (the most common), subglenoid, and subspinous, based on the final resting position of the humeral head (Paterson et al, 2010).

Early diagnosis is essential to prevent long-term complications. The affected arm is hold across the chest in adduction and internal rotation. The normal shoulder contour is replaced by a flat, squared appearance, and the coracoid process is prominent and easily palpated. The humeral head may be palpable posteriorly beneath the acromion process. Abduction is significantly limited; external rotation is completely blocked and there may be restricted forearm supination (Jacobs, Meredyth & Michelson, 2015).

True or standard anteroposterior radiographs can appear deceptively normal in the presence of posterior dislocations. In radiography, standard anteroposterior radiograph should show loss of the half-moon elliptic overlap of the humeral head and glenoid fossa. The humeral head is seen in internal rotation. A true anteroposterior film shows abnormal overlap of the glenoid fossa with the humeral head. The axillary lateral view or apical oblique view also identifies associated fractures of the inferior edge of the glenoid fossa (Figure 7). If an adequate radiograph cannot be obtained, shoulder CT should be considered (Rouleau, Hebert-Davies & Robinson, 2014). Bedside ultrasonography is used in diagnosis of posterior shoulder dislocation in emergency department (Rouleau and Hebert-Davies, 2012).

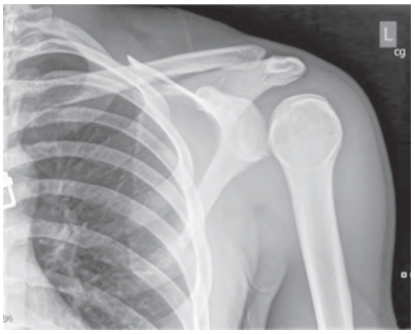

Figure 7: Posterior Humerus Dislocation

Closed reduction can be performed with procedural sedation in emergency department. The technique incorporates internal rotation and lateral traction to detach the humeral head from the glenoid edge. If there is no fracture of the humerus neck, then the Stimson technique can be also used. If it fails, the reduction is performed with the patient under general anesthesia. Various reduction techniques are shown in Table 1. After the reduction, the shoulder should be immobilized in external rotation with slight abduction until the orthopedic follow-up within 1-2 weeks (Jacobs, 2015).

Reduction Technique	*Description*
Stimson (Hanging Weight)	The patient is placed in prone position with the dislocated arm hanging over the edge of the examination table. A 4, 5 or 7-kg weight is attached to the wrist or forearm to provide traction in forward. Reduction occurs over 20-30 minutes.
Traction/ counter traction	While traction is applied to the abducted arm, an assistant applies countertraction using a folded sheet wrapped across the chest.
External Rotation	It does not include traction. When the patient is in sitting or supine position, the affected arm is slowly and gently adducted to the side. The elbow is flexed to 90 degrees, and gentle external rotation is applied to achieve reduction.
Scapular manipulation	Reduction is performed by repositioning the glenoid fossa rather than the humeral head. Manipulation is combined with other techniques.
Not recommended techniques	The Hippocratic and Kocher methods are no longer recommended since associated complications are severe.

Table 1: Various Techniques for Shoulder Dislocations

Inferior Glenohumeral Dislocation (Luksasyo Erekta)

Luxatio erecta is a rare type (<0.5%) of glenohumeral dislocation in which the superior aspect of the humeral head is forced below the inferior

edge of the glenoid fossa (Beck and Chilstrom, 2013).

Clinically, the patient's arm is locked overhead in 10 to 160 degrees of abduction. The elbow usually is flexed, and the forearm typically rests on top of the head. The inferiorly displaced humeral head may be palpable along the lateral chest wall. A thorough neurovascular examination should be performed to evaluate accompanying injuries (Beck and Chilstrom, 2013).

Luxatio erecta may be misdiagnosed and treated as subglenoid anterior dislocation. Standard antero-posterior radiographs show that the articular surface is further inferior to the glenoid fossa (Figure 8). In addition, the humeral shaft characteristically lies parallel to the spine of the scapula on the anteroposterior view. This radiographic feature helps distinguishing luxatio erecta from a subglenoid anterior dislocation (Beck and Chilstrom, 2013).

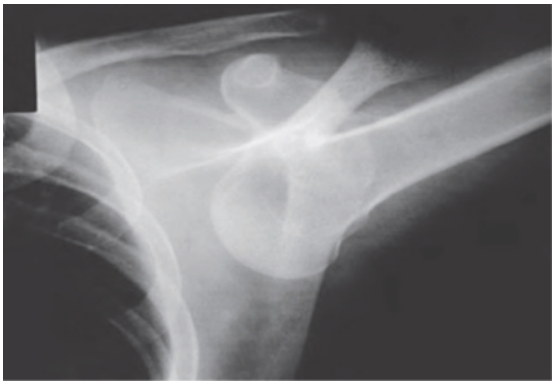

Figure 8: Inferior Humerus Dislocation

Reduction usually can be performed by traction-countertraction maneuvers under procedural sedation. It was reported that local anesthesia in the form of interscalene block under guidance of ultrasonography is effective in facilitating reduction (Groh, Wirth & Rockwood, 2010). An alternative approach is the two-step closed reduction maneuver, in which the inferior dislocation is first converted into an anterior dislocation before the reduction (Brant-Zawadzki and Herring, 2015).

Scapulothoracic Dislocation

Scapulothoracic dissociation is a rare and severe injury which is characterized by complete disruption of the scapulothoracic articulation and may be considered as a partial or complete closed internal forequarter amputation of the upper extremity (Patel, Zuckerman & Egol, 2011). Approximately half of the reported cases involve motorcycle accidents. The dislocation may be overlooked at first, because most patients have concurrent traumas. Massive local soft tissue swelling of the shoulder, along

with more than 1 cm of displacement of the scapula on the anteroposterior chest radiograph, is pathognomonic for scapulothoracic dissociation (Flanigin and Leslie, 2013). Vascular and neurologic injuries can be confirmed by immediate angiography and MRI. Outcomes are mostly poor, with death in 11% of cases, and complete brachial plexus injuries result in upper extremity or above-elbow amputations (Patel, 2011).

Soft Tissue Conditions

Rotator Cuff Tears

Rotator cuff serves as a dynamic stabilizer of GHJ. Restricted supply of blood to rotator cuff, abusive tensile overload, and chronic wear under the coracoacromial arch predispose it to age-related degenerative changes and shoulder impingement. The advanced stage of this process is characterized by complete rupture. Rotator cuff tears typically affects the dominant arm and are seen in men aged over 40 years. Tears can be classified according to being chronic, their size, integrity, location and duration. Acute tears (10%) are often associated with a certain traumatic event. The patient usually has no history of shoulder conditions. The most common mechanism of injury is forced abduction against resistance. This usually occurs when the patient attempts to prevent a fall with an outstretched hand (Hegedus, 2012).

In acute tears, patients report a sudden tearing sensation in the shoulder and then a severe pain radiating into the arm. Shoulder movement is limited by pain and muscle spasm. Physical findings depend on the integrity, size and location of the tear. Point tenderness is usually present over the rupture site. Patients with a large tear cannot initiate shoulder abduction. A discrepancy between active and passive range of motion indicates a rotator cuff tear. The drop-arm test, which is performed by passively abducting the arm to 90 degrees and asking the patient to hold the arm in this position, is positive in significant tears (Hegedus, 2012).

Plain radiographs appear normal in acute and chronic tears and usually are not indicated. The characteristic finding of a complete tear is the superior displacement of the humeral head and is best viewed in external rotation. Outpatient ultrasound examination and MRI can confirm the diagnosis (4 Hegedus, 2012).

Acute tears should be immobilized in a sling for the patient comfort and the patient should be referred for orthopedic follow-up. Early surgical repair (before 3 weeks) is preferred for certain lesions in young or active individuals (Hegedus, 2012).

Rupture of Biceps Tendon

Ruptures of the biceps tendon can be classified as proximal and distal types. Distal ruptures are rare. The rupture can occur spontaneously or

following a traumatic event involving either forced extension or resisted supination and flexion (Yablon, 2013).

The classic history of an acute rupture is a sudden snap or pop, followed by pain and ecchymosis along the arm (Yablon, 2013).

Radiographic findings usually are not remarkable, and the preferred confirmation test is MRI. In emergency department, bedside ultrasonography can be used to determine whether there is tendon in the bicipital groove and there is a fluid within the fiber (Yablon, 2013).

The injured arm should be immobilized in an arm sling with the elbow in 90 degrees of flexion. The patient should be referred to an orthopedic surgeon for a more comprehensive evaluation and treatment within 72 hours (Yablon, 2013).

Conclusion:

In shoulder and upper arm injuries, the anatomy of the injury site should be well known. A patient with trauma should be diagnosed using medical history, physical examination and imaging methods. Possible special injuries should be kept in mind and specific treatments for such injuries should be administered.

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Chapter 8

OPHTHALMIC OPTICS LENSES, FITTING APPLICATIONS AND DISPENSING OPTICAL PRESCRIPTION: A REVIEW OF OPTICIANRY

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1. Optics

Optics examines the light, its characteristics, reflection, refraction, and other properties as well as its interaction with other substances. It can be also defined as a sub-branch of physics that provides information on the measurement and classification of light. Light is one of the basic needs of living creatures and is a source of life. 2015 was declared by United Nations as International Year of Light and Light-Based Technologies with a view to put emphasis on the importance of vision technologies in people's lives and development of the society (UNESCO, 2015). Light and related definitions such as refractive index, reflection, diffraction, scattering of light, interference, lenses, aberrations in lenses, prisms are among the basic topics related to optics (Özer, 2005). Ophthalmic optics lens theory is explained by the laws of geometric optics. Geometric optics explains the geometric behavior of light, its speed in the subject medium, wavelength and frequency, scattering, propagation, diffraction, absorption, polarization, wave fronts, simple wave properties with point sources. In addition, it provides important information about electromagnetic radiation, reflectance, abbe value, light diagrams, refractive defects, optical axis, focal point, dioptric power, spherical and cylindrical lenses, convex/concave lenses, prism and prismatic glasses and Prentice rule. Lenses and correction of refractive errors, emmetropia, ametropia, myopia, hyperopia, astigmatism, astigmatic systems, vertex powers, aberrations, visual acuity are among the subjects of vision optics (Keating, 2002).

The aim of this study is to summarize the features of the ophthalmic lenses and the steps of lens fitting applications. Prescription-reading, fitting, and dispensing of eyeglasses are given in detail with references to the corresponding optical devices and tools. Eyeglasses are ophthalmic lenses specially mounted and fit on frames for personal use. The properties of ophthalmic lenses used in the correction of refractive errors and vision problems and the points that should be considered during the assembly of optical lenses are explained in this study together with visual optics and geometrical optics.

2. Eye and Eye Health

The eye consists of a cornea, crystal lens, iris, and retina as given in Figure 1. Cornea and crystal lens both have convergent structures, and they are in convex form. The rays coming from the external environment are collected on the retina from where the image is transmitted to the brain to enable vision. In the case of emmetropic eyes, images are expected to form a clear focal point on the retina for distant objects. When objects are close to the eye, non-aged eyes will remain focused on the images with changes in the dioptric power of the crystalline lens which is known as accommodation (Artal, 2011). The total refraction of the eye is nearly

+ 62.00 D diopter (D) and cornea constitutes approximately 70% of this refraction. The front surface of the cornea has + 48.80 D and the back side has a power of -5.80 D. In total, the cornea is the most important refractive medium of the eye with a refractive power of +43.00 D. The total refractive power of the lens is nearly +19.00 D. The refractive power of the lens can increase in the range of 14.00 D - 15.00 D with the maximum accommodation process (Acaroğlu, et al. 2011).

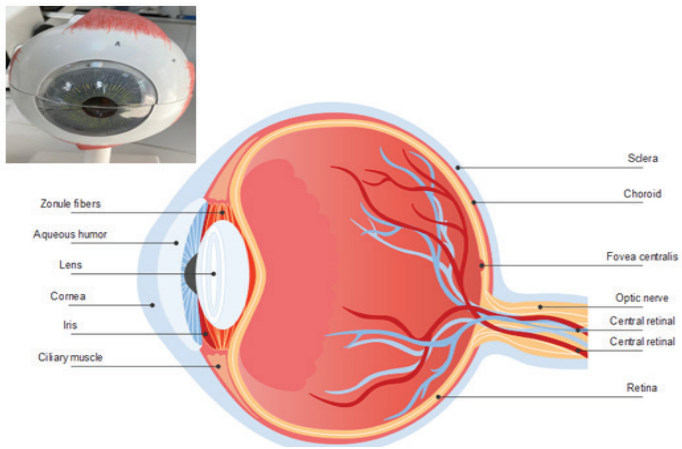


Figure 1. *The eye anatomy (E-drawSoft program, Eye Model, 2020)*

Eye health has a great influence on the quality of our lives. Therefore, care of eye health particularly in early ages is of great importance. As a result of the wide usage of electronic devices in our daily lives, further importance should be placed on eye care. The usage of personal computers and mobile phones for extended periods have resulted in an increased prevalence of refractive defects as from early ages. Thus, selection of appropriate eyeglasses for personal use has thus become even more important. Also, working in sufficiently illuminated environments, relaxing our eyes via increased blinking and taking reading breaks will help us relax our eyes during prolonged activities. Keeping an adequate distance between the subjects of sight such as books or screens will prevent diseases such as dry eye and related issues such as head and neck pain which are related to excessive eye strain.

2.1. Refraction errors

Ophthalmology is a branch of medicine that deals with eye diseases and eye treatments. Ophthalmologist is a doctor who specializes in the diagnosis, medical and surgical treatment of eye diseases. The visual acuity and visual function of patients in terms of eye health are evaluated by ophthalmologists by determining the patient's vision via objective and

subjective examination methods. The eye consists of a complicated optical system. During vision, the light is first refracted from the cornea and then from the lens, subsequently focusing on the retina at the back of the eye to create a clear image.

"Emotropy" can be defined as focusing of light rays coming parallel to the optic axis on the retina plane in an eye that does not accommodate during vision (Meister and Sheedy, 2008). Refractive error of the eye is a general term used for an ametropic eye. There are four different refractive defects that impair visual acuity, namely, myopia, hyperopia, astigmatism, and presbyopia (McCleary, 2008). Myopia occurs when rays coming parallel to the eye form a focus in front of the retina. Hyperopia, on the other hand, occurs when rays coming parallel to the eye form a focus behind the retina. Myopia and hyperopia are corrected with (-) and (+) powered spherical lenses, respectively. For spherical lenses with spherical lens surface, the dioptric power of the lens in all meridians is equal as shown in Figure 2. Astigmatism is the case where the rays coming parallel to the eye are refracted differently in different meridians, creating more than one focus without the retinal plane (Özer, 2005). Astigmatism is a non-spherical refractive error and corrected with cylindrical lenses. Spherocylindrical lens consists of a combination of spherical and cylindrical surface sections and has different dioptric powers in different meridians as shown in Figure 2. There are five types of astigmatism depending on the composition of the refractive defects in the main meridians of the eye. The first is "mixed astigmatism" where the focal lines are both before and behind the retina. The second one is the "compound myopia astigmatism" in which case both are in front of the retina. The third one is the "compound hyperopia astigmatism" where both are located behind the retina. In the fourth case of "simple myopia astigmatism", one is in front of the retina and the other is on the retina. The fifth case is "simple hyperopia astigmatism" where one is located on the retina and the other is behind the retina.

Presbyopia is another refractive defect such as myopia, hyperopia, and astigmatism, that occurs when the lens loses its flexibility and images of nearby objects cannot be formed on the retina. Presbyopia usually starts in the forties (Gücükoğlu, 2012).

3. Ophthalmic Lenses

Ophthalmic means "of or pertaining to the eye". Ophthalmic optics is a branch of optics that is related to vision (Meister and Sheedy, 2008). Lenses are classified according to their properties. Lenses are classified according to their geometric shapes, diopters, chemical properties, indices, focal, color and diameter. They are also classified based on their edge shapes as thin-edged lenses and thick-edged lenses. According to their surface shapes, ophthalmic lenses are divided into verre plan (VP),

spherical (SPH), cylindrical (CYL), plan cylindrical (PL-CYL), spherical cylindrical (SPH-CYL), cylindrical-cylindrical (CYL-CYL) and prismatic lenses. They are also classified according to their chemical structure as mineral and plastic (organic). They are classified based on their refractive indices as mineral (1.52), CR-39 (n = 1.49), higher index (n =… 1.60 / 1.71 / 1.74…), polycarbonate (n = 1.59), trivex (n = 1.53). They can be also classified according to the number of focal points as monofocal, bifocal, trifocal and multifocal. They are separated according to their color as colorless, colored, UV darkened (colormatic / photochromic) glasses. Lenses are classified according to their diameter/size as small (50-55 mm), medium (60-65 mm) and large (75 mm) (Özdemir and Kabak, 2018). Ophthalmic lenses are divided into three basic categories: single vision lenses, split multifocal lenses, and progressive addition lenses as shown as Figure 3. In single vision lenses, the entire surface of the optical lens has the same dioptric power. In split multifocal lenses (Bifocal and Trifocal), there is more than one dioptric power. In progressive addition lenses in the class of multifocal lenses, lens power gradually increases. The single vision lens (monofocal lens) is a spherical or spherocylindrical lens. The multifocal lens is a multi-vision lens and is grouped into bifocal, trifocal, progressive lens. As the most widely used material for ophthalmic lenses, CR-39 (Allyl diglycol carbonate/ADC) is a non-thermoplastic and organic synthetically produced plastic polymer (Özdemir, 2016). The characteristics of ophthalmic lenses are classified as mineral lenses (crown, flint, borosilicate, heavy flint lenses) and plastic/organic lenses (CR-39, high index, polycarbonate and trivex).

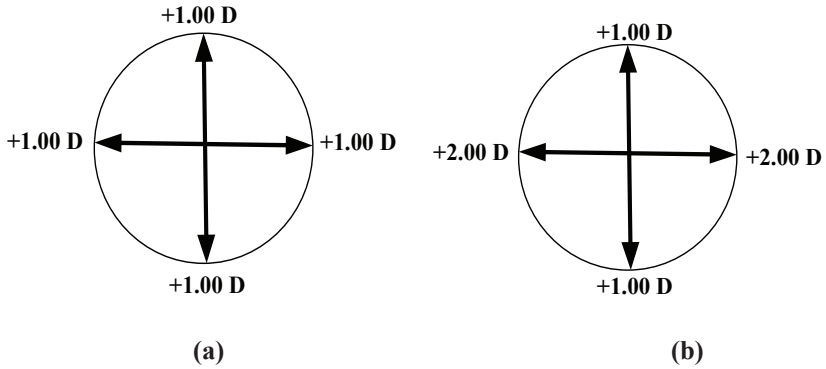


Figure 2. *The dioptric power of optics lenses in meridians*

	Monofocal		Multifocal		Prismatic
	Organic	Mineral	Bifocal	Progressive	
Lenses					

Figure 3. *Monofocal and multifocal optical lenses*

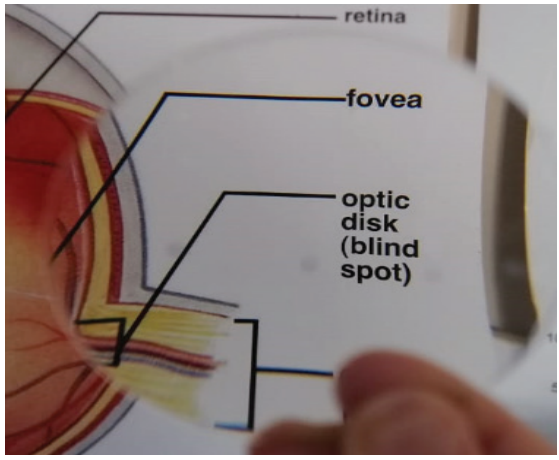


Figure 4. *Image of optical lens with SPH: +1.75 D*

"Focal length" and "diopter" are two important concepts in optical glasses. The total dioptric power of the lens is equal to the sum of the two surfaces and the dioptric power of the surfaces. The dioptric power of the lens varies inversely with its focal length D=1/f(m). The refractive power of the lens increases as the focal length decreases, and the refractive power of the lens decreases as the focal length increases. The unit of dioptric power is (D) diopter. All optical lenses are used to change the path of light. Optical properties vary depending on the radius of curvature, diopter, and refractive index of the glass as per the following Equation 1:

$$D = \frac{1}{f} = 1000\,(n-1)\left(\frac{1}{r_1} - \frac{1}{r_2}\right) \qquad (1)$$

The prismatic effect in ophthalmic lenses was initially determined by the famous scientist Charles F. Prentice. This rule is thus known as the "Prentice Rule" (Δ =D x C). Decentration of the center point of any lens from the correct PD of the patient can reduce the unwanted effects of prism

as headaches and double vision (McCleary, 2008). Optical lens diameter (R), basic curvature (BC), diopters (D), sagittal depths (S), surface properties including plano-convex, plano-concave, bi-convex, bi-concave, meniscus lenses, spherical surfaces (SPH), spheroidal surfaces, edge thicknesses (ET), optical centers (OC), focal points (F), refractive indices (n) are among the physical properties. Lenses are divided into two as spherical and astigmatic lenses (cylindrical lenses, toric lenses) according to their geometric properties. Spherical lenses are grouped as spherical convex lens and spherical concave lens. Spherical convex lenses are lenses produced with a base-to-base prism system. They are convergent, convex, and thin-edged lenses with positive (+) dioptric power as shown in Figure 4. Spherical convex lenses have thicker centers and convergent structure. They make things look bigger. When a convex lens is moved to the right and left, the image of the object to be viewed moves in the opposite direction of the movement direction. These lenses are used to correct hyperopia and presbyopia. Spherical concave lenses are produced with a peak-to-peak prism system. They are divergent, concave, and thick-edged lenses with negative (-) dioptric power. Spherical concave lenses have thinner centers and divergent properties. They make things look smaller. When a concave lens is moved to the right and left, the image of the object to be viewed moves in the same direction of its movement direction of the lens. These lenses are used to correct myopia refraction error. Cylindrical lenses have a significant dioptric power in a single direction and axis value. Cylindrical lenses are used to correct astigmatism defect. Spherocylindrical lenses, on the other hand, consist of a combination of a sphere and a cylinder cross section. In addition, the lens has refractive power on both meridians. Spherocylindrical lenses are described by spherical power, cylindrical power, and axis of the lens (Aksak and Küçüker, 2005).

In bifocal lenses, the «segment» section provides clear vision. This part of the lense is relatively smaller and it is located close to the bottom-nasal side. “Segment” section is smaller located near the bottom-nasal side. Accordingly, the lenses intended for left and right eyes are separately prepared. There are two types of bifocal lenses in terms of the segment form, namely, straight or s-type lens and curved or c-type lens. Prior to the fitting of the lens on the frame, parameters such as addition, dioptric power, axe in addition to near and far regions of the lens should be checked and confirmed by an optician using a lensmeter. After the verification of the values, bifocal lenses that fail to meet the required dimensions specified in manufacturer’s instructions should not be used (Demir, 2002).

Progressive addition lenses have surface curvature values that gradually vary between the minimum value in the “distance region” and the maximum value in the “near region”. When writing progressive addition lens prescriptions, it is important to consider the patient’s eye health

requirement and the extent of the refractive error. During the mounting of these lenses frame adjustment, pupillary distance measurement and determination of parameters such as the pantoscopic angle, fitting height, vertex distance and wrap angle are also important.

Exposure to ultraviolet radiation may lead to serious eye health problems and diseases at early ages such as cataract. Therefore, protection of eyes from the harmful effects of ultraviolet radiation is an important issue that should be taken seriously. Eyeglasses used in daily life for general or special purposes are expected to provide protection against UV radiation. There are several studies on ophthalmic lenses in the literature. Ozdemir et al. (2016), reported the spectral transmittance of optical eye-lenses (Özdemir et al, 2016). Ozdemir Oge (2019) explains the assembly steps with ophthalmic optical glasses used for the correction of eye errors (Özdemir Öge, 2019). The effect of light on ophthalmic optical lenses was investigated, and light transmittance analysis was performed to determine the physical and optical properties of ophthalmic lens materials. The dioptric power values, diameters, center thicknesses, edge thicknesses and indices of the lenses are given in physical measurements. As a result of the study, it was observed that the light coming into the ophthalmic lens was reflected by approximately 5-10% in the visible range. The low reflection rate and high light transmittance value in the visible light region are important for the healthy use of ophthalmic optics lenses (Özdemir and Özdemir, 2016).

UV 3600 Plus Shimadzu UV-VIS-NIR spectrophotometer device is equipped with three detectors. The device uses the PMT (photomultiplier tube) detector in ultraviolet and visible region, InGaAs and cooled PBS detectors in the near infrared region. In Figure 5, the first eyeglass (-0.75 D Antireflective eyeglass) has a UV protection of 380 nm, the second (black colored sunglasses) has a UV protection of 400 nm and the third (sunglasses) have a UV protection of 600 nm. In 400-600 nm region; the sunglasses were found to have lower light transmittance as compared to -0.75D spectacle lens (Figure 6).

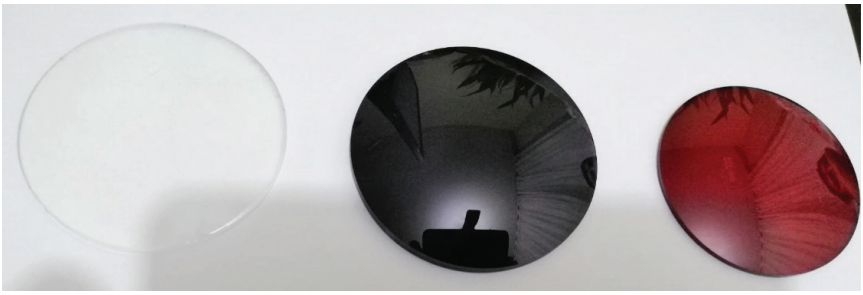


Figure 5. *Various lenses*

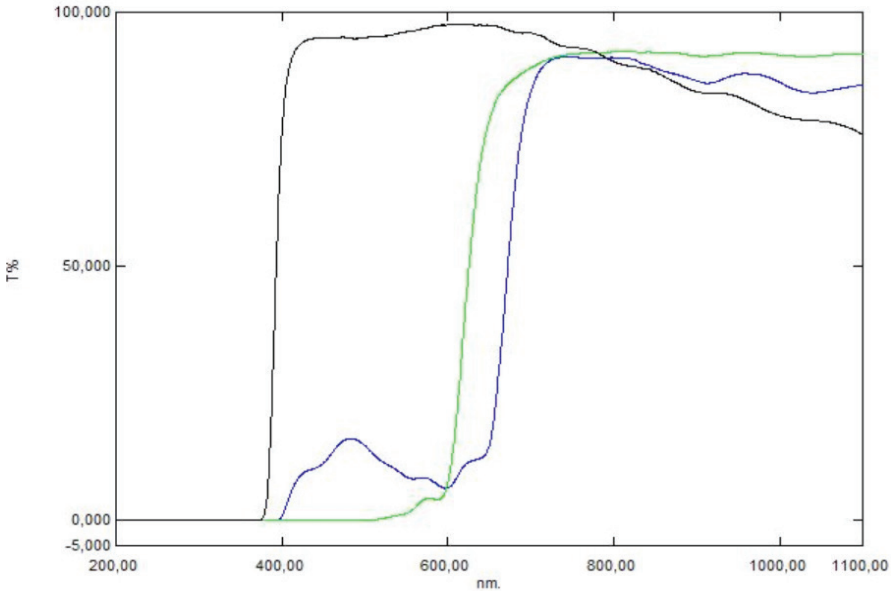


Figure 6. *Transmittance values of eyeglasses a) -0.75 D Antireflective eyeglass b) Black colored sunglasses-UV400 c) Sunglasses-UV600*

4. The Assembly Steps of Ophthalmic Lenses

a) Initial Fitting Step

An example of a prescription used in eye refraction defects mainly consists of four parts: spherical power, cylindrical power, cylinder axis (axis) and addition power. Spherical value gives the dioptric power of myopia and hyperopia. Cylindrical power value is the amount of astigmatic power given as (+) or (-) power. The addition power is the additional power of the lens. Axis is the orientation of the cylindrical power line (McCleary, 2008).

A patient's prescription is examined in detail before dispensing the spectacle lens as shown in Figure 7 and Figure 8. If the patient has a previous prescription, this is compared with the prescription of the new eyeglasses. Patients with high diopter values should prefer eyeglasses with higher index by an optician's advice. Suitable coating types are recommended to the patient/user to provide comfortable vision.

	Göz	SPH (D)	CYL (D)	AKS	ADD (D)	PD (mm)
Distance	OD (Oculus Dexter)	-2.00	-1.00	30	+2.50	32
Distance	OS (Oculi Sinistri)	-2.50	-1.25	150	+2.50	32

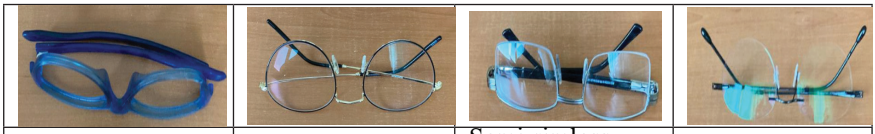
Figure 7. *A prescription sample used in eye refraction errors*

	OD			OS			
	SPH (D)	CYL (D)	AXIS	SPH (D)	CYL (D)	AXIS	PD (mm)
Distance	-0.75	-	-	-0.50	-	-	64
Near	+1.75	-	-	+2.00	-	-	62

Figure 8. *A prescription sample used in eye refraction errors*

b) Frame and Face Shape

Frames are classified as full-frames (plastic frames and metal frames), semi-rimless frames (string mounted frames/nylon supras) and rimless frames as shown in Figure 9. Full frames are metal or plastic frames that fully enclose the eyeglasses. A herringbone pattern is engraved inside full-frames for mounting eyeglasses, whereas monofilament fishing lines (nylon fishing cord) are used for mounting eyeglasses on semi-rimless frames. In rimless eyeglasses, lenses are connected to each other with the nose bridge and the temples are directly fixed to the lenses by pins glued or pegged in the through hole of the lens without the use of any metal or plastic covering (Şen, 2017).



Plastics frame	Metal frame	Semi-rimless frame	Rimless frame

Figure 9. *Frame types*

Main facial shapes can be listed as oval, oblong, round, square, triangular, inverted triangular, diamond facials. The eyeglass frame should be chosen in accordance with the user's face.

Selection of the frame is another important subject for the wearer. It is important to choose the right frame according to the patient's refractive error and face shape. The wearer's eye frame should fit comfortably over the nose and behind the ear.

It is important to make the eyeglass adjustments (metal or plastic temple settings, screw adjustment, nose pad adjustment) and to ensure that the glasses fit perfectly on the face. After the frame settings are made, it should be checked on the person's face.

c) Settings

Pupillary distance (PD) measurement, fitting height, vertex distance measurement and pantoscopic angle settings are the other measurements.

And the optical center of the lenses should be positioned in front of the pupils to obtain for optimal vision.

In spectacle lens fitting, PD (pupillary distance) measurement is of great importance. The undesired prismatic effects can be reduced and thus a comfortable vision is enabled for the patient (Özdemir Öge, 2019).

As illustrated in Figure 10, vertex distance is the distance between the imaginary axis tangent to the back surface of the corrective lens mounted on the frame, and front of the cornea. Fitting height is the vertical distance between the pupil center and the deepest point of the lens. Pantoscopic angle is the angle between the lens-mounting plane and the vertical plane.

Pantoscopic and retroscopic tilt are illustrated in Figure 11. As shown in the figure, pantoscopic tilt is the rotation of the lens plane (or the bottom of the rim) around the vertical axis towards the patient's face, and retroscopic tilt is the rotation of the lens plane around the vertical axis away from the patient's face.

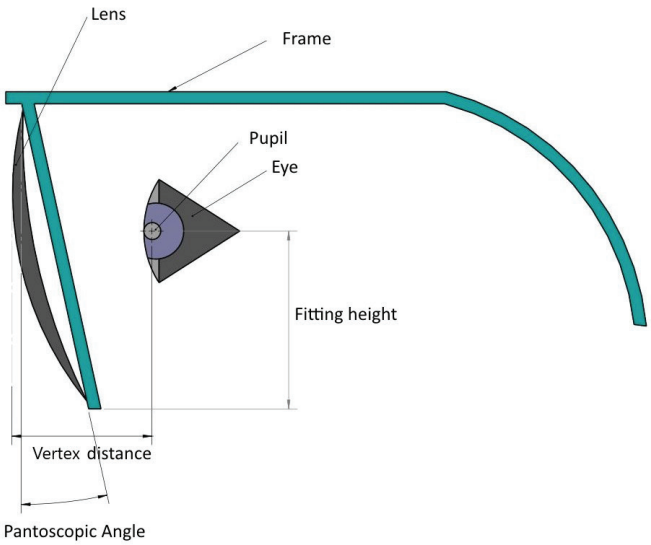


Figure 10. Eyeglass fitting parameters

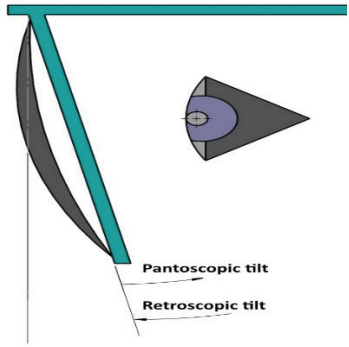


Figure 11. Illustration of pantoscopic and retroscopic tilt

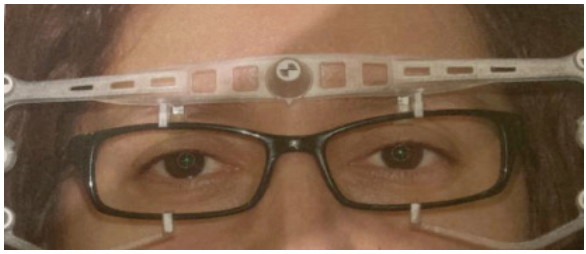
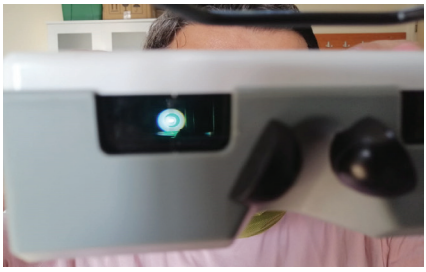
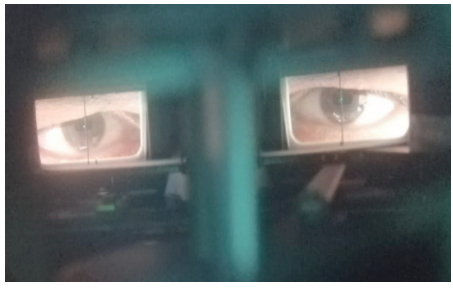


Figure 12. Frame adjustment

The right PD (R-PD) of the patient as shown in Figure 12 is 31.9 mm and left PD (L-PD) of the patient is 30.4 mm as shown in Figure 12. Frame length is 51.8 mm and frame height is 29.2 mm.





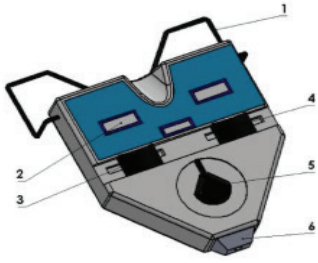


Figure 13. Pupilometer and PD measurement

Pupillary (or interpupillary) distance is the distance measured between the centers of the patients' pupils. When the eyes focus at the infinity, the measured distance is far pupillary distance, and when the eyes focus an object that is about 25 cm away from the patient, the measured distance is called the near pupillary distance. Interpupillary distance (PD) measurement is strictly required before the lens is mounted on the frame as shown in Figure 13 (Özdemir Öge, 2019).

By using the frame pupillary distance device, the total and monocular PD of the frame are also calculated. As shown in the Figure 14 and Figure 15, the lens dimension is 49 mm, the nasal distance is 19 mm, and the temple length is 145 mm. These values are generally marked or inscribed on the frame. Patients and suppliers can learn about a frame's dimensions by using these values. During the lens fitting process, the optician should make sure that the patient looks through the optical center of the lens (Özdemir Öge, 2019).

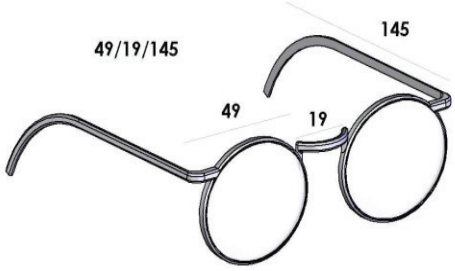


Figure 14. *Eye-frame dimensions*

Figure 15. *Here is a temple marked with a temple length of 145*

The boxing system is used to define lens and frame dimensions. In this measurement system the value A defines the horizontal lens size, B is the vertical lens dimension, DBL (distance between lenses) is the bridge size and MRP (major reference point) is the point on the lens on which the prismatic value equals to the one written on the prescription (Brooks, 2003).

Decentration is applied in case the frame's pupillary distance differs from that of the patient. In this case the optician should make sure that the wearer looks through the optical lens center via decentration.

Total Decentration=(A+DBL)-PD (2)

Horizontal Decentration=(DBC/2)-Monocular PD (3)

Vertical Decentration= MRP Height-B/2 (4)

where; G is the geometric center of the lens, DBC (A+DBL) is the distance between centers, DBL is Distance Between Lenses or Bridge Size, A is the Eye-Size, B is the eye height as given in Figure 16. Decentration means that the decentration per lens. If the calculated decentration value is positive, it is decentered towards the nasal direction of the frame. If the calculated decentration value is negative, it is decentered towards the temporal direction. Horizontal decentration is the shifting of the optical center towards the nose or the temple. Vertical decentration is the process of moving the optical center upward or downward from its standard state.

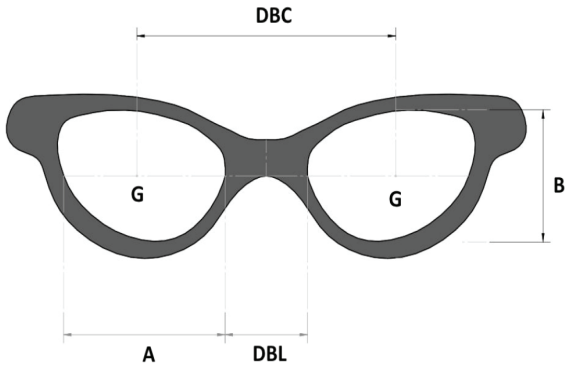


Figure 16. *Frame sizes in boxing system*

d) Ordering

Opticians order suitable ophthalmic optics lens specified in prescriptions from ophthalmic laboratories, lens manufacturers or ophthalmic lens distributor companies.

e) Spectacle Lens Dispensing

Eyeglasses given to the patients depending on the refractive error and its degree provide clear vision. While mounting spectacle lenses, it should be ensured that the prescription lenses are mounted on the correct frame and assembled correctly. The pupillary distance of patient and optical center height defines the position of the lens in the frame. The lens' optical center should coincide with the patient's pupil to enable the maximum vision. Therefore, spectacle lens mounting on the frame should be performed correctly and meticulously.

f) Dispensing Steps

When measuring in the lensmeter, we first adjust the ocular according to our eyes. As the values in the lensmeter become clear, the ocular is adjusted according to our eyes. While looking through the ocular in the lensmeter, we see two and three lines inside the lensmeter as given in Figure 17. The value of two lines gives the spherical value (SPH), while the value of three lines gives the spherical+cylindrical (SPH + CYL) value. SPH is found by subtracting the value of 2 lines from that of 3 lines. If there is a CYL value on the prescription, the axis value on the lensmeter is marked. The lens is marked with marking pins after the lens diopter values are measured.

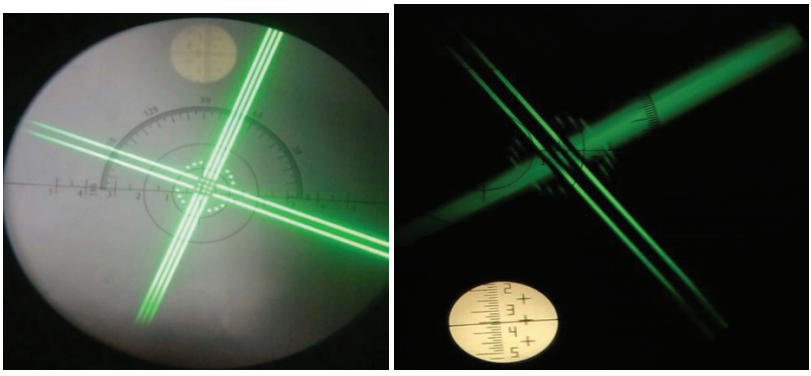


Figure 17. *The inside view of the lensmeter showing the narrow and wide cross lines and the power scale*

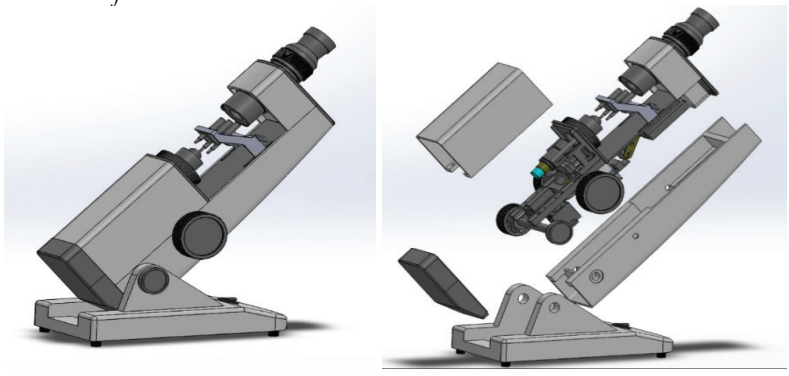
A lensmeter is used to measure the vertex power of the lens. During the measurement of a cylindrical or spherical lens, the image of the target is seen as a ring of dots (Bhootra, 2009). In clinical applications, the subject of the measurement is the power of a lens with respect to its front or back surface. The power associated with the back surface (or the ocular surface in an ophthalmic lens), is called the back-vertex power, which is abbreviated as

$$\text{Fv}\ (F_V = \frac{F_1}{1-\left(\frac{t}{n}\right)F_1} + F_2) \qquad (5)$$

When using a lensmeter to neutralize the distance prescription in a patient's spectacle lenses, we typically measure the back-vertex power. It can be calculated with the following formula where F1 is the front surface's power, F2 is the second surface's power, t is the thickness of the

lens in meters, and n is the lens's index of refraction as shown in Equation 5 (Schwartz, 2013). The relationship between the target movement and the power of the unknown lens can be deduced from Newton' s relationship as $f^2 = -x.x^{\dot{y}}$ (Fowler and Petre, 2001). Spectacle prescriptions can be read and written in two value sets. Cylinder value in the prescription is read as a minus and a plus cylinder (Brooks and Borish, 2007; Brooks, 2003). The lensmeter uses a system of rings inside the lensmeter to indicate the amount of prism being measured. If the lensmeter is focused, the lines on the target cross at the location of the optical center of the lens is clearly visible. If the target lines are not centered, the place on the lens where the lensmeter is measuring creates a prismatic effect. The amount of prism is measured by reading the location of the intersection of the target lines (Brooks, 1992).

Eyepiece, lens holder lever, ink marker, inkwell, spectacle table lever, spectacle table, power drum, lens stop, and cylinder axis wheel are exterior design parts of the device (McCleary, 2008). The light source, color filter, marker reticle, measuring objective lens, aperture, objective lens, the angle reticle, cross reticle, ocular, reading division board, reflective lens, front lens, right angle prisms, and back lens are the optical components constituting the interior design. Main components of a lensmeter is given in Figure 18. In a lensmeter, one of the most important optical elements is the objective lens that gathers light coming from the object and focuses the light rays to obtain a real image. Ocular lens (Eyepiece) ensures parallel incidence of light rays to the eye. This system of pairs is called a telescope. The target image is obtained through a lens of focal length. The user axially shifts the target until it is simultaneously in focus with the reticle. The target position d is related with the power of the spectacle lens ϕ ($\Phi = \frac{d-f}{f^2}$) by (Schwiegerling, 2004).



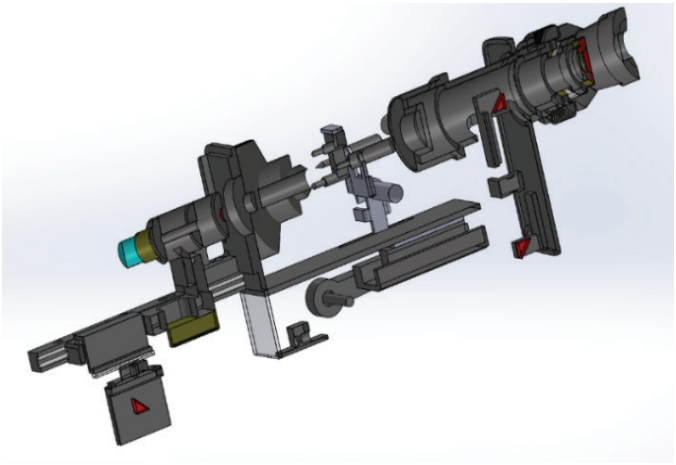


Figure 18. *Normal view and exploded view of a lensmeter assembly (SLM-1 Lensmeter Manual, 2020).*

Generally, there are automatic, semi-automatic and non-automatic lensmeter models. They have different technical specifications. Their properties include the measuring ranges determined at the minimum and maximum range. Measuring range, astigmatic axis angle of cylindrical lens that has 1-degree minimum scale value are $0\text{-}180^0$. Prism diopters range between $0 \sim 20\Delta$. Prism basal angle is between $0\text{-}180^0$. The diameter of the lens to be measured is in 16 mm-80 mm range. The optical system of a lensmeter involves mirrors, lenses and prisms directing the light on the optical path of the lensmeter. Focusing system includes the light source, target (cross hairs), standard lens, lens stop and power wheel (SLM-1 Lensmeter Manual, 2020). The ordered ophthalmic optical lenses are examined on the lensmeter (focimeter) device and their focus is marked (Figure 19).

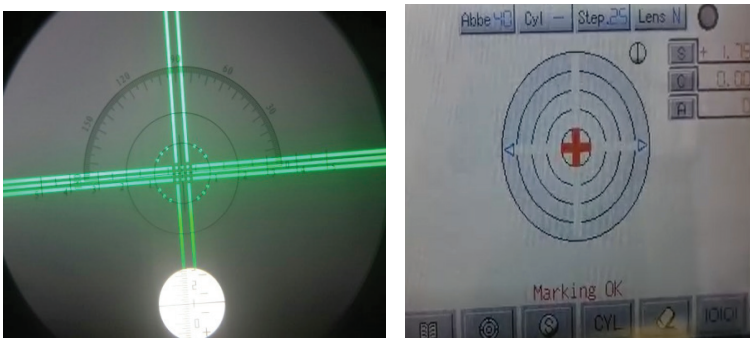


Figure 19. *The reading diopters of lenses*

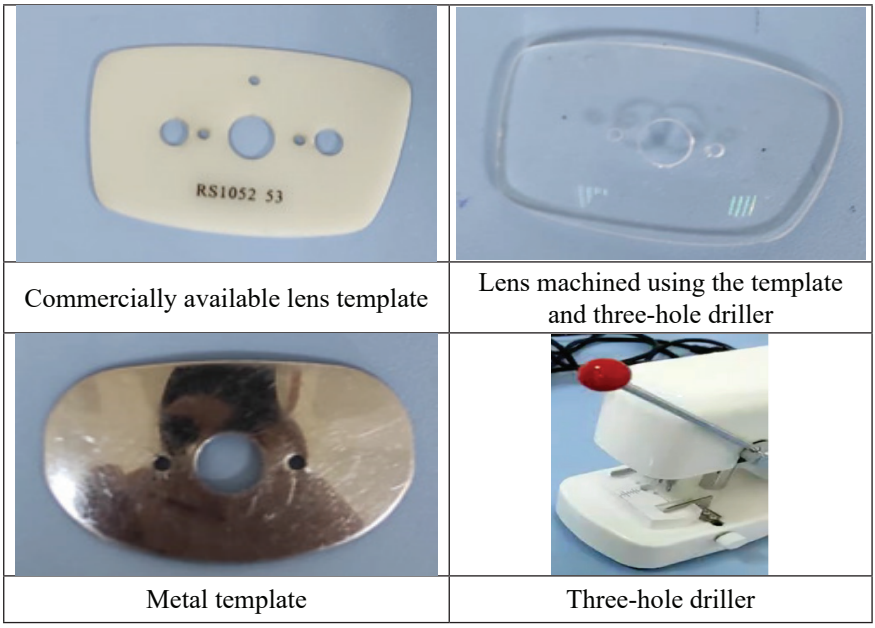


Figure 20. *Templates and three-hole driller*

Before the edging of the lens in automatic lens edger, a template lens should be prepared for use in the device (Figure 20).

Figure 21. *The marking on the lensmeter of the lenses*

Optical center of the lens is marked with the optical center device (Figure 22). If the alignment of lens' optical center fails, image displacement may happen due to the induced prism.

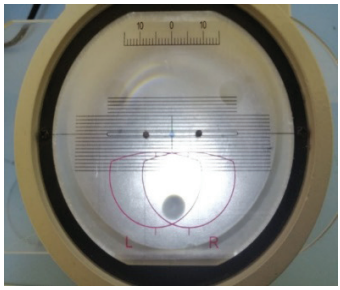


Figure 22. *The optical centering measurement*

Suction cups are attached to the adjusted optical centers of the eyeglasses with the adhesive tape (Figure 23).

Figure 23. *Optical center device, decentration adjustment and suction cups*

The diopters of the optical lenses that are supplied and prepared for edging are checked by a lensmeter device (Figure 21) and their focus is adjusted (Figure 19), marked and the suction cup is glied in the optical centering device (Figure 23). For the lens edging process, optical lenses are placed in a lens edger device (with or without template) and the fitting dimensions are defined on the machine (Figure 24).

Figure 24. *Patternless lens edger*

Edging is the process of cutting or machining the optical ophthalmic lens to fit into the frames, to obtain the pair of eyeglasses (Figure 24).

Automatic lens edging process takes place which is followed by chamfering using a manual lens edger when smoothing is required (Figure 25).

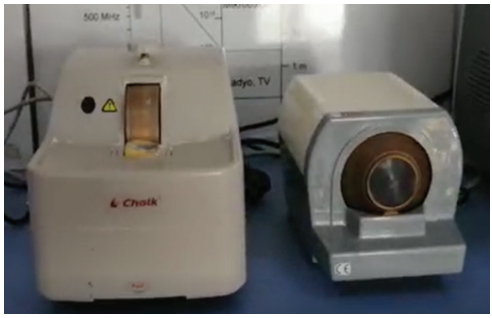


Figure 25. *Manuel lens edger*

If it is a full-frame, a herringbone pattern is engraved to mount the lens. In the case of semi-rimless frames, grooving process takes place (Figure 26).

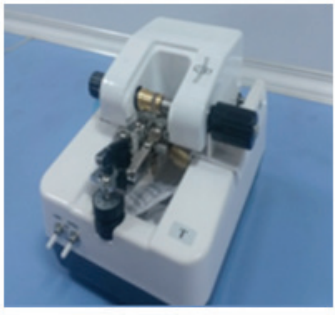


Figure 26. *Lens groover*

Drilling and notching are applied on rimless frames (Figure 27).

Figure 27. *Manuel drilling machine and multifunctional drilling machine*

g) Final inspection of the assembled eyeglasses

The machined (or edged) lenses are mounted on the frame and the mounting step is thus completed (Figure 28). A final inspection of eyeglasses should be made after the lenses are mounted on the frame. In this stage, parameters such as the axis value, PD, segment height, fitting height, vertex distance, pantoscopic tilt should be checked and verified. The prepared eyeglasses should be in correct alignment on the patient's face. Also, eyeglasses should provide comfortable usage and clear vision for both two eyes.

Figure 28. *Final inspection of spectacle lenses.*

The cleaning of eyeglasses is an important issue in terms of a healthy vision for patients. Therefore, patients should be informed as to how they should clean their eyeglasses to avoid scratches and other damages to their eyeglasses. For cleaning purposes, eyeglass cleaning cloths, specifically manufactured for this purpose, should be used. These cotton-free and hydrophilic (water-retaining) clothes can be used along with special sprays and smoothly moved on eyeglasses without impairing both lenses and patients' vision.

5. Conclusion

In opticianry programs, students are provided with the specialized knowledge and skills required to dispense ophthalmic lenses, in addition to a basic knowledge of optics, vision optics, geometric optics and eye anatomy, and equipped with the capability to use optical devices. This study aims to provide an overview of the properties of ophthalmic optical lenses, design, fitting, and dispensing of corrective lenses for the correction of a person's vision, mounting applications for eyeglasses, PD measurement, prescription of eyeglasses and the use of optical machines, for use by individuals studying and working in the field.

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Chapter 9

HEAD TRAUMA

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INTRODUCTION

Head trauma, today is evaluated as an important life-threatening health problem with high morbidity and mortality rates, which constitutes more than half of the deaths due to trauma. Head trauma is one of the most common reasons for admission to the emergency room. It is more common in pediatric patients. Traumatic brain injury (TBI) is defined as the impairment of brain function by a blow to the head, penetrating injury, or a concussion. This dysfunction may be temporary or permanent and may or may not result in underlying structural changes in the brain (Thurman, Miller, & Hayes, 2001). TBI is one of the main problems that physicians have to overcome in emergency services during the first aid process. Since it is an important cause of death and disability in people of production age, it causes severe social and economic losses (Baker, Baker SP, Ginsburg, Li, & O'Neill, 1992). Approximately 1/3 of all trauma deaths are due to head trauma (Heegaard,& Biros, 2007). Clinical severity in TBI is classified according to Glasgow Coma Scale (GCS) (**Table 1**). Based on GCS, TBI is divided into severe (GCS 3-8), moderate (GCS 9-13), and mild (GCS 14-15) degree (Thurman et al, 2001).

Table 1. *GCS Scale*

Eye Opening	4	Spontaneous eyes open
3		With sound
	2	With pain
	1	No answer
Verbal Response	5	Oriente
4		Confused
3		Inappropriate Words
	2	Meaningless sounds
1		No answer
Engine Response	6	Fits order
	5	Localizes the pain
	4	Pull
	3	Abnormal flexion
	2	Abnormal extension
	1	No answer

Mild degree TBI accounts for approximately 80% of head injuries. Moderate TBI constitutes approximately 10% of head injuries and the mortality rate is lower than 20%. Mortality in severe TBI is approximately 40% and deaths occur mostly within the first 48 hours (Wright & Merck, 2011).

Anatomy

The outermost layer of the scalp consists of 5 layers; skin, subcutaneous tissue, galea, areolar tissue and pericranium from the outside to the inside. Since the scalp is rich in blood vessels, it plays a major role in temperature regulation and may cause serious blood loss after injuries. The skull is like a closed box and consists of eight major bones. Since the skull base is the entry and exit point of cranial nerves and blood vessels, these structures are at risk in base fractures. The occipital ridge is the anatomical junction of the frontal, sphenoid, temporal and parietal bones. At this point, skull fracture may cause an epidural hematoma by causing a rupture in the lower middle meningeal artery. The brain consists of three main structures: the cerebral hemispheres, the cerebellum, and the brain stem. In the cerebral hemispheres, the falx divides the cerebri vertically into right and left. Tentorium cerebelli separates the brain stem from the cerebrum at the cerebellum and base. The inner edge of the tentorium cerebellum is the most common brain herniation syndrome, the uncal herniation site. The cerebrum is divided into major lobes according to the bony structure under which it is located: frontal, temporal, parietal, occipital. The brain is surrounded by multiple anatomical layers and potential intervals. the dura mater ,the outermost layer, firmly adhered to the skull and cranial sutures. There is a thin connective tissue arachnoidmatter underneath. It pierces the dura from the venous sinuses, these points are the points where cerebrospinal fluid and blood drain from the brain. The arachnoidmatter is loosely attached to the piamater forming the potential subarachnoid space. Cerebrospinal fluid (CSF) circulates in the subarachnoid space and is approximately 150 ml around the brain and spinal cord in adults, this fluid is produced from the choroid plexus and is 500 ml daily (Ainsworth & Brown, 2020**).**

Physiopathology

The brain consumes 20% of the body's total oxygen demand and 15% of the total cardiac output. The brain is very sensitive to ischemia and hypoxia. Cerebral blood flow changes and adapts according to the regional needs of the tissue, which is called autoregulation. Regional cerebral blood flow is maintained through the broad scope of cerebral perfusion pressure (BPE) (between 50-150 mmHg in a normal functional system). Since accurate measurement of cerebral blood flow is difficult, especially due to regional differences and needs, BPE is used as an indicator for monitoring. SBP; is the pressure difference required by cerebral tissue perfusion. SPB is calculated as the difference between mean arterial pressure (MAP) and intracranial pressure (ICP):

SPB = OAB- KIB

OAB is calculated by the formula = [Systolic blood pressure + (2 X Diastolic blood pressure)] / 3.

Factors affecting local cerebral blood flow are microvascular changes in blood volume, pH, PO2 and PCO2, oxygen supply and metabolism (Wright & Merck, 2011). Autoregulation is impaired in most patients with TBI, however, even a moderate drop in blood pressure results in cellular hypoxia. Increased in ICP decreases BPE and cerebral blood flow more.

Typically in adults, the intracranial volume is about 1500 ml. It makes up 80-85% of the brain, 10% of the intravascular cerebral blood volume and <3% of the cerebrospinal fluid (CSF). When a severe head injury occurs, cerebral edema often develops, the relative volume of the brain increases. Since the intracranial volume is constant, the pressure in this compartment increases without some compensatory effects such as a decrease in the volume of another intracranial component. Cerebral vessels maintain the desired SPB by changing the physological state with the ability of vasoconstriction and vasodilation. Vasoconstriction occurs with hypertension, hypocarbia, and alkalosis, and hypotension, acidosis, and hypercarbia cause cerebral vasodilation (Wright & Merck, 2011; Ainsworth & Brown, 2020). As PCO2 decreases with hyperventilation, cerebral vasoconstriction occurs. This vasoconstriction results in a decrease in parenchymal blood flow that buffers the effects of hematoma in the rigid cranial space and increased cerebral edema. However, prolonged hyperventilation causes severe decrease in systemic PCO2, injured brain tissue neighborhood and severe vasoconstriction in the penumbra, and brain ischemia and cell death. Therefore, proactic and prolonged hyperventilation is not recommended (Heegaard,& Biros, 2007). TBI is divided into two as primary and secondary brain injury. Primary brain injury is the initial brain injury that results from direct trauma (Ainsworth & Brown, 2020). Primary injuries are contusion (crushing of the brain parenchyma), hematoma (subdural, epidural, intraparenchymal, intraventricular and subarachnoid), diffuse axonal injury, direct cellular damage (neurons, axons and other supporting cells), tissue ruptures and cuts, disruption of the blood-brain barrier, these include impairment of neurochemical homeostasis and electrochemical functions (Wright & Merck, 2011). Secondary injury results from the biochemical consequences of systemic hypotension, hypoxia, anemia, hyperglycemia, increased intra-cranial pressure (ICH), or various physiological changes induced by the original trauma. Treatment of TBI is to prevent or minimize secondary brain injury (Ainsworth & Brown, 2020). Hypotension (systolic blood pressure <90 mmHg) and hypoxemia (PaO2 <60) are associated with two-fold mortality, and anemia (hematocrit <30%) causes an increase in mortality as it decreases oxygen carrying capacity (Rajajee, Moreira, & Rabinstein, 2020). Increased in ICP decreases BPE and cerebral blood flow more (Wright & Merck,

2011; Ainsworth & Brown, 2020). Rapid increase in ICP causes Cushing reflex (hypertension, bradycardia and respiratory irregularity). This triad is classic for an acute increase in ICP, but occurs in only one-third of cases and is more common in children than adults.

PRE-HOSPITAL EVALUATION AND INTERVENTION

Because secondary injury has an impact on the brain, early management can have enormous effects on the final outcome. Therefore, hypoxia and hypotension should be detected rapidly in the field. Management and rapid transport to the appropriate facility is critical for patients with moderate to severe head injury. Management proper airway cervical spine stabilization and circulation control is the first priority for all trauma patients. Prehospital intubation is controversial, and data on whether rapid sequential intubation is appropriate in the field is not entirely clear. While the results of other studies are controversial, some studies have shown worse outcomes with rapid sequential intubation before hospital. One potential cause for worse outcomes is post-intubation hyperventilation during transport, avoid hyperventilation (Esnault et al, 2019).

EMERGENCY SERVICE MANAGEMENT

In the anamnesis, how and when the injury occurred, the type of force, the location of the injury in the head are questioned.It is questioned whether it was a direct hit to the head, a motor vehicle accident or a fall, for falls; Did it fall from a height? Or did it fall on the ground where it was? Or did his foot slip off? Or he is questioned whether he fell due to a syncope and suffered a head injury. In this process, the presence of clinically important traumatic brain injury (TBI) is investigated in patients, but sometimes intracranial pathology may give little or no clinical findings. The goal of resuscitation in the emergency service is to prevent secondary injuries and slow the spread of the underlying injury (Wright & Merck, 2011).

Airway and Respiration

As hypoxia increases the mortality rate, aggressive airway and respiratory intervention is required. All severe TBI patients (GCS ≤8) require rapid airway control. Using short-acting induction agents with limited effects on blood pressure and ICP, orotracheal rapid sequential intubation (RSI) is preferred (Wright & Merck, 2011).

Table 2. *Drugs recommended in RSI*

Induction agents (sedatives / hypnotics)
Etomidate, 0.3mg / kg iv
Propofol, 1-3mg / kg iv
Neuromuscular blocking agents

(Long-acting drugs are not recommended)

Succinylcholine, 1-1.5mg / kg iv

Rocuronium, 0,6mg / kg iv

Circulation

Hypotension followed by ischemia in damaged neuronal tissue accelerates the secondary injury process and results worsen. Even a single episode of hypotension increases mortality in severe TBI. Hypotension and hypoxia alone increase the mortality rate by 150% (Wright & Merck, 2011). Therefore, aggressive fluid therapy may be required to prevent hypotension. The guidelines recommend keeping systolic blood pressure> 90 mm Hg. There is no recommended value for OAB. Hypotension rarely develops in isolated head injuries. If fluid resuscitation is ineffective, vasopressor therapy can be initiated with MAP around 80 mm Hg (Heegaard,& Biros, 2007; Wright & Merck, 2011).

Neurological Examination

Neurological vital signs evaluation is made according to GCS score. GKS is used for grading the severity of TBI. Complete GCS evaluation must be made before sedation and intubation and after resuscitation (Wright & Merck, 2011). Another important part of the neurological examination is pupil assessment (size, activity and anisocoria). In unanswered patients, unilateral fixed and dilated pupils, uncal herniation and ipsilateral intracranial hematoma may be signs. Direct eye trauma should also be considered. Bilateral fixed and dilated pupils support the increase in ICP by poor perfusion, bilateral uncal herniation, drug effect or severe hypoxia. Bilateral pinpoint pupils (ping pong pupils) suggest opiate use or pontine lesions. Altered motor functions may indicate injury to the brain, spinal cord or peripheral nerve. The decorticated posture indicates supra-midbrain injuries, while the decerebent posture indicates more caudal injuries and has poor results. Respiratory patterns and eye movements provide more information about brainstem functions in completely unresponsive patients (Wright & Merck, 2011). Raising the head of bed by 30 degrees increases CSF flow from the skull base.

CLINICAL SITUATIONS IN TRAUMATIC BRAIN INJURY

1. Mild Degree Traumatic Brain Injury

Mild degree TBI (sometimes called concussion) is characterized by associated signs and symptoms after GCS 14-15 injury. Most minor head injury episodes occur during sports injuries and other activities. Loss of consciousness is not the determining factor of mild degree TBI (Heegaard,& Biros, 2007). In AS, the diagnosis of mild TBI is mainly based on the history of the injury, ie any mental state change during or after

the event, to the story around. This definition includes, so to speak, 'ringing bells', 'seeing stars' or being stunned or confused as a result of injury. The presence of amnesia makes the diagnosis stronger and may be associated with more serious injury (Lovell, Collins, & Bradley, 2004; Cantu, 1998; Iverson, Gaetz, Lovell, & Collins, 2004). Loss of consciousness remains an important risk factor for detecting lesions on CT, but is only one of several risk factors (others are vomiting, moderate-to-severe headache, focal neurological complaint, age> 65, skull base fracture physical examination findings, coagulopathy and dangerous injury mechanism) (Wright & Merck, 2011).

The key decision for the emergency room physician is whether a brain tomography should be performed in cases of minor head injury. CT is performed according to clinical criteria in patients with mild TBI. Two types of criteria have been developed; It supports neuroimaging indications if it complies with the Canadian Brain Tomography guidelines (**Table 3**), New Orleans Criteria (**Table 4**) and Nexus II (**Table 5**) (Evans & Whitlow, 2020; Stein, Fabbri, Servadei, & Glick, 2009). Both have been validated in prospective studies and are 100% sensitive in detecting patients who require neurosurgical intervention, but their selectivity is limited (3% and 37%, respectively) (Haydel, 2000). If the intracranial lesion is taken as the final target point in diagnosis, the Canadian Brain CT Rule is less sensitive (83%). The disadvantage of these two rules is that patients must have loss of consciousness and memory loss in order to be used. These rules should not be applied to patients using anticoagulants or children, because they were excluded from validity studies (Stiell, Laupacis, & Wells, 2000; Collins, 2002).

Table 3. *Canadian IT Rules*

Criteria for using these rules
1. GKS 13-15
2. Age > 16
3. No history of coagulopathy or coagulation
4. If there is no open skull fracture.
According to Canadian Rules Brain CT is not required if the following criteria are not available
➢ Age > 65 years
➢ Vomiting> 2 times
➢ Skull fracture, which may be open, suspicious or displaced
➢ Signs suggesting a basal head fracture:
❖ Hemotympanium
❖ Raccoon eye finding
❖ Otorrhea and nosebleeds
❖ Battle's sign (ecchymosis on the mastoid bone)
➢ GCS <15 2 hours after injury
➢ Retrograde amnesia for more than 30 minutes
➢ Dangerous mechanism of trauma
❖ Pedestrian-powered vehicles hit
❖ Kicking out of the motor vehicle
❖ Fall:> 90 cm or falls from 5 ladder-steps.

Table 4. New Orleans Rules

Patients included in this rule
Age> 18
GKS score is 15
Loss of consciousness or amnesia and disorientation within 24 hours after blunt head injury
CT is not indicated if patients do not have any of the following criteria.
➢ Age> 60 and over
➢ Headache (diffuse or local)
➢ Vomiting
➢ Alcohol or drug intoxication (Alcohol intake determined clinically or by level)
➢ Short-time memory loss (Persistent antregrade amnesia)
➢ Trauma finding in the area above the clavicle (contusion, abrasion, laceration, deformity, facial and skull bone fracture findings)
➢ Seizure (Post-traumatic seizure or suspicion of seizures)

Table 5. Nexus II Rules

Brain Tomography is not required if All of the following are not available
➢ Age≥ 65 years
➢ Evidence of skull bone fracture
➢ Scalp (scalp) hematoma
➢ Neurological deficit
➢ Variable mental state
➢ Abnormal behavior
➢ Coagulopathy
➢ Repetitive or strong vomiting

Most low-risk minor head trauma patients can be discharged from the emergency room after a normal physical examination, but effective observation for 4-6 hours is recommended. All patients should be educated in terms of delayed symptoms and signs of head trauma. The patient should be awakened every two hours on the first night and should avoid stressful activities for at least 24 hours. In the presence of any of the following symptoms, urgent medical assistance should be sought (Evans & Whitlow, 2020).

- Unable to wake up the patient.
- Severe or ingravescent headache
- tendency to sleep
- clouding of consciousness, seizure

- Visual impairment, double vision
- Vomiting, fever, or stiff neck
- Urine or stool incontinence
- Numbness or weakness in any part of the body.

Concussion

Also known as mild TBI, it is a brain injury as a result of force or impact applied directly or indirectly to the head, with or without loss of consciousness that constitutes a range of possible symptoms. Impairment of brain function is associated with loss of neurometabolic function rather than structural injury, and is typically associated with normal structural neuroimaging findings. Normal result in brain CT scan excludes injury requiring neurosurgical intervention, but the findings are negative in most patients with mild TBI. Concussion results in a cluster of cognitive, somatic, emotional, and sleep-related symptoms. The duration of the symptoms is variable and may last within a few minutes, and in some cases, last for days, weeks, months or longer (Heegaard,& Biros, 2007; Wright & Merck, 2011).

It is absolutely necessary to detect concussions in athletes. Athletes in general do not want to quit and tend to downplay their findings. A tension may arise between the team's need and the athlete's health. The decision to return to the game should be made very carefully and preferably by medical personnel trained in concussion management. Repetitive concusions may worsen the result and lead to second strike syndrome (Iverson,2004; Collins, 2002). Treatment of symptoms includes rest, "brain rest" and includes therapies that target specific symptoms (NSAIDs, hydration, etc.). Aspirin should be avoided to prevent any bleeding complications (Ingersoll, 1993).

2. Moderate Head Trauma

GKS 9-13 and its clinical admissions are highly variable. Patients may have temporary loss of consciousness, brief posttraumatic seizure, and confusion, but most patients follow orders when they come to the emergency room. The most important clinical picture in moderate TBI is the "talking and worsening" patient. These patients may speak initially, but it worsens within 48 hours. In general, 40% of patients with moderate TBI have abnormal findings in CT and 8% require surgical intervention (Thurman, Miller, & Hayes, 2001). 75% of the patients who speak and worsen have an extra-axial hematoma such as subdural or epidural. Brief hyperventilation and osmotic therapy should be initiated as an emergency intervention in patients presenting with moderate TBI and worsening in the emergency room, in the absence of a neurosurgeon and developing

signs of herniation. CT should be performed in all moderate TBI patients and they should be hospitalized for observation even if the CT is normal (Heegaard,& Biros, 2007).

3. Severe Head Trauma

It defines severe TBI patients as GCS 8 or less. Orotracheal intubation is preferred using short-acting induction agents with limited effects on blood pressure and intracranial pressure (ICP). 10% of TBI cases are severe TBI. 25% of severe TBI patients require neurosurgical intervention. Mortality in adults is about 60%; mortality is lower in children. Cushing re minus or Cushing phenomenon is seen in severe TBI, which is associated with increased ICP and herniation syndrome, and requires urgent aggressive ICP management including hyperventilation and maximum osmotic therapy (Heegaard,& Biros, 2007). Hyperventilation decreases ICP with cerebral vasoconstriction, its effect starts within 30 seconds. However, vasoconstriction induced by hyperventilation may cause secondary ischemia and worsen the results. Hyperventilation should be seen as a short-term life-saving intervention and should be used in the emergency department only for patients with herniation findings or worsening neurological findings (Heegaard,& Biros, 2007; Rajajee, Moreira, & Rabinstein, 2020). Hypotension is a terminal event in TBI and is a sign of herniation. Therefore, other sources of hypotension should be considered in patients who have not developed herniation. Aggressive fluid therapy should be initiated to prevent hypotension and secondary brain injury. Isotonic fluids should be used in the continuation of euvolemia. Fluid and blood products do not increase ICP in hypotensive patients. Systolic blood pressure should be kept> 90 mm Hg (Heegaard,& Biros, 2007; Wright & Merck, 2011). If the patient is unresponsive to intubation and hyperventilation and has signs of space-occupying extra-axial hematoma or emergency herniation, the use of osmotic diuretics such as mannitol or hypertonic saline (HTS) should be considered. Osmotic agents can be life-saving in case of deepening of coma, pupil inequality or other neurological examination deterioration (Heegaard,& Biros, 2007). Mannitol is the most commonly used agent in ICB control, it is a free radical scavenger, it has been shown to improve cerebral blood flow, it also expands plasma volume, first of all, hypotension decreases and oxygen transport capacity improves. It reduces the ICP within thirty minutes and its effect is variable up to 6-8 hours. If necessary, 0.25-1 g / kg iv bolus is given every 4-6 hours (Rajajee, Moreira, & Rabinstein, 2020). Corticosteroid Therapy: Their use after TBI has been widely studied and has been found to be of no benefit. In large meta-analyzes, negative effects such as immunosuppression effects, induction of diabetes and delayed wound healing have been demonstrated. Posttraumatic seizures occur in 12% of patients with blunt head trauma and 50% of patients with penetrating head trauma. Posttraumatic seizure is not

a precursor to epilepsy. Treat the seizure immediately, seizure may increase subarachnoid hemorrhage and intraparenchymal volume. Benzodiazepines are fast acting, first choice anticonvulsants. diazepam (0.1 mg / kg, 5 mg IV, total 20 mg every 5 minutes) is used. For long-term anticonvulsant activity, phenytoin (13-18 mg / kg IV) or fosphenytoin (13-18 mg / kg) can be given (Heegaard,& Biros, 2007).

Epidural Hematoma

Epidural hematoma is associated with middle meningeal artery rupture. Blood accumulates in the potential gap between the skull and dura mater. It is usually unilateral, and the deterioration of epidural hematoma patients due to arterial bleeding is rapid and dramatic. It is rare in the elderly and children under 2 years of age as the dura adheres very tightly to the skull (Heegaard,& Biros, 2007). It mostly occurs as a result of blunt trauma to the temporal or temporoparietal area. The classic symptom of epidural hematoma is seen in the lucid intervale in the first period, which is the change in consciousness and perception that occasionally opens and closes. The diagnosis of epidural hematoma is based on physical examination findings and CT scanning. On CT scan, epidural hematoma appears more in the temporal region, biconvex (**Figure 1, 2**). High pressure arterial bleeding of an epidural hematoma can cause a herniation within hours after injury. Therefore, early detection and evacuation are key to reducing morbidity and mortality. Underlying injury to the brain parenchyma is often absent, so full recovery can be expected if the hematoma is evacuated prior to the development of neurological damage or herniation (Heegaard,& Biros, 2007; Wright & Merck, 2011).

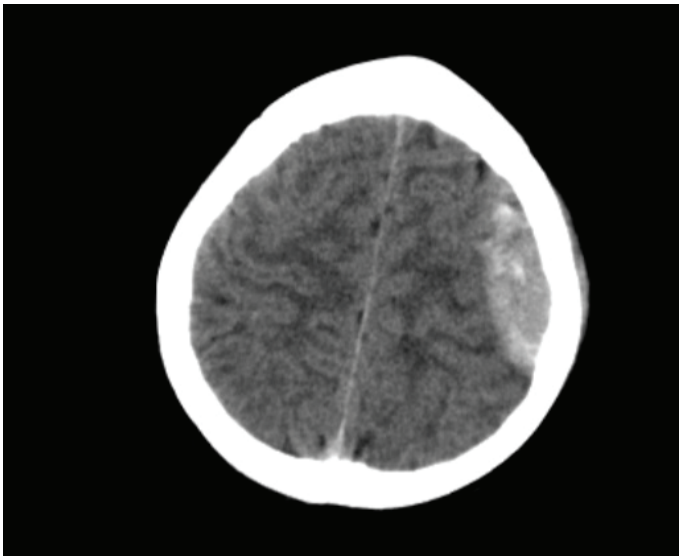

Figure 1 *(From Dr. Pasahan R archive)*

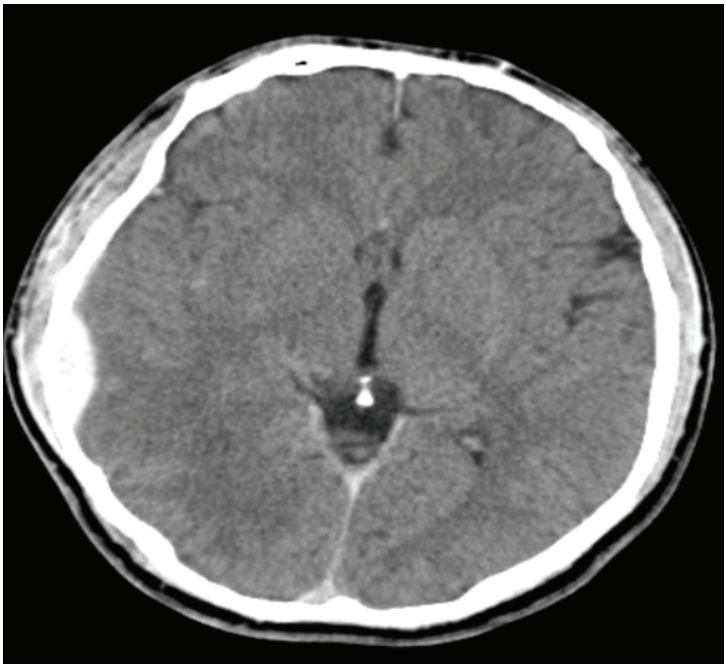

Figure 2 *(From Dr. Pasahan R archive)*

Subdural Hematoma

Subdural hematoma develops as a result of ruptured bridge veins. Blood accumulates between the dura mater and the arachnoid. Since blood is of venous origin, it tends to collect more slowly than an epidural hematoma. However, subdural hematoma is often associated with other brain injuries and underlying parenchymal damage. Extremely atrophic brains, the elderly, and alcoholics are more prone to acute subdural hematoma. Children under the age of two are also at high risk of subdural hematoma. Traditionally, subdural hematoma is divided into acute, subacute and chronic according to the time elapsed since the onset of active bleeding. Acute subdural hematoma often comes immediately after severe trauma, and the patient is often unconscious. Its symptoms usually develop within 14 days after injury. The occurrence of symptoms after two weeks is called chronic subdural hematoma. On CT, acute subdural hematoma appears as hyperdense, crescent-shaped, past the suture lines (**Figure 3**). Subacute subdural hematoma is not detectable (**Figure 4**), intravenous (iv) contrast-enhanced CT scan or MRI helps to detect subacute subdural hematoma. Chronic subdural hematoma appears hypodense (Wright & Merck, 2011). (**Figure 5**)

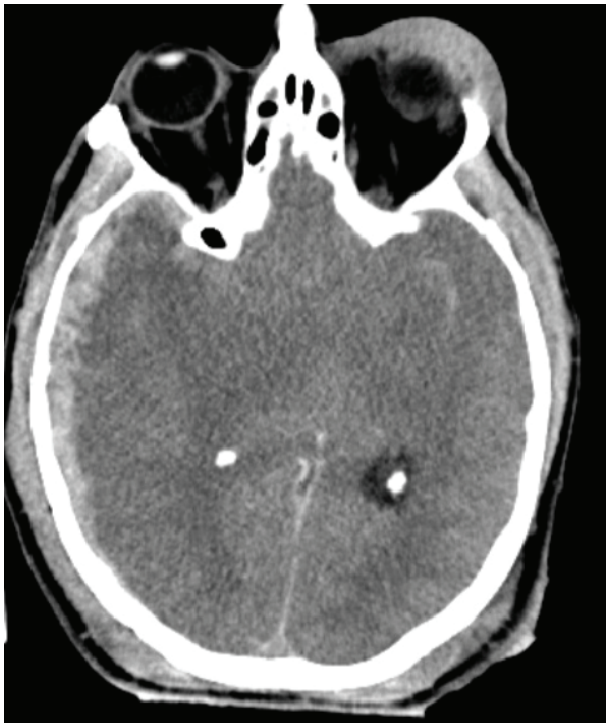

Figure 3 *(From Dr. Pasahan R archive)*

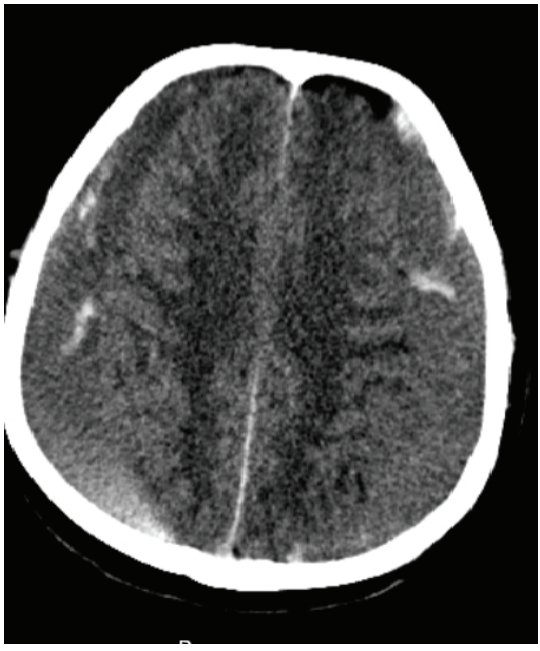

Figure 4 *(From Dr. Pasahan R archive)*

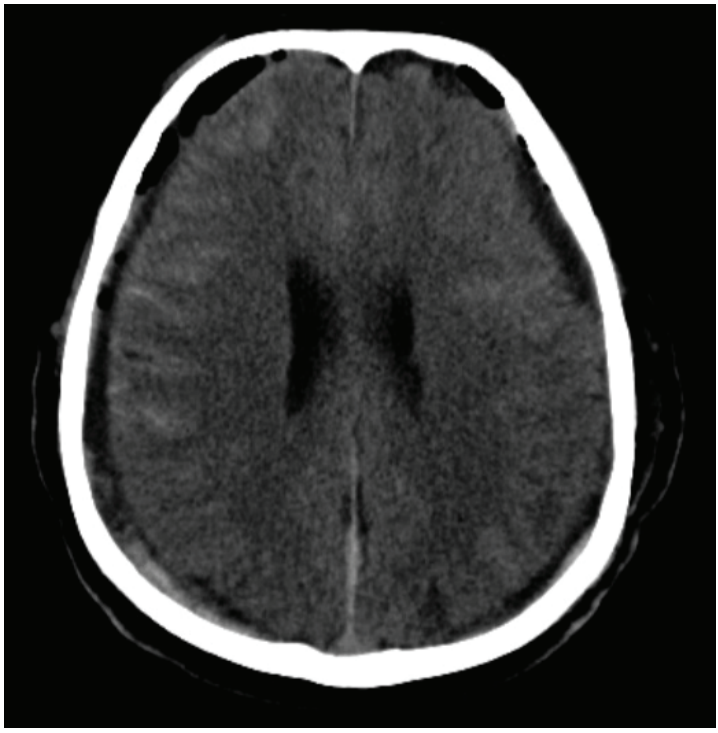

Figure 5 *(From Dr. Pasahan R archive)*

Subarachnoid Hemorrhage

Traumatic subarachnoid hemorrhage (SAH) is caused by rupture of parenchyma and subarachnoid vessels and there is blood in the CSF (**Figure 6**). Patients with traumatic subarachnoid hemorrhage alone may show signs of headache, photophobia, and meningeal irritation. Patients with traumatic subarachnoid hemorrhage have high morbidity and mortality (Friedman, Ebersold, & Quast, 2001). Some traumatic subarachnoid hemorrhages may be overlooked on early CT scans. In the presence of suspicious clinical conditions, a control brain CT scan performed 6-8 hours after injury is more sensitive to detect traumatic subarachnoid hemorrhage. Treatment is limited, but secondary injuries should be prevented by reducing cerebral edema.

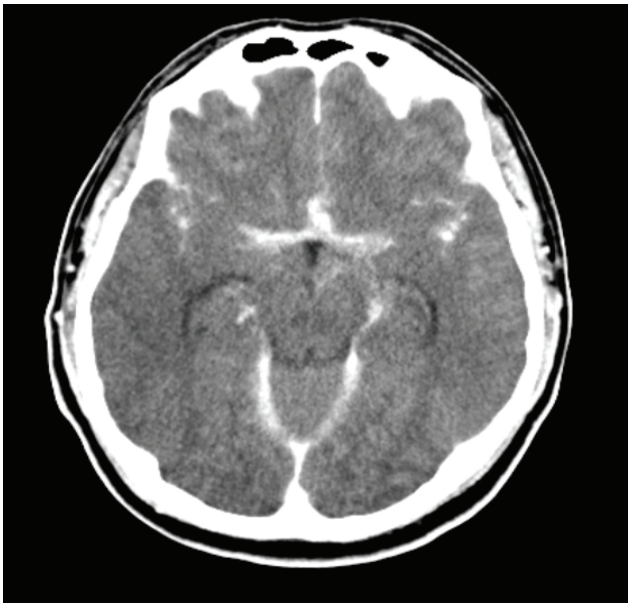

Figure 6 (*From Dr. Pasahan R archive)*

Diffuse Axonal Injury

Diffuse axonal injury is the rupture of axonal fibers in the white matter and brainstem. Predatory forces in neurons due to sudden deceleration are the cause of diffuse axonal injury. Classically, diffuse axonal injury occurs after blunt trauma such as a motor vehicle accident. Shaken baby syndrome in infants is well defined as the cause (King, MacKay, & Sirnick, 2003). In severe diffuse axonal injury, edema may develop rapidly. The underlying injury can result in devastating and often irreversible neurological damage. CT scan of patients with diffuse axonal injuries may show normal findings, but classical CT shows punctate bleeding injury along the gray-white junction of the cerebral cortex, and deep structures of the brain. Diffuse axonal injury treatment options are quite limited, the aim should be to reduce cerebral edema, and prevent secondary damage by limiting the pathological increase in ICP.

SPECIAL HEAD INJURIES

Scalp Incisions

Since the scalp has a rich vascular bed, its incisions may cause intense blood loss and should be checked as quickly as possible. Wounds should be carefully examined prior to closure to detect underlying fractures or galeal incisions. Large galeal separations should be repaired, and tetanus prophylaxis should not be forgotten.

Skull Fractures

The skull is the bone structure that provides protection for the brain. Brain CT is required for all patients with skull fracture or suspected skull fracture. Skull fractures are often classified by location (pedestal, skull convexity), structure (linear, collapsing or fragmented), and whether it is open or closed. Skull fractures that cause open or collapse, involving the sinuses and intracranial air infiltration (pneumocephaly) should also be treated with antibiotics. The most common skull base fracture involves the petrous part of the temporal bone, the external auditory canal, and the eardrum. Signs and symptoms associated with skull base fracture. CSF leak includes otorrhea or rhinorrhea, mastoid ecchymosis (Battle sign), periorbital ecchymosis (raccoon eye), hemotympanum, vertigo, hearing impairment or deafness, and seventh nerve palsy. Meningitis may develop in patients with acute CSF leak, and broad-spectrum antibiotics may be initiated (Friedman, Ebersold, & Quast, 2001).

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Chapter 10

ABDOMINAL TRAUMAS

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ANATOMY

Anatomically, abdominal trauma is defined as the trauma of the region from the nipples to the inguinal folds in anteriorly, from the lower end of the scapula to the gluteal fold in posteriorly. The abdominal area is divided into two parts: the peritoneal cavity and the retroperitoneal. Therefore, abdominal traumas are classified as follows: (Ronald M. Stewart, 2018)

1. Intraperitoneal

Trauma-related hemorrhage accumulates in the peritoneal cavity. Diaphragm, liver, spleen, gall bladder, small intestine, transverse and sigmoid colon injuries can be seen due to abdominal trauma. (Nichols JR & Puskarich MA, 2018).

2. Retroperitoneal

Retroperitoneal trauma is more difficult to diagnose because of it gives few symptoms. Injuries of the aorta, vena cava inferior, most of the duodenum, pancreas, colon and upper urinary tract can be seen due to abdominal trauma (Ronald M. Stewart, 2018).

MECHANISM OF TRAUMA

1. Blunt abdominal trauma

Blunt abdominal trauma is usually seen as a result of multiple injuries. It should be considered in unexplained shocks in trauma patients. In addition, physical examination findings can be misleading. Therefore, determining early diagnosis and treatment priorities in blunt abdominal trauma may be difficult and late. A single physical examination (PE) is not sufficient in these patients, serial PE should be performed. Therefore, diagnostic tools such as USG, CT and DLP should be used (Calder et al., 2017; French LK et al., 2016; Wang YC et al., 2012).

It is generally seen after traffic accidents, falls and assaults. While solid organ injuries are generally seen in direct trauma, tissues and vascular structures are disrupted in slowing down trauma, such as in high speed traffic accident and falling from height (Al-Mudhaffar & Hormbrey, 2014; Nichols JR & Puskarich MA, 2018). In order of frequency, the spleen, liver, small intestine and retroperitoneal organs are most commonly injured in blunt abdominal trauma.

2. Penetrating abdominal trauma

Penetrating injuries are the injuries to the small intestines, stomach and colon, inn order of frequency. This type of trauma is easy to diagnose, but it is difficult to determine whether peritoneal penetration is present. In this case, diagnostic laparotomy (DL), which is the most reliable examination, should be performed (French LK et al., 2016; Ronald M. Stewart, 2018).

Penetrating abdominal trauma should be examined in two groups as gunshot and stab wounds. In gunshot injuries, the probability of injury to the abdominal organs is 95-98%. It should also be kept in mind that, bullet may cause damage not only in the entrance area, but also in the surrounding tissues due to the blast effect. Important factors that need to be determined in the anamnesis such as the time of injury, the type of gun, the distance to be hit, the number of bullets hit and the estimated amount of blood at the scene should be kept in mind. In gunshot wounds, the entrance hole is small and the exit hole is larger, and the bullet usually changes direction in the abdominal area. In this type of trauma, if the physician is sure that the bullet has passed the peritoneum, it should be immediately performing an emergency laparotomy without losing time. In cases where it is not sure that there is an access to the abdominal area, a DL should be performed to detect penetration (Nichols JR & Puskarich MA, 2018).

In cutting-piercing injuries, it is necessary to determine whether the wound has passed through the peritoneum or not. Accordingly, peritoneum involvement should be detected by wound exploration or DL. Wound exploration is often performed because it can be applied more easily. However, when it is detected that the wound has passed the peritoneum, there is no exact indication for laparotomy as in gunshot wounds. Because, in injuries of the abdominal region by sharp cutting-piercing tools, with a probability of 60% of abdominal organ injuries can be seen (French LK et al., 2016). Therefore, a selective approach is required in stab injuries to avoid negative laparotomy. In such patients, DPL or DL should be performed, and laparotomy should be performed in those who have evidence of organ damage.

Laparotomy should be performed in the presence of sharp cutting-piercing tool injuries, or in accompanying shock, free air detection, blood in the nasogastric catheter or in rectal examination, or large evisceration in abdominal organs. Hematuria is not a definite indication for laparotomy. Furthermore, in renal penetrating injuries, if the patient is stable, if there is no urinary extravasation and there is no possibility of other organ injury, conservative treatment can be applied (Barnett, Love, Sepulveda, & Cheadle, 2014; Brenner & Hicks, 2018).

CLINICAL FINDINGS

In abdominal trauma, the clinic of the patients is usually insidious. Young and healthy patients may not be symptomatic even if they lose 50-60% of their blood volume. Head trauma, alcohol or substance abuse along with abdominal trauma can hide the patient's clinic. In addition, when severe intra-abdominal hemorrhage is experienced, PE findings may not develop. In fact, no symptoms may be seen in the early stages of cases with hemoperitoneum. Therefore, PE alone is not reliable, patients should

be evaluated with a comprehensive and systematic approach (Barnett et al., 2014; Nichols JR & Puskarich MA, 2018).

The most obvious symptom of abdominal trauma is abdominal pain. This pain occurs as a result of hematic, infectious, enzymatic and acidic irritation of the peritoneum. Pain can be suppressed due to spinal cord injury, another underlying medical problem, and distracting pain elsewhere in the body. Reflected pain on the shoulder tips or neck may occur as a result of hepatic or splenic injury due to abdominal trauma (Kehr sign) (El-Matbouly et al., 2016). Pain due to retroperitoneal injury such as urogenital or duodenal may be reflected in the testicles. Weakness, fatigue, dizziness, confusion, orthostatic hypotension and hypovolemic shock may be seen due to excessive blood loss. Nausea and vomiting may develop due to peritoneal irritation. Even respiratory system symptoms due to diaphragmatic irritation can also be seen (French LK et al., 2016).

Solid organ injuries

Solid organ damage often causes symptoms related to blood loss. When 30% blood loss occurs in patients, hypotension, tachycardia and confusion can usually be seen. In blunt solid organ injury, hemorrhage may have a slow course and early hemodynamic changes and peritoneal irritation findings may not be seen. However, sudden and profound hypovolemic shock may occur in the late period (French LK et al., 2016).

Hollow organ injuries

Hollow organ injuries appear with blood loss and peritoneal irritation findings. As a result of stomach, small intestine and colon injury, peritoneal contamination occurs and causes symptoms (French LK et al., 2016; Nichols JR & Puskarich MA, 2018).

Diaphragm injuries

Although diaphragm injuries are rare during abdominal trauma, it is difficult to diagnose. Hemodynamic stability of the patients may not change or they may present with a picture of deep shock. Symptoms in these patients may vary depending on the rate of displacement of the abdominal organs towards the thorax. The most common symptoms are shortness of breath, orthopnea and chest pain. In diaphragm injuries, the spiraling of the nasogastric catheter on chest X-ray may be diagnostic. (Biffl & Leppaniemi, 2015; Nichols JR & Puskarich MA, 2018).

DIAGNOSIS

Blunt abdominal trauma

In blunt abdominal trauma, no test alone is enough. Diagnostic tests, hemodynamic stability, and accompanying injuries should also be

evaluated in these patients with PE. In the first PE, it should be checked whether there is an abrasion or contusion in the abdomen. The lateral parts of the abdomen, back, lower thorax, abdominal anterior wall and pelvic region should be examined. Urogenital and anal area examination should not be forgotten in PE. In addition, PE should be repeated for at least 16-24 hours, and should be evaluated with vital signs and hematocrit values (Biffl & Leppaniemi, 2015; Nichols JR & Puskarich MA, 2018; Wang YC et al., 2012).

In abdominal trauma, the patient who is conscious and hemodynamically stable, having abdominal pain, tenderness and signs of peritoneal irritation are mostly reliable and can be detected in 90% of abdominal injuries (Calder et al., 2017).

Laboratory: Hematological and biochemical parameters have limited use in patients with acute abdominal trauma. It can be used to aid diagnosis, but cannot replace clinical evaluation. Hematocrit shows the extent and time of bleeding, exogenous fluid administration, and endogenous plasma return. Therefore, not a single, but serial hematocrit measurements should be done. White blood cell, which is an acute phase reactant, does not have any distinctive feature. It can increase in the absence of intra-abdominal injuries as a result of demarcation caused by trauma-related stress (Al-Mudhaffar & Hormbrey, 2014; Badru et al., 2019).

Lipase and amylase, which are among the biochemical parameters, may indicate pancreatic injury, but normal does not mean that there is no pancreatic injury. It can also be high in alcohol, drug intoxication and hypotension. Likewise, serum transaminases may be elevated in liver trauma, but cannot distinguish minor contusions from serious injuries. Metabolic acidosis in trauma patients indicates hemorrhagic shock. Increased lactate and base deficit indicate hypoperfusion and may be interpreted in favor of the presence of injury. (Al-Mudhaffar & Hormbrey, 2014; Nichols JR & Puskarich MA, 2018).

Direct graphics: In trauma patients, resuscitation and stabilization come first, before imaging. Chest radiographs in unstable patients may show extraperitoneal causes of hypotension. Plain abdominal radiographs can show the location of the bullet and its predictive path. It can also provide free air detection but cannot show where it originates from. Since all these can be easily detected in CT, patients are usually conducted to have a CT scan.

Ultrasonography (USG): In abdominal trauma, Focused Assessment with Sonography for Trauma (FAST) can be used to detect intraabdominal and intra-thoracic free fluid in bedside evaluation. Contrary to USG, FAST is an application that is used only for the evaluation of free fluid, not for the

detection of routine pathologies. With FAST, Morrison's pouch (between liver and kidney), splenorenal space, and Douglas pouch (between uterus and rectum) are evaluated. (Nichols JR & Puskarich MA, 2018; Stengel, Rademacher, Ekkernkamp, Güthoff, & Mutze, 2015).

FAST should be repeated twice with 6 hour intervals in a patient with abdominal trauma. By means of FAST performed by emergency physicians, the use of DPL decreases, the time of patient operation is shortened and medical cost is reduced. FAST is a non-invasive, bedside and repeatable method. There is no use of contrast agents and no radiation exposure. Pleural fluid can also be examined in the diagnosis of pericardial fluid and pneumothorax. FAST has not only advantages but also disadvantages. In these, it cannot detect intraperitoneal fluid etiology, its specificity and sensitivity changes depending on the practitioner, it is difficult to use in obese and subcutaneous emphysema, it may not be able to distinguish retroperitoneal pathologies well (Stengel et al., 2015).

Computed tomography (CT): It is the gold standard in abdominal trauma and is the first diagnostic imaging test. CT is the first option for organ-specific damage diagnosis, to detect retroperitoneal damage, duodenal and pancreatic pathologies. It can also detect injuries that do not require surgery. Therefore, it is used as the first diagnostic tool in most trauma centers. DPL has been put in the second strategies. It can indicate the source of intraperitoneal bleeding and determine the approximate amount of bleeding (Nichols JR & Puskarich MA, 2018).

Although abdominal CT has high sensitivity and specificity in trauma patients, use of nephrotoxic contrast media and exposure to radiation are its disadvantages. In addition, the patient needs to be transported to the CT unit and the cost is higher.

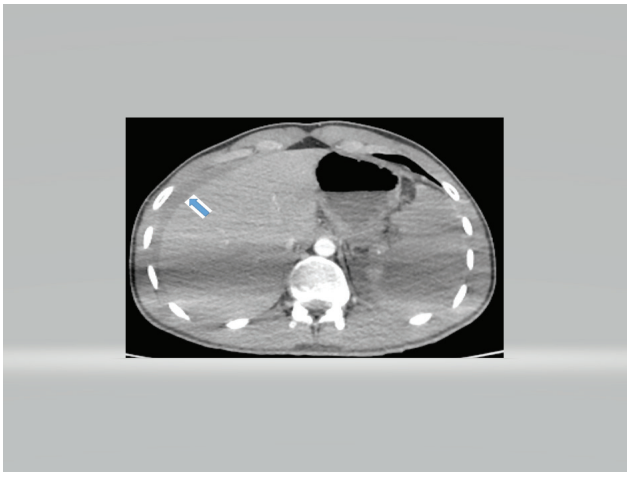

Figure 1. *Free fluid*

Diagnostic peritoneal lavage (DPL): During DPL, 10 mL of blood in the first aspiration was defined as positive. In cases where there is no blood in the first aspiration, 1 L isotonic is injected into the abdomen and should be left to free flow with a lavage catheter. Examinig 100,000 erythrocytes per microliter in the collected fluid is in favor of positive diagnosis. However, it is no longer the first choice due to the widespread use of CT and USG in emergency services today. It was observed that 30% of the cases with erythrocyte in DPL did not result in a positive diagnosis. The presence of leukocytes in DPL is not specific (Feliciano, 1991).

Local wound exposure: It is used to evaluate the integrity of the peritoneal cavity in stab injuries of the anterior abdominal wall. It is not very useful in obese patients. Local anesthetic is given to the wound site. Then, peritoneal penetration is examined by carefully examining the tissue layers. Blind evaluation with fingers, instruments or cotton-tipped swabs is not appropriate. After ensuring the integrity of the peritoneum by local wound exploration, and if FAST is negative, there is no intraperitoneal damage and appropriate primary repair is performed and discharged. If peritoneal integrity is impaired or the end of the wound trace is not detectable, an abdominal CT should be performed. In a patient with multiple injuries, local wound exploration is not preferred and requires intense effort. It is more rational to perform a CT scan in these patients. Deep exploration should not be performed in thoracic injuries in order to avoid neovascular and pleural damages. Superficial exploration is safer (Menichini, Sessa, Trinci, Galluzzo, & Miele, 2015).

Laparoscopy: Laparoscopy is used limitedly for diagnostic purposes in abdominal trauma in the emergency room. Hemodynamically unstable patients should be urgently performed laparoscopy in the operating room. In hemodynamically stable patients, awake laparoscopy can be performed under local anesthesia. However, it should be done by experienced physicians. There is a risk for the incidental invasive and iatrogenic injury. It is not suitable for evaluating non-surgical and retroperitoneal injuries (Cocco, Bhagvan, Bouffler, & Hsu, 2019).

Penetrating abdominal trauma

Penetrating-cutting tool injury: It may not be possible to detect the damage with PE and local wound exploration can be performed. Also, PE should be repeated serially. Internal exploration may be an effective method, especially in penetrating injuries in the anterior abdominal region. Radiographic examination performed by finger examination or contrast agent injection instead of local exploration can often lead to misdiagnosis or underdiagnoses. If the integrity of the anterior fascia is intact, the patient can be discharged easily in abdominal trauma caused by penetrating-cutting tool injury. However, if the integrity of the anterior fascia is impaired,

serial PE should be performed and laparoscopy should be performed with advanced diagnostic examinations (Nichols JR & Puskarich MA, 2018; Verdonck et al., 2016).

CT has gained widespread use in the diagnosis of penetrating abdominal injuries and is the first choice in most centers. Clinical findings may not occur within hours, especially in retroperitoneal organ injuries such as colon and rectum. We can easily detect contrast leakage or free fluid with free air on CT performed with oral rectal and intravenous triple contrast material. In addition, CT can be used more easily in the evaluation of side and back trauma (Verdonck et al., 2016).

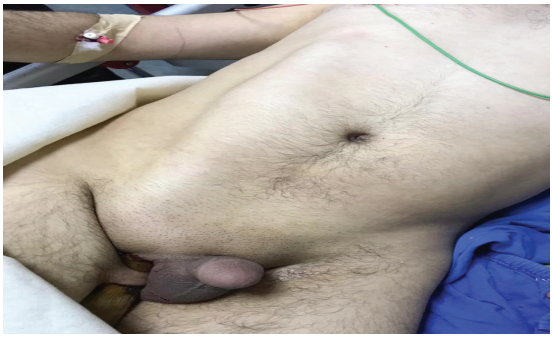

Figure 2. *Foreign body*

Gunshot injury: The most important problem in firearm injury is to determine whether the bullet has passed into the peritoneal space. The presence of a bullet hole in the front and back of the abdomen facilitates the determination of the bullet path. If the patient has only an entry hole, posterioanterior and lateral radiographs of the thorax, abdomen and pelvic regions may be useful in determining the bullet trajectory. Nevertheless, exploratory laparotomy in firearm injury is the most reliable and life-saving method (Vlček, Jaganjac, Niedoba, Landor, & Neumann, 2018).

FAST can assist in evaluating these patients, but cannot rule out abdominal injury if it is negative. CT is an effective and reliable method in hemodynamically stable patients and is used by most centers as the first choice. Bullet tract, organ damage, free fluid and air can be seen easily with CT. In addition, perivascular damage, retroperitoneal and urogenital damage can be detected easily. Patients with hemodynamically unstable patients should be urgently performed laparotomy for diagnosis and treatment (Verdonck et al., 2016; Vlček et al., 2018).

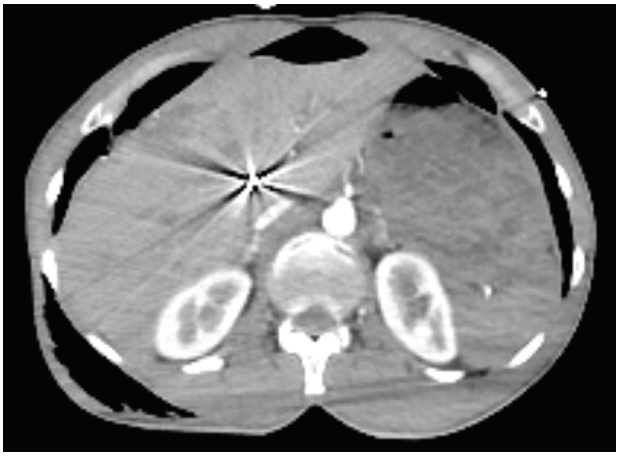

Figure 3. *Liver injury and the bullet*

PATIENT MANAGEMENT

In the management of the patients, whatever the reason, the safety circle and stabilization steps should be applied first. After these steps, the diagnosis should be made and the underlying cause should be tried to be eliminated (Fedor, Burns, Lauria, & Richmond, 2018).

Unnecessary fluid resuscitation should not be performed in order not to aggravate mild hypotension (mean arterial pressure> 50 mmHg), coagulopathy, hypothermia and not to activate unstable thromboembolic foci occurred secondary to trauma. Instead, it is necessary to apply the permissive hypotension protocol (Kurowski, Timler, Evrin, & Szarpak, 2014; Tran, Yates, Lau, Lampron, & Matar, 2018).

If the abdomen is distended in trauma patients, a nasogastric tube should be placed in intubated patients at high risk of stomach and duodenal injury. Thus, abdominal decompression is provided and the risk of aspiration is reduced. Orogastric tube should be inserted in patients with skull base fracture or suspected maxillofacial fracture. Foley catheter should be placed on patients who are unconscious, whose organ perfusion and urine output need to be monitored. In trauma patients, prophylactic antibiotics should be given to reduce the possibility of intraabdominal sepsis (Fedor et al., 2018; Nichols JR & Puskarich MA, 2018).

Blunt abdominal trauma

In blunt abdominal trauma, the patient's hemodynamic stability and clinical condition are considered. Despite the 55-65% diagnosis with PE performed in an unconscious patient, it is a crucial method in the initial evaluation of the blunt trauma patient. Diagnostic testing should

be performed as long as the patient's hemodynamic stability allows. CT scanning is a good choice for these patients (Nichols JR & Puskarich MA, 2018; Wang YC et al., 2012). While evaluating the patient with blunt abdominal trauma, if the motor vehicle accident is at the scene, it should be questioned whether there is a person dead at the scene, the type and speed of the vehicle, whether the seat belt is used, whether the airbags are deployed, and the patient's location in the vehicle. In case of ecchymosis or seat belt traces in the lower abdominal area, it indicates that 33% of patients have abdominal injury (Calder et al., 2017; El-Matbouly et al., 2016).

The decision for emergency laparotomy as a result of blunt trauma should be made according to the following. These;

1. If hemoperitoneum is detected by FAST in an unstable patient, without pelvic fracture, and in the presence of resistant hypotension,

2. Presence of peritonitis in a patient with FAST positive,

3. Detecting intraabdominal injury with FAST

4. Significant gastrointestinal bleeding as a result of blunt trauma,

5. Evidence of diaphragmatic laceration,

6. Patients with unstable hemodynamics.

In blunt abdominal trauma, if the patient is conscious and hemodynamically stable, abdominal pain, tenderness and presence of peritoneal findings are generally reliable findings for the evaluation of the patient. In retroperitoneal injuries, signs of peritonitis may not be present even in the presence of serious injury. Therefore, further investigation and observation should be performed in these patients, even if these findings are not present. The patient with stable hemodynamics should first be performed FAST and the presence of intraabdominal free fluid should be investigated. If there is free fluid in FAST, IV contrast-enhanced CT should be performed. If solid or hollow organ damage is detected in CT, one of the laparotomy or conservative treatment should be applied. If no finding is detected in FAST, PE is reliable and if no pathology is detected, the patient will followed up. If the patient is unconscious, the PE findings won't be reliable and CT should be performed. If severe trauma is suspected or if there is suspicion of an abdominal injury, CT should be performed regardless of the FAST result (Ozkan S; Wang YC et al., 2012).

When evaluating a patient with unstable hemodynamics in blunt abdominal trauma, first of all, it should be checked whether surgery is required or not. Primarily, FAST should be performed on the patient and the peritoneal cavity should be evaluated. If there is a positive finding in FAST, extra-abdominal causes such as long bone fractures, pneumothorax,

hemothorax and scalp lacerations should be reviewed. If any of these are detected, they must be intervened urgently. If the patient can be stabilized with intervention, CT should be performed. If pathological findings are detected in CT, laparotomy or conservative treatment should be applied preferably. If there is no bleeding focus in the patient and if the suspicion of an abdominal injury whose hemodynamics does not improve despite resuscitative procedures and the patient cannot be taken to CT, DPL or paracentesis should be performed. If a positive finding is detected, laparotomy should be performed (Ozkan S).

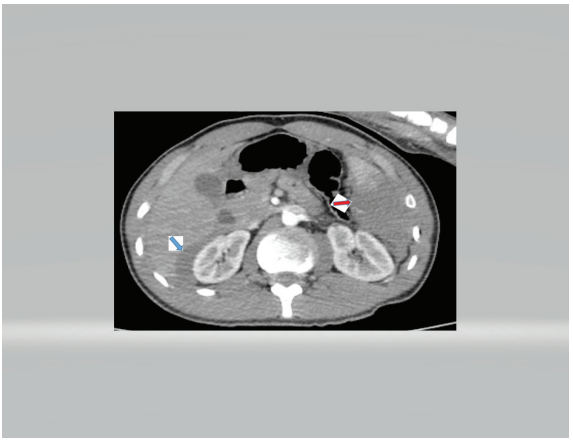

Figure 4. *Blue arrow: Perihepatic free fluid, Red arrow: Spleen laceration*

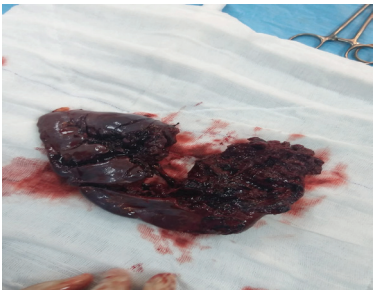

Figure 5. *Splenic laceration after an in-vehicle traffic accident*

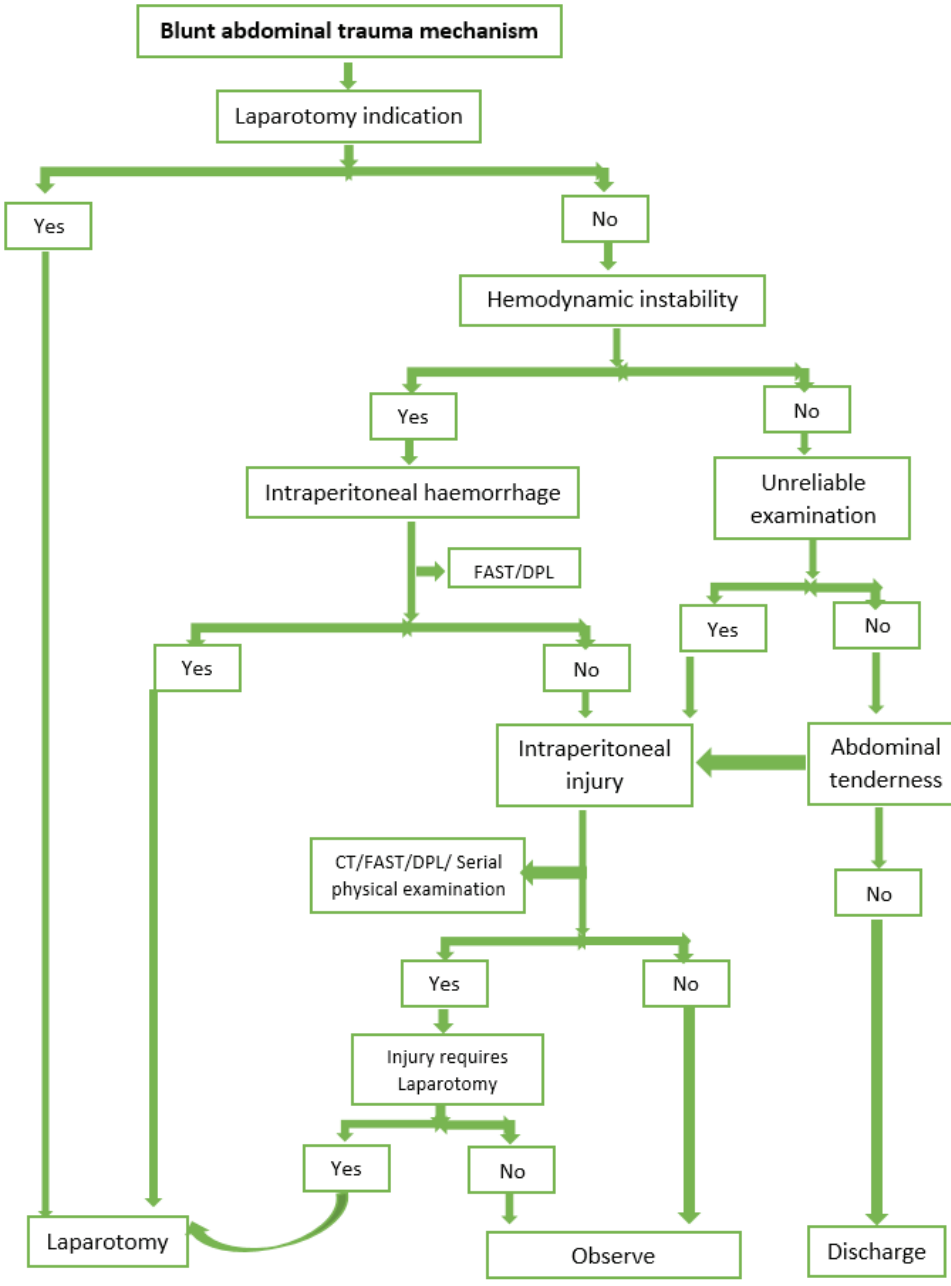

Figure 6. *Blunt abdominal trauma algorithm*

Penetrating abdominal trauma

Penetrating tool injury

1. Abdominal anterior wall injuries

In the anterior abdominal wall injury, it is first necessary to decide whether there is an indication for emergency laparotomy. If the patient is not indicated for an emergency laparotomy, secondly, it should be decided whether the peritoneal integrity is complete. If it is concluded that the peritoneal integrity is complete, the patient is discharged after appropriate repair and wound care. If peritoneal integrity is not preserved or if it is doubtful, it should be checked for intraabdominal injury. (Nichols JR & Puskarich MA, 2018).

Emergency laparotomy indications;

1. Hemodynamic instability

2. Peritoneal findings

3. Evisceration

4. Left-sided diaphragmatic injury

As soon as any of the emergency laparotomy indications are detected, the patient should be transported to the operating room immediately. The diagnosis of left-sided diaphragmatic injury is made by visualizing the stomach and intestines in the left thorax on bedside chest radiography.

Indications for laparotomy with additional clinical findings;

1. Gastrointestinal bleeding

2. Foreign body in tissue

3. Intraperitoneal air

4. In abdominal trauma, if blood is detected as a result of nasogastric tube or vomiting, it may indicate stomach or duodenal damage. Foreign objects in the abdomen should be removed under operating room conditions.

In order to evaluate whether the peritoneum is intact;

1. Peritoneal strength is impaired in the presence of evisceration,

2. Intraperitoneal free air indicates intestinal perforation.

3. Local wound exploration is a reliable method used to determine whether the peritoneal cavity has been penetrated. Accordingly, it is a reliable method used to determine whether it has been deeply penetrated. Accordingly, superficial wounds are primarily repaired and the patient is discharged from the emergency room.

4. USG can detect hemoperitoneum, pneumoperitoneum, and pericardial effusion, and demonstrate peritoneal penetration and damage. However, negative FAST does not exclude peritoneal damage. The presence of intraperitoneal hemorrhage can hinder local wound exploration and prevent the patient's time loss. Hollow organ and occult diaphragmatic injuries can often be overlooked on CT. To prevent this, repetitive PE should be made with series FAST.

5. In the operating room, laparoscopy or thoracoscopy can be used to evaluate the wound trace, but are procedures that require experience. Therefore, the risk of complications is high (Nichols JR & Puskarich MA, 2018).

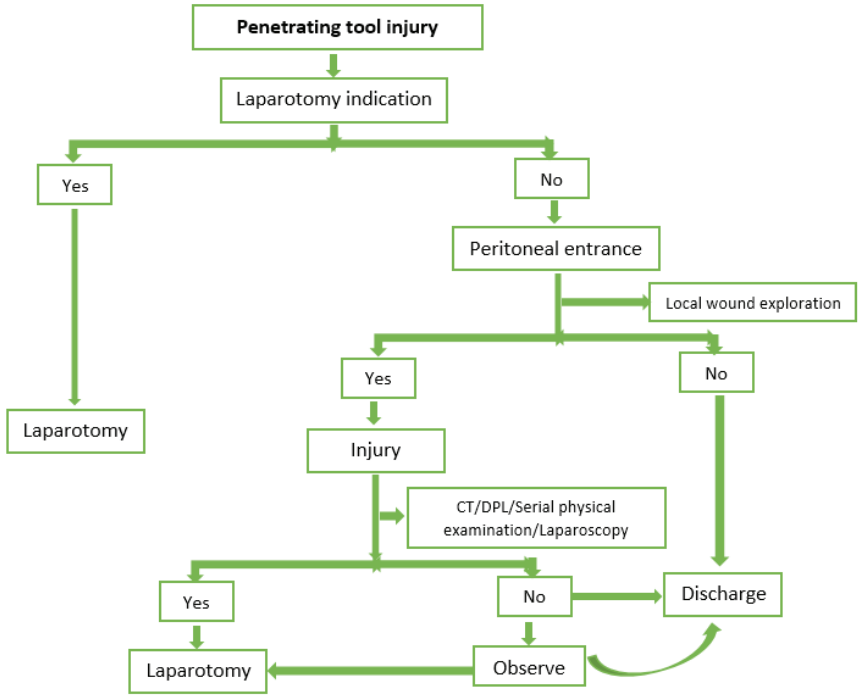

Figure 7. *Penetrating tool injury algorithm*

2. Thoracoabdominal penetration injuries

Penetrating injuries in the lower thoracic region can cause damage to the thoracic cavity, diaphragm, peritoneal and retroperitoneal region. If the patient has hemodynamic instability due to injury, laparotomy and / or thoracotomy should be performed. If the patient's hemodynamics is stable and there is no indication for laparotomy / thoracotomy, the presence of hemopericardium and hemoperitoneum can be rapidly evaluated by USG (Segalini et al., 2019). Local wound exploration can also be performed in these patients, but may not clearly show intrathoracic and intraabdominal

damage. In addition, another problem in these patients is the difficulty in detecting diaphragmatic injury. Even the sensitivity and specificity of CT in diaphragm injuries is around 90%. Despite all advanced examinations, more precise methods such as laparoscopy or thoracoscopy can be used in suspected cases (Nichols JR & Puskarich MA, 2018; Ozkan S).

3. Flank and Back penetration injuries

In this area injuries, the probability of retroperitoneal damage is higher than in other regions. In addition, the probability of intraabdominal injury is around 40%. Contrast-enhanced CT is the preferred method in evaluating the injury in these patients. Even if the CT scan is negative, the patient should be followed for at least 24 hours and serial PE should be performed.

Gunshot wounds

Almost all firearm injuries cause intraperitoneal injury, leading to multiple organ damage, in contrast to stab wounds. Mortality risk may be high depending on the bullet velocity and the route it takes. In bullet injuries that hit the lower thorax, both the thorax and the abdominal area, including the diaphragm, are likely to be injured (Lee et al., 2017). Therefore, the management of gunshot injuries is limited and the laparotomy indication is mandatory. First of all, it should be decided whether there is an emergency laparotomy indication in these patients. It should then be decided whether the peritoneal integrity is compromised. If the peritoneal integrity is impaired, the patient should be taken to laparotomy immediately.

To detect whether the lead has entered the peritoneal cavity;

1. The path followed by the bullet is tried to be determined. If it only scrapes the superficial tissue in the abdominal wall, it is not a penetrating injury.

2. In anteroposterior and lateral abdominal radiography, the location of the bullet in the peritoneal area is tried to be determined. However, it is not a safe method for patients with both entry and exit holes, and in multiple gunshot injuries.

3. Peritoneal cavity integrity can be checked by performing local wound exploration in injuries that are outside the peritoneum, or where the bullet has grazed or pass through the lateral area.

4. Presence of intraabdominal fluid with FAST indicates bullet penetration.

5. Peritoneal integrity can be checked with laparoscopy.

6. CT has very high sensitivity and specificity in detecting intraabdominal injury in bullet injuries. In addition, bullet trace, organ

damage and vascular injury can be easily detected with CT (Lee et al., 2017; Nichols JR & Puskarich MA, 2018).

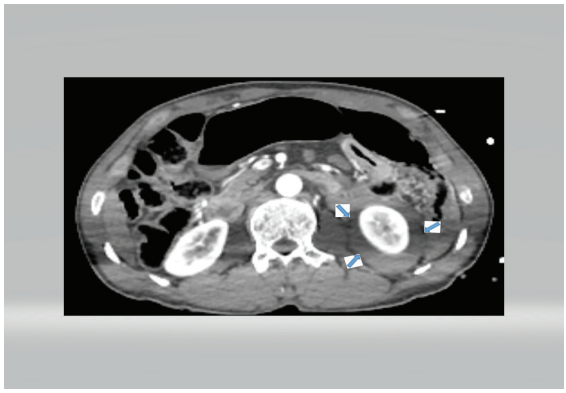

Figure 8. *Hematoma in the left perirenal region as a result of a firearm injury*

Shotgun injuries cause multiple organ injuries, shock, and hemostasis and tissue loss requiring extensive debridement. Emergency laparotomy is indicated in these patients in the presence of instability or peritoneal findings.

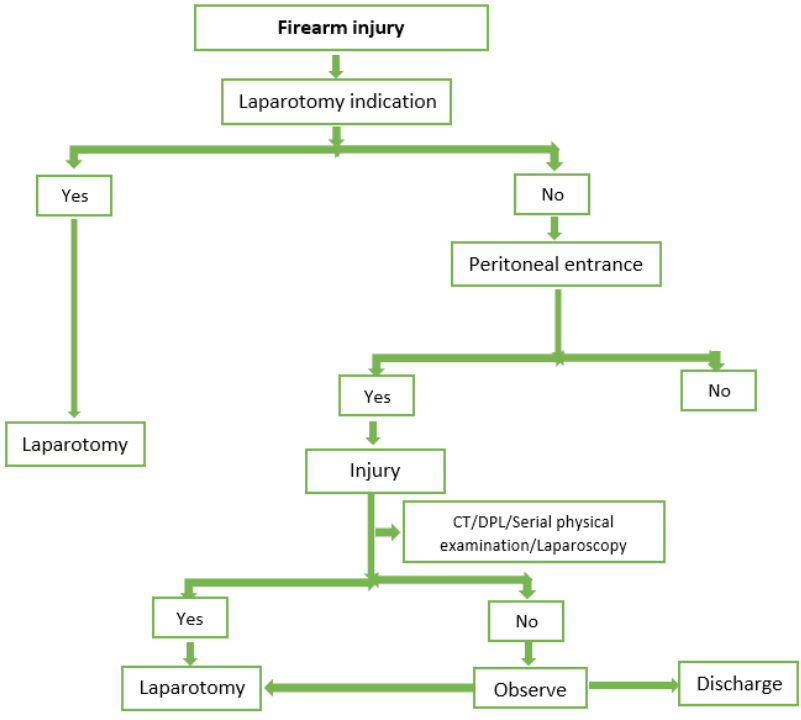

Figure 9. Firearm injury algorithm

Figure 9. *Firearm injury algorithm*

Thoracoabdominal injury

The patient, who was injured with a gunshot from the lower thorax, also has abdominal damage. It is tried to determine whether there is an indication for emergency laparotomy. If the patient's hemodynamics are stable, the damage is detected by performing both thoracic and abdominal CT scans and taken to the operating room.

Flank and Back injuries

If the patient is unstable, an emergency laparotomy should be performed. CT scanning is the first choice for a stable patient for retroperitoneal injury. Later, the patient is taken to the operating room. If the lead trace in CT does not damage any important anatomical structure, the patient can be kept observed, and followed. (Lee et al., 2017).

Pelvic fracture

In pelvic fracture, the presence or absence of hemoperitoneum determines patient management. Mechanical pelvic stabilization should be applied to these patients in the early period. FAST can be used to evaluate hemoperitoneum, but its sensitivity is reduced due to pelvic fracture. If the unstable patient is FAST negative, therapeutic angiography should be performed in terms of both retroperitoneal bleeding and pelvic bleeding. CT scan should be performed in patients with pelvic fracture with multiple organ injuries and if possible, pelvic angiography and embolization should be applied in the early period (Burkhardt et al., 2014; Nichols JR & Puskarich MA, 2018).

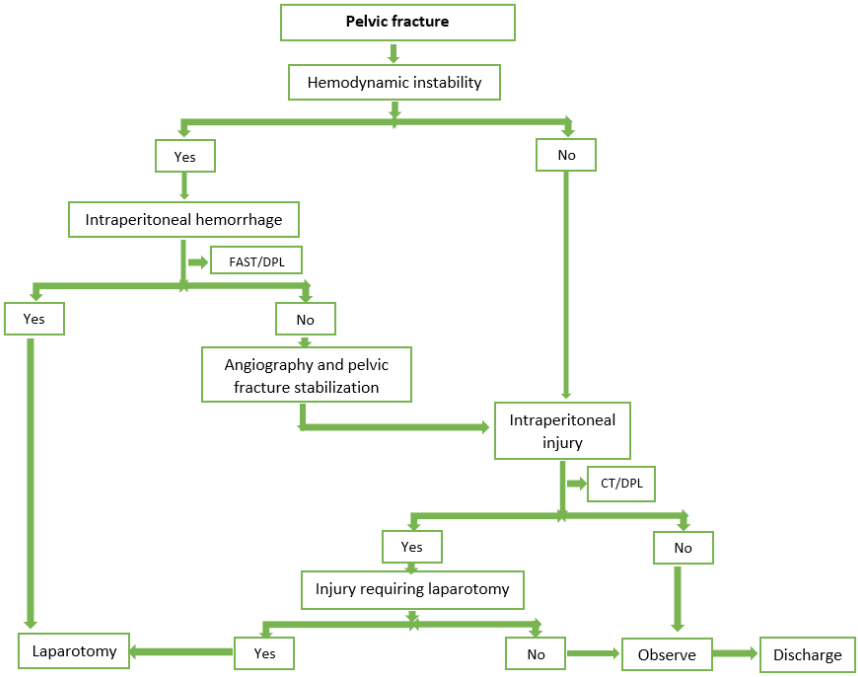

Figure 10. *Pelvic is the difference and blunt abdominal trauma algorithm*

Multiple trauma

The likelihood of other system traumas to be seen together with abdominal trauma is considerably high. Depending on the mechanism and severity of the trauma, abdominal injuries can be accompanied by head, chest or pelvic injuries. A multisystemic approach is required in these traumas and simultaneous intervention may be required in the injured areas. The important point here is that when hemoperitoneum is detected in an unstable patient, the patient is urgently taken to laparotomy (Nichols JR & Puskarich MA, 2018; Segalini et al., 2019).

Patient stabilization should be provided first in multiple trauma. Once the patient is stabilized, the presence of intraperitoneal hemorrhage should be investigated by FAST and / or DPL. Laparotomy should be performed to detect hemorrhage. If intraperitoneal hemorrhage cannot be detected, scanning should be performed with head, thorax and abdominal CTs according to PE findings. It is based on the patient's stability, PE and findings in diagnostic procedures (Ozkan S).

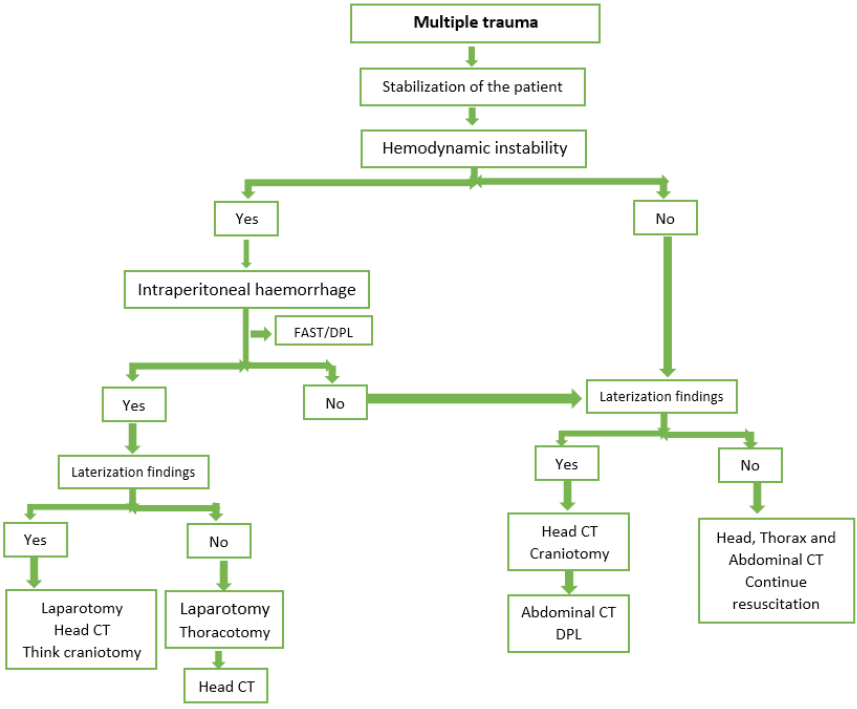

Figure 11. *Multiple trauma management algorithm*

Therapeutic angioembolization

It is an application in which unstable patients with blunt trauma embolize large bleeding vascular structures in the presence of pelvic fracture. This application takes time and should be done by experienced people. It is a nonoperative method also used in blunt solid organ injuries. It is rarely used in the management of intraperitoneal and retroperitoneal hemorrhage in penetrating trauma. (Skitch & Engels, 2018; Tsilimigras et al., 2019).

Discharge

It may vary according to the patient's PE and clinical findings. The patient with blunt or penetrating trauma without laparoscopy indication and in whom no peritoneal access injury is detected can be safely discharged. Patients whose peritoneal integrity is compromised should be hospitalized and followed up with serial PE. Depending on the situation, he should be taken to the operating room immediately.

Conclusion

Abdominal traumas are divided into blunt and penetrating injuries. PE is not reliable in cases where orientation and cooperation cannot be

established in patients with abdominal trauma, together with the head trauma, alcohol or drug intoxication, and psychiatric problems. In these cases, advanced diagnostic procedures should be started. After PE and FAST, contrast-enhanced CT should be performed if there is an indication. Thus, an accurate diagnosis is achieved in the majority of patients with blunt and penetrating abdominal trauma. Combinations of lung, diaphragm, mediastinum, intraperitoneal and retroperitoneal injuries can often be seen in stab wounds and gunshot wounds (Al-Mudhaffar & Hormbrey, 2014; French LK et al., 2016; Ozkan S).

Emergency laparotomy should be performed in the presence of unstable hemodynamics, signs of peritoneal irritation, evisceration or left-sided diaphragmatic injuries. Local wound exploration should be performed in other patients to obtain information about the condition of the wound. In addition, CT scanning, FAST and serial PE should be performed (Al-Mudhaffar & Hormbrey, 2014; French LK et al., 2016).

In the patient who is hemodynamically unstable, the presence of intraperitoneal hemorrhage in the pelvic fracture should be investigated. While laparotomy is indicated when hemorrhage is detected with FAST, CT and DPL, diagnostic and therapeutic angiography should be performed when hemorrhage is not detected (Nichols JR & Puskarich MA, 2018).

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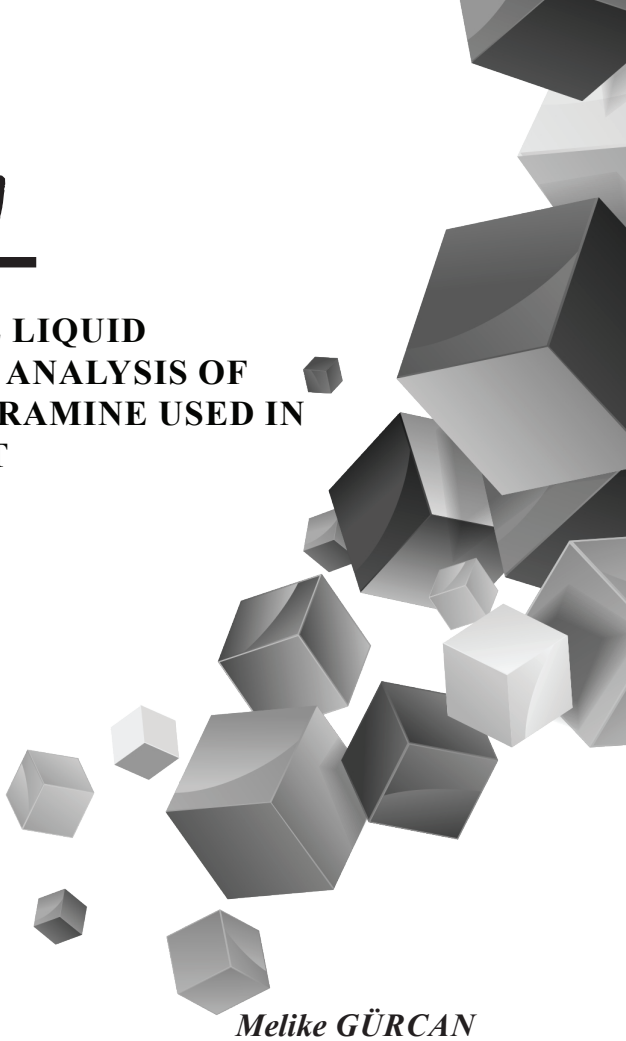
Chapter 11

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF ORLISTAT AND SIBUTRAMINE USED IN OBESITY TREATMENT



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INTRODUCTION

Obesity is recognized as the most common health condition affecting low, middle and high-income countries with epidemiological concerns, with 400 million clinically obese subjects and more than 1.5 billion overweight adults, which greatly affects global health in the 21st century. Obesity can simply be defined as a phenomenon where body weight is higher than the amount predicted for a certain height of an individual [1].

The incidence of diseases such as hyperlipidemia, hypertension, diabetes mellitus, colorectal cancer, atherosclerotic cerebrovascular disease, coronary heart disease is quite high in relation to obesity [2].

Although the causes of the disease are multiple, it is not well known the reasons. Obesity is seen partly as a result of higher calorie diet consumption than energy use. Other factors that can cause obesity are cited as food addiction, depression, side effects of drugs or personality traits [3].

Childhood obesity is emerging as another rapidly growing problem in the world. It is estimated that 10% of children (5 to 17 years) who attend school are obese over the world. Insulin resistance, dysglycemia, fatty liver disease, hypertension and dyslipidemia are the effects of obesity in childhood. It also can affect organ systems to a great extent. As the main cause of the increasing prevalence of overweight and chronic noncommunicable disease (NCDs), obesity accounts for 44% of diabetes, 23% of ischemic heart disease, and 7-41% of certain cancer burden [4].

The energy value of food consumed by humans today is very high. Fatty food intake yields 9kcal / g compared to 4kcal / g carbohydrates. Therefore, foods with higher fat content may result in higher energy content. It is reported that when consuming a high-fat diet, the thermogenic effect caused by the diet is lower compared to a diet containing high carbohydrate and protein, that is, it causes lower energy expenditure. That's why the reason for its ability to induce less efficacy than carbohydrate and protein, dietary fat often points to an increase in energy intake. Increasing prevalence of obesity is due to high fat diets, so it can trigger the development of hyperglycemia. Normally, an individual consumes dietary fats and consumes a large number of refined carbohydrates that cause the development of visceral fat and weight gain. Hence, many diseases such as diabetes and cardiovascular disease (CVD), which are manifestations of obesity-related disorders, are supported by high sucrose intake. Lipids contribute to the increased amount of calories in the diet [3].

Obesity and Body Mass Index

Obesity is explained as a medical condition caused by excessive fat storage in the body and causing harmful effects on the health of the individual. BMI or Body Mass Index is widely used to determine obesity, includes weight-height parameters that indicate the amount of body fat, and is used for classification among overweight and obese adults. Obesity increases the vulnerability to various illnesses like type 2 diabetes (T2D), bone and joint disease, CVD, osteoarthritis and depression. CVD and T2D are commonly associated with a higher BMI with higher total and central fat for a given body weight due to higher waist circumference [5].

Waist, hip friction and waist-hip ratio are other indicators used to measure the regional distribution of fat [3].

The division of a person's weight in kilograms by the square of his height in meters is defined as his Body Mass Index (BMI). Higher BMI indicates higher fat levels. By measuring the height and weight of an individual, BMI can be determined [3].

Table 1: *Weight classification according to Body Mass Index [3]*

Weight class	**BMI**
Underweight	<18.5
Normal	18.5-24.9
Overweight	25.0-29.9
Obese	>30

Table 2: *Classification of Obesity according to Body Mass Index [3]*

Type of Obesity	**BMI Index**
Class I obesity	30.0- 34.9
Class II obesity	34.9-39.9
Class III obesity	>40

Class 3 obesity is generally classified as extreme or severe obesity. Waist circumference measurement is becoming more important and is used to decide the measure of overweight or obesity. The fact that fat around organs is metabolically active, is associated with deregulation in metabolism, and individuals are prone to CVD and related conditions are considered irrational [6].

There are some risk factors involved in the development of obesity such as incorrect eating habits, insufficient physical activity, age, gender, education level, socio - cultural factors, hormonal and metabolic

factors, genetic factors, smoking-alcohol use status, some drugs used (antidepressants, etc.)

According to internationally used metabolic syndrome guidelines, the classification of adults per aggregate of dysmetabolic conditions that makes abdominal fat responsible for CVD, a component, is defined as a waist circumference (WC)> = 94 cm, resulting in increased cardiovascular risk. European males and European females = 80 cm, while different criteria are set for individuals belonging to other ethnic groups (for example, males and females> = 90 and> = 80 cm, respectively, among most Asian people). Although body weight classification in children is different from adults, body composition changes greatly during the development phase. There is more difference between boys and girls, because of the difference in sexual development and maturation [3].

Awareness levels are insufficient when it comes to risks associated with obesity. It is reported that the media does not see obesity as a threat even after it is proven by the available evidence. A study conducted in the USA between 2001-2002 among children and adolescents reveals that there is a 31.5% risk for overweight, whereas 16.5% are already obese. In another study in UK, girls aged 7-11 Symptoms of obesity and overweight were found at 23.6% and 17% for men of the same age group [3].

Epidemiology of Obesity

Obesity threatens public health as a individual health problem that is frequently seen in almost all societies around the world. The prevalence of obesity is becoming a more and more serious problem with the rapid change of lifestyle in our country [7].

Turkey in 1997-98 include examining 24 788 people age 20 and over 540 centers in order to determine the prevalence of obesity Turkey Epidemiology of Diabetes (TURDEP-1) study was conducted. In this study, the prevalence of obesity is reported as 22.3%. Twelve years after this study, in the TURDEP-2 study conducted as a repeat of the same study in 2010, the prevalence of obesity was obtained 35% in the people. According to the detailed results, it is stated that obesity has increased by 34% in Turkish women and 107% in Turkish men in 12 years [7].

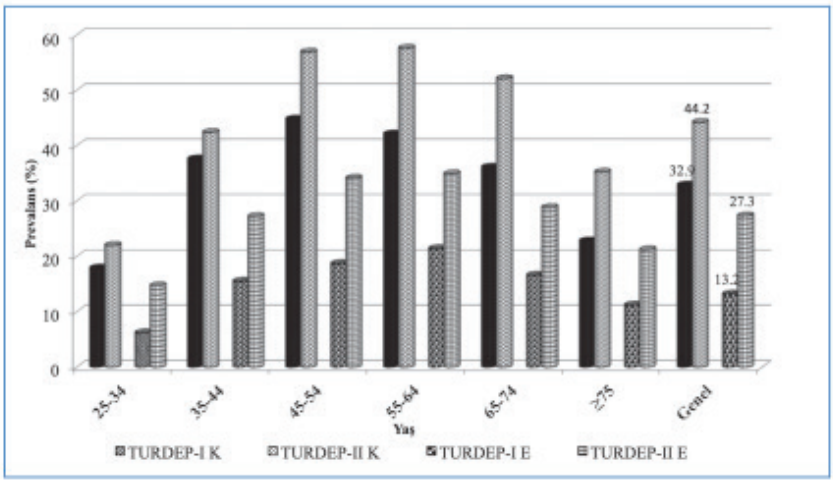
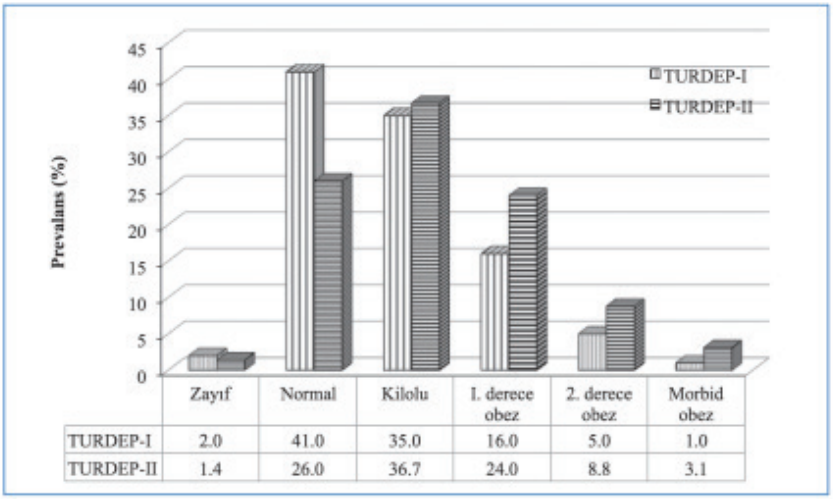


Figure 1: *Change in the frequency of obesity in different age groups and genders according to the results of TURDEP-1 and TURDEP-2 studies [8].*



	Zayıf	Normal	Kilolu	I. derece obez	2. derece obez	Morbid obez
TURDEP-I	2.0	41.0	35.0	16.0	5.0	1.0
TURDEP-II	1.4	26.0	36.7	24.0	8.8	3.1

Figure 2: *TURDEP AND TURDEP-2-one according to the working adult RDA groups in Turkey distribution [8].*

Effects of Obesity on the Health

Various diseases and conditions are associated with excessive body weight, resulting in reduced life expectancy. Increased blood flow, hypertension and cardiac output have been associated with obesity-related disorders. Increased sympathetic tone, hyperinsulinemia (increased insulin levels in the blood), structural changes in the kidney, activation of

the renin-angiotensin system (RAS) and preparation of pokines such as leptin (fat-produced hormones) are cited as some of the few changes in the pathophysiological state induced by obesity [3,9].

Obesity effects many serious illness such as high blood pressure, heart failure, diabetes especially T2D, sleep apnea, different kinds of cancers, skeleton and muscular system damages, coronary, complications during pregnancy, menstrual irregularity, infertility, urinary bladder diseases and psychiatric diseases [10-14].

Heart disease - Atherosclerosis is 10 times more common in obese people than non-obese people. Atherosclerosis starts with hypercholesterolemia and this causes risk factor formation among other CVDs. The highly specific array of molecular and cellular responses to the vascular endothelium initiates chronic inflammatory diseases. Coronary artery disease develops when fat accumulates in the arteries that carry blood to the heart. Decreased blood flow and narrowed arteries can cause chest pain and even heart attack. Due to given rates of obesity, researchers have recently been examining the impact of obesity in early life and following adulthood. Owing to childhood or adolescent obesity, the probability of adult hypertension, coronary heart disease and stroke is stated twice or greater [15].

Joint problems as extra weight is gained, stress is placed on the joints, which can affect the hips and knees. It is not recommended for patients who have undergone joint replacement surgery because of the high risk of joint damage. Many studies have linked obesity with the presence, development, and severity of osteoarthritis. From joint replacement surgery to complication in recovery, obesity has been reported to be a risk factor for OA [11].

Obese people may not be able to breathe for a short time because of sleep apnea. As a result, their sleep can be interrupted, causing insomnia throughout the day. The person may also start snoring heavily. The weight added to the chest of an obese person can compress the lungs and prevent breathing. It is adversely affected by obstructive sleep apnea associated with multiple organs and systems, especially CVDs [13].

Cancer is an increasing threat to overweight in women as it can cause various cancers, including the colon, breast, gall bladder and uterus. For obese men, the risk of colon cancer increases [9].

As psychological effects, obese or overweight people always have a disadvantage in a society where extremely thin people are attractive. They can be considered weak-willed or lazy and may be discriminated against [3].

Obesity Treatment

The growing prevalence of overweight and obesity underline the require for advanced intervention plans to counter this important public health problem. For this purpose, it seems that rises in energy cost through training and other forms of physical activity can be a great part of interventions that effectively affect initial weight loss and prevent weight gain. Nevertheless, so as to gain these results, efficient exercise and levels of physical activity seem to be required. Hence, 60 to 90 minutes / day is recommended. For this reason, counseling should be given to overweight and obese adults to step by step accomplish these levels of training and physical activity. In addition, there is a substantial proof that although an overweight or obese adult may not be able to achieve this level of activity, remarkable health benefits can be reached by taking part in at least 30 minutes of daily activity of moderate intensity. Thus, it is important to have interventions targeting these levels of physical activity to improve health outcomes and facilitate long-term weight control [16].

As a result of the increase obesity, pharmaceuticals prescribed for treatment have become widespread and their numbers have increased. Drugs prescribed for weight loss are divided into 2 classes in terms of mechanism which appetite suppressants and lipase inhibitors. Appetite suppressants are also divided into lower classes according to the neurotransmitters that act on [17].

Antiobesity drug therapy is often beneficial for patients who cannot achieve enough weight loss through diet, lifestyle, and behavioral modification. These drugs are prescribed by the Food and Drug Administration (FDA) for use in obese patients with at least one additional obesity-related disease in overweight patients with a BMI of 30 kg / m2 or higher and a BMI between 27 and 29.9 kg/m^2 approved. Most are only approved for short-term use, but SBA and ORL are also approved for weight loss and weight control in long-term use [18].

Table 3: *Pharmacological properties of ORL and SBA [19]*

	ORLISTAT (ORL)	**SIBUTRAMINE (SBA)**
Mechanism action	Gastric and pancreatic lipase inhibitor	Norepinephrine and serotonin reuptake inhibitor
Daily dosage	120 mg three times a day	10-15 mg once a day
Absorption	Minimal	%77
Binding to plasma proteins	%99	%94
Peak time	8 hours	1-2 hours
Elimination	%96 with faeces	%77 with urine

There are different treatment methods against obesity, which is very important for human health. These treatment methods such as non-pharmacological treatment with nutrition-diet, physical activity, behavioral changes, pharmacological treatment, antidiabetic therapy and antihypertensive therapy with ACE Inhibitors and Ang II Antagonists, Diuretics, β-blockers and Calcium channel blockers [20].

Mechanisms Action of Antiobesity Drugs

a) Combined norepinephrine and serotonin reuptake inhibition: Sibutramine (SBA)

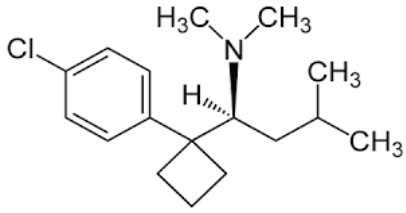


Figure 3: *Chemical structure of SBA [21].*

b) Inhibition of fat absorption - Fat absorption lipase inhibition: Orlistat(ORL)

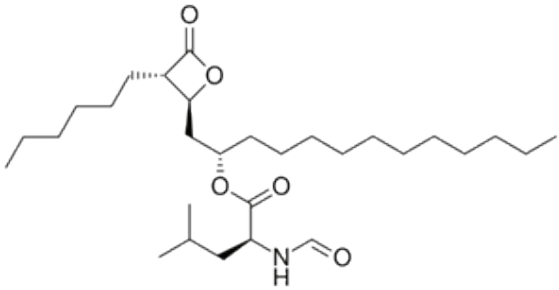


Figure 4: *Chemical structure of ORL [22].*

a) Combined norepinephrine and serotonin reuptake inhibition: SBA

SBA is used in obesity as a b-phenethylamine derivative which contains the cyclobutyl group. Both demethylation metabolites of SBA are biologically active similar to SBA [23].

SBA, a combined norepinephrine and serotonin selective reuptake inhibitor, provides weight loss in a dose-dependent manner [23].

SBA, a serotonin and norepinephrine transporter blocker, is used as an auxiliary obesity treatment. As a result of studies in healthy subjects, it has

been claimed that SBA may have adverse effects on peripheral and central sympathetic activity, causing an increase in blood pressure. In addition, cardiovascular side effects such as mildness, paraetshesia, tachycardia, palpitations and increased blood pressure are seen. Direct measurements of muscle sympathetic nerve activity (MSNA) have not been performed in patients treated with SBA [24,25].

SBA is contraindicated with coronary disease. Increased heart rate and blood pressure caused SBA to be discontinued in 5% of patients [26].

Although many obesity medications have been suggested to increase the feeling of fullness in humans, only a small number of studies have shown little effect of these drugs on people's eating habits. The effects of a 7-day dose of SBA 10 mg and 15 mg daily on appetite and energy balance in 30 obese women (BMI 34.6 + - 3. 3 kg / m2. Age 46.0 + - 12.9 years) double-blind, It was investigated using a Universal Eating Monitor (UEM) and indirect calorimetry in a placebo-controlled crossover study. On day 7, SBA 10 mg and 15 mg reduced food intake by 16 ($p < 0.001$). SBA reduced eating rate compared to placebo instead of meal length 10mg $p < 0$. (15 mg $p < 0.001$). In addition, 10 mg of SBA was found to significantly reduce postprandial hunger ($p < 0.05$) and 15 mg SBA increased fullness at early meal ($p < 0.01$) consistent with increased satiety in the meal. SBA had a little effect on resting metabolic rate, but 15 mg significantly reduced the respiratory system [27].

Some results were obtained by determining several time point segments during the test day. These results provided new evidence that the consumption of a test meal was reduced. SBA causes a decrease in eating rate, which increases the cumulative slowdown in food intake within a meal associated with the development of satiety. Changes occur in the meal appetite grades. It has been reported that it is particularly sensitive to drug-induced satiety and can provide key indices to evaluate the therapeutic potential of new anti-obesity drugs [27].

b) Inhibition of fat absorption - Fat absorption lipase inhibition: ORL

ORL is a gastrointestinal lipase inhibitor used in obesity control. ORL facilitates weight loss when taken with a diet that minimizes calorie intake [28].

ORL, the first new group of drugs, does not show systemic effects; It prevents the absorption of dietary fat with its local effect in the gastrointestinal tract [29].

ORL is a potent, specific, reversible and long acting inhibitor of gastrointestinal lipases. It provides weight loss by inhibiting the absorption

of fats in foods. It shows that therapeutic effect by forming a covalent bond with serine, which is in the active site of gastric and pancreatic lipases in the lumen of the stomach and small intestine. Thus, the body absorbs less fat since inactivated lipase enzymes can not hydrolyze the fat taken as triglycerides with foods into free fatty acids and monoglycerides. Ultimately, reduced calorie intake leads to negative energy balance and weight loss. Therefore, it does not need to be absorbed systemically to achieve weight loss [30].

ORL is an FDA approved drug for the treatment of obesity. By inhibiting gastric and pancreatic lipases, it reduces the systemic absorption of fat taken with food. In a study that lasted 2 years, it was revealed that ORL was more effective in losing weight than losing weight with diet alone. ORL treatment has also been observed to have positive effects on cholesterol, LDL, blood pressure, fasting glucose and insulin concentrations [31].

Mild-to-moderate gastrointestinal side effects such as oily stools, diarrhea, abdominal pain and stool stains can be seen with ORL use. Serious hepatic side effects such as cholelithiasis, colostatic hepatitis, and subacute liver failure have also been reported. Moreover, ORL may have side effects on blood pressure, heart rhythm, musculoskeletal system and kidney functions [32].

It is contraindicated during pregnancy and lactation, in patients with cholelithiasis (gallstones), patients with malabsorption syndrome and those with sensitivity to ORL [30].

ORL ought to be taken with a diet rich in vegetables and fruits, well-balanced, calorie-controlled and taking an average of 30% of its calories from fat. Daily intake of fat, carbohydrate and protein ought to be distributed over three main meals. Accordingly, one capsule should be taken at breakfast, one capsule at lunch, and one capsule at dinner. In order to get the maximum benefit from ORL, eating foods with high fat content such as biscuits, chocolate, or appetizing snacks should be avoided. ORL works only if taken with dietary fats. Therefore, if you miss the main meal or if a meal containing no fat is taken, ORL is not required [33].

REVIEW OF ANALYTICAL METHODS

E. Deconinck et al., have validated UHPLC-DAD method so as to identify and quantify pharmaceutical preparations containing molecules like SBA commonly detected in illicit weight loss products (WLP). HT C18-B column was used in the proposed method (2 mm × 100 mm, 1.5 μm). $C_2H_7NO_2$ buffer pH 5.0 was used as the aqueous phase and C_2H_3N as the organic modifier. The method was verified depends on the measurement uncertainty (accuracy profile). Calibration curves of all

components examined at specific concentrations were found linearly. It was observed that the relative bias and relative standard deviations for all components were less than 3.0% and 1.5%, respectively. In addition, the β-expectation tolerance limits did not exceed 10% acceptance limits. It was found that all relatively expanded uncertainties were less than 3%. The methodology of UHPLC-DAD attained in order to identify and quantify of such pharmaceutical preparations was validated for quantitation following ISO17025. It is stated that this method will remarkably decrease the analysis time and workload of laboratories [34].

Rebiere et al., have conducted by UHPLC-DAD method was carried out to detect dangerous WLP in additive slimming formulations. The availability of illegal products on the market for weight loss or overweight treatment is considered a health problem. These products might comprise illegal chemicals to increase their efficacy. Some of these WLPs may be responsible for adverse effects, such as fatal consequences. This method was used in order to detection and quantification of 34 components such as SBA in weight loss preparations for the control of weight loss formulations on the market, including over the internet. A rapid UHPLC, a solvent gradient (phosphate buffer and ACN), a C18 end cap column and a DAD (diode array detector) were used. This system lets dual identification according to RT (retention time) and UV spectra. It has been reported that the analytical method is easy, rapid and selective, as 34 WLPs can be found in a 15-minute RT. Therefore, 32 commercial slimming formulations were analyzed by this method and the determination of potentially dangerous active substances were studied [35].

Song et al., have developed a new method using flow injection MS/MS to semi-quantitatively screen WLPs in dietary supplements, including SBA. Positive determination of these drugs in the samples was further verified and measured by LC-MS / MS. The degradation products of SBA were detected and identified by LC-MS / MS containing N-desmethylsbutramine, N-didesmethylbutramine, N-formyldimethylbutramine and N-formyldidesmethylsibutramine [36].

Kevin Tran et al., have studied a validation for the detection and quantification of multiple WLPs in dietary supplement materials. Sample preparation as described in LIB 4549 was monitored and mass spectral measurements were made using ESI-MS / MS. A method validation was studied for 11 drug compositions like SBA at various supplement levels (250 and 1000 micrograms / gram). The average of the total recoveries with standard deviation was 93.8% ± 6.65%, with the majority of individual recoveries between 76% and 110%. The total quantification limits (LOQ) for average analytes at 10 ng / g, the mean of the overall linearity index (R2) were 0.9993 (0.0250.0 ng / mL). The accuracy of the five replicates of

the 50 ng / mL level was averaged from <1% to 98.4 ± 0.41 [37].

Adela Krivohlavek et al. have studied SBA, which is available in the Croatian market and used for slimming food supplements, was detected by validated HPLC - ESI-MS/MS method. Drugs were isolated using simple methanol (MeOH) extraction and using a Zorbax SB C18 gradient column (0.1% formic acid (FA) in ACN and 0.1% FA in water as a mobile phase), using reverse phase liquid chromatography (RPLC). According to the study, it is reported that one fifth of the samples tested over a period of six years contained SBA. It is reported that regular market control using the method described can prevent health risk for the population [38].

Ying Shi et al. have developed using HPLC - ESI - MS / MS and HPLC for the detection of 8 substances containing 2 appetite suppressants, 2 energy-increasing drugs, 1 diuretic and 3 cathartic in attenuation of functional foods. After the samples had been ultrasonically dissolved and centrifuged with 70% (v / v) MeOH, the compounds in the sample solution were prepared using a programmed gradient elution (2.1 mm × 150 mm, 5 μm Gold column). 0.02% (v / v) FA - HCO_2NH_4 buffer solution (pH = 3.50) and MeOH were used for elution with a flow rate set to 250 μL / min and a column temperature of 25 °C. Qualitative analysis was relied on characteristic ion pairs and RT of targeted compounds using the SRM (selective reaction monitoring) mode. Clenbuterol and ibuprofen were used as internal standards (IS). Average recoveries at 3 different concentrations were between 80.2% and 94.5%. LODs were reported between 0.03 and 0.66 mg / kg (excluding chrysoptanol 1.6 mg / kg). The linear dynamic range (excluding chrysoptanol 50–5000 μg / L) ranging from 1 to 500 μg / L was analyzed. Thanks to the advanced method, the mixtures in four different weight loss functional foodstuffs were determined and adequate outcomes were gained. These experimental outcomes suggested that mixing SBA and fenfluramine were the main mixing ingredients ranging from 6.1 to 1.3 x 10 3 mg / kg and 1.9 to 9.7 x 10 3 mg / kg, respectively. Furthermore, 4 cathartic compounds were determinated in 6 of these samples tested, and ephedrine, norpseudoephedrine and clopamide were not determinated in the all samples [39].

Zohreh Abolfathi et al., have conducted studies so as to characterize the food impact on the pharmacokinetics of SBA and its pharmacologically active metabolites. A single dose, crossover method was used in this study on 6 healthy men. Plasma concentrations of SBA and its metabolites were detected by LC / MS / MS by orally administering a single dose of 15 mg SBA under fasting and fed circumstances: Non-compartmental pharmacokinetic and statistical analysis were carried out using SAS. Food intake did not affect the M2 metabolite, although the AUC and C (max) of SBA and M1 metabolite increased significantly. When SBA was administered with

food, the T (max) for SBA, M1 and M2 metabolites were delayed by 2 to 4 hours, as stated in the literature. The outcomes of this investigation shows that SBA can be measured by a sensitive bioanalytical method. In contrast, informed in the product monograph, this study showed that the bioavailability of SBA and its M1 metabolite is remarkably raised by food administration. The results confirm that there is no food impact on the pharmacokinetics of the M2 metabolite. It is reported that this relatively large food impact observed for SBA and M1 metabolite may have an impact on the efficacy and safety of the drug [40].

Souri et al., have developed a HPLC-UV method for the detection of ORL. The chromatographic system consists of a Nova-Pack C18 column, 0.1 % H_3PO_4 - ACN (10:90, v / v) isocratic mobile phase and 205 nm UV detector. The ORL was eluted about 6 minutes before the mixture peak of the excipients used to prepare the dosage form. The method is in the linear range of 10- 160 µg / ml ORL (r^2> 0.9999). In addition, the intraday and between days precision values range from 0.10% to 0.59%. Appropriate dissolution conditions were detected and performed to determine the dissolution profile of ORL capsules. The standard condition was 1000 ml of water containing 3% SLS and a flow rate of 100 per minute for the dissolution medium. The suggested method was successfully carried out in the determination of ORL components in capsules and in vitro dissolution studies. This study has described the development of an accurate HPLC method with rapid, accurate and isocratic elution for the determination of quality control of ORL in pharmaceutical preparations and dissolution media. The developed method was validated and performed for content determination of Xenical capsules containing ORL and samples obtained from in vitro dissolution studies [41].

Mohammadi et al., have improved a stable HPLC method for detection of ORL. Perfectsil® ODS-, 250mm × 4.6mm i.d. and an isocratic separation was performed by a 5 mcm particle size column with a 0.7ml / min flow rate and using a UV detector to display the eluate at 210 nm. The mobile phase contained MeOH: acetonitrile (ACN): trifluoroacetic acid (TFA) (82.5 : 17.5 : 0.01, v / v / v). Separation was achieved completely by an analytical run of about 15 minutes. The method has linear in the concentration range 0.02-0.75mg / ml (r = 0.9998). The method has the accuracy, selectivity and sensitivity required for the determination of ORL in capsules. Degradation products resulting from stress tests did not interfere with the determination of ORL, therefore the test demonstrated stability [42].

Schneider et al., have studied ethanol extracts of the reference (Xenical) product and the generic (Lipiblock) product for comparative impurity profiles in HPLC-UV and LC-MS / MS studies. While the generic

product contained higher levels of impurities, no appreciable impurities were found in the reference product. Since the effects of impurities are not known, Xenical, a fully synthetic product, has a greater share of reliability than the generic product [43].

Sun et al., have investigated quantitative magnetic resonance (qNMR) method was used to determine the ORL content in tablets. The method was found simple, reliable, accurate and efficient. In this study, while phloroglucinolanhydrous was used as IS, dimethylsulfoxide-d6 (DMSO-d6) was used as a solvent. The qNMR method includes linearity, limit of detection (LOD), limit of detection (LOQ), stability, sensitivity and accuracy. When comparing the qNMR method and the HPLC method, the content determination results in the three groups of ORL tablets were found nearly the same. The proposed method has correctly corrected the shortcomings of the existing HPLC method for determining the ORL content and proved complementary to conventional analysis for the purity measurement of ORL in some pharmaceutical preparations [44].

Liu et al., have developed a HPLC method to examine the components of o ORL tablets. Isocratic separation was performed with the Agela Venusil XBP-CI8 C18 column at a flow rate of 1.5 ml / min. The mobile phase contained 89:11 (v / v) MeOH - H_2O containing 0.1 part FA. The UV detector was set at 210 nm. The results indicated the complete separation of ORL with tablet impurities. The method is linear (r^2> 0.999) in the concentration range 0.4-0.6 mg / ml. Therefore, the HPLC developed method can be used effectively to accurately determine the ORL content in tablets [45].

Yang et al., have studied the impurities contained in ORL capsules. A new reverse phase high performance liquid chromatographic (RPHPLC) method was developed and validated by a UV spectrophotometric detector with high sensitively and selectively. The suggested HPLC method was found suitable for the analysis of pharmaceutical dosage forms of ORL [46].

Rao et al., have studied an easy and precise RPHPLC method for the determination of 50% ORL pellets. Inertsil ODS 3V column and MeOH, ACN and TFA (82.5 : 17.5 : 0.01) were used as mobile phase. Flow rate was 1 ml / min and was observed at 210 nm. It has been reported that the proposed method does not require very simple, fast and complicated sample preparation [47].

Schneider et al., have studied a HPLC-UV and MS / MS methods in order to analyze the impurity profiles of the original (Xenical) and two generic (CobeseTM and Orsoten) products containing ORL. The samples were dissolved in EtOH. While the impurity profiles of the generic products are similar among themselves, the impurity profile of the original product is different. D-Determined impurities in generic products are 14

for CobeseTM and 13 for Orsoten. For the original product, Xenical, the impurity number was found 3. According to this analysis, analytical quality order follows each other as Xenical, Orsoten and CobeseTM [48].

Ahamad et al., have developed and validated a sensitive, accurate, easy and specific, RPLC method for the detecrion of ORL in various dosage forms. ACN: MeOH and distilled water as the mobile phase were in the ratio of 75:15:10 and maintained at pH 3 by the addition of ortho phosphoric acid. The method was carried out with an Agilent ZORBAX SB-C18 column at a flow rate of 1.0 ml / min at 210 nm. The RT of ORL was obtained 1542 minutes. The linearity range of ORL is 10-60 µg / ml. The specificity, precision, linearity, accuracy and robustness of the method are confirmed. The proposed method can be applied to evaluate ORL in pharmaceutical capsule dosage form belonging to different trademarks and to calculate the system suitability parameter [49].

CONCLUSION

The presence of illegal products used for weight loss in the market is considered as a serious health problem. In order to increase the effectiveness of these products, chemicals can be illegally added to their ingredients. Some of these compounds can cause serious negative consequences that can even cause death. Qualitative and quantitative analysis of such illegal products and legal food supplement products on the market is of great importance.

SBA and ORL are the compounds used in WLPs, are also used as an aid in the treatment of obesity. SBA, a combined norepinephrine and serotonin reuptake inhibitor, tries to reduce the calories taken by suppressing appetite in obese patients and does this by inhibiting absorption. SBA also causes a decrease in the rate of eating, which further slows down the cumulative eating at a meal related to the development of satiety.

ORL is a reversible gastrointestinal lipase inhibitor. It is used in obesity treatment. It inhibits the absorption of fats taken with food and allows it to be thrown out without being absorbed by the body. The use of ORL and concurrent diet during weight control provides greater weight loss than diet alone.

In this review, methods such as HPLC method, UHPLC-DAD method, HPLC-ESI - MS / MS), LC-MS / MS method, which are developed or available for the determination of SBA and ORL, are presented. It is understood that the HPLC used in the determination of SBA and ORL is the most widely used method among all analytical separation techniques. The reasons for the widespread use of the method include its sensitivity, accuracy, being easily adaptable to quantitative analysis, suitability for the separation of non-volatile species or easily degradable species, and above all, its wide applicability to substances of interest to industry, many disciplines and the public.

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Chapter 12

ULTRASONOGRAPHIC FEATURES OF GASTROINTESTINAL EMERGENCIES IN PEDIATRIC PATIENTS

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Introduction

Gastrointestinal non-traumatic emergencies are the most common causes of acute abdominal pain in the pediatric population (Zurynski et al., 2020). An urgent radiological examination is required as any delay in the diagnosis is associated with increased morbidity and potential mortality in children (Vasavada, 2004).

There are a number of gastrointestinal diseases causing acute abdominal pain from mild illnesses such as acute gastroenteritis to life-threatening conditions such as perforated appendicitis. The most common clinical features of abdominal emergencies are vomiting, abdominal pain, and fever (Guney & Coskun, 2019). A detailed physical examination is necessary to reach an accurate diagnosis.

The abdominal ultrasonography (US) is an initial imaging technique used for evaluating pediatric patients with acute abdominal pain, due to its cost-effectiveness, non-invasiveness, no radiation and easy availability (Vasavada, 2004). Although US examination may not always contribute to the definitive diagnosis, it allows the elimination of a significant number of differential diagnostic dilemmas and therefore directs further diagnostic investigations (Chen, Wang, Hsu, Huang, & Lin, 2000).

This article discusses the US imaging features of the gastrointestinal diseases that cause acute abdomen in pediatric patients.

Ultrasonography technique

Ultrasonographic evaluation of the gastrointestinal system in pediatric patients is performed in a quiet environment with a high-resolution ultrasound system (Vasavada, 2004). A 3.5-6 MHz convex probe is commonly used in older children while 10–18 MHz linear probe is preferred in infants and for imaging of bowel diseases in children. Overall, the child should be examined with the highest frequency transducer that will provide the anatomy to be imaged.

The patients are usually examined in the supine position with arms extended above the head (Yip, Tay, & Wong, 1985). An agitated child should be examined in the arms of a parent. Sedation is almost never necessary.

Emergencies of the gastrointestinal tract

Emergencies of the gastrointestinal tract in pediatric patients include hypertrophic pyloric stenosis, intussusception, acute appendicitis, necrotizing enterocolitis, midgut volvulus, duplication cysts, Meckel's diverticulum, and foreign body ingestion.

1) Hypertrophic Pyloric Stenosis (HPS)

Hypertrophic pyloric stenosis (HPS) is defined as an abnormal thickness of the muscle of the pyloric antrum of unknown etiology and is the most common urgent_surgical entity affecting children during the first 6 months of life. The overall incidence is 2-5 in 1000 births and it is four times more frequent in males than in females (Hernanz-Schulman et al., 1994). HPS is more common in first-born boys and in families with a familial predisposition. Ninety-five percent of this disease manifest between 3 and 12 weeks of age, most commonly at 4 weeks. HPS is very rare in patients younger than 10 days(Ohshiro & Puri, 1998).

HPS manifests as occasional non-bilious vomiting which is often confused with gastroesophageal reflux. Pyloric muscle hypertrophy causes complete gastric obstruction resulting in "projectile" vomiting after breastfeeding and leads to dehydration, weight loss, and hypochloremic alkalosis(Chandran & Chitkara, 2008). The presence of bile in the vomited contents indicates more distal obstruction of the gastrointestinal tract including intestinal malrotation and midgut volvulus which is related with high morbidity and mortality(Chandran & Chitkara, 2008).

US is the approach of choice in children with suspected HPS. It allows dynamic analysis of the pyloric canal peristalsis. The first step is to identify the pylorus which is usually located medially and posteriorly to the gallbladder(Ball, Atkinson, & Gay, 1983). The second step is devoted to the measurement of the pyloric canal. The thickness of the normal muscular layer of the pylorus is approximately 2 mm. The third step is to visualize the passage of the gastric contents. Dynamic analysis is essential as a wide-open pylorus with normal passage of the gastric contents excludes the diagnosis of HPS(Weiss, Leixner, & Brandesky, 1984).

The main ultrasound criteria for the diagnosis of HPS are: thickened, nonrelaxing antropyloric muscle measuring ≥3 mm, length of the pyloric canal over 14 mm, lack of relaxation and peristalsis of the pyloric muscle, retained gastric contents, and increased Doppler flow signal of the mucosal and muscular layer(Hussain, 2008) (Figure 1).

Thickening of the pyloric muscle may be transient as a result of pylorospasm. In such cases, a prolonged dynamic ultrasound analysis is essential for confirming the opening of the pyloric canal at some point. If the muscle layer is 2-3 mm thick and does not show signs of relaxation during the US examination, a follow up is recommended(Jackson, Holden, Doering, & Lappas, 1985). Unskilled sonographers are encouraged to remove the air from the stomach by nasogastric tube, to inject 20mL of saline, and to examine the child in a right-sided position(Blumhagen, Maclin, Krauter, Rosenbaum, & Weinberger, 1988).

HPS is treated surgically via pyloromyotomy. Balloon dilatation has been reported to be successful after failed pyloromyotomy or recurrent pyloric stenosis in a limited number of patients. If vomiting persists after surgery, a control US examination should be performed(Nasr, Ein, & Connolly, 2008). However, it may take up to 6 months for the pylorus to regain the normal thickness(Assefa, 2002).

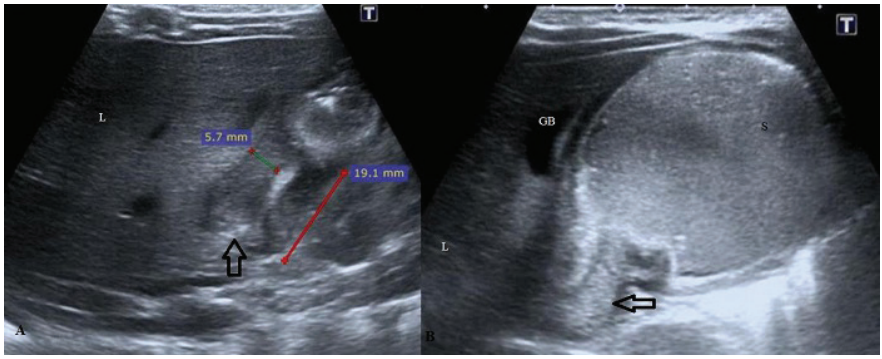

Figure 1. Hypertrophic pyloric stenosis (black arrows). Transverse views of the upper abdominal ultrasonography show (A) thickened pyloric muscle measuring ≥3 mm (green), and length over 14 mm (red). (B) Dilated stomach with retained gastric contents. L – liver. GB – gallbladder. S - stomach.

2) Intussusception

Intussusception is a condition in which a segment of the intestine invaginates into another part of the intestine and is the most frequent reason for gastrointestinal obstruction in children (Trigylidas et al., 2019). Intussusception most commonly occurs in children between 6 months and 2 years of age with an incidence of 38 per 100.000 cases in the first year of life and 31 per 100.000 cases in the second year of life(Park, Rabiner, & Tsung, 2019).

Abdominal pain, red currant jelly stool, and palpable abdominal mass are classic clinical features of acute intussusception. About 20% of patients with intussusception are asymptomatic, and only 30-68% of patients with suspicious clinical features have intussusception(Santos, Espinosa, & Lucerna, 2019).

Intussusception may cause intestinal obstruction and a reduction in the vascular flow that leads to the development of intestinal ischemia and necrosis. Therefore, early diagnosis and treatment of this disease are critically important. Intussusception is idiopathic in nearly 90% of pediatric cases. About 5% of children with intussusception have pathologic lead points such as MD, polyp, duplication cyst, lymphoma, or Henoch – Schönlein purpura(Sharma et al., 2019).

Ultrasound sensitivity in detecting intussusception is 97.9%, specificity 97.8%, positive predictive value 86.6%, and negative predictive value 99.7%. US examination of intussusception is performed using high-resolution linear probes (5-12 MHz). On transverse images, intussusception appears as a round mass with concentric hypo and hyperechoic layers known as a "target sign" or a "doughnut sign". On longitudinal views, a "pseudokidney sign" is observed(Lee, Kim, Choi, Lee, & Ryu, 2020) (Figure 2).

Small bowel intussusception is located mostly in the periumbilical region or right lower quadrant. It is usually transient with a short length (<3 cm), thin diameter (<2.5 cm), preserved wall motion, and absence of a lead point. Ileocolic, ileo-ileocolic, or ileocolocolic intussusception is often located in the right quadrant with a longer length (>3 cm), and larger diameter (>2.5 cm). The ratio of hyperechoic inner fat core diameter to wall thickness is >1.0 in ileocolic and <1.0 in small-bowel intussusception. The presence of lymph nodes inside the lesion is more often seen in ileocolic intussusceptions(Sharma et al., 2019).

Small bowel intussusception in the absence of a lead point or bowel obstruction is treated symptomatically and serial US imaging should be performed to confirm the spontaneous resolution. The standard treatment procedure for ileocolic intussusception is hydrostatic (barium, water-soluble contrast, saline) or pneumatic reduction performed under fluoroscopic or ultrasonographic guidance. After nonsurgical treatment, the intussusception recurrence rate is 10-15% (Fahiem-Ul-Hassan et al., 2020). When non-surgical interventions are not sufficiently effective, surgery is considered an appropriate treatment option.

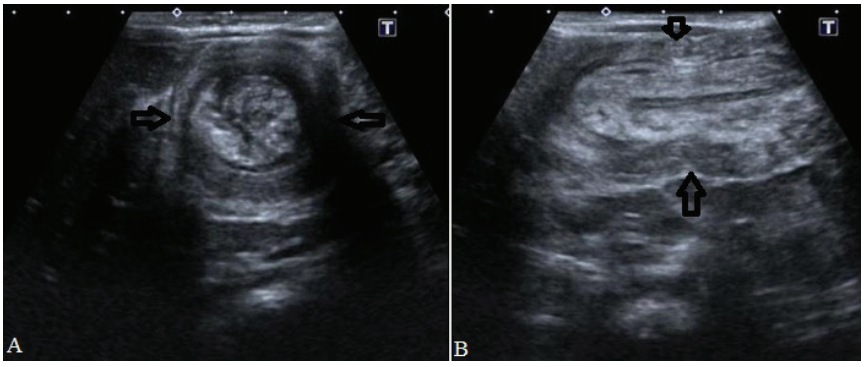

Figure 2. Ileocolic intussusception (black arrows). Right lower abdominal ultrasonography (A) transverse view shows a "target sign"; (B) Longitudinal view shows a "pseudokidney sign".

3) Acute Appendicitis (AA)

Acute appendicitis (AA), an inflammation of the appendix, is the most common indication for surgery in the pediatric population (Addiss, Shaffer, Fowler, & Tauxe, 1990). It affects approximately 70.000 to 90.000 pediatric cases annually with an overall incidence of 75 to 233 per 100.000 children. The incidence increases with age with a peak incidence around the second decade of life. After that, the incidence decreases with age. AA is uncommon under two years of age. (Addiss et al., 1990).

The etiology and pathogenesis of AA are controversial. AA is usually related to mechanical obstruction of the lumen of the appendix by various causes, such as fecaliths, lymphoid hyperplasia, foreign bodies, parasites, and primary (carcinoid, adenocarcinoma, lymphoma) or metastatic (usually colon or breast) tumors. In approximately 60% of pediatric cases, the obstruction is due to the hyperplasia of the submucosal lymphatic tissue most often caused by acute respiratory infections, infectious mononucleosis, or other diseases that cause a generalized reaction of lymph tissue(Koepsell, 1991). In 35% of pediatric cases, the obstruction is caused by fecaliths. AA is caused by a foreign body (residual barium, fruit or vegetable seeds) or intestinal parasites in 4% of cases, while in 1% of children and adults the AA is caused by a tumor of the wall of the appendix or caecum(Koepsell, 1991).

The diagnosis of AA is made on the basis of accurate anamnesis and physical examination. The classic clinical feature is initial pain in the periumbilical region with subsequent migration into the right lower quadrant. This classic presentation of AA is found in only 50-60% of patients(Chen et al., 2000).

The US examination of the AA is performed using the technique of gradual compression of the right quadrand. This technique allows us to distinguish compressible intestin from an inflamed, uncompressible appendix. To find the location of the appendix, it is necessary to identify several anatomical landmarks such as the caecum, terminal ileum, and right psoas muscle(Vasavada, 2004). The ascending colon is shown as a nonperistaltic intestinal structure containing gas and liquid. The next anatomical landmark is the terminal ileum, which is presented as an easily compressible intestinal structure showing peristaltic movements. The appendix is usually located 1-2 cm below the terminal ileum. The right psoas muscle, external iliac artery, and external iliac vein are located medially from these intestinal structures(Kessler et al., 2004).

Transverse US images of the right lower abdomen show a target sign characteristic of AA which is formed by a hypoechoic center corresponding to the intraluminal fluid and with concentric hyper (submucosa) and

hypoechoic (muscle layer) parietal layers. The diameter of the appendix is greater than 6mm and the appendix is noncompressible. The longitudinal US images demonstrate a fluid-filled, blind-ending intestinal structure connected to the caecum(Krishnamoorthi et al., 2011). Surrounding soft tissue hyperechogenicity, reactive lymph nodes, presence of an appendicolith, local fluid collections, and an abscess may be associated. Conditions that may cause a thick-walled appendix are inflammatory bowel diseases, cystic fibrosis and Henoch–Schönlein purpura(Fox et al., 2008) (Figure 3).

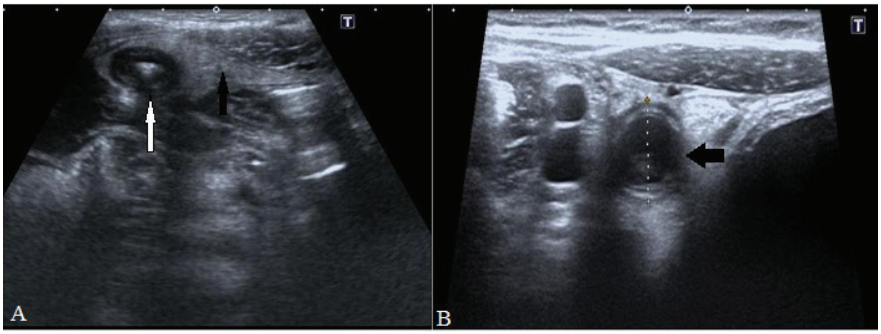

Figure 3. Acute appendicitis (AA). Transverse views of the right lower abdominal ultrasonography show (A) A "target sign", intraluminal appendicolith (white arrow), and surrounded soft tissue hyperechogenicity (black arrow); (B) The diameter of the appendix is greater than 6mm.

Appendiceal dilatation may cause interruption of the the submucosal layer that leads to perforated AA. US findings suggestive of perforation include surrounding soft tissue hyperechogenicity, local fluid collections, abscess, and intraperitoneal free fluid (Mittal et al., 2013). The perforated appendix may appear decompressed with a diameter less than 6 mm. The absence of visualization of an appendix may be due to a retrocecal location, obscuration by superimposed bowel gas, or limited US penetration in obese patients(Mittal et al., 2013) (Figure 4).

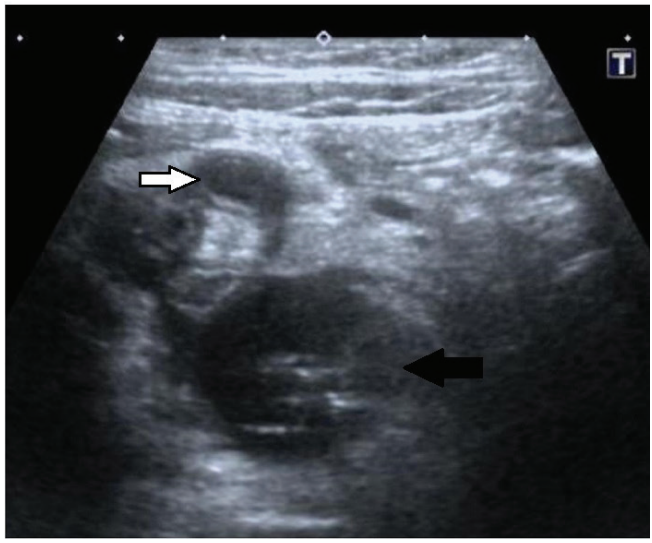

Figure 4. Perforated acute appendicitis. Transverse view of the right lower abdominal ultrasonography shows a decompressed perforated appendix (white arrow), and a local fluid collection (black arrow).

The differential diagnosis of AA includes infectious enterocolitis, inflammatory bowel diseases, mesenteric lymphadenitis, neutropenic colitis (typhlitis), diverticulitis, Meckel's diverticulitis, epiploic appendagitis, and omental infarction. Infectious enterocolitis is presented with a bowel wall thickening (> 3mm) and surrounding free fluid (Wessling, 2018) (Figure 5).

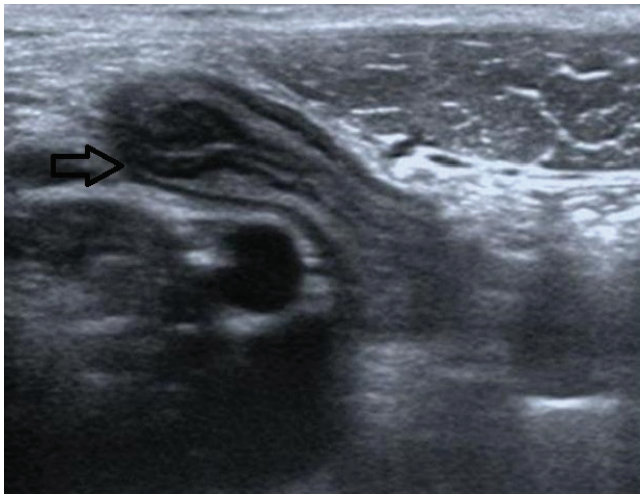

Figure 5. Infectious enterocolitis. Transverse view of the right lower abdominal ultrasonography shows bowel wall thickening (> 3mm) of the ileum (black arrow).

Mesenteric lymphadenitis, or right lower abdominal quadrant lymphadenopathy, is defined as a cluster of 3 or more lymph nodes in the right lower abdominal quadrant with a short-axis diameter more than 8-10 mm. The bowel and appendix appear normal (Binagia & Levy, 2020) (Figure 6).

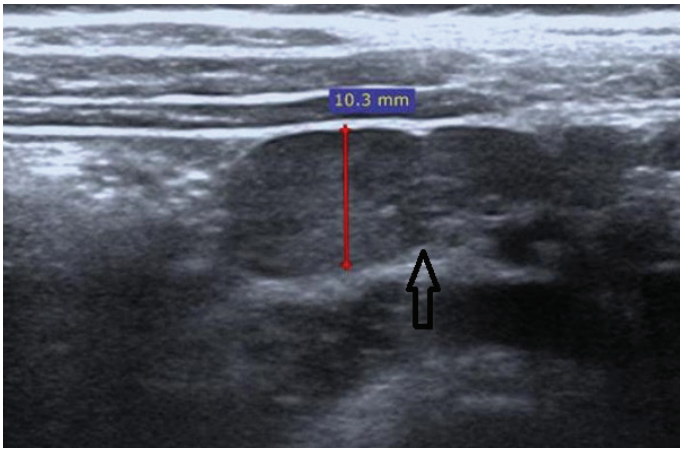

Figure 6. Mesenteric lymphadenitis. Transverse view of the right lower abdominal ultrasonography shows a lymph node with a short diameter more than 10 mm (black arrow).

Inflammatory bowel diseases (IBD) including Crohn's disease (CD), ulcerative colitis (UC), and undifferentiated colitis are idiopathic, inflammatory, chronic diseases with autoimmune etiology (Rajbhandari et al., 2020). Family history increased the risk of IBD by >15%. These diseases mainly occur in younger adults. One-third of all patients are under 20 years of age. The reported incidence and prevalence rates are 4.8 - 6.8 / 100.000 and 17.9 - 30.7 / 100.000, respectively (Gong et al., 2020).

CD most often affects the terminal ileum. Complicated CD may be associated with perforation, the formation of an abscess, fistula, or bowel obstruction due to chronic inflammatory adhesions. On abdominal US, the CD is usually presented as segmental bowel wall thickening > 3 mm of the terminal ileum. Surrounding soft tissue hyperechogenicity, lymphadenopathy, and free intra-abdominal fluid is suggestive of associated active inflammation (Wilkens, Novak, Lebeuf-Taylor, & Wilson, 2015). Formation of an abscess and fistula may be seen at the subacute and chronic stage of CD. Chronic inflammation is presented with bowel fibrosis that may cause bowel obstruction (Lu, Merrill, Medellin, Novak, & Wilson, 2019) (Figure 7).

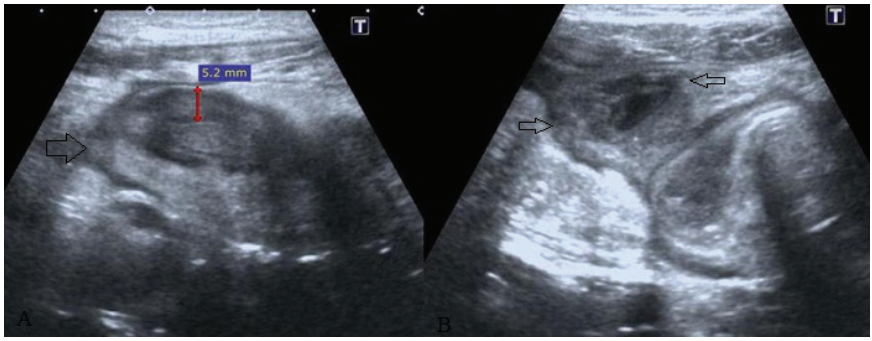

Figure 7. Crohn's disease. Transverse views of the right lower abdominal ultrasonography show; (A) Segmental bowel wall thickening > 3 mm of the terminal ileum (red), surrounded soft tissue hyperechogenicity (black arrow), and (B) formation of an abscess (black arrows).

UC involves the colon and rectum. On US wall-thicked, hyperemic colon loops may be detected. The bowel contour appears smooth in UC, and irregular in CD. Sclerosing cholangitis, cirrhosis, hepatitis, and calcium oxalate urolithiasis may be associated with IBD (Rosenbaum et al., 2017).

Laparoscopic appendectomy is the preferred surgical approach for AA (Slomski, 2020). Some studies suggest that emergent appendectomy may not be necessary and that IV antibiotics with appendectomies delayed for 12 to 24 hours after presentation do not significantly increase the rate of perforations, operative time, or length of hospital stay. Stabile patients with perforated AA usually undergo antibiotic therapy followed by interval appendectomy several weeks later. If an abscess is present, image-guided percutaneous drainage is recommended. If the patient is septic, urgent surgery is preferred ("Surgery or antibiotics for appendicitis?," 2020).

4) <u>Necrotizing Enterocolitis (NEC)</u>

Necrotizing enterocolitis (NEC) is a major reason for morbidity and mortality in premature infants. About 90% of cases occur in preterm infants with a history of premature rupture of the fetal membranes and perinatal asphyxia. Children with congenital cyanotic heart anomalies and enteral nutrition are at risk of NEC(Daneman, Woodward, & de Silva, 1978).

The most important risk factors for the development of NEC are previous ischemic damage of the bowel, bacterial colonization, and enteral nutrition. Ischemia causes intestinal mucosal injury that leads to increased intestinal permeability and bacterial colonization(Zhu, 1981). The bacteria penetrate the damaged intestinal wall causing pneumatosis intestinalis, pneumatosis portalis, and pneumoperitoneum. Perforation most often occurs in the terminal ileum. The colon and the proximal part of the small

intestine are less affected. Sepsis occurs in 1/3 of children and can be fatal(Kliegman, Walker, & Yolken, 1993).

Children may have ileus manifested by enlargement of the abdomen, bilious vomiting, and blood in the stool. Sepsis is manifested by lethargy, temperature instability, apnea, and metabolic acidosis. The diagnosis is made clinically and confirmed by imaging.

Abdominal radiographs have been the mainstay of imaging evaluation of infants with NEC. The X-ray images of infants with mild NEC are nonspecific and may show a mild ileus. In infants with a moderate NEC ileus and focal intramural gas can be seen. Severe stages of NEC are presented with extensive pneumatosis intestinalis, portal venous gas, ascites, and pneumoperitoneum. Close follow-up with early repeated imaging is suggested for these patients(Buonomo, 1999).

US is a complementary imaging modality to abdominal radiography. US may provide information regarding bowel wall thickening or thinning, absent bowel wall perfusion, ileus (bowel dilatation, absent peristalsis), pneumatosis intestinalis, pneumatosis portalis, intraperitoneal fluid, and pneumoperitoneum. Pneumatosis intestinalis is defined as the presence of intramural gas that wraps around the intestinal subserosa. Multiple intrahepatic echoes with linear distribution are pathognomic for pneumatosis portalis(Zhu, 1981). Moreover, small amounts of free abdominal air may be detected between the liver and anterior abdominal wall. However, pneumoperitoneum may not be present in 37% of infants with perforation. The presence of ascites is correlated with bowel perforation and the need for surgery(Morrison & Jacobson, 1994).

Treatment is primarily supportive and includes nasogastric suction, parenteral fluid administration, complete parenteral nutrition, antibiotics, isolation in cases of infection, and often, surgery(Bradshaw, 2009).

5) Intestinal Malrotation (IM) and Midgut Volvulus (MV)

The midgut extends from the second part of the duodenum to the distal third of the transverse colon. The term intestinal malrotation (IM) describes a wide spectrum of anomalies associated with abnormal position and fixation of the midgut. IM affects approximately 3.9 per 10.000 children(Fernandez Sanchez, Lopez Pereira, Diez Pardo, & Utrilla, 1987).

US features of IM include inverse relation of the superior mesenteric artery (SMA) and superior mesenteric vein (SMV), absence of the horizontal portion of the duodenum, and abnormal position of the cecum and ascending colon. Plain abdominal radiography may show an absence of the normal bowel pattern in the right lower quadrant. In the case of nonrotation, all colonic bowel segments may be seen in the left half of the

abdomen(Long, Kramer, Markowitz, & Taylor, 1996).

Upper gastrointestinal series are often preferred in cases of suspected IM. On the frontal view, the normal duodenojejunal junction is located at the same level as the bulb. The lateral view shows the parallel orientation of the ascending and descending portions of the duodenum. The abnormal cecal position is suggestive for IM (Applegate, Anderson, & Klatte, 2006).

The acute midgut volvulus (MV) is a life-threatening complication caused by IM and is characterized by intestinal obstruction and ischemia. About 60% to 80% of patients with acute MV in the first month of birth are presented with bilious vomiting and crampy abdominal pain. Vascular compromise leads to hematochezia which may be seen in only 15% of patients with MV(Kumar, Brereton, Spitz, & Hall, 1988). Preterm infants usually present nonspecific symptoms such as apnea, abdominal distension, and shock with gangrenous bowel segments. Chronic MV is defined as a partial or intermittent MV due to the impairment of the venous and lymphatic flow which leads to malabsorption(Blanc et al., 1986).

On US, a whirlpool sign is detected due to the twisting of the duodenum and SMV around the axis of the SMA. In patients with bowel ischemia, the whirlpool sign may not be detected. In children with chronic MV, the US presentation is similar with additional findings of dilated duodenum(Long, Kramer, Markowitz, Taylor, & Liacouras, 1996). Upper gastrointestinal series may demonstrate the spiral twisting of the duodenum. In the case of complete intestinal obstruction, the contrast material cannot proceed further and terminate with a beaklike configuration(Applegate et al., 2006).

Emergency surgery is required in cases of acute MV. Surgery is also indicated in symptomatic children with IM and without MV. The role of surgery is controversial in asymptomatic patients with IM(Ingoe & Lange, 2007).

6) Duplication Cysts (DCs)

Duplication cysts (DCs) most often originate from the terminal ileum and stomach, however, they can occur anywhere along with the gastrointestinal system(Liu & Adler, 2014). They are located in the mesenteric side of the bowel and usually do not communicate with the intestinal lümen. DCs are frequently symptomatic in patients younger than 1 year(Kumar, Ramanathan, Haider, Khanna, & Otero, 2015).

Two sonographic signs are diagnostic for DCs: (1) a double-layered wall known as “bowel wall signature” and (2) peristalsis in the cyst. They usually appear as anechoic cysts or hyperechoic lesions due to infection or hemorrhage from associated ectopic gastric mucosa. Small DCs may cause intestinal obstruction and may be a lead point for intussusception(Ponder & Collins, 2003).

Mesenteric cyst, lymphatic malformations, ovarian cysts, and cystic teratoma can mimic DCs. DCs are treated by surgical resection (Figure 8).

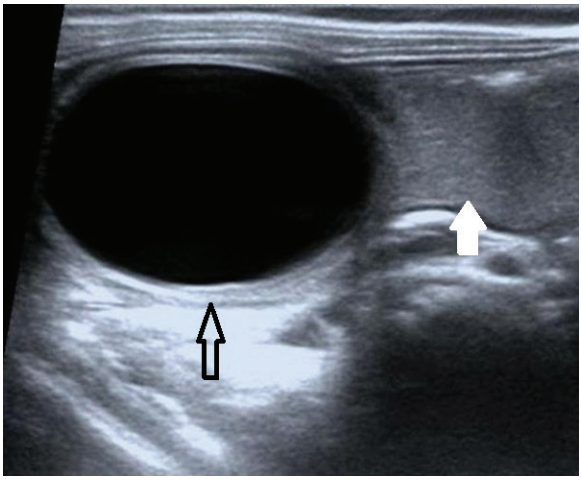

Figure 7. Duplication cyst. Transverse view of the right upper abdominal ultrasonography shows a double-layered anechoic cyst (black arrow) causing an intestinal obstruction (white arrow).

7) <u>Meckel's diverticulum (MD)</u>

Meckel's diverticulum (MD) occurs as a result of incomplete obliteration of the omphalomesenteric duct during embryogenesis and usually lies on the antimesenteric side of the terminal ileum(Grall, Granier, & Vibert-Guigue, 1997). It occurs in 2% to 3% of the population and is the most common developmental anomaly of the gastrointestinal tract. MD is generally recognized by the 'rule of twos' affecting 2% of the population, located two feet (60cm) from the ileocecal valve, usually two inches (5 cm) long, and may contain two types of ectopic tissue (gastric or pancreatic tissue)(Hawkins, Slavin, Levin, & Spencer, 1996).

MD is often asymptomatic in children. The most common complications of MD are gastrointestinal bleeding, intestinal obstruction, diverticulitis, and rarely neoplasm. Gastrointestinal bleeding (melena or hematochezia) is the most frequently encountered complication of MD in children(Yamauchi et al., 2020). Meckel's diverticulitis occurs more frequently in adult patients. Complicated diverticulitis may be associated with perforation, the formation of an abscess, fistula, or bowel obstruction due to inflammatory adhesions. Intestinal (closed-loop) obstruction may develop due to intussusception, volvulus, and internal hernia. MD may act as a lead point causing ileo-vitelline, ileo-ileal, and ileocolic type of intussusceptions. Volvulus and internal hernia of the small intestine occur due to the congenital mesodiverticular fibrous strips extending from the ileum to the umbilicus. Incarceration of MD in the inguinal hernia (so-

called Littre's hernia) is a very rare cause of intestinal obstruction(Ajmal, Majid, Tahir, & Sagheer, 2020).

MD tumors are very rare. The most common tumor type is carcinoid tumor, followed by adenocarcinoma, intraductal papillary mucinous neoplasm, gastrointestinal stromal tumor (GIST), melanoma, lymphoma, and leiomyosarcoma(Araki et al., 2012).

On abdominal US, MD is usually presented as an intestinal blind-ending, cystlike structure in the periumbilical region or right lower quadrant. Surrounding soft tissue hyperechogenicity, reactive lymph nodes, and free intra-abdominal fluid are suggestive of associated inflammation(Grall et al., 1997).

8) Foreign Bodies

Foreign body ingestion is often seen in children aged 6 months to 3 years. In approximately 80% to 90% of cases, foreign bodies pass spontaneously, 10% to 20% need endoscopic intervention, and 1% require urgent surgical removal(Coco & Leanza, 2020). Anteroposterior (AP) and lateral radiography of the neck, chest, and abdomen are required. Radiopaque foreign bodies (coins, batteries, magnets) are clearly evident on X-ray(Yang & Postma, 2020).

Ingested foreign bodies may cause intestinal perforation, the formation of an abscess, fistula, or bowel obstruction. They may act as a lead point causing small bowel and ileocolic type of intussusceptions. The complications such as intussusception, bowel obstruction, and an interloop abscess can be detected by US(Hintzen, Pieracci, & Storfa, 2019).

Conclusion

Gastrointestinal non-traumatic emergencies require an urgent radiological examination as any delay in the diagnosis is related with high morbidity and potential mortality in children.

Early detection and appropriate management are crucial for preventing progressive and irreversible consequences. Therefore, the radiologists take an essential role in identifying the gastrointestinal emergencies on imaging, and the initial diagnosis is an integral part of a comprehensive multidisciplinary approach.

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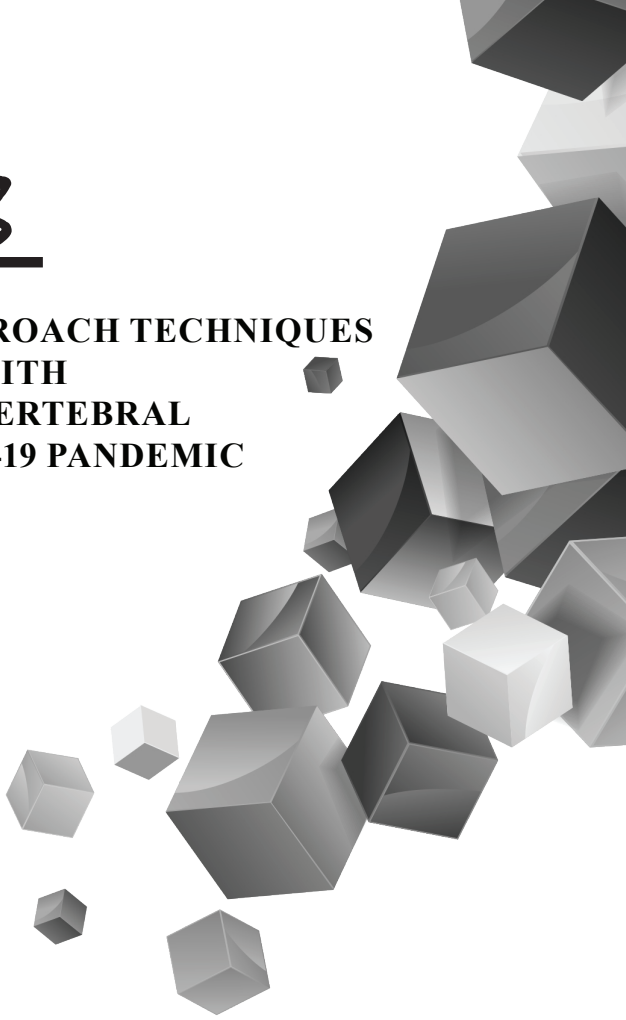
Chapter 13

PERCUTANEOUS APPROACH TECHNIQUES FOR THE PATIENTS WITH THORACOLUMBAR VERTEBRAL FRACTURE IN COVID-19 PANDEMIC



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Most of the vertebral fractures occur in the thoracolumbar region due to the anatomy and biomechanical characteristics of the spine. Although traffic accidents and falls are among the leading causes of trauma, osteoporosis and malignancies are the other factors predisposing to fracture etiology.

Depending on the type of fracture, when the patient's neurological examination is evaluated, the type of surgical approach is decided. The method and frequency of minimally invasive procedures have increased as time progresses. In minimally invasive interventions to the thoracolumbar region, it is aimed to achieve diagnosis and treatment by the percutaneous approach. This area's approach by percutaneous procedure causes minimal damage to muscle, joint, and bone structures. In this way, the patient's need for medical treatment is less after the operation, and the short hospital stay also contributes to the reduction of medical expenses.

Since 11th March 2020, the COVID-19 global pandemic has been continuing rapidly all over the world. This pandemic has adversely affected the health system around the world. Precautions are taken in hospitals, and these include stopping or postponing elective surgery in many countries. There are studies aimed at spending minimum time in the operating room and accelerating the discharge from the hospital environment for emergency cases. It is aimed to complete the treatment of the patient as soon as possible. Percutaneous approaches have gained more importance today due to these and similar reasons.

Under this topic, vertebroplasty-kyphoplasty, transpedicular screw applications will be discussed in thoracolumbar fracture patients' emergency intervention.

Vertebroplasty and Kyphoplasty

With this percutaneous technique, polymethylmethacrylate (PMMA) is injected into the vertebral corpus, which is weakened for a reason, and it is aimed to strengthen the corpus. With the increasing prevalence of osteoporosis-related fractures in recent years, their use has become more widespread, and they are less invasive techniques compared to open surgery (Berlemann, Franz, Orler, & Heini, 2004; Garfin, Yuan, & Reiley, 2001; Grohs, Matzner, Trieb, & Krepler, 2005).

In the historical development stage, vertebroplasty has been used before and was applied in the late 1980s. The first kyphoplasty was performed in 1998. In both methods, PMMA is injected into the vertebral corpus. In the kyphoplasty technique, in addition to vertebroplasty, a balloon is inflated in the vertebral corpus where the fracture occurs, and the vertebrae are elevated. Afterward, cement is filled into the cavity formed by the balloon (Ploeg, Veldhuizen, The, & Sietsma, 2006). The procedure aims to treat the pain of the patient in both techniques. In kyphoplasty, as the balloon

is inflated and the height is preserved, the spine biomechanics are also preserved.

- Patient Selection

Vertebroplasty and kyphoplasty techniques are very effective, especially in the period of acute pain due to vertebral collapse. The procedure can be applied to new fractures for up to 6 weeks to treat the pain (McGraw et al., 2002). These procedures are generally not performed in fractures older than this period, as the pain has decreased or stopped (Garfin, Buckley, Ledlie, & Balloon Kyphoplasty Outcomes, 2006). Analgesic and rest can be recommended as well as conventional treatment methods. However, if early mobilization and labor loss are desired to be prevented, percutaneous vertebral strengthening techniques should be applied if there is an indication for pain relief (Berlemann et al., 2004). The application of this treatment prevents patients from staying in bed for a long time, using analgesics, and wearing corsets (Garfin et al., 2001).

If the vertebral fracture is suspected in patients with primary malignancy, osteoporosis, whether due to trauma or not, percutaneous vertebral augmentation procedures can be easily applied for these patients. These techniques are also helpful on the way to diagnosis in terms of both pain relief, and vertebral biopsy can be applied to patients during the percutaneous procedure. The technique of the procedure will be discussed in the next topics.

- Indications and Contraindications

Percutaneous vertebral augmentation techniques are procedures used to treat vertebral compression fracture pain resistant to conventional treatments and in terms of diagnosis and treatment of vertebral metastases in malignancy patients (Evans et al., 2003). Vertebroplasty can also be applied prophylactically to the vertebrae between two damaged vertebrae. It is a useful application in terms of pain reduction (Togawa et al., 2006).

Vertebroplasty and kyphoplasty can be applied especially in osteoporotic vertebral fractures, non-osteoblastic vertebral tumors such as multiple myeloma, osteolytic metastases, for pain relief, vertebral strengthening, and vertebral biopsy for diagnosis and treatment (Ploeg et al., 2006).

This technique, which has fewer complication rates, is contraindicated in cases such as vertebral-plana, fractures compressing on the spinal cord, presence of infection, cement allergy, and invisible pedicles radiologically (Evans et al., 2003).

- Diagnosis Methods

2-way plain radiography should be performed first in the patient admitting with back and low back pain. It will give us information about the fracture' s location and the pedicle′ s condition on plain radiographs (Garfin et al., 2001).

CT examination is useful in seeing the vertebrae' s detailed structure to be operated, determining the needle' s diameter to be operated, examining the pedicle structure, and seeing the vertebrae status in the postoperative period. MRI can diagnose with fat-suppressed (Short TI Inversion Recovery-STIR) sequence up to 6 weeks after fracturing of the spine (Figure-1) (McGraw et al., 2002). Other pathologies of the spinal canal can also be distinguished in MRI. Disc herniation, foraminal narrowing, spinal stenosis, and spondylolisthesis are critical for retraction.

Bone scintigraphy, which diagnoses the metabolic activity in the pathological region, is also an essential diagnosis method (Fourney et al., 2003).

- Comparison of Kyphoplasty and Vertebroplasty, Materials Used

In both methods, cement is injected into the vertebral corpus. What is different in the kyphoplasty technique is that the volume of cement in the vertebrae can be determined with the balloon, which is inflated before, and the vertebrae are elevated. This situation both minimizes cement leaks and helps to protect the stabilization of the spine, and prevents the development of kyphosis in the advanced stage (Grohs et al., 2005).

The material used is polymethylmethacrylate (PMMA). It has been found that PMMA injection between 15-17% of the corpus volume is sufficient to provide vertebral stiffness (Evans et al., 2003). As the corpus volume increases as descending from the thoracic to the lower lumbar vertebrae, the amount of PMMA administered also increases. Although more than one vertebrae can be treated in the same session, it is recommended in the literature to apply a maximum of 3 levels in the same session (Garfin et al., 2006).

- Surgical Procedure

Percutaneous transpedicular procedures can be performed under local or general anesthesia. The application under sedation is another option. The patient is placed in a neutral position on the operating table. The preoperatively determined level is centered with fluoroscopy, and fluoroscopy is adjusted. The operating table should be radiolucent. A large room is required for access to the patient from both sides of the table and for obtaining anterior-posterior (A-P) and lateral (sagittal) imaging efficiently by the fluoroscopy (Foley & Gupta, 2002; Foley, Gupta, Justis, & Sherman, 2001; Gaines, 2000).

When taking fluoroscopy images, the endplates must be overlapped in AP and lateral views, and the margins of the pedicles are clearly defined in the AP image. Therefore, a clear view is provided by giving an angle to the fluoroscopy (Figure-2). Adjusting this angle in patients with scoliosis is vital in determining the pedicle entrance (Idler, Rolfe, & Gorek, 2010).

After the image is taken, the patient is stained sterile and covered. The surgical field remains open. In the meantime, the anesthesia team is requested to apply antibiotics to the patient. Publications have shown that it is beneficial to administer antibiotics before surgery to prevent infection after cement injection. After the injection of local anesthesia at the insertion point, a hole is made in the skin with a scalpel, and the procedure is started. The bone periosteum is reached by passing through the skin, subcutaneous, and muscle tissues with the insertion needle. The insertion point to the pedicle is critical. Both AP and lateral views should be taken to confirm that it is at the pedicle' s lateral border with fluoroscopy image controls. The patient may feel pain when the needle touches the pedicle insertion site. At this stage, the anesthesiologist should be asked to administer an analgesic to the patient. The needle is advanced with fluoroscopy controls. Meanwhile, the important thing is the angle of insertion of the needle into the pedicle and the needle's advancement (Evans et al., 2003; Garfin et al., 2006; Ploeg et al., 2006).

While the needle is advanced in the pedicle, the target should be the anterior and middle part of the corpus in the lateral view. In the AP image, the upper corner of the pedicle' s lateral wall should be targeted (Figure-3). While advancing the needle, it is necessary to stay away from the medial wall of the pedicle. Because the medial wall is in contact with neural structures and the spinal cord. With controlled hammer blows, when the middle of the pedicle is reached in the AP image, the pedicle's middle point should be reached in the lateral view as well. When it is advanced a little more, when the pedicle' s medial margin is touched in the AP image, we must lean against the corpus' s posterior margin in the lateral view so that the pedicle is passed. With gentle hammer blows, we need to align 1/3 anterior-lower part of the corpus in lateral views as we approach the midline in the AP image. If the procedure is to be performed bilaterally, the same procedures should be performed for the opposite side.

After reaching the target place with the needle, the cone wire passed through it is used as a guide, and the needle is removed. The operation cannula is sent over the Kirschner wire. From now on, a biopsy can be taken through the operation cannula, balloon inflation, and cement injection can be performed. With the help of a drill sent through the operation cannula, we have the chance to take samples from the bone tissues in the broken vertebrae. Care should be taken that the drill does not pierce the front

edge of the vertebrae. If necessary, bone tissue pieces should be taken by rotating around itself without advancing it. Taking biopsy is important, even if it is an osteoporotic or traumatic fracture. Because there may be a metastasis of malignancy detected incidentally due to future pathology (Uzunoglu et al., 2019). If kyphoplasty is planned after the biopsy is taken, the balloon inflation procedure should be started. As mentioned before, balloon inflation is an advantageous procedure in terms of determining the cement volume in the vertebral corpus and adding height to the vertebrae (Figure-4) (Belkoff, Mathis, Jasper, & Deramond, 2001). The risk of cement leakage is also minimized in the balloon-inflated vertebrae. The most crucial point to be considered during the balloon inflation process is that the inflated balloon does not break the vertebral endplate.

After the balloon inflation process, the balloon is removed, and cement is injected through the operation cannula with cannulas. The cement volume of the cannulas used is usually 1.5 cc. The PMMA used has the fast freezing feature, and therefore, it should be injected in cement consistency to prevent cement leakage. Powder and liquid parts are mixed and left for about 2-3 minutes. The injection is started with simultaneous fluoroscopy control. The process should be terminated when a leak is detected. Recommended PMMA volume is 1-2 ml more than the balloon volume inflated. The needle should be withdrawn a little, and cement should be given to the volume covered by the needle. The aim is to fill the front 2/3 of the vertebrae. As mentioned before, these operations can also be done bilaterally. However, studies have not found a significant difference in vertebral corpus stiffness when performed unilateral or bilateral (Evans et al., 2003; Majd, Farley, & Holt, 2005; McGraw et al., 2002; Ploeg et al., 2006). The cement ratio to provide stabilization is important. If the cementum does not spread bilaterally in the vertebral corpus in unilateral procedures, the procedure should be performed bilaterally; because the amount of cement that falls short on one side causes collapse fractures in the weak area in the future (Belkoff et al., 2001; Garfin et al., 2006; Garfin et al., 2001).

Cement provides vertebrae strengthening as well as has a vital function in vertebral stabilization. At the same time, studies indicate that malignant cells in the vertebrae decrease with cement in collapses due to malignancies, and cement has a burning effect on tumor cells (Uzunoglu et al., 2019).

- Postoperative

Patients are subjected to CT control after the procedure, and the cement state and vertebrae status are examined. The distribution of cement within the vertebrae is essential. A neurological examination of the patient should be done. Patients can be discharged on the same day or the next day

after they are mobilized. If the patient has a new onset of spinal pain, chest pain, neurological loss, elevated fever, the patient should be observed for treatment and observation (Belkoff et al., 2001; Evans et al., 2003; Garfin et al., 2001).

- Complications

The most common complication that develops due to these procedures is cement leakage. The leaks to the lateral and anterior do not cause a symptom, whereas leaks to the posterior cause compression on the spinal cord and may lead to neurological complications. Radiculopathy or leaks that cause spinal cord injury usually occur in fractures due to malignant metastases or due to malignancies of the bone itself (Belkoff et al., 2001; Grohs et al., 2005; McGraw et al., 2002).

Increased pain, complications of neurological compression, infection, and rib fractures in thoracic fractures are other complications. To prevent these complications, the amount of cement to be given should be calculated correctly, the insertion point to the vertebrae and the advancement of the needle should be made in detail with fluoroscopy control, antibiotics should be given before surgery, and hammer blows should be performed in a controlled manner.

Pulmonary embolism may occur after PMMA embolism. This rare complication can be seen due to perivertebral venous migration. To reduce the risk of embolism, it is not recommended to perform more than three levels in a single session. To reduce this complication, attention should be paid to the consistency of the cement, care should be taken not to break the vertebral borders of the inflated balloon, and cement leakage into the spaces other than the balloon should be prevented (Majd et al., 2005; Ploeg et al., 2006).

Although rare, vertebral osteomyelitis can be seen after percutaneous cementing (McGraw et al., 2002).

Due to the pedicle' s thinness in the thoracic region, a lateral approach to the corpus can be preferred, and complications related to this intervention may be observed. Cement leakage is slightly higher at these levels. Again, pedicle fracture, rib fracture, and neural structure damage can be observed (Belkoff et al., 2001; Garfin et al., 2001).

When radiculopathy due to cement leakage is seen, the patient should be supported with steroids and anti-inflammatory drugs. The rate of radiculopathy requiring surgical intervention is around 2% (Evans et al., 2003).

Adjacent vertebral fractures due to vertebral strengthening can be seen. Cement leaking to the disc distance predisposes to this situation.

Hardened bone and disc distance with leakage affect the biomechanics of the spine and change the power distribution. This situation causes fractures in an adjacent vertebra (Garfin et al., 2001).

PMMA can also leak into the paravertebral tissue. This situation is mostly seen in the psoas muscle, causing pain in that area (McGraw et al., 2002).

- Evaluation and Conclusion

Patients can be evaluated with VAS (visual analog score) after the procedure. Postoperative radiological evaluation is done with CT. Kyphosis angle, comparison of pre and postoperative vertebral height, cement volume, and cement localization can be calculated in CT. Patients should be followed up in terms of adjacent vertebral fractures and relapse in lytic lesions.

Percutaneous Thoracolumbar Instrumentation

With the emergence of minimally invasive interventions, screw insertion techniques with the percutaneous procedure have improved. Correction of instability is aimed in this way in degenerative or traumatic pathologies of the vertebrae. For this purpose, preoperative preparation should be done carefully, pedicle diameters and angles should be calculated correctly (Glaser et al., 2003; Taylor et al., 2002). This will shorten the operation time and minimize the risk of complications.

- Preoperative Planning

In the percutaneous screwing technique, it is essential to understand the pedicle structure well and to choose a screw suitable for the pedicle diameter. The screw length can be calculated with the help of CT by taking the corpus size into account. Pedicle diameter measurement should be done to minimize complications; if not possible, screw definitions suitable for all levels in the literature should be examined. If the calculations are not made correctly, damage to the pedicle walls may occur (Lowery & Kulkarni, 2000; Ravi, Zahrai, & Rampersaud, 2011; Wang, Pineiro, & Mummaneni, 2010).

Scoliosis radiographs should be taken in addition to CT in patients with scoliosis to make appropriate decisions beforehand.

It should be noted that the instruments to be used before percutaneous procedures in morbidly obese patients are of sufficient length. A large area should be prepared for obese patients because the skin' s starting points may have translocated laterally.

A special set is required for percutaneous screw placement. Tubular retractors are used to avoid extensive dissection and denervation (Kim, 2010).

After the vertebral anatomy and variations of the patient are kept, the AP and lateral fluoroscopy images should be interpreted correctly, and the procedure should be started. The use of neuromonitorization is vital to increase screw insertion accuracy in structural anatomical disorders, especially in scoliotic patients (Wang et al., 2010).

- Surgical Technique

After the cannula is placed as in vertebroplasty and kyphoplasty procedures, a wire is sent through the cannula as a guide to the vertebral body. This process is repeated for every level to which screws will be sent. The cannulas are removed after the guidewire is sent. The skin incision can be enlarged, or dilators can be used to reach the lower vertebrae. Local anesthetics with epinephrine can be used to reduce blood loss during this procedure.

Care should be taken not to bend the wire. The area is expanded by performing successive dilatation, and the wire should not be bent at this stage. Afterward, while the guidewire is inside, the previously calculated and dimensioned screws are sent to the vertebral corpus over the wires (Figure-5). Screws should be sent in the same direction as the wire to prevent the wire from breaking. Wires can be removed after screws are advanced (Idler et al., 2010; Ravi et al., 2011).

All these steps should proceed under fluoroscopy control. Rods, endcaps, and other devices can be attached with a special set, and properly selected tools, stretching or compression can be applied to the system (Idler et al., 2010; Ravi et al., 2011; Wiesner, Kothe, & Ruther, 1999).

- Complications

As in all surgeries, there are complications in screw surgery. The complication rates decrease with the increasing use of imaging methods. The most important complication of pedicle screws is the wrong positioning of the screw (Gaines, 2000; Lowery & Kulkarni, 2000).

When the screw is placed in the wrong position, the nerve root, spinal cord, and vascular structures may be damaged. Transient radicular pain or permanent neurological sequelae may occur in patients (Kim, 2010; Wang et al., 2010).

Large vessel injuries may occur if the screws are removed from the anterior alignment of the corpus. Vertebral anatomy should be mastered in the preoperative period to reduce screw-related complications, and appropriate screw placement should be made after appropriate measurements and imaging methods calculations.

The weakening of the screw system and screw fractures are other complications (Ravi et al., 2011; Wiesner et al., 1999).

Complications may also occur in insufficient bone quality. Over time, the deformation of the bone tissue causes the screw places to loosen and the system to weaken. Bone strengthening methods should be used when placing thicker screws to be placed in their places. These methods include using bone cement or translocating the screw. While inserting the new screw, attention should be paid to the weakened bone tissue's pedicle structures, and walls, especially the medial wall and inferior wall of the pedicle, should not be damaged. The protrusion of the screw from the corpus anterior may cause other organs to penetrate and damage the organs. Therefore, care should be taken in cases where bone density is insufficient (Foley et al., 2001; Glaser et al., 2003; Ravi et al., 2011).

COVID-19 and Percutaneous Techniques

On 31st December 2019, the World Health Organization reported cases of pneumonia of unknown etiology in Wuhan, China. On 7th January 2020, a new coronavirus type was reported in humans as a factor. The global pandemic was identified on 11th March 2020, with this factor, called COVID-19, which started to be seen worldwide. An increasing number of cases and the increase in deaths caused changes in countries' health policies and health centers. The primary purpose of these changes was to postpone elective or postponable operations as much as possible and shorten the patient′ s hospitalization period by approaching the operations as minimally invasive as possible in terms of emergency operations and oncological pathologies.

Minimally invasive procedures in thoracolumbar surgery have become more available with the advancement of technology and possibilities. This is advantageous for both patients and the health system during the pandemic period. Vertebroplasty, kyphoplasty, percutaneous screw system can be applied to the patient in fractures caused by osteoporosis, malignancy, or trauma in the thoracolumbar region if the patient has no neurological deficits and open surgery is not required.

These procedures, which are applied percutaneously, shorten the duration of the patient' s stay in the hospital, allow a rapid discharge time, and prevent a long time under anesthesia. Simultaneously, the patient' s use of analgesics is reduced, and the loss of workforce is minimized. Thus, during the pandemic period, patients are provided to spend a short time in health centers, and the risks of COVID-19 infectiousness are minimized.

The fact that percutaneous procedures can be performed with local anesthesia or sedation saves patients from intubation and prevents them from staying in the prone position for a long time.

Patients prefer the kyphotic posture due to pain in fractures occurring in the thoracic region. In this case, there is a decrease in thorax volume

capacity, especially in elderly patients with comorbid diseases. Capacity reduction contributes negatively to the treatment response of patients in possible COVID-19 infectiousness. Minimally invasive interventions for the treatment of pathology are promising for patients.

In patients with thoracolumbar fracture, immobilization increases the risk of possible embolism, and this risk increases with COVID-19, which causes hypercoagulapathy. The return of the patients to their daily life and being mobilized minimizes this risk.

Thanks to the advancing technology and advancing percutaneous systems, minimally invasive interventions gain more importance during the epidemic period, and the probability of patients getting an epidemic is minimized.

Figures

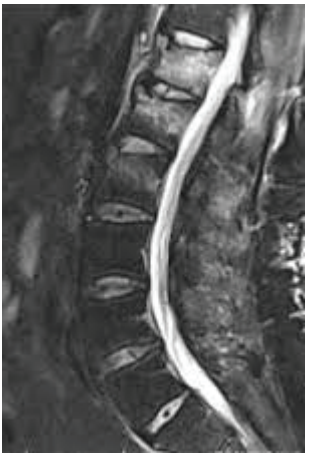

Figure-1: *STIR Lumbar MRI*

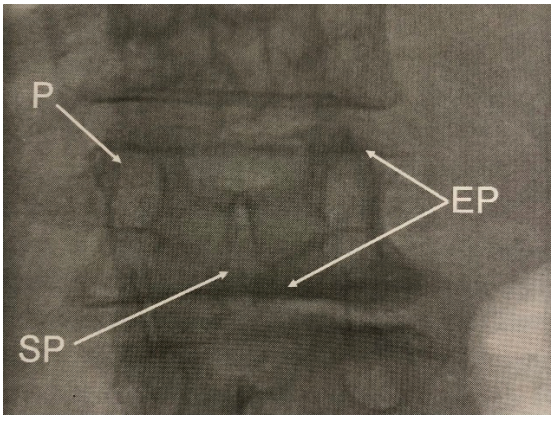

Figure-2: *AP C-arm image. SP: Spinous process, EP: End-plate, P: Pedicle*

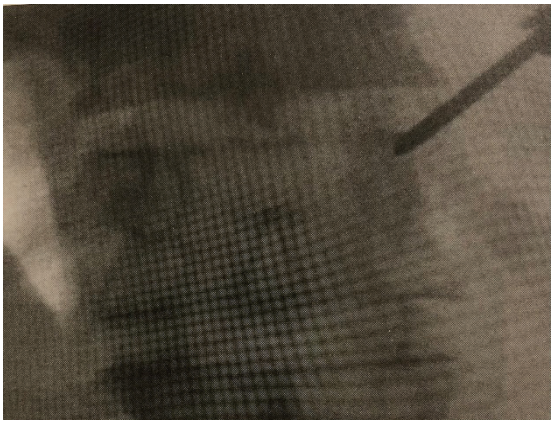

Figure-3: *Superior-lateral corner of pedicle*

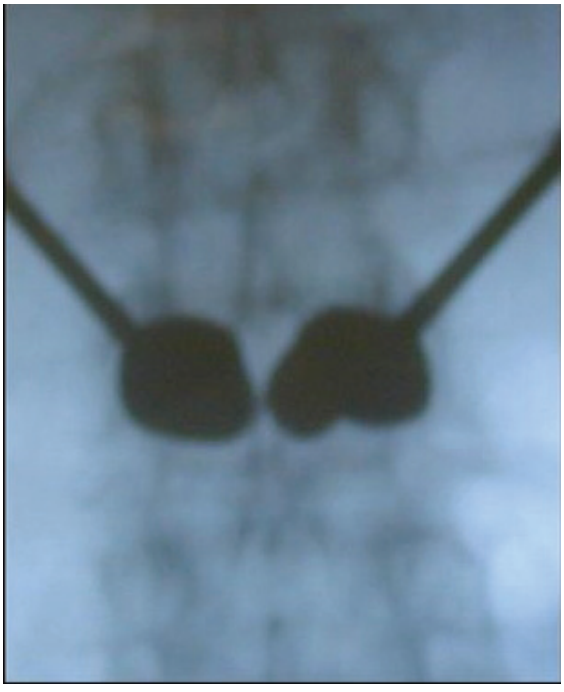

Figure-4: *Baloon kyphoplasty*

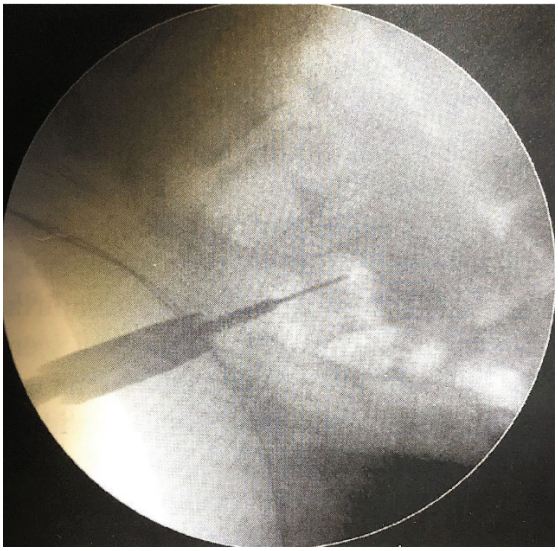

Figure-5: *Percutaneous lumbar screw*

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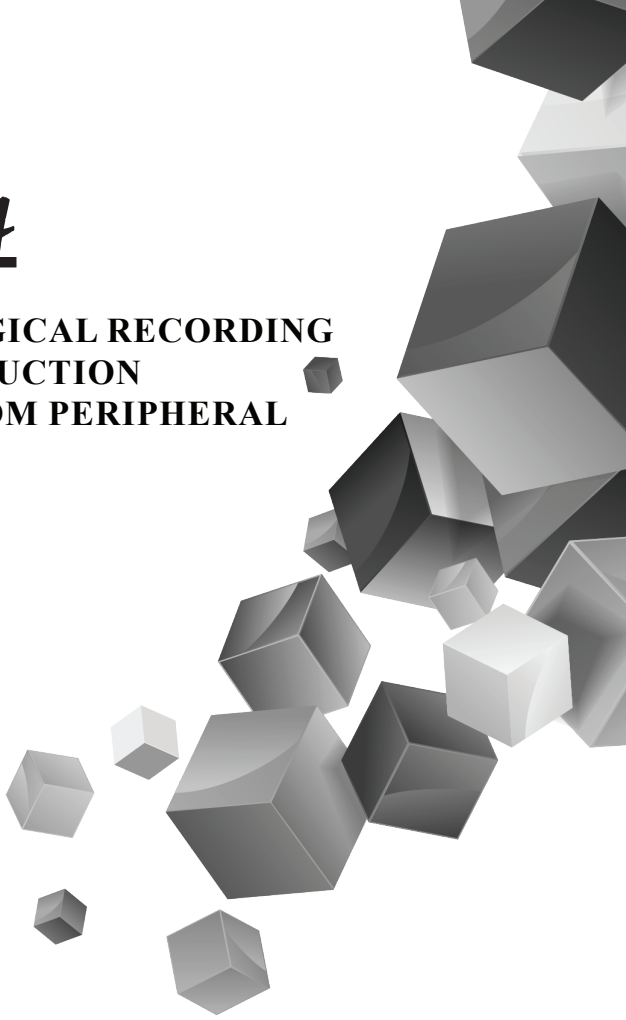
Chapter 14

ELECTROPHYSIOLOGICAL RECORDING METHODS AND CONDUCTION MEASUREMENTS FROM PERIPHERAL NERVES IN VITRO



Seçkin TUNCER[1]

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Transmission of information from one point to another distant point in the human body is possible by nerve cells. This vital event has often been life-saving throughout the evolutionary process. Both the transmission of information processed in the central nervous system to the target organ and the transmission of environmental information to the central nervous system involve electrophysiological processes. In the resting state where the nerve cells do not conduct any information, the concentrations of ions inside and outside are different. There is an electrical potential difference between the inside and outside of the cell resulting from these concentration differences. In other words, the cell membrane acts like a battery. This potential difference, called the resting membrane potential (RMP), is distorted by the impact of a stimulus and the membrane can be reverse polarized for a short time due to processes involving ion transitions. This potential difference change, which has a specific pattern over time, is called the action potential (AP). This change, which lasts a few milliseconds, is the result of active processes occurring in the axons of nerve cells. Opening of the ion channels in the axon membrane which has a voltage-dependent behaviour, initiates this irreversible active process. Hence, cells show an "all or none" behavior in this process. Basically, this process is completed with the movement of Na^+ ions into the cell and K^+ ions out of the cell under the effect of driving forces. RMP is maintained by the energy (ATP) dependent Na-K pump. APs occured by the stimulus propagate along the axon of the nerve cell in an autowave nature. This propagation may occur from the central nervous system to the target organ via efferent nerves or through afferent nerves to the central nervous system.

In the nerve cell, conduction takes place in axons. In this area, there is a myelin sheath formed by folding the schwann cells. The myelin sheath reduces the capacitance of the axonal membrane and allows the propagation to take place in leaps by being interrupted in certain regions which is called nodes of Ranvier. In this type of propagation that is called saltatoric conduction, the conduction velocity is much higher than in an unmyelinated axon (Pehlivan, 2015).

Another structural feature that has a determining effect on conduction velocity is the axon diameter. The large diameter of the axon causes less intracellular resistance (R_i). Therefore, conduction is faster in neurons with large axon diameters. It is known that the relationship between conduction velocity and axon diameter in myelinated axons is linear (Rushton, 1934). In unmyelinated axons, this relationship is directly proportional to the square root of the axon diameter (Bullock, Horridge, Bern, & Hagadorn, 1965). The g-ratio is defined as a parameter containing information on both of the aforementioned structural properties. In this parameter, which is the ratio of the inner diameter of an axon to its outer diameter, the inner diameter represents only the diameter of the axon, and the outer diameter

represents the diameter of the axon with myelin. It has been shown that the optimum value of the g-ratio in peripheral nerves is 0.6 (Rushton, 1934). The change in this parameter causes the nerve conduction velocity to slow down. Axon diameter is a major structural property for nerve cells and does not change. However, myelin sheath thickness may vary in relation to many neuropathological conditions. This condition taht is called demyelination, is caused by demyelinating diseases. Demyelinating diseases can occur in the central nervous system such as Multiple Sclerosis (MS) or in peripheral nerves such as Guillain-Barré Syndrome (GBS). In addition, some metabolic diseases such as Diabetes Mellitus (DM) cause neuropathies secondary to demyelination (Yagihashi, Kamijo, & Watanabe, 1990). Factors other than myelinization and axon diameter also determine the conduction properties of peripheral nerves. All of these factors related to the cell membrane can be called the electrical properties of the cell membrane.

Electrical Properties of Cell Membrane

In order to better comprehend the electrical events related to conduction occurring in the cell membrane, the theory known as the "cable theory" must be understood (Hodgkin & Rushton, 1946). In this theory, which is the subject of passive propagation of an electrical impulse along the axon membrane, the axons and dendrites where propagation takes place are represented by an electrical circuit as in Figure 1.

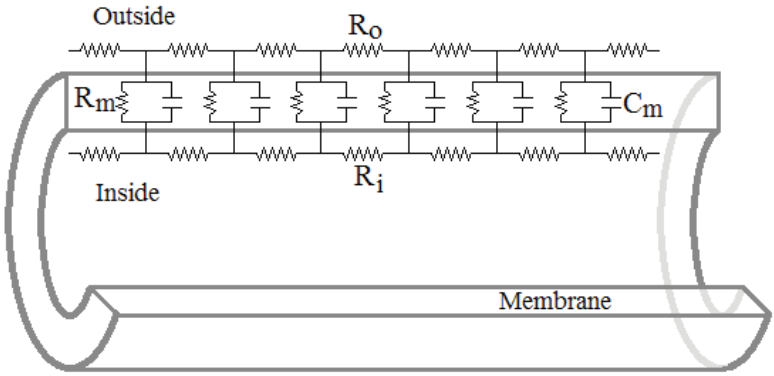

Figure 1. *Schematic representation of the electrical equivalent circuit representing the axon according to cable theory.*

In this model, which is called the passive membrane model, the word "passive" is used in the sense that the response does not change depending on the potential change. In the cylindrical structure shown in the figure 1, the cell membrane consists of small units formed by a parallel connected capacitor (C_m) and a resistor (R_m). Considering that this cylindrical structure represents an axon or dendrite, intracellular resistance (R_i) and

extracellular resistance (R_o) are also parameters that determine the passive electrical properties of the nerve cell. If there is an electrical stimulus in the cell membrane, it means that current ($_{Im}$) is passing through the circuit in the equivalent circuit model. Meanwhile, the time dependent change of the potential difference between the two ends of the circuit for each unit can be expressed with a function as

$$V_z(t) = I_z \,.\, R_z \left(1 - e^{\frac{-t}{\tau}}\right)$$

The time constant "τ", is a parameter related to the loading time of the membrane and is given by the following equation.

$$\tau = C_z \,.\, R_z$$

This parameter, which determines the conduction velocity of the action potential as well as the probability of triggering an action potential in the postsynaptic membrane, is very important for conduction.

When stimulation occurs in any part of the cell membrane, if active processes do not begin, the stimulus propagates in the membrane by losing its intensity. This reduction is exponential and expressed as a function of V_z.

$$V_z(x) = V_0 \,.\, e^{\frac{-x}{\lambda}}$$

"λ", called the space constant, is a parameter that is related to distance and is given by the following equation.

$$\lambda = \sqrt{\frac{R_z}{R_i + R_d}}$$

This parameter, which determines how effectively the propagating will take place in cell membranes where there is no myelination, is also very important in terms of conduction. Since the structural differences of nerve cells cause changes in these parameters, their conduction velocities are also different (Waxman, 1980).

Compound Action Potential (CAP)

Nerve cells with different conduction velocities undertake the task of conducting different types of information. Peripheral nerves are formed by the collection of axons of many nerve cells in a sheath and form a bundle-like structure. These structures are called nerve bundles. While information is conducted in these structures, action potentials are generated and conducted in more than one axon. (von During & Fricke, 2007). The sum of single fiber action potentials (SFAP) formed by each of the

nerve fibers that make up the nerve bundle is called the compound action potential (CAP). Behavioral characteristics of CAP are quite different from SFAP. Since the threshold values of the axons within the nerve bundle are different from each other, it does not show an "all or none" behavior. As the intensity of the stimulation increases, the amplitude of the CAP increases. However, a stimulus that is strong enough to stimulate all fibers that is called supramaximal stimulus will increase the CAP amplitude to a fixed value. In this case, a CAP is the sum of the SFAPs of all fibers, that is, it contains the activities of all axons in the nerve bundle. (Hirose, Tsuchitani, & Huang, 1986). As the distance through which the conduction takes place increases, the CAP amplitude decreases and the duration increases. The activity of the conduction velocity groups formed by the fibers with different conduction velocities begins to disperse as the conduction distance increases (Figure 2). This dispertion may also cause the formation of hump mounds in CAPs. Each of the humpes that become prominent in this way represents the activity of a particular conduction velocity group. In this phenomenon called temporal dispersion, the area under the CAP curve remains constant (Dalkilic & Yuruten, 2004).

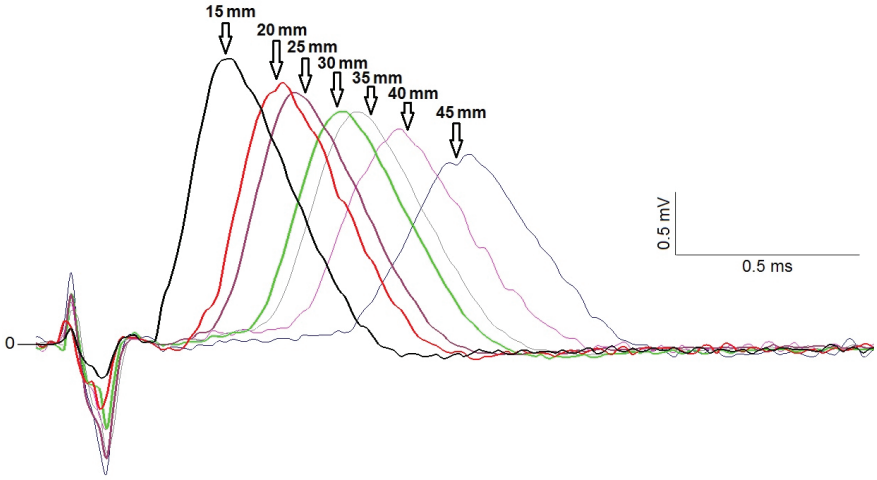

Figure 2. *CAP signals recorded from different distances (15-45 mm) in the isolated rat sciatic nerve.*

Compound Action Potential Recording Techniques

CAP recording from peripheral nerves is made from isolated peripheral nerves in order to eliminate the contribution of the activities of muscle and other tissues and the effect of volume conductor. In order to work with isolated nerves *in vitro*, peripheral nerves from experimental animals should be dissected precisely under light anesthesia without impairing their viability (Figure 3). There are three methods generally preferred in

experimental studies performed with peripheral nerves isolated from an experimental animal.

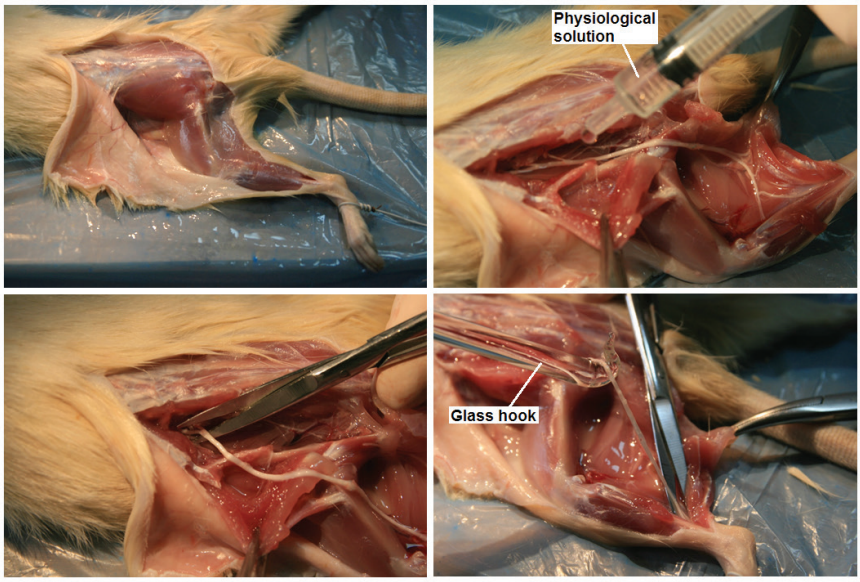

Figure 3. *Many important steps of isolation of rat sciatic nerve.*

1. Extracellular Technique

In the extracellular recording technique, the peripheral nerve isolated from the experimental animal is rapidly transferred to the in vitro environment where the conditions closest to physiological conditions are provided. The apparatus in which both stimulating and recording electrodes are built-in is generally called organ bath or nerve chamber. Inside the Nerve chamber, there is a chamber in which a solution that has the closest properties to the extracellular environment and is kept at body temperature is perfused at a certain speed. The electrodes are designed to stay level above the solution. Isolated nerve bundle is kept in the solution, but it is placed on the electrodes only when recording will be taken. Since the electrical current will prefer to pass through the conductive solution, stimulation cannot be performed in the solution. Square shaped stimuli are delivered from the proximal to the isolated nerve, recording is performed from the distal. A large number of selectable electrodes are placed in the nerve chamber in order to change the distance at which the recording is performed (Figure 4).

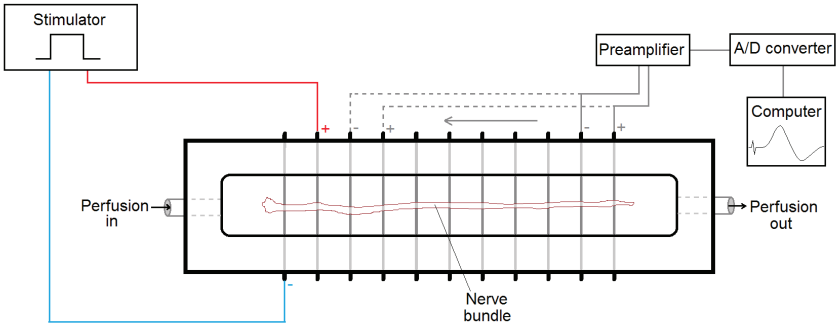

Figure 4. *Schematic representation of the apparatus used in the extracellular recording technique.*

CAP can be observed in two ways in the extracellular recording technique. Nerve bundle is crushed from the recording point in order to observe the monophasic CAP signal. The observed signal is monophasic, as the cells in the crushed spot will be in a depolarized state (Figure 5A). The CAP observed from a normal nerve bundle is biphasic (Figure 5B) (Kimura, 1989). The fact that the second phase of this signal is negative is similar to the hyperpolarization of SFAPs, but completely different reasons are effective in the occurrence of this situation (Tuncer, Dalkilic, Esen, & Avunduk, 2011).

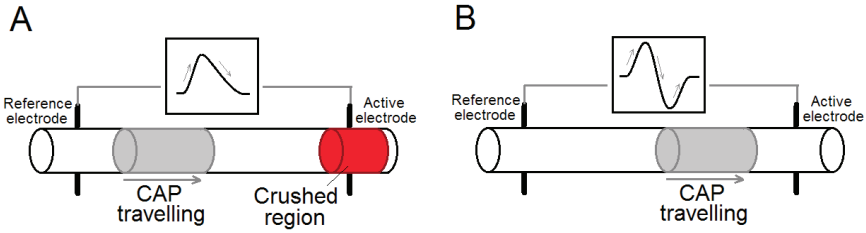

Figure 5. *Monophasic (A) and biphasic (B) CAP recording.*

Although the extracellular recording method is the most basic and preferred method, it also has some disadvantages. The most important of these is that the entire tissue must be separated from the environment that mimics the physiological environment at the time of recording. This causes alterations in conduction by causing deterioration of temperature and stability of the extracellular environment. In addition, although it is taken out of the solution while recording, some solution remains around the tissue. Completing the current circuit through this path whose resistance is less than intracellular resistance makes the stimulation difficult and reduces the amount of information carried by the signal.

2. Sucrose-Gap Technique

The sucrose-gap technique was first used by Stampfli in 1954 to observe the electrical activity of tissues containing neighboring cells in a cable-like structure (Stampfli, 1954). The peripheral nerve isolated from the experimental animal is rapidly transferred to the recording chamber where physiological conditions such as temperature and extracellular environment are kept constant, ensuring that the tissue remains alive. This technique can also be used to record from tissues that is also composed of excitable cells other than peripheral nerves such as muscle strips. All electrodes are built into the apparatus. In the organ bath, which usually contains an extracellular solution pool and two salt solution pools, there are chambers with sucrose solution between these pools. The solution pool and sucrose chambers are continuously perfused (Figure 6). In the solution pool, there can be a solution that is closest to the physiological conditions of the tissue to be studied, as well as a solution containing a drug if a drug study is being conducted (Shi & Blight, 1997). In addition, if there is a study about mechanical effect such as crush injury and/or the treatment of this injury, the nerve bundle region where the effect is expected is placed in the solution pool (Sakai, Honmou, Kocsis, & Hashi, 1998). In salt solution pools, isotonic KCl (Iso-KCl) solution is generally preferred. However, in some studies, physiological solution may be preferred. Especially the reason for putting iso-KCl in the chamber where the active electrode is located is to be able to record monophasic CAP by imitating the intracellular ion concentration (Blaustein & Goldman, 1966; Jirounek & Straub, 1971).

Electrical square stimuli are applied with the cathode in the iso-KCl pool and the anode through the electrodes in the solution pool, usually from the proximal of the nerve bundle. While the active one of the recording electrodes is usually located in the iso-KCl pool, the reference electrode is located in the solution pool. It is assumed that there is no membrane potential due to the presence of iso-KCl in the area where the active electrode is located.

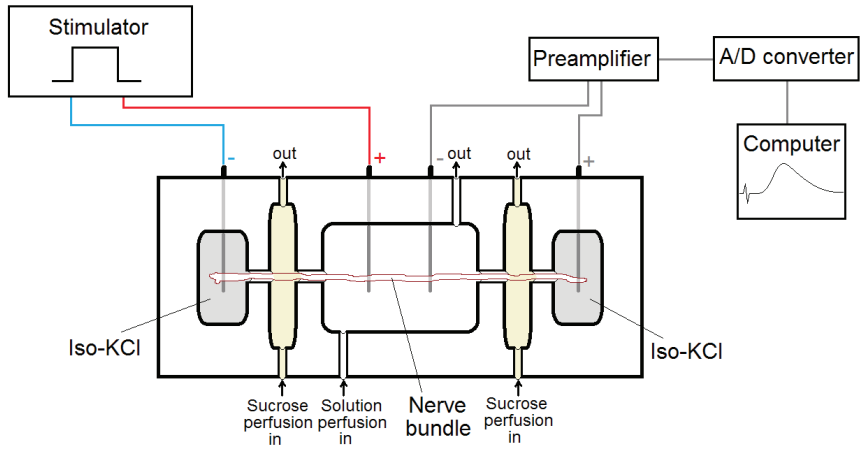

Figure 6. *Schematic representation of the apparatus used in the sucrose-gap recording technique.*

The basic principle of the sucrose-gap technique is that thanks to the presence of sucrose chambers, the shunt effect between the electrodes is eliminated and the membrane potential can be observed. In other words, the high resistance of the isotonic sucrose solution, around 106 Ω.cm, prevents electrical short circuit between the segments of the nerve. One of the most important problems of the sucrose-gap method is that it is very difficult to prevent mixing of isotonic sucrose solution and physiological solution or iso-KCl solutions. In addition, the most important limiting factors are that CAP amplitudes are half of the real ones, the absence of sodium in the sucrose chambers affects the conduction velocity and the failure to record when the space constant (λ) is close to zero (Mert, 2007).

3. Suction Electrode Technique

The suction electrode can be used as an active recording electrode for CAP recordings from peripheral nerves, as well as as an electrode where electrical stimuli are applied (Bostock & Grafe, 1985; Carley & Raymond, 1987). In addition, there are also studies using monophasic action potential recordings from cardiac tissues (Cotoi & Dragulescu, 1975). It has even been used in some studies to record SFAP from the axon of a single nerve cell (Baker, Bostock, Grafe, & Martius, 1987). As in other techniques, the peripheral nerve isolated from an experimental animal is quickly transferred to a specially designed recording apparatus that provides the conditions closest to physiological conditions. In this apparatus, in addition to the stimulating electrodes, there is also a built-in suction recording electrode. There is a single solution pool in which an equivalent solution with the extracellular medium is perfused at a constant rate at a constant temperature. The stimulating electrodes are positioned above the solution level in order to provide an efficient stimulation by completing the current

circuit over the nerve bundle to be stimulated. A large number of stimulus electrodes are placed at certain intervals in order to stimulate at different distances or to apply more than one stimulus at a time (Figure 7).

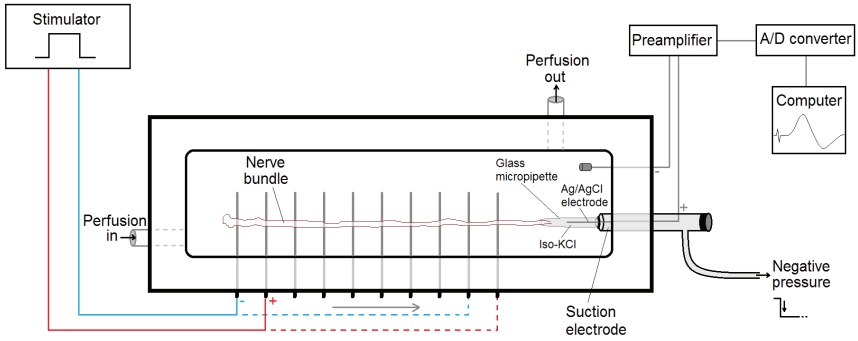

Figure 7. *Schematic representation of the apparatus used in the suction electrode recording technique.*

The most important component of the apparatus used is the suction electrode. Because of their fragile properties, electrodes are manufactured just before each recording experiment in the laboratory where the recording experiment will be conducted. Borosilicate glass pipettes are preferred when manufacturing suction electrodes due to their low thermal expansion coefficient and their insulating properties. The tip diameter of these pipettes is reduced according to the diameter of the tip of the nerve to be studied using a microelectrode puller. An Ag/AgCl wire electrode is immersed after filling it with isotonic KCl solution (Easton, 1993). The glass pipette thus becomes an electrode and is connected to the recording system component with the appropriate input impedance. The internal environment of the electrode must share the same medium as a piston that allows negative pressure to be applied. In this way, the end of the nerve to be recorded can be pulled into the electrode and it is ensured to fit properly. The point of the nerve that is inside the tip of the suction electrode but closest to the outside can be considered as the point where the active electrode is located. Similarly, the point outside the tip of the electrode, but closest to it, is the reference point. The reference electrode is considered to be there because it is made possible by the silver pellet electrode placed in the solution pool and the low resistance of the pool medium (Figure 7).

It can be modeled with an electrical equivalent circuit in order to better understand how the potential difference change can be observed when the nerve is placed inside the suction recording electrode (Figure 8).

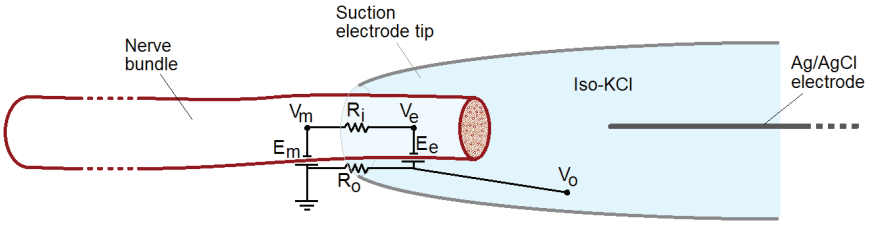

Figure 8. *Electrical equivalent circuit model of the suction recording electrode with a nerve end inside.*

Although a nerve bundle in the suction electrode consists of axons of thousands of nerve cells, we can think of it as a single nerve fiber for ease of understanding. Based on this assumption, the intracellular potential of the nerve inside the suction electrode is V_e, and the intracellular potential just outside it is V_m. The reference for these potentials is Ground. Accordingly, it is clear that,

$$V_m - V_e \neq 0$$

for current to flow through the circuit. The resistances encountered by the current flow in this circuit are internal resistance (R_i) and external resistance (R_o). External resistance is the sum of many resistances. The most important of these is the resistance caused by the space between the tip of the glass pipette and the nerve. Others can be listed as the resistance of the connective tissue around the nerve, the resistance of glial cells, if any, and the resistance of extracellular space. Transmembrane potential is indicated with E_e inside the suction electrode and E_m outside. The current passing through the circuit (I) can be written as,

$$I = \frac{V_e - V_m - V_o}{R_i - R_o}$$

where the potential difference measured by the suction electrode is V_o. In this case, the potential to be measured can be expressed with an equation such as

$$V_o = \frac{R_o(V_m - V_e)}{R_i}$$

Based on this statement, two important inferences can be made; Potential Vo can only be measured if the potential difference between V_e and V_e is a different value from zero, the value of V_o is directly related and inversely proportional to R_i. This means that the smaller the R_i, the greater the potential measured by the suction electrode (Stys, Ransom, & Waxman, 1991).

The fact that the amplitude of the potential measured by the suction electrode is so dependent on R_i emerges as an important disadvantage of this recording technique. In other words, the amplitude of the CAP directly correlated with how tightly the tip of the nerve fits the suction electrode. CAP amplitude is observed to be greater when sucked it tightly. The most viable method to overcome this is to apply an amplitude correction. Amplitude correction coefficient can be determined by measuring the resistance R_i on the fly with a setup addition. The corrected CAP is obtained by multiplying the obtained coefficient by the CAP signal (Dalkilic & Pehlivan, 2002).

The suction electrode recording technique is highly preferable for use on peripheral nerves isolated from warm-blooded experimental animals. Because in the recording area, the nerve is in the pool solution. Thus, the solution, which is gassed with a suitable gas mixture and whose temperature is kept constant, provides the closest environment to physiological conditions in the recording site. Therefore, the most accurate records can be obtained. Thanks to this advantage that the method has, it stands out among other recording techniques. For this reason, it has recently been preferred in both nerve conduction studies (Tuncer & Celen, 2019) and nerve excitability studies (Tuncer et al., 2017).

Nerve Conduction Measurement

Conduction velocity calculations from isolated peripheral nerves are generally based on latency measurements. Latency can be defined as the time it takes for the CAP departing from the stimulation point to arrive at the recording point, in other words, the travel time. By simply dividing the distance (x) between the stimulation point and the recording point by the latency, the nerve conduction velocity (NCV) can be calculated (Figure 9).

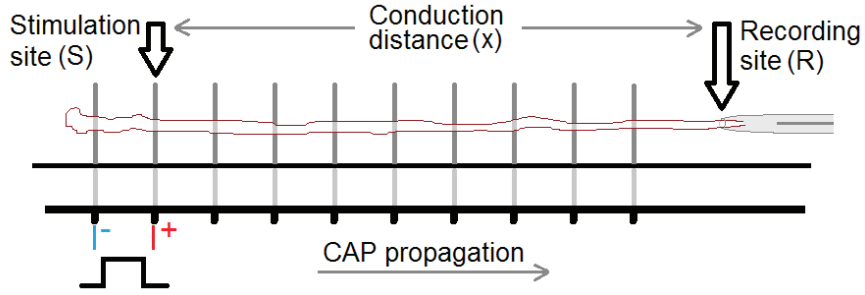

Figure 9. *Parameters used in nerve conduction velocity calculation in suction electrode recording chamber.*

While latency is determined in traditional studies, the time until the onset of CAP is measured with the stimulus artifact representing the moment when the stimulus is applied. Unfortunately, the conduction velocity information calculated by that method only belongs to the axons having the

highest velocity in a nerve bundle. However, in nerve conduction velocity distribution (NCVD) studies, it has been shown that fibers having medium and slow conduction velocities are primarily affected by neuropathological conditions (Tuncer et al., 2011) and neurotoxicity (Tuncer, Dalkilic, Akif Dunbar, & Keles, 2010). Therefore, two different latencies have been defined in nerve conduction studies; L_{onset} is the time delay between the moment the stimulus is delivered and the onset of CAP, and L_{peak} is the time delay between the moment the stimulus is delivered and the CAP amplitude reached its maximum value (Figure 10). Conduction velocity values, CV_{onset} and CV_{peak} are calculated using each latency measurement. The conduction velocity corresponding to the values of the fastest conducting nerve fibers constituted the nerve bundle is CV_{onset}. On the other hand, CV_{peak} corresponds to the velocity of the majority of nerve fibers that constitutes the nerve bundle (Tuncer et al., 2011). In some studies, it is also stated that CV_{peak} corresponds to the velocities of fibers having medium conduction velocities (Dalkilic, Tuncer, Bariskaner, & Kiziltan, 2009).

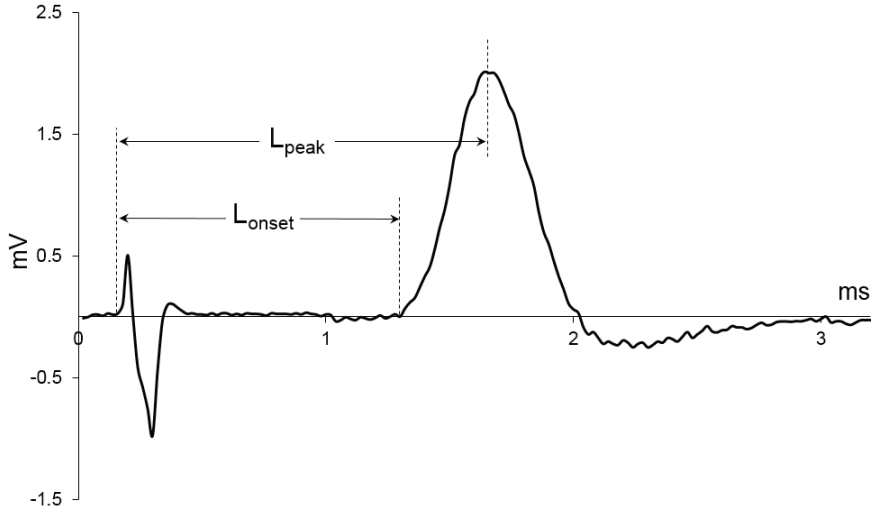

Figure 10. *Two different latency measurements (L_{onset} and L_{peak}) shown on a sample CAP recording.*

Although traditional velocity calculations with latency measurements are practical, since the CAP signals are biphasic, these measurements performed by applying a single stimulus have errors. Especially, the shorter the distance between the stimulation point and the recording point, the higher the error rate. For this reason, the conduction velocity measurement method, which uses two co-stimuli, is generally preferred to make more accurate and precise measurements. A supramaximal stimulus with equivalent characteristics is applied to the Nerve bundle from two different points, one from the proximal (S_p) and the other from the distal (S_d) (Figure 11).

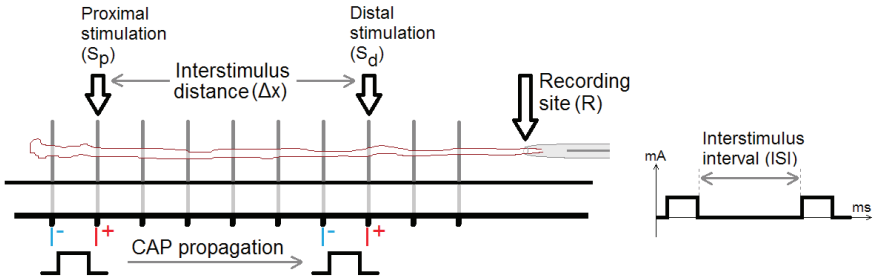

Figure 11. *Parameters used in nerve conduction velocity calculation performed by applying two co-stimuli shown on suction electrode recording apparatus.*

In this method, stimuli are not applied simultaneously. The time between stimuli is at least the sum of the time it takes for the CAP to reach the recording electrode from the point where the first stimulus is given (latency) and the duration of the CAP. This delay time is called the Interstimulus interval (ISI) (Figure 11). CAP responses generated by stimuli (S_p and S_d) are recorded separately (Figure 12).

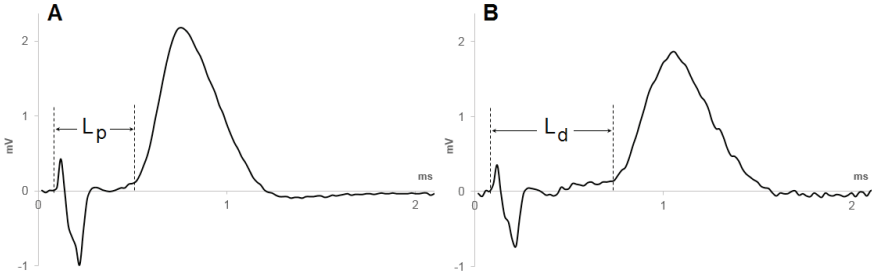

Figure 12. *CAP responses generated by supramaximal co-stimuli applied proximally (Sp) (A) and distally (Sd) (B). The sample CAP recordings shown in the figure were recorded by stimulating at a distance of 25 mm (S_d) and 40 mm (S_p) from the stimulating electrode.*

Conduction velocity (NCV) calculation is made by using the,

$$NCV\ (^{m}/_{s}) = \frac{\Delta x\ (mm)}{L_d - L_p\ (ms)}$$

equation, where L_p is the onset latency of the response to the stimulus given from the proximal (S_p), L_d is the latency of the response to the stimulus given from the distal (S_d), and Δx is the distance between the two stimuli. It has been reported that increasing the distance between the two stimuli as far as the length of the isolated nerve allows, increases the measurement sensitivity (Kimura, 1989).

Nerve conduction velocity (NCV) calculations are clinically preferred for showing the presence of many neuropathologies and neurotoxicity. In addition, it is used in the elucidation of many neuropathies with unknown

aspects and even in research studies in which applications for their treatment or prevention are tested. In vitro CAP recording and NCV calculation from peripheral nerves in order to eliminate the bioelectric contributions and volume conductor effects of peripheral tissues is still seen as the most important option in such research studies.

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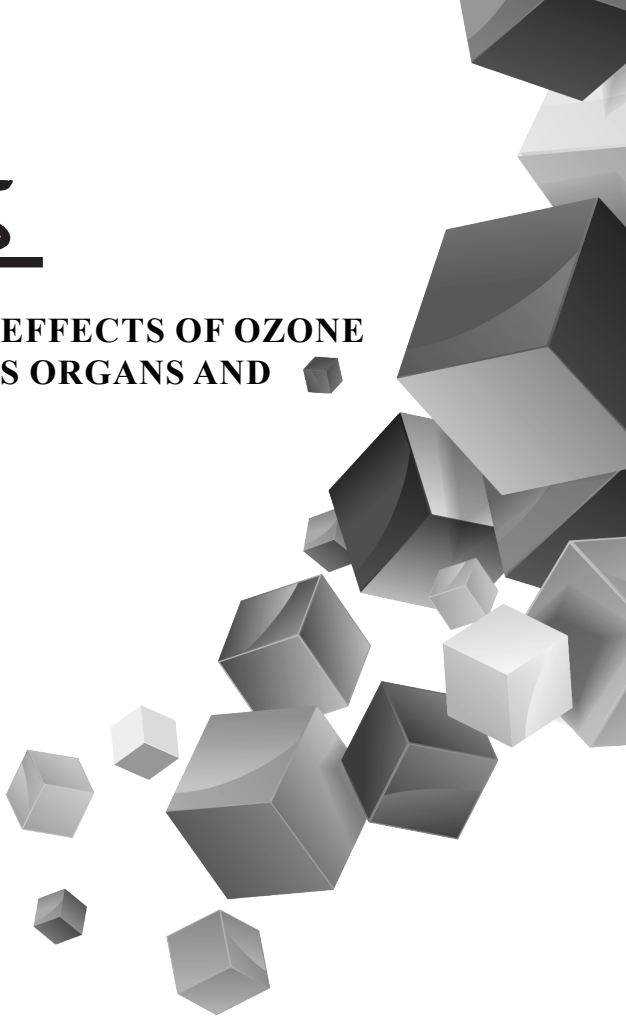
Chapter 15

EVALUATION OF THE EFFECTS OF OZONE THERAPY ON VARIOUS ORGANS AND SYSTEMS - PART 2



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Ozone (O3) is a gas composed of three oxygen atoms (1). Ozone, which has no color and has a characteristic smell, has a high reactivity. Therefore, its half-life is short (2). Synthesized by ultraviolet rays in the atmosphere, ozone has therapeutic effects (3). In order to benefit from these effects, ozone is produced in medical devices and applied with determined oxygen-ozone ratios (4). These applications can be performed in different ways such as autohemotransfusion (minor or major), injection (intramuscular, intradiscal, paravertebral or intradermal), rectal insufflation, ozone bag, ozonized liquid or ozonized oil. The method of application varies according to the disease, local or systemic application option and the effect desired to be revealed (5). Its application in rheumatology and oncology in the form of major autohemotransfusion and its use in the form of ozonized fluid for wound healing are examples of this situation (6).

Ozone therapy has a therapeutic effect when applied at the appropriate dose and correctly. The effect of ozone is mainly revealed through the antioxidant mechanism. Bocci et al. defines this use of ozone as shock therapy to restore homeostasis. It starts with the reaction of ozone given to the body with both antioxidants and polyunsaturated fatty acids. As a result of these reactions, reactive oxygen species (ROS) and lipid oxidation products (LOP) are released. Since these products must be removed as soon as possible, the antioxidant system is activated. In other words, ozone activates the antioxidant system in the body by creating oxidative stress. As a result, increases in the synthesis of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, glutathione transferase and glucose 6 phosphate dehydrogenase are observed (7-9).

In addition to its antioxidant effect, ozone may also exhibit anti-inflammatory, antifungual, antimicrobial, antiapoptotic, analgesic and immunomodulatory effects. It has been determined that these effects are revealed by stimulating different signal pathways in the body or by changing different molecular ratios. Ozone shows its antiinflammatory effects by decreasing pro-inflammatory cytokine levels and increasing cytokine levels and antioxidant system activity. The immunoregulatory effect is revealed by the NTAF (nuclear factor activated T cells)/AP-1 (Active Protein-1) signal. Due to all these effects, it is widely used in clinics in the treatment of diseases such as vascular diseases, wound healing, viral diseases, autoimmune diseases and musculoskeletal diseases. In preclinical studies, beneficial effects of ozone therapy for many different diseases have been determined (5, 10-12).

When the contraindications of ozone therapy are examined, it is seen that it is very few. Coagulation disorders, organ bleeding, thrombocytopenia, hemorrhagic and apoplectic stroke, glucose 6- phosphate dehydrogenase

deficiency, uncontrolled hyperthyroidism, ozone allergy and ozone intolerance are contraindications (5, 6). In addition to its high benefits, ozone therapy is considered to be a useful treatment method, given the few contraindications, low cost and reliability (12). In the light of this information, in this article, the efficiency and usage areas of ozone in terms of respiratory system, cardiovascular system, urinary system and genital systems will be examined.

When studies involving ozone in the respiratory system are evaluated, septic shock has an important place in experimental studies. It has been reported that in rats with septic shock, ozone therapy decreases the total pro-oxidant activity in the lung and increases the total antioxidant activity (13). Again, in a study conducted in septic rats, it was reported that different doses of ozone administered with antibiotherapy may cause tissue damage histopathologically, but still had positive effects in terms of decreasing inflammation (14). In a study comparing the effects of hyperbaric oxygen application and ozone, which is another method that is being used increasingly today as a complementary treatment, it was reported that ozone or hyperbaric oxygen therapy decreased inflammation in the lungs in septic rats, but ozone was more effective (15). With a different damage mechanism, it has been suggested that ozone may have positive effects as an adjuvant treatment modality in lung damage caused by Paraquat overdose (16). It has also been reported that ozone therapy may be protective in early lung damage caused by radiation histopathologically and biochemically (17). Not only sepsis or toxic injuries, but also the effects of ozone in lung transplantation have been studied. There are studies showing that ozone may be experimentally effective in ischemia reperfusion injury observed in the lung (18) and that ozone administration may delay chronic lung transplant rejection (19). Finally, when asthma is examined with its increasing frequency, it has been found that ozone is effective in reducing IgE levels and inflammatory mediators with the induction of antioxidant elements in asthmatic patients (20).

In experimental studies on the cardiovascular system, in a study investigating the effects of ozone on oxidative stress, cardiac functions and clinical findings in patients with low ejection fraction in heart failure, it was found that ozone therapy significantly reduced nitric oxide (NO) and malondialdehyde (MDA) serum levels; and significantly increased SOD, catalase (CAT), glutathione (GSH), glutathione peroxidase, serum levels (21). In a study conducted on ischemic heart diseases, which is one of the most important causes of death in advanced age, it was stated that ozone increased antioxidant capacity by reducing mitochondrial damage and myocardial apoptosis in heart ischemia reperfusion damage and protects myocardium against ischemia reperfusion damage; ıt has also been stated that ozone can be used in ischemic conditions such as cardiovascular

surgery, cardiopulmonary bypass procedures or transplantation (22). It has been reported that the use of prophylactic ozone (23), which is detected as a potential agent for antiaging, reduces morphometric changes such as elastic laminin irregularity, disruption of endothelial cells, vacuolization and bleeding caused by vasospasm, and therefore, it will be effective in post-hemorrhagic vasospasm (24).

It is remarkable that there are many studies for the urinary system in the studies. Especially, kidney related clinical conditions emerge as an area where the effects of ozone application are widely evaluated. In a study examining infective conditions, it was reported that ozone therapy combined with antibiotherapy in experimental acute pyelonephritis induced by Escherichia coli corrected renal dysfunction and histopathological findings more effectively than pure antibiotherapy (25). In an experimental study investigating the effect of ozone on the renal damage that may occur in extracorporeal shock wave (ESW) lithotripsy, which is the frequently used method in urinary stone treatment, it has been reported that ozone therapy improves nitro-oxidative stress and reduces the severity of pathological changes (26). When toxicity studies are examined, there are studies evaluating acute and chronic damage in renal toxicity studies. It has been reported that in chronic cadmium exposure, ozone therapy reduces cadmium accumulation in the kidneys and as a result, metallothionein expression in the tissue decreases (27). In chronic renal failure induced by adenine, it has been reported by various studies that ozone decreases inflammation over tubulointerstitial inflammation observed in the kidney (28, 29). In renal damage caused by acetaminophen, ozone therapy has been reported to significantly reduce the MDA level, increase SOD and GSH-Px activities, and normalize renal histology (30). In another study, it was reported that ozone decreased MDA levels and increased tissue GSH levels and had positive histopathological effects in reducing the damage caused by acetaminophen in nephrotoxicity (8). Similarly, in an experimental model in which the effects of ozone and N-acetyl cysteine (NAC) on acetaminophen nephrotoxicity were examined, it was reported that the use of these two agents together reduced renal damage and inflammation (31). It has been shown that gentamicin-induced nephrotoxicity, another nephrotoxic agent, can improve biochemical disorders and histopathological changes with ozone administration (32). Contrast-associated nephropathy is a condition that should be kept in mind due to the increasing use of imaging methods and the availability of accessibility. In a study, the effects of ozone and NAC in nephropathy caused by contrast material were evaluated; positive effects have been observed in terms of increasing the total antioxidant capacity and reducing oxidative stress, biochemical and histopathological harmful effects (3).

There are studies evaluating the effectiveness of ozone in different conditions that cause chronic damage, not just toxicity. In an experimental study conducted to evaluate the effectiveness of ozone in diabetic kidney, it was reported that ozone therapy decreased the expression of apoptotic genes in diabetic kidney tissue and corrected the damage histopathologically (33). Similarly, low concentration ozone therapy in chronic renal injury has been shown to improve renal function and renal morphological damage in 5/6 nephrectomized rats. In addition, it was reported that the expression of NLRP3, ASC and caspase-1-p10 in the kidney of these rats decreased with ozone treatment, and kidney inflammation caused by IL-1β was significantly reduced by ozone therapy (34). In a study evaluating age-related oxidative stress changes in rats, it was stated that ozone treatment had potentially positive effects in neutralizing chronic oxidative stress associated with aging in the kidney (35). Transplantation and ischemia reperfusion process has an important place in today's world where the number of kidney transplants is increasing. Numerous studies evaluating the effects of ozone on perfusion, draw attention. In vivo and in vitro studies in ischemia reperfusion injury, the study reporting that ozone protects the kidney from damage by decreasing pyroptosis in kidney cells is only one of these studies (36). Similarly, it has been suggested that ozone administration in renal transplantation reduces the inflammatory reaction and oxidative stress damage in the renal allograft, which may be related to strengthening the anti-oxidative system and suppressing the inflammatory reaction (37). It has been reported that ozone therapy has strong anti-inflammatory properties by modulation of the TLR4-NF-κB pathway in kidney tissue ischemia reperfusion injury, where not only the effect but also the cause-effect relationship is investigated (38). In addition, it was reported that the efficiency of ozone in renal ischemia reperfusion injury was evaluated experimentally and that ozone has a protective effect on ischemia reperfusion injury on the kidney and serum neopterin levels can be used as a marker in the follow-up of ischemia reperfusion injury (39). Another organ where ozone therapy is frequently evaluated in the urinary system is the bladder. Especially ozone therapy is involved in experimental studies in various inflammatory conditions. For example, it has been suggested that ozone can be used as an effective agent to protect against oxidative/nitrosative stress in E.coli-induced cystitis experimentally (40). In addition, it has been reported that ozone therapy increases microcirculation in the mucosa with chronic cystitis and has positive effects on structural reorganization (41). In addition, it has been reported that ozone applied with tamsulosin in the treatment of cystitis is more effective than ozone therapy applied alone and can be used safely in patients with cystitis (42). When chemical-based bladder inflammations are examined, it has been reported that ozone prevents urothelial damage by reducing oxidative stress, inflammation and NO levels in the bladder in cyclophosphamide-induced

bladder intoxication (43). Similarly, in a study conducted in rabbits, it was found that intravesical ozone administration provided mucosal integrity and decreased inflammatory infiltration in chemical cystitis (44). Similar to chemical damage, it has been reported that intravesical ozone therapy applied in progressive hematuria triggered by radiation control hematuria (45). When the male genital system is examined, the use of ozone in clinical and experimental studies draws attention. Although there are fewer clinical studies, it was reported that intraprostatic ozone injection helped to reduce the prostate volume to some extent in a study conducted on 30 cases (46). It is observed that experimental studies focused more on testicular damage. When ischemic models were evaluated, it has been reported that ozone application in testicular torsion reduces the damage biochemically and histopathologically (47). It has been reported that in ischemia reperfusion injury, ozone applied together with Taurine or alone in the testis may play an important role in preventing germ cell degeneration (48). In addition, intratesticular ozone application was found to be more effective than intraperitoneal ozone application in testicular damage caused by torsion and detorsion (49). In a study evaluating the effects of ozone on testicular damage in the cryptoorchidic rat model, it was reported that ozone reduced apoptosis (50). In testicular damage induced by radiotherapy, which is one of the direct damaging effects, ozone application has been observed to increase the antioxidant enzymes and testicular weight (51). In the case of chemical toxicity, it has been reported that in adriamycin-induced testicular damage, ozone almost completely reverses the toxic effects caused by adriamycin (52).

When the female genital system is evaluated, clinical and experimental studies, such as the male genital system, draw attention. When clinical studies are reviewed, a study showing that recanalization with ozone perfusion effectively increases the postoperative pregnancy rate and reduces re-occlusion of the fallopian tube in 116 cases who were intervened for tubal uterine obstruction (53). When experimental studies are evaluated, it has been reported in various studies that intraperitoneal ozone application has a positive effect on histological and biochemical markers in ischemia reperfusion injury caused by ovarian torsion (54-56). In a study evaluating postoperative adhesions and ovarian functions, ozone had positive effects on fibrosis, congestion and hormonal parameters, although it was not statistically significant (57); in an experimental uterine adhesion model, it was reported that it prevented postoperative uterine adhesions by modulating TNF-α levels and oxidative/antioxidative state (58). In addition, intraperitoneal ozone administration has been reported to improve pelvic inflammatory disease (PID) conditions by inhibiting the necrosis of endometrial epithelial cells and at the same time reducing inflammatory reactions (59).

Finally, when the infectious conditions are examined; in a study conducted on human papillomavirus 16 (HPV16) transgenic mice for HPV16 involved in anogenital and oropharyngeal carcinogenesis, ozone was reported to progress with a markedly reduced inflammation (60). In one of the clinical study examples, it was reported that ozone application would be a valid supportive treatment for exhaustion in cancer patients both during cancer treatment and in a palliative environment without any significant side effects (61).

Together with those examined in the previous book chapter (62), when all these studies are evaluated together, it is concluded that ozone application is a promising complementary method and may even become one of the routine treatment protocols over time.

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Chapter 16

MANUFACTURING PROCESSES AND SURFACE MODIFICATION TECHNIQUES OF DENTAL IMPLANTS

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Dental implant surgery is performed by the oral and maxillofacial surgeons very well. But the success of implant survival is not limited to surgeon's surgical ability. It also depends on the material is used. Therefore, good material should be chosen. For this, it is needed to know the properties of the dental implants' manufacturing processes and surface modification techniques. This chapter aims to have a relevant idea.

Dental implants are the most frequently used material for the restoration of missing teeth with high patient satisfaction. The most important factor affecting the success of dental implants is osseointegration. Osseointegration was defined as direct contact between the bone and the surface of the loaded implant by BRANEMARK (Brånemark & Chien, 2005). Osseointegration depends on the design, shape, geometry, material, and surface properties of the implant.

Dental implants varied over time according to the material used, the shape of the implant, the place where it was placed, and the surface properties. Based on the classifications made in classical books, a classification can be made to the material used, by place of placement, according to implant shape, and according to surface properties (Table 1). Nowadays, dental implant body is mostly produced from titanium, zirconium, tantalum, and their alloys (Piglionico et al., 2020).

1. Classification according to the material used		
a- Metal and alloys	b- Ceramic and carbons	c- Polymers and Composites
* Titanium and titanium-6 Aluminum-4 Vanadium	* Aluminum-Titanium-Zirconium oxides	* Polytetrafluoroethylene
* Cobalt-Chromium-Molybdenum alloys	* Calcium phosphate sources	* Polymethylmethacrylate
* Iron-Chromium-Nickel alloys	* Hydroxyapatite	* Ultramolecular weight Polyethylene
* Other metals and alloys (gold, platinum, palladium,..)	* Carbon and Carbon Silicone Compounds	* Polypropylene, Polysulfate
2. Classification by place of placement	**3. Classification according to implant shapes**	**4. Classification according to surface properties**
a- Intraosseous (intraosseous) implants	a- Cylinder	a- Unmachined surface implants
b- Subperiosteal implants	b- Screw	b- Machined surface implants
c- Intramucosal (Subdermal) implants	c- Needle	c- Coated surface implants
d- Transmandibular implants	d- Root	d- Combined surface implants
e- Transdental (Endodontic) implants	e- Straight (Blade)	

Table 1. *Classifications of dental implants*

What is the adventure of a dental implant starting from a titanium rod? How it is done from the beginning to the end?

1. MILLING/ TURNING

To make an implant, it starts with a solid titanium rod (Figure 1). More than 200 implants can be produced by a rod by a CNC machine. Chip removal by giving a movement to the implant body in the direction of the cutting tool is called turning. At first, the rod is being gone through highly accelerated processing externally. The titanium rod spins as a computerized tool up and down. In minutes the solid metal cylinder becomes a threaded implant. Special oils are used during milling to prevent the metal from overheating (Figure 2).

Figure 1. *Titanium rods*

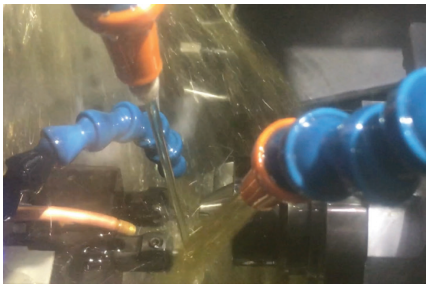

Figure 2. *Processing titanium on CNC machine*

The surface layer is plastically deformed and abraded. Some surface properties arise from the turning process. Turned surfaces depend on the speed of the working insert and the pressure applied, the properties of the lubricant solution used (Brunette, Tengvall, Textor, & Thomsen, 2012).

After the machining, it is checked whether the length and diameter of the implant body are within the tolerable deviations. Shape, depth, width, pitch, and helix angle of the thread are also controlled (Figure 3).

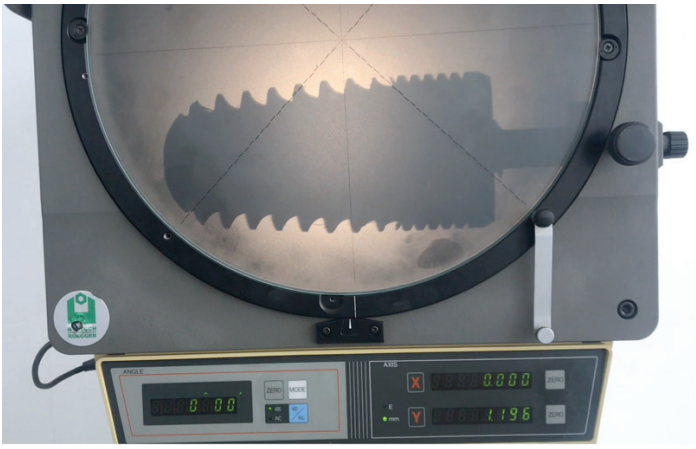

Figure 3. *Geometric checks of the implant exterior*

2. GRINDING AND POLISHING

Grinding and polishing processes are material removal with abrasive media on the material surface. In the grinding process, faster material loss and rougher topographies are obtained by using coarse sized plates containing abrasive particles. In the polishing process, when plates containing increasingly thinned abrasive particles are applied using lubricant solutions in different directions, very smooth surfaces are obtained (Brunette et al., 2012).

3. CLEANING

Residual lubricant oils used in cutting process and the burrs on the implant are cleaned by various solvents. After the cleaning, the inside of the implant is checked by various measurements (Figure 4). After the controls, surface processing of the implant is performed.

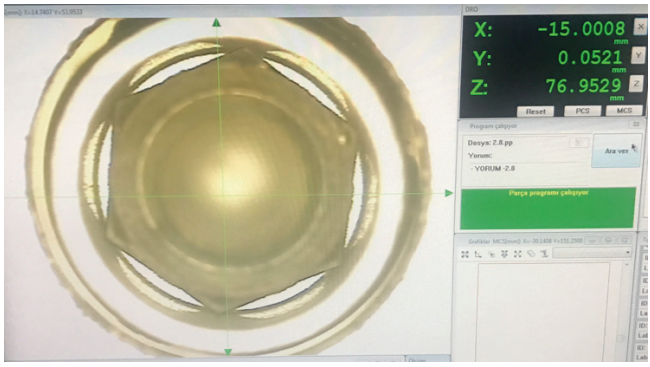

Figure 4. *Checks of the inside of the implant*

4. SURFACE PROCESSING

The surface has an important role in healing time for osseointegration and, ultimately, the success of implant treatment. It is the only part of the implant exposed to the surrounding oral environs, and its chemical, physical, mechanical, and topographic characteristics are all crucial to maximizing the likelihood of successful osseointegration. Wennerberg (Wennerberg, 1998) classified implant surfaces based on the surface roughness as 1. Smooth (Sa < 0.5 μm), 2. Minimally rough Sa = 0.5-1 μm), 3. Moderately rough (Sa = 1-2 μm), 4. Rough (Sa > 2.0 μm)

It is thought that implant surface properties have an important function in ensuring osseointegration. Because the response of the bone tissue is different according to the surface properties of the implant used. It is stated that the implant surface should have an enhancing effect on bone healing (Buser et al., 1991). The main objective of the development of implant surface modifications is to promote Osseointegration, with faster and stronger bone formation. Furthermore, it accelerates bone healing and thereby allowing immediate or early loading.

The roughing techniques are classified in many different ways like concave and convex texture mechanical, chemical, and biological techniques, ablative, etc. There are 20-25 roughening techniques (Table 2).

1. Sand blasting
2. Grit blasting
3. Shot peening
4. Acid etching
5. Dual acid etching
6. Sand blasting and acid etching SLA
7. Other chemical treatments
• Solvent cleaning
• Alkaline etching
• Passivation
8. Electrochemical treatments
• Anodic oxidation /anodization is the chief anodic technique.
• Biocoat – colour anodization
• Biodize – alkaline grey anodization
• Biobright -- electropolishing
• Electrophoresis and Cathodic HA depositions are the cathodic techniques
9. Laser treatments – Laser peening
10. Vacuum treatment
• Plasma treatments - plasma deposition method and plasma surface modification
• Ion implantation method
11. Thermal treatments
• Plasma spraying -- Titanium plasma spray (TPS)
• Sputter deposition -- Radio frequency sputtering (RF),Magnetron sputtering
• Sol-gel coated implants
• Biomimetic precipitation
• Electrolytic deposition
• Ultrasonic spray pyrolysis

Table 2. *Roughing techniques of dental implant surface*

However, today's manufacturers use 8-9 of these techniques commonly. Implant manufacturers create different surfaces with variations of the same techniques and give different names (Figure 5). For example, SLA is the abbreviation of Sand-blasted Large grit Acid etched, while SAS is the abbreviation of Sandblasted Acid-etched Surface. AB/AE is the abbreviation of Alumina Oxide Blasted Acid Etched. 3 definitions are actually the same (Figure 6).

Figure 5. *Implant manufacturers and different surfaces*

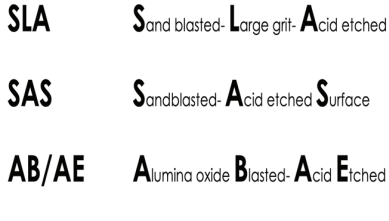

Figure 6. *Different abbreviations but the same surface*

Many methods have been developed to increase the surface roughness of dental implants and to improve osseointegration. These methods in 3 main classes were specified: A. Mechanical (Physical) methods, B. Chemical methods, C. Biochemical methods (Bagno & Di Bello, 2004).

A. Mechanical Surface Modification Processes

It is the processing, shaping, or material loss of the material by applying mechanical forces to the surface with another solid material (Brunette et al., 2012).

1. Roughening with Titanium Plasma Spray

Titanium plasma spray method (TPS) is used to create a rough implant surface. In this method, titanium particles are heated with plasma flame on

the titanium body, sprayed with high temperature and speed, and a layer of approximately 30 μm is formed. The microscopic surface increases 6-10 times and becomes 3-dimensional, stimulating adhesion osteogenesis (Buser et al., 1991; Sammons, Lumbikanonda, Gross, & Cantzler, 2005; Xue, Liu, Zheng, & Ding, 2005).

In the study conducted on pigs, it was stated that a faster implant-bone contact occurred on a TPS surface compared to a flat surface. However, titanium particles were found adjacent to these implants in the bone (Urban et al., 2000). In other animal studies, after the implant was placed, this layer was separated and titanium residues were found (Franchi et al., 2004).

2. Blasting

Roughening the titanium implant surface by spraying hard ceramic particles is called blasting. The particles create deep slits by cutting effect on the surface and roughen them by breaking small pieces from the surface. Various particles have been used such as Alumina (Corundum), Titanium oxide, Silicium oxide, and Calcium phosphate particles. Particles on these surfaces can be removed by dissolving with a nitric acid bath (Novaes Jr, Souza, de Oliveira, & Souza, 2002). The material frequently used in the roughening process is Alumina (Al_2O_3) (Mehmet Emre Yurttutan & Keskin, 2018). The alumina blasted implants were osseointegrated well although the implant sockets were oversized drilled (Mehmet Emre Yurttutan, Kestane, Keskin, & Dereci, 2016). However, these particles are often embedded in the implant surface and may remain on the surface as residues even after ultrasonic cleaning, acid passivation, and sterilization (Aparicio, Gil, Fonseca, Barbosa, & Planell, 2003). Titanium dioxide (TiO_2) is also used to roughen dental implants. According to clinical studies, a higher marginal bone level and success rate were found in implants roughened with TiO_2 than polished surfaces (Astrand et al., 1999; Van Steenberghe, De Mars, Quirynen, Jacobs, & Naert, 2000). Similar bone-implant contact was obtained in the sandblasting system with TiO_2 and Al_2O_3 particles, and more biomechanical fixation was observed compared to the flat surface (Abron, Hopfensperger, Thompson, & Cooper, 2001).Other materials used in roughening are osteoconductive and resorbable calcium phosphate products, hydroxyapatite, beta-tricalcium phosphate, silicon dioxide (SiO_2), and bioactive glass (Novaes Jr et al., 2002; Piattelli et al., 2002). Compared with the polished surface, more bone-implant contacts were seen on these surfaces (Müeller et al., 2003).

Sandblasting can be done automatically with a blasting machine or manually (Figure 7, 8). However, it is very difficult to provide standardization in manual sandblasting.

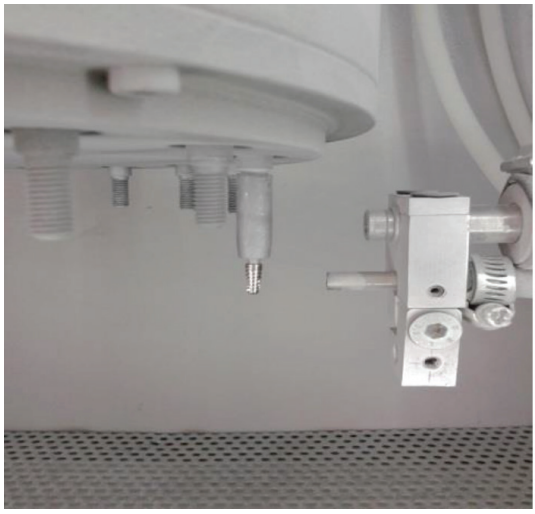

Figure 7. *Sandblasting by machine*

Figure 8. *Manual sandblasting*

B. Chemical Methods

Chemical methods are methods applied to make modifications in the chemical structure of the titanium surface.

1. Cleaning with Solvents

It is the cleaning of oils and oily surface contaminants resulting from production with organic solvents (aliphatic hydrocarbons, alcohols, ketones), surfactant detergents (trichloroethylene), and alkaline solutions, without targeting the material under the surface oxide layer(Matsuura, Hosokawa, Okamoto, Kimoto, & Akagawa, 2000; Rønold & Ellingsen, 2002; Wennerberg, 1998)

2. Acid-Etching

Another method used to roughen titanium dental implants is etching with strong acids such as Hydrochloric, Sulfuric, Nitric and Hydrofluoric acid. After this procedure, micro pits with a diameter of 0.5-2 μm are formed (Massaro et al., 2002; Trisi, Lazzara, et al., 2003). It has been found that double acid-etched surfaces increase the osteoconductive process through fibrin and osteogenic cell attachments resulting in direct bone formation on the implant surface (Park & Davies, 2000; Trisi, Lazzara, et al., 2003). In experimental studies; double acid-etched surfaces; It has higher bone-implant contact and less bone resorption compared to polished surfaces and TPS surfaces (D. L. Cochran et al., 2002; Trisi, Marcato, & Todisco, 2003). Acid etching can reduce the mechanical properties of titanium. Titanium forming micro-cracks that can reduce fatigue strength on the implant surface may cause the loss of hydrogen embrittlement (becoming

easily brittle, softening) (Yokoyama, Ichikawa, Murakami, Miyamoto, & Asaoka, 2002). The type, concentration, temperature, and processing time of the acid solution are effective in etching the surfaces (Sykaras, Iacopino, Marker, Triplett, & Woody, 2000; Szmukler-Moncler, Perrin, Ahossi, Magnin, & Bernard, 2004).

3. Sandblasted, Large-grit, Acid-etched (SLA)

In this method, which has been studied since the 1990s, the irregular and rough macro structure obtained by sandblasting is transformed into microstructure with acid. The sandblasted and etched surfaces were compared with only the etched surfaces and it was reported that the sandblasted and etched surfaces gave significantly higher extraction torque values (Buser et al., 1999; Mehmet E Yurttutan, Oncul, & Alkaya, 2018). SLA-surfaced implants (SLA, Institute Straumann, Switzerland) are obtained by roughening the titanium surface with large grit blasting process and then applying acid. As a result of this process, the surface area of the implant increases by approximately 33%. SLA-surfaced implants are mechanically more durable because they do not have a coating process as in TPS-surfaced implants (Lindhe, Lang, & Karring, 2003; Wang, Yan, Hayakawa, Tsuru, & Osaka, 2003).

When commercial implant systems are examined, it is observed that different sandblasting and etching processes are applied. The surfaces of implants with ITI® SLA surface (Straumann) are obtained by applying a double etching process with HCl / H_2SO_4 acid solution after sandblasting with 250-500 μm Al_2O_3 particles (Zinger et al., 2004). The surfaces of implants with Osseotite® surface (3i / Implant Innovations) are obtained by applying a second etching process with HCl / H_2SO_4 acid solution after HF etching (Cordioli, Majzoub, Piattelli, & Scarano, 2000). The surfaces of the Spline Twist MTX surface implants (Centerpulse Dental) are sandblasted with HA particles and obtained by etching the surface with HNO_3 (Mazor & Cohen, 2003).

4. Chemically Modified Sandblasted and Acid Etched Hydrophilic Surface (modSLA- SLActive)

After sandblasting with large particles (0.25-0.5mm) and pickling with HCl / H_2SO_4, the implants are rinsed under nitrogen protection to prevent exposure to air. Thus, the wettability of the surface is increased and osseointegration is accelerated (Rupp et al., 2006).

5. Anodization

Anodized surfaces; It is obtained by applying a high voltage to the titanium model in the electrolyte (strong acids such as H_2SO_4, HNO_3) (D. Cochran, Schenk, Lussi, Higginbottom, & Buser, 1998; Le Guéhennec,

Soueidan, Layrolle, & Amouriq, 2007) (Figure 9). The dissolution of the oxide layer along the convection current creates micro or nanopores on the titanium surface (Huang et al., 2005; Y. Sul et al., 2001).

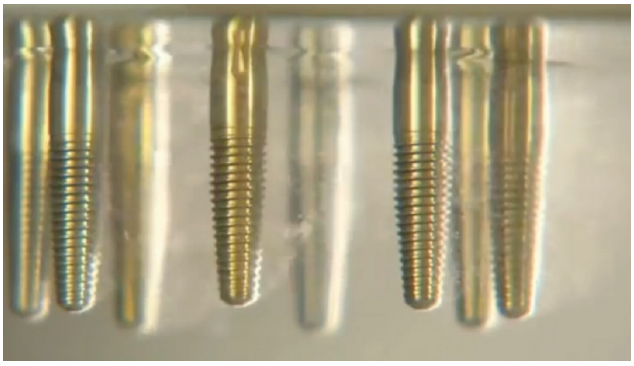

Figure 9. *Anodization of implants*

Anodization creates modifications in the microstructure and crystallinity (transparency) of the titanium oxide layer (Y. T. Sul, Johansson, Jeong, Wennerberg, & Albrektsson, 2002). Anodized surfaces result in enhancing bone response with higher values in mechanical and histomorphometric tests compared to untreated surfaces (Rocci, Martignoni, & Gottlow, 2003; Y. T. Sul, Johansson, Röser, & Albrektsson, 2002).

C. Biochemical Methods

1. Coating with Calcium Phosphate

Calcium phosphate ($CaPO_4$) can be used for coating the titanium surface due to its similarity to bone mineral content, its ability to form carbonate hydroxyapatite on the implant surface, its ability to act as a suitable skeleton for bone formation, to bind and store endogenous bone morphogenetic proteins (LeGeros, 2002).

2. Coating with Hydroxyapatite (HA)

It is a technique developed to increase implant anchorage and bone growth by taking advantage of the osteoconductive effect of hydroxyapatite in Type 3 and 4 bones with low bone quality (Balshi, Allen, Wolfinger, & Balshi, 2005). An implant can be coated by spraying, dipping, and dripping with Hydroxyapatite. Sprayed HA ceramic particles combine with the previously roughened titanium surface to form a film layer of 1-2 μm - 1-2 mm thick. There are positive short-term results for HA coatings. However, ruptures at the implant-coating junction due to the delamination (fatigue) of the coating are the release of foreign matter and the consequent clinical failure of the implants. It is reported that HA coating increases microorganism retention and negatively affects the success of the implant (Le Guéhennec et al., 2007).

3. Coating with Fluorine

Titanium is very reactive to fluorine ions and forms soluble TiF_4 (Le Guéhennec et al., 2007). Fluorine increases bone regeneration, increases the number of growth factors that cause calcification, and acts on osteoprogenitor cells or undifferentiated osteoblasts that synthesize most growth factors (Ellingsen, 2006). This chemical treatment may have the potential to improve implant anchorage placed in bone by increasing implant surface bioactivity.

4. Laser Ablation

The laser ablation technique focuses on improving the integration of dental implants in the surrounding soft tissue. Therefore, the nanoscale surface manufacturing technique has been transferred. Microchannels have been proposed to act as a biological seal by eliciting the attachment and bone and inhibiting epithelial down growth (Linkevicius, Puisys, Svediene, Linkevicius, & Linkeviciene, 2015).

In figure 10, the techniques that have just mentioned and the implant companies produced with these techniques can be seen.

5. PACKING & STERILIZATION

After the surface treatments are completed, the implants go into a tank full of hot purified water and are again subjected to a series of cleaning operations and dried. And then, the implants are packed in sterile terms in a special clean room. (Figure 11). And implants are sent to gamma sterilization.

SURFACE TREATMENT	IMPLANT SYSTEM/SURFACE [29]
Acid-etched Etching with strong acids increases the surface roughness and the surface area of titanium implants.	BIOMET 3i OSSEOTITE® and NanoTite™
Anodized This electrochemical process thickens and roughens the titanium oxide layer on the surface of implants.	Nobel Biocare TiUnite®
Blasted Particles are projected through a nozzle at a high velocity onto the implant. Various materials, such as titanium dioxide, aluminum dioxide and hydroxyapatite (HA) are often used. HA treatments also include microtextured (MTX) surface treatments and RBM surface treatments *(Figs. 2a, 2b)*.	DENTSPLY Implants ASTRA TECH TiOblast™, Zimmer Dental MTX™, Inclusive® Tapered Implants
Blasted and acid-washed/etched Implants undergo a blasting process. Afterward, the surface is either washed with non-etching acid or etched with strong acids.	CAMLOG Promote®, DENTSPLY Implants FRIALIT® and FRIADENT® plus, Straumann® SLA®
Hydroxyapatite (HA) HA is an osteoconductive material that has the ability to form a strong bond between the bone and the implant.	Implant Direct (various), Zimmer Dental MP-1®
Laser ablation High-intensity pulses of a laser beam strike a protective layer that coats the metallic surface. As a result, implants demonstrate a honeycomb pattern with small pores.	BioHorizons® Laser-Lok®
Plasma-sprayed Powdery forms of titanium are injected into a plasma torch at elevated temperatures.	Straumann® ITI® titanium plasma-sprayed (TPS)

Figure 10. *Implant companies and their surface treatments*

Figure 11. *Clean room*

CONCLUSION

In conclusion; geometrical design, surface treatment, and surgical technique are also essential in evaluating the performance of a specific implant. The implant/tissue interface is influenced by numerous factors, including surface chemistry and surface topography of the foreign material. There is a number of surfaces commercially available for dental implants. Various methods modifying the titanium implant surface have greatly influenced the quality of clinical service in implant prosthodontics. Implant surface characterization and working knowledge about how surface and bulk biomaterial properties interrelate to implant osseointegration represent an important area in implant-based reconstructive surgery.

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Chapter 17

EMBRYONIC MORTALITY IN DAIRY COW

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PREFACE

Although tremendous progress has been achieved in milk yield in the last fifty years in dairy farming, fertility has experienced a dramatic decrease. One of the reasons for this decline in fertility is embryonic deaths. Especially in high-yield dairy cows, an increase in embryonic death rates has been observed. Embryonic death is the loss of offspring within the first 45 days of pregnancy and appears as a factor affecting milk production, economic efficiency, and fertility. The etiology of embryonic deaths is multifactorial, and for new treatment options to be developed, its etiology and diagnostic methods must be known. The aim of this compilation is to review and summarize new opinions about the etiology and diagnostic methods of embryonic deaths as an important cause of infertility in dairy cows.

EMBRYONIC MORTALITY IN DAIRY COWS

INTRODUCTION

Embryonic deaths are shown as an important cause of economic losses and decreased fertility in dairy cow breeding (Baranski et al., 2012; De Rensis et al., 2015; Gädicke et al., 2010). Embryonic deaths seen in cows directly affect production efficiency and profitability by decreasing the conception rate (Diskin et al., 2011).

The loss of offspring before the 45th day of pregnancy in cows is called embryonic death. It has been reported to be between 10% and 40% after the first insemination and as much as 65% in repeat breeder cows (Bilodeau and Kastelic, 2003). Embryonic deaths in cows are examined in three periods: very early deaths (0-7 days), early deaths (7-24 days), and late deaths (24-45 days). Deaths after the 45th day are considered fetal death (Walsh et al., 2011). The rate of early embryonic deaths occurring within the first 3 weeks of pregnancy is higher than the rest. Although late embryonic deaths are less common than early embryonic deaths, related economic losses are more serious (Inskeep and Dailey, 2005; Diskin and Morris, 2008; Walsh et al., 2011).

1- Very Early Embryonic Deaths (0-7 days)

Low oocyte quality and a poor environment for uterus are shown as the causes of embryonic deaths occurring within the first 7 days after insemination. It has been reported that cows with high genetic value and cows with low body condition score (BCS) have low embryo development (up to day 7) in oocytes and that their chance of reaching blastocyst stage decreases (Snijders et al., 2000). It has been proven that oocytes developing from large antral follicles have a higher rate of developing blastocytes than oocytes developing from small antral follicles (Lonergan et al., 1994).

In recent studies, the rate of conception at first insemination has been reported to be 32% in lactating cows and 50% in non-lactating heifers (Sartori et al., 2009). It has been reported that lactation affects the quality of embryos, that there is a relationship between milk yield and embryo quality, that Holstein cows in lactation have lower quality embryos than heifers and beef cattle, and that the chance of survival of these embryos is less (Leroy et al., 2005; Sartori et al., 2009).

It has also been reported that in high-yield dairy cows, embryonic losses occur due to the deterioration of early embryonic development during the first week after insemination and that high milk production output negatively affects oocyte quality and embryonic development, thus causing embryonic deaths (Sartori et al., 2009; Sartori et al., 2002).

According to Robinson et al. (2008), three important factors affect the embryo's survival in the first week after insemination.

These are as follows:

1- In order to ensure the development of the embryo up to the morula stage, the cow must be provided with a suitable environment and secretion in the oviduct. These are nutrients such as ions, amino acids and glucose, and insulin-like growth factors (IGF-1 and IGF-2) (Pushpakumara et al., 2002).

It has been reported that the circulating IGF levels in the cows with negative energy balance (NED) decrease in the early lactation period and the IGF availability in the egg canal is impaired, thereby disrupting the embryo development and contributing to high embryonic death rates (Fenwick et al., 2008). Insulin-like growth factors affect embryo development directly through the embryo, while indirectly affecting oviduct secretions and muscle activity (Pushpakumara et al., 2002). It has also been reported by Jousan and Hansen (2007) that IGF protects the embryo against heat stress.

2- They are the disorders that may occur in oviduct epithelial cells, which play a role in the transport of the embryo to the uterus. It is the coordinated movements of the cilia and smooth muscle contractions of the oviduct, which enable the embryo in the Morula stage to reach the uterus in a timely manner (Robinson et al., 2008). The Oviduct epithelium contains two types of cells, namely secretory cells and ciliary cells, and these cells play important roles in the first days after insemination. While secretory cells produce various molecules that support embryo development, ciliary cells facilitate the transport of the zygote through their specific coordinated movements. The proportions and functions of the two cell types show a dynamic change during the estrus cycle. The number of secretory cells increases with the formation of the corpus luteum (CL) (Ito et al., 2016).

3- It is the formation of corpus luteum after ovulation and the ability to secrete progesterone. Progesterone (P4) secreted from the corpus luteum is critical for the onset and sustainment of pregnancy. Progesterone plays an important role in the regulation of endometrial secretions needed to stimulate and mediate the changes and the growth of the embryo during early pregnancy in ruminant animals (Lonergan et al., 2016).

The delay in progesterone secretion after ovulation or the low progesterone secretion during the luteal phase causes the development of weak embryos that may or may not produce too little interferon tau (IFNt). Such embryos cannot hinder luteolysis and are the main cause of pregnancy loss in cows. In the presence of a high concentration of progesterone, the embryo prevents luteolysis by inhibiting the development of oxytocin receptors on the oxytocin-induced secretion of PGF2α and the lumen epithelium of the endometrium, and through induction of a prostaglandin synthesis inhibitor in the endometrium (Bajaj and Sharma, 2011; Mann et al., 1999). In short, the embryos of cows with high progesterone concentrations have proven to develop better in the early embryonic period (4-7 days after insemination) (Green et al., 2005; Diskin and Morris, 2008).

Recent studies (Batistaa et al., 2019) make mention of two mechanisms that lead to the early regression of the corpus luteum. These are the development of a hereditary low-life CL and early regulation of the estrogen and oxytocin receptor genes in the endometrium associated with PGF2α synthesis. The estrogen hormone induces oxytocin release, allowing PGF2α production and completion of luteolysis (Silvia et al., 1991).

2- Early Embryonic Deaths (7-24th days)

Early embryonic deaths occurring between the 8th and 27th days of pregnancy in dairy cows have an important prevalence, as they constitute 32% of all embryonic deaths (Wiltbank et al., 2016). Bovine embryo reaches the uterus between 3 and 4 days after insemination and is in the form of a 16-32 cell morula during this period. The embryo takes the form of blastocyte after the 7th day. There are two groups of cells in the blastocyte, namely internal cell clusters and trophoblast cells (Noakes, 2009; Senger, 2005). Mononuclear trophectoderm cells of trophoblast synthesize and secrete IFNt, which is the pregnancy recognition signal. Binuclear giant trophoblast cells also begin to secrete Chorionic Somatotropin 1 (CSH1 or placental lactogen). These two are responsible for maternal recognition of the pregnancy. It suppresses the release of estrogen and oxytocin, which triggers the production of PGF2α leading to lysis of interferon tau CL. Thus, the corpus luteum continues to produce progesterone, which is necessary for pregnancy (Bazer, 2013). Progesterone hormone plays a role in promoting the adhesion, growth, and differentiation of the embryo to

the endometrial epithelium by inducing the secretion of IFNt and CSH1 (Spencer et al., 2008). It has been reported that 25% of pregnancy losses in dairy cows are caused by early embryonic deaths due to pregnancy not being recognized maternally (Sreenan and Diskin, 1983).

It has been proven by Ribeiro et al. (2016) that both uterine and non-uterine inflammatory diseases increase pregnancy loss by negatively affecting the IFNt secretion.

Expression of homozygous recessive genes causes early embryonic deaths in genetic factors resulting from errors in chromosome number or structure and lack of significant developmental genes, and this constitutes 5% of all embryonic losses (Peters, 1996; Van Raden and Miller, 2006).

It has been reported that the age of the mother also affects embryonic deaths. Khun et al. (2006) proved that heifers that are 15-16 months old have the highest conception rates, while in heifers that are 26 months old and older, the conception rate decreases (13%) due to embryonic deaths.

Nutritional factors act individually or in combination on the hypothalamic-pituitary-ovarian axis, oocyte maturation, embryo survival, and uterus (Bilodeau-Goessels and Kastelic, 2003). Nutritional deficiencies in mammals impair embryo quality and cause early embryonic losses (Valazquez, 2015). A low level of blood glucose in dairy cows slows down the embryo growth rate and causes embryonic deaths (Lucy et al., 2014).

Heat stress appears as a factor that reduces conception rate in cows. It is known that the mother raises her body temperature and causes embryonic deaths. As a result of the mother's exposure to high environmental temperature, the embryo cannot adequately secrete IFNt, which is necessary for maternal recognition, resulting in the inability to inhibit PGF2α synthesis and a decrease in progesterone concentration, all of which lead to embryonic deaths (Sakatani, 2017; Shaham-Albalancy et al., 2001).

3- Late Embryonic Deaths (24-45th days)

Late embryonic deaths cause significant economic losses as they reduce reproductive efficiency, cause a long delay in conception, and affect the economic efficiency of milk production (Baranski et al., 2012; Diskin et al., 2016; Silke et al., 2002).

It has been reported that the rate of late embryonic deaths in cattle can vary from 3.2 to 42.7% (with an average of 12%) depending on the bovine species, environment, and whether the embryo is produced in-vivo or in-vitro (Vasconcelos et al., 1997, Cartmill et al., 2001; Aono et al., 2013; Pereira et al., 2013; Ribeiro et al., 2013; Wiltbank et al., 2016).

Information about the causes of late embryonic deaths is not yet sufficient. However, placental insufficiency is shown as a potential cause (Wiltbank et al., 2016). Pregnancy-related proteins (bPAG) are secreted from the giant trophoblast cells of the placenta in cows. They are found in the blood circulation of the mother by the 24-26th day of pregnancy; they can be used as a biomarker of pregnancy diagnosis and placental functions (Breukelman et al., 2012; Pohler et al., 2013; Wallace et al., 2015). It has been reported that circulating PAG concentration is low in cows with embryonic deaths seen between the 31st and 59th days of pregnancy (Pohler et al., 2016).

It has been reported that the embryonic mortality rate is higher in cows with a body condition score (BCS) of less than 2.5 days between the 28th and 42nd days of pregnancy, where the incidence of late embryonic death is also associated with BCS (Rodríguez et al., 2019).

It has been reported that one of the causes of late embryonic death is oxidative stress and, with high rates of late embryonic deaths, is the high oxidative stress parameters in dairy cows (Celi et al., 2011).

Grimard et al. (2006) reported the late embryonic mortality rate to be 25%, highlighting the effect of high milk output (> 39), high VKS (> 2.5), and the season (high in autumn).

Many pathogens such as bacteria, viruses, protozoa, and fungi have also been shown as the cause of late embryonic deaths in cattle (Givens and Marley, 2008). Specific pathogens can be transmitted through the vagina or artificial insemination (BVDV) through the hematogenous route (Toxoplasma gondii) or during natural erosion (Campylobacter fetus). Non-specific pathogens are generally bacteria and are transmitted to the uterus by ascending via insemination (Rani et al., 2018). While specific bacterial infections are caused by Trueperella pyogenes and Campylobacter fetus (Adler, 1959), trichomonas fetus, which is a flagellated protozoon, plays a role in the etiology of embryonic deaths by infection via a venereal transmission (Onyango, 2014). Bovine herpes virus-1 group, which includes IBR and IPV, is also responsible for abortion and embryonic losses. BVDV also plays a role in the early embryonic loss, but it is not a major factor in cows (Whitmore et al., 1981).

It has been reported by Cartmill et al. (2001) that high environmental temperature and temperature stress increase the embryonic death rate between the 27th and 30th days of pregnancy, which causes late embryonic deaths.

Diagnostic Methods of Embryonic Deaths

Knowing the economic losses caused by embryonic deaths in dairy herds requires any and all precaution against this situation and treatment as soon as possible. In situations where routine reproductive control programs are suspected in dairy herds, the detection of embryonic deaths will ensure within this regard that the herd remains within the fertility parameters as much as possible by applying the necessary measures and treatments on time.

Immunologic methods

In the determination of embryonic deaths, bPAG-1 (Bovine pregnancy-associated glycoprotein-1) and progesterone measurements are used. Both biomarkers are determined on the basis of double-antibody measurement by RIA or ELISA techniques. Bovine pregnancy-associated glycoprotein 1 concentration is 0.8 ng/ml in border-level plasma for pregnancy detection. It has been reported that the embryos of cows with serums below this value are not healthy (Kaufmann et al., 2009). It has also been reported that the level of progesterone is 6.48 ± 0.38 ng/ml (3.93-7.68 ng/ml) in pregnant animals on the 21st day (Hadiya et al., 2015). The positive and negative predictive values of progesterone in pregnancy diagnosis have been reported as 79.55% and 100% for the 21st day, while the positive and negative predictive values on the 24-25th days as 91% and 88%, respectively (Chung ve Kim, 1980). In cases where the embryo is lost, a gradual decrease in the level of progesterone occurs on the 35-60th days (Chaffaux et al., 1986). Due to this change in the endocrine pattern and progesterone profile of the sexual cycle, it has been reported that very early and early embryonic deaths do not yield results with progesterone level measurements. However, a significant decrease between the progesterone values on the 21st or 24th days and the progesterone values measured after the 30th day may give clues in the diagnosis of embryonic death (Rani et al., 2018).

Ultrasonography

Early pregnancy in cows can be diagnosed on the 21-25th days after insemination with 5-7.5 MHz linear probe B-mode real-time transrectal ultrasonography (Fricke et al., 2002). However, in early studies (23rd, 28th, and 35th days) pregnancy diagnosis has been reported to have less accuracy than pregnancy examinations performed on the 42nd day (Hadiya et al., 2015). Embryonic heartbeat can be monitored on ultrasonography on the 21st day of pregnancy. In the transrectal ultrasonographic examination performed in these periods, symptoms such as the observation of embryonic sac, fluids, embryo and heartbeat, the impaired embryonic sac in the repeated embryonic examination performed on the 30th-35th days,

absorption of fluids, inability to see the heartbeat, and impaired integrity of the embryo show that embryonic death has occurred (Szenci et al., 1998; Rani et al., 2018).

CONCLUSIONS

Embryonic deaths in dairy cows are one of the main causes of reproduction-related losses and economic losses. Although many factors play a role in embryonic deaths, the main reason is thought to be disorders related to the maternal recognition of pregnancy and luteal dysfunction. In this review, it is aimed to guide veterinarians and researchers in the search for new treatment protocols to be determined to prevent or reduce embryonic deaths and to present potential issues that should be paid attention to.

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Chapter 18

EFFECTIVE FACTORS IN THE CLINICAL ASSESMENT AND BIOANALYSIS OF BIOTHERAPEUTICS

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1. INTRODUCTION TO PROTEIN AND RECOMBINANT PROTEIN BASED PHARMACEUTICALS

A protein-based therapeutic can be defined as drugs that are partially or completely protein in structure. According to European Medicines Agency (EMA), therapeutic protein molecules can be small or large in size (Committee for Medicinal Products for Human Use (CHMP) 2007). The first approved pharmaceutical recombinant protein by U.S. Food and Drug Administration (FDA) was "Recombinant Human Insulin for The Treatment of Diabetes" in 1982 (Pham 2018). Since then, numerous protein therapeutics based on recombinant protein technology have been produced such as hormones, interferons, growth factors, vaccines, monoclonal antibodies etc. (Dimitrov 2012).

Due to the advancement in technology and our better understanding of the structure of protein-based pharmaceuticals, improvements have been made in biopharmaceuticals. Three generations existed with these developments. The first generation contains the proteins in their nature states. The second-generation protein therapeutics shows improvements in their specificity, efficacy, biodistribution and especially pharmacokinetic (PK) profiles. Third one displays higher efficiency and increased safety due to new administration routes and novel formulations (Pham 2018).

Based on their functions, it can also be assessed as pharmacological classification, therapeutic proteins classified in 4 major groups.

Table 1. *Examples of protein products representing each group.*

API	Trade Name	Group	Indication(s)
Alglucosidase-α	Myozyme	Ia	Pompe disease (glycogen storage disease type II)
Tenecteplase	TNKase	Ib	Acute myocardial infarction
Botulinum toxin type A	Botox	Ic	Many types of dystonia, particularly cervical; cosmetic uses
Trastuzumab	Herceptin	IIa	Breast cancer
Natalizumab	Tysabri	IIa	Relapsing multiple sclerosis
Enfuvirtide	Fuzeon	IIa	Adults and children (at least 6 years old) with advanced HIV infection
Ibritumomab tiuxetan	Zevalin	IIb	Relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma (NHL), including rituximab-refractory follicular NHL
Hepatitis B surface antigen (HBsAg)	Recombivax HB	IIIa	Hepatitis B vaccination

Anti-Rhesus (Rh) immunoglobulin G	Rhophylac	IIIb	Routine antepartum and postpartum prevention of Rh(D) immunization in Rh(D)-negative women
Talimogene Laherparepvec	T-VEC (Imlygic®)	IIIc	Melanoma
Capromab pendetide	ProstaScint	IV	Prostate cancer detection

Group 1 divided into 3 three sub-groups which consists from protein therapeutics with enzymatic or regulatory activity. Group Ia contains replacement products that is deficient or abnormal. Those products are being used for the treatment of endocrine and metabolic disorders with defined molecular cause. Group Ib contains products that augmenting an existing pathway. Although its molecular mechanism is not known exactly, products that increase the hematologic and endocrine pathways and immune responses are used in Group Ib. Group Ic contains products that provides a novel function or activity. The products of Group Ic are used to modify naturally occurring proteins to modify the pathophysiology of diseases. Group II products are protein therapeutics with special targeting activity and divided into 2 sub-groups. Group IIa products function by specifically binding to their target and blocking their function, labeling them for destruction or regulating by stimulating a signal pathway. Group IIb therapeutics deliver other compounds such as toxins or proteins to a particular site. Group III consists of protein vaccines. Group IIIa represents vaccine products used against infectious agents, group IIIb represents autoimmune diseases and Group IIIc represents cancer vaccines. Group IV is a class of proteins used in diagnosis and they are very important because they are effective in decision making (Leader, Baca, and Golan 2008)(Akash et al. 2015).

2. CONSIDERATIONS ON PHARMACOKINETICS OF BIOTHERAPEUTICS

Due to the large number of protein therapeutics with such different pharmacological effects and the complexity of their structures, pharmacokinetic (PK) studies can also become complex. Generally, evaluation of pharmacokinetics procedure for the conventional products is similar with specific considerations related to the protein characteristics. Some of the factors that may affect the efficacy and safety of a therapeutic protein are their sizes, the protein folding state such as secondary, tertiary and quaternary structures, post-translational modifications, aggregation. Besides their unique properties, administration route is also important. They are mainly administered parenterally such as intravenously (iv), subcutaneously (sc) and intramuscularly (im). For instance; im and sc

administrations mostly result with higher T_{max} compared to conventional drugs. Protein therapeutics also affected by formation of neutralizing anti-drug-antibodies (ADA) which may strongly affect their pharmacokinetic profile via increased sustain or clearance. (Zhao, Ren, and Wang 2012) (Vandivort, Horton, and Johnson 2020).

According to the EMA guidanline, ADME (absorbtion, distribution, metabolism, excretion) profile should be characterized during single-dose and under steady-state conditions. However, special consideration must be taken depending on the type of protein and its intended use. If the clinical study is conducted in healthy volunteers, the rationale for the extrapolation of the pharmacokinetics obtained from the study with healthy volunteers and the pharmacokinetics in the target population should be presented. Because elimination may differ for some protein therapeutics depending on the target expression level difference under healthy and disease conditions which can cause different elimination half-life (Committee for Medicinal Products for Human Use (CHMP) 2007).

2.1. Non-Target Related Clearance of Biotherapeutics

Since special consideration is needed for PK, it is important to understand the mechanisms affecting the PK profile. Despite all the R&D expenditure of the developed protein therapeutics, the success of 8-10% in clinical studies has shown that the relationship between PK-PD (pharmacokinetic and pharmacodynamic) and protein structure should be understood. Therefore, in recent years, studies on the causes affecting PK-PD profiles have succeeded in dividing the factors affecting PK and disposition characteristics of protein therapeutics into two categories as target-mediated drug disposition and non-target related clearance. It has been determined that the factors associated with non-target related clearance are factors such as molecular structure, route of administration, dose and frequency. Many studies have determined that the properties related to the structure are molecular weight, three-dimensional structure of the molecule, post-translational modifications, charge and hydrophobicity, thermal and catabolic stability, interaction with proteins such as human serum albumin and neonatal fragment crystallizable receptor (FcRn) and chemical modification etc. Besides the relationship with physicochemical properties of protein therapeutics, the route of administration is also important. Until now, mAbs (monoclonal antibodies) in particular have been administered to the patient iv. However, it is necessary to understand its properties such as adsorption kinetics, solubility and permeability depending on the application route, due to reasons such as providing ease of use and improving PK-PD profile.

Molecular weight or in other words hydrodynamic size is one of the parameters that most affect PK and disposition in blood and tissues.

Protein pharmaceuticals with a molecular weight of less than 50 kDa are known to undergo renal clearance. Therefore, renal clearance is tried to be prevented by modification of protein/peptide molecules smaller than 50 kDa with polymers such as polyethylene glycol (PEG). Thus, their period in circulation is extended. On the other hand, approaches such as conjugation with plasma/serum proteins, increasing the ability to bind to albumin by conjugation with lipids, and fusion with Fc are different strategies used for the same purpose (Datta-Mannan 2019).

Another factor that affects protein therapeutics disposition is their ability to interact with FcRn. Protein therapeutics such as monoclonal antibodies, Fc-fusion proteins or albumin conjugated proteins/peptides have longer circulation period (longer elimination half-life) because FcRn helps them to escape from lysosomal degradation. Therefore, the ability and potency of FcRn and protein therapeutics to interact is important in determining the dose-response relationship. This is the main reason why Fc engineering has gained importance in recent years. On the other hand, since FcRn is also responsible for albumin turn-over there is a chance to compete of albumin (or any other protein that reacts to FcRn) with protein therapeutics. Also, there is another possibility such as impairment of FcRn function relating with a disease such as cancer. In this case, the expression of FcRn may be down-regulated or its function may be impaired which leads the change in disposition of protein therapeutics. Change in plasma protein levels, especially reduction of albumin levels during disease may also affect in the same manner. Therefore, the quantitative information regarding FcRn may help to adjust effective dose (Datta-Mannan 2019) (Abdallah and Zhu 2019)(Cadena et al. 2020).

Another feature that is effective in the ADME profile is post-translational modifications. These modifications result in structural and physicochemical consequences such as change in aggregation tendency, conformational change and change in charge distributions. Examples of such modifications are pyroglutamate formation involved in N-terminal heterogeneity; deamidation, oxidation, glycation of amino acids; N-glycosylations such as fucosylation, galactosylation, sialylation, and amidations that result in C-terminal heterogeneity. Especially modification related to glycation and N-glycosylations have an impact on PK profile of protein therapeutics. In addition to FcRn, asialoglycoprotein and mannose receptors can bind to glycoproteins, but contribute to clearance (Goetze et al. 2011; Higel et al. 2016). Goetze et al. found that if a mAb contains M5 (mannose type), it is eliminated faster from the circulation in a dose-independent manner. Therefore, it is important to know glycan structure of protein pharmaceutics to understand and predict the PK profile.

Charge, which is among the physicochemical properties affecting the pharmacokinetic profile of biological molecules, is another feature that should be taken into account. Each protein has its own isoelectric point (pI) depending on primary structure and post-translational modifications. For instance, native antibodies have pI above 6.0 and plasma proteins below 5.5. Several studies regarding modifying charge of therapeutic proteins revealed that cationization resulted with decreased plasma half-life and increased tissue distribution. On the other hand, anionization resulted with decreased plasma half-life and tissue distribution. Neutralization is another approach to alter charge of therapeutic proteins but mostly yielded with altered biological behavior (Boswell et al. 2010). Therefore, engineering the charge of protein pharmaceuticals should be carried out carefully and in line with the intended use.

2.2. Target Mediated Drug Disposition (TMDD) of Biotherapeutics

Most of the therapeutic proteins show high affinity to their target. Due to this high affinity, they exhibit non-linear pharmacokinetics. This profile of such drug molecules with high affinity for its target is terminologically called Target Mediated Drug Disposition (TMDD). Although the interaction between the protein pharmaceutical and its target is related to the pharmacodynamic process, this affects the PK profile.

While showing high affinity to the pharmacological target of the molecule, non-specific binding should be low in order to show TDMM. In addition, the molecule must be able to saturate its pharmacological target at the dose range of interest. The protein molecule binds to its target and forms the drug-target complex. This complex either releases the drug and target by disrupting the interaction, or the complex is eliminated by entering into the cell for proteolytic degradation.

In the case of TDMM, a PK profile associated with the concentration of the molecule with high affinity for its target is observed. As the molecule will saturate the target at low doses, the drug-target complex will be dominant in determining PK parameters. On the other hand, since the target is already occupied in high concentration plasma, the amount of free drug will play a role in determining PK parameters (Glassman and Muzykantov 2020).

Protein therapeutics other than monoclonal antibodies such as pegfilgrastim and epoetin alfa also cause a process called PD-PK loop. When such drugs bind to their pharmacological targets, they stimulate the cells resulting in the formation of more target cells. Also, the drug-target complex formed undergoes degradation. High drug concentration causes more target formation, hence a loop that leads to the formation of the drug-target complex and faster drug elimination.

Different methods are used to determine whether the non-linear PK profile is related to TDMM. In the first method, different doses of molecules administered to the pharmacological target is to knock out and wild type animals. Linear PK results in knockout animals and non-linear PK results in wild type animals show its relation with TDMM. In the second method, called *in vivo* displacement, when the low-dose drug reaches the terminal phase, high-dose displacer is administered and, if associated with TDMM, the first drug dissociates from its target and gives a second peak. The third method is the same as the second, with only the first drug bearing a radioactive label (Dayanand 2018).

2.3. Immunogenicity and PK/PD Relationship in Biotherapeutics

Since most biotherapeutics are antigenic, the immunogenic response to them affects the PK profile. This immunogenic response is mostly due to the development of anti-drug antibodies (ADAs). ADAs against biotherapeutics mostly increase the elimination of biotherapeutics or prevent their efficacy on their targets. While all biopharmaceuticals have the potential to induce ADA, those that mimic endogenous molecules exhibit less ADA triggering potential than those without endogenous analogs such as antibody-drug conjugates or bi-specific molecules. Therefore, it is essential to evaluate the immunogenicity of biotherapeutics to both predict side effects and impact on PK profile.

When evaluating immunogenicity, it is necessary to determine whether ADA is formed in terms of adverse events and its titer, as well as to be careful in terms of the accuracy of the analysis performed for PK profile as a result of conjugation of developed ADA molecules and free drug in serum. In addition to the antigenic nature of biotherapeutics, the route of administration of the drug also affects the formation of ADA. In the sc way, which is one of the preferred application routes because it is more patient-friendly and economically more advantageous; since the absorption time of the drug depending on the molecule size will be prolonged, there is a risk due to uptake by dendritic cells and triggering the formation of ADA.

The resulting ADAs can be neutralizing or non-neutralizing. Neutralizing ADAs act by binding to the biotherapeutic itself or the region that will interact with its pharmacological target. Thus, they have a total effect on the efficacy of the therapeutic. Mostly, biotherapeutics complexed with ADA are rapidly eliminated. However, both neutralizing or non-neutralizing ADAs can shorten or prolong the half-life of biotherapeutics. Since it is possible to change the PK profile due to the effect of immunogenicity on the elimination of biotherapeutic, it is important to determine the immunogenicity profile and to examine its possible effects on PK, in terms of the success and efficiency of the biotherapeutic (Smith et al. 2016). Beyond the effect of immunogenicity on the PK profile, ADAs

that develop against the biotherapeutic which has endogenous analogue cause immune complexes by interacting with endogenous molecules, leading to deficiency syndromes and/or anaphylaxis/hypersensitivity reactions, creating a serious safety problem.

PK parameters, immunogenicity and physicohemical properties of some biotherapeutics can be seen from Table 2 below. Below mentioned data obtained from biological licence application forms or package inserts of individual products.

Table 2. *Some PK parameters, immunogenicity and molecular weight of exemplary biotherapeutics.*

API	CL	V_{ss} (mL/kg)	$t_{1/2}$	C_{max} (ug/mL)	Immunogenicity (%)	MW (kDa)
Alglucosidase-α	21.4 mL/h/kg	66.2	2.8 d.	178.2	-	109
Tenecteplase (30 mg)	100 mL/min	6.1	22 min.	7.5	-	70
Botulinum toxin type A	Clinical pharmacokinetic studies were not conducted due to the limitations in time tests for theoretical concentrations of 0.2 picogram / mL (200 femtograms / ml) and lack of evidence that efficacy is related to blood levels.					150
Trastuzumab (500 mg)	0.241 L/d	44	12 d	377	5.9	148
Trastuzumab-Emtansine	7.8 mL/d/kg	29.5	3.7 d	83.4	5.3	148.5
Natalizumab (3 mg/kg)	0.0043 mL/min/kg	53	250 h	71.8	12	149
Enfuvirtide (90 mg)	24.8 mL/h/kg	5.5	3.8 h	4.6	-	4.6
Ibritumomab tiuxetan	7.2%	-	59.8 h	271	1.3	148
Hepatitis B surface antigen (HBsAg)	Not applicable (NA).					24
Anti-Rhesus (Rh) immunoglobulin G (iv)	0.2 mL/min	Vd:8.59 L	16	0.084	-	NA
Talimogene Laherparepvec	No traditional pharmacokinetic studies were performed.					
Antihemophilic factor (recombinant), glycopegylated-exei (>18 years old)	1.2 mL/h/kg	37.3	21.7	197.9 IU/dL	2	166 protein + 40 PEG

2.4. First in Human (FIH) Dose Determination

First-in-human (FIH) studies are the first step in the transition from pre-clinic to clinic. In biological products, especially in recombinant protein-based pharmaceuticals, it is difficult to find a suitable *in vivo* model that represents the human disease. Dose information obtained from the preclinical model should be used with caution due to problems such as antibodies developed mainly due to immunogenicity and the availability of the appropriate pharmacological target in the animal model. Therefore, both FDA and EMA have published guidelines for FIH studies. The FDA published the new FIH draft guideline for oncological drugs and biologics in 2018 and EMA published in 2017 (FDA, ucm616325, 2018; EMEA/CHMP/SWP/28367/07 Rev. 1, 2017).

According to the EMA, no observed adverse effect level (NOAEL) determination should be obtained from preclinical studies. For this purpose, the most relevant or sensitive animals should be used, and the most up-to-date models and/or allometric factors should be used to adapt them to humans. However, using data from preclinical pharmacology studies to provide information about PD, the minimal anticipated biological effect level (MABEL) for humans should be determined and also the pharmacologically active dose (PAD) and/or anticipated therapeutic dose range (ATD) should be inferred. The dose to be used in the FIH study should generally be a dose below the PAD with minimal pharmacological effect, but without safety issues (EMEA/CHMP/SWP/28367/07 Rev. 1, 2017).

In determining the FIH dose from the NOAEL, the NOAEL obtained from the preclinical study is firstly converted to the human equivalent dose (HED) by applying the Body Surface Area Conversion Factor (BSA-CF). Then the maximum recommended starting dose (MRSD) is obtained by multiplying by the safety factor.

After the tragic clinical trials of TGN1412 and BIA 10-2474, EMA has included the MABEL approach in its guidelines. MABEL normally gives a lower dose than NOAEL. It is almost a necessity to use transgenic animal models that express the same homology with human receptors and have metabolic profile in the selection of the most appropriate animal model for MABEL detection. It is recommended to determine the most accurate dose by comparing the MRSD value obtained using NOAEL with MABEL, PAD and ATD (Mishra, Sarangi, and Reeta 2020; Zhao et al. 2012).

3. BIOANALYSIS OF BIOTHERAPEUTICS

Correct selection and application of bioanalytical methods are crucial for an accurate PK/PD and immunogenicity assessment. Myler et al. prepared a case-by-case review of bioanalytical methods in PK/PD and

ADA analysis of 50 different biotherapeutics in 2011 (Myler et al. 2011).

Since antibody-drug conjugates are highly complex structures, PK analysis is equally complex. For this reason, 4 different assays were used in the PK analysis of trastuzumab-DM1. ELISA for total antibody, ADC and circulating HER-2 extracellular domain in serum and LC-MS/MS methods for free DM1 in serum were used. ELISA method was used for PK evaluation of recombinant Apo2/TRAIL. However, ELISA does not have the capacity to separate the trimer, dimer and monomer structure of TRAIL from each other. Therefore, combining the ligand binding-based assay ELISA with a technique such as LC-MS/MS is recommended. ELISA and flow cytometry were used in the Rituxan PK/PD study. The free Rituxan level (PK) was determined by ELISA, and by flow cytometry before and after the study, tumor CD20 expressing cells and CD positive B cells of interest in the blood were determined, respectively. However, the problem with this drug is human anti-murine antibodies (HAMA) that develop against rituxan. Because HAMAs complexed with rituxan may cause false serum levels measurement. For this reason, attention should be paid in studying the specificity and selectivity during the analytical method development and validation phase, and applications such as pH or ionic strength change during the sample extraction should be evaluated when necessary. ELISA was preferred in the GLP-1-like peptide exenatide and recombinant human erythropoietin PK study. Interferon immunoradiometric assay was used initially for interferon-α PK analysis. Although the same method was used for Intron A at the beginning, the analyzes continued with enzyme immunoassay and electrochemiluminesence (ECL) methods. For IFN-β used to treat MS disease, the situation is a little more complicated. Since the mechanism of action of IFN-β in the treatment of this disease has not been fully elucidated, cytopathic effect or antiviral bioassay methods that are not specific to the drug have been used for PK analyzes. However, with the increase in knowledge about the disease, ELISA or radioimmunoassay (RIA) for biomarker detection, and thanks to a personalized treatment approach mRNA with RT-PCR and flow-cytometry for T-cells analysis began to be used (Myler et al. 2011).

As can be seen from the review, ligand-binding-based assays (LBA) have been used mostly in PK / PD studies related to biotherapeutics until recently. With the advancing technology, mass spectroscopy has entered our lives and has led to significant improvements in bioanalytical methods. However, technological developments have brought more sensitive and automated methods based on ligand binding, such as surface plasmon resonance (SPR), as well as mass spectroscopy, yet, LBA still remains the gold standard (Zhang and An 2017).

In addition to the bioanalytical studies performed in the tissue to determine off target toxicity, the level of antigen expression in the target tissue and parameters such as penetration/disposition, bioanalysis in the tissue has become very important, especially with the introduction of the MABEL approach into the guidelines. LBA and RIA based methods are still frequently used for this purpose, but LC-MS/MS-based approaches have been used for a while due to their high selectivity and precision. LC-MS/MS is also affected by complex biological matrix such as LBA and RIA. For example, contamination of tissue with blood causes high bias in analyzes. Therefore, it is essential to optimize the perfusion conditions in order to decontaminate the tissue from blood without loss of tissue-related drug. After the tissue is decontaminated from blood, it is possible to increase the sensitivity of the analysis by using target and tissue specific sample extraction, sample enrichment by immunoaffinity or chromatographic procedures alone or in combination. However, care should be taken to remove bias from sample enrichment by using a stably tagged internal standard (Fu et al. 2017).

LC-MS assays combined with immunoaffinity enrichment can provide greater sensitivity and selectivity and are known simply as IA LC-MS assays. Sensitivity can be easily achieved in high-dose non-clinical and clinical studies. Therefore, LBA, LC-MS / MS or IA LC-MS alone provides reliable results. LBA analyzes are highly affected by the biological matrix and also the reagents used must be highly consistent and of high quality. It requires good expertise and has relatively high costs. LC-MS/MS techniques are also affected by the biological matrix, and they can be highly dependent on sample preparation. This drawback is corrected by using immunoaffinity in the sample preparation step and thus high sensitivity/specificity can be achieved. Therefore, in any case, in studies using low concentrations, there is a need for an expert who is erudite about the purpose of the method, biological product and matrix and, has a good command of the technique. In the IA LC-MS technique, instead of the whole molecule, it is possible to perform the analysis by obtaining the signal peptide initially by enzymatic digestion and obtaining this signal peptide directly with immunoaffinity enrichment in the sample preparation stage.

The complexity of biological drugs with neutralizing antibodies such as ADA or soluble receptors in biological matrices and the strength of this complex necessitates the use of an appropriate analytical method for an accurate PK/PD or immunogenicity assessment. For example, LC-MS/MS method is not suitable for determining total and free mAb amount in biological matrix with a high affinity mAb-ADA complex since it uses direct digest method in sample preparation. On the other hand, LBA or IA LC-MS would be a more suitable technique. If the effect from the biological matrix cannot be eliminated in the LBA, or suitable key reagents are not available, the use of IA LC-MS should be considered.

Since there are no guidelines available on which method should be used for particular analysis, the expert's knowledge and experience should be relied upon, but the guidelines of the existing and recently updated (ICH M10 bioanalytical method validation guidelines) for LBA and chromatography based bioanalytical method validation need to be followed (Kaur et al. 2020).

Multi-tier approaches regarding the validation requirement of bioanalytical methods used in PK/PD, toxicology, immunogenicity, toxicokinetic studies from the preclinical stage to the end of Phase III have been discussed for about the last 10 years. The first of the 3 suggested tiers is regulatory validation, the second is scientific validation and the third is research validation. In regulatory validation, all validation parameters recommended by regulatory guidelines are performed as specified. It should be used in pre-clinical studies performed under GLP conditions, phase studies such as pivotal studies and dose determination. Scientific validation studies are recommended to be used in studies such as pre-clinical studies in non-GLP conditions, early-phase clinical trials, and examination of the biological matrix effect in cases where PK is not the primary or secondary outcome. Therefore, although the scope of characterized parameters can be reduced, the acceptance criteria are the same as in regulatory validation. Research validation is recommended to be used in early drug discovery, candidate drug selection, or research-oriented PK/PD studies. Since it is used for research purposes, the number of experimental studies can be reduced and acceptance criteria may be less stringent (Watson et al. 2017).

Regardless of the tiers, it is important to consider the validation requirements and acceptance criteria outlined in ICH M10. This guideline updated in 2019 and contains separate sections for LBA and chromatography based analysis. However, depending on the innovation of the technique, it does not contain any specific information for the analysis performed with the IA LC-MS.

The guideline makes recommendations for the validation of the bioanalytical method developed for the quantitation of both synthetic and biological drug molecules or related metabolites found in biological matrices (e.g., blood, plasma, serum, other body fluids or tissues). According to the guideline, the primary matrice(s) necessarily expect full validation, but this rule is considered as partial validation for secondary matrices. Bioassays and immunogenicity assessments of biomarkers are excluded from this guideline (EMA/CHMP/ICH/172948/2019, 2019). Recommendations regarding the validation characteristics and acceptance criteria of LBA and chromatography-based bioanalytical methods are summarized in the Table 3.

Table 3: *Summary of bioanalytical method validation characteristics recommendations in ICH M10 guideline.*

Parameter	Chromatography-based		LBA	
	Procedure	**Acceptance Criteria**	**Procedure**	**Acceptance Criteria**
Reference Standards	It should be identical with the analyte or established form. In addition, internal standard (IS) (radioisotope marked if MS detection is available) should be used.	A certificate of analysis (CoA). (Not required for IS if suitability of usage demonstrated)	It should be identical with the analyte.	A certificate of analysis (CoA).
Critical Reagents	NA	NA	Reagent performance should be evaluated using the bioanalytical assay. Minor changes can be assesd with a single comparative accuracy and precision studies. Major changes must be assessed with additional validation experiments.	Data sheet containing identity, source, batch/lot number, purity (if applicable), concentration (if applicable) and stability/storage conditions.
Quality Control Samples	QCs should be prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within three times of the LLOQ (low QC), around 30 - 50% of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC).	Should be prepared from separate stock solutions in order to avoid biased estimations.	QCs should be prepared at a minimum of 5 concentration levels within the calibration curve range: the LLOQ, within three times of the LLOQ (low QC), around the geometric mean of the calibration curve range (medium QC), and at least at 75% of the ULOQ (high QC) and at the ULOQ.	QCs should be completely independent from dilution series for the preparation of calibration standard samples.
Selectivity	At least 6 individual sources/lots (non-haemolysed and non-lipaemic). Using blank samples (matrix samples processed without addition of an analyte or IS)	For each matrix; <20% of the analyte response at the LLOQ And <5% of the IS response at the LLOQ	Blank samples obtained from at least 10 individual sources and by spiking the individual blank matrices at the LLOQ and at the high QC level with other "unrelated compounds".	Response; <80% at LLOQ of the individual source Accuracy; ±25% at the LLOQ and ±20% at the high QO level of the nominal concentration in at least 80% of individual sources evaluated.

Specificity	Comparison of the molecular weight of a potential interfering related substance with the analyte and chromatographic separation of the related substance from the analyte.	For each interfering component; <20% of the analyte response at the LLOQ And <5% of the IS response at the LLOQ	Spiking blank matrix samples with related molecules at the maximal concentration(s) of the structurally related molecule anticipated in study samples.	Accuracy; ±15% of nominal value for target analyte in presence of related molecules
Matrix Effect	At least 3 replicates of low and high QCs, each prepared using matrix from at least 6 different sources/lots.	Accuracy; ±15% of nominal value and Precision; <15% in all individual matrix sources/lots.	NA	NA
Calibration Curve and Range	Blank sample, a zero sample (blank spiked with IS) and at least 6 concentration levels including LLOQ and ULOQ.	Accuracy of back-calculated calibration standards; ±20% of nominal concentration at the LLOQ and ±15% within all other levels.	Blank sample and at least 6 concentration levels including LLOQ and ULOQ.	Accuracy of back-calculated calibration standards; ±25% of nominal concentration at the LLOQ and ULOQ and ±20% within all other levels.
Accuracy and Precision	Within-run accuracy: at least 5 replicates at each QC concentration level in each analytical run Between-run accuracy; each QC concentration level in at least 3 analytical runs over at least 2 days.	Accuracy; ±15% of nominal value of each concentration level except LLOQ (±20%) Precision; <15% in all concentration levels except LLOQ (<%20).	At least 3 replicates per run at each QC concentration level (LLOQ, low, medium, high, ULOQ) in at least 6 runs over 2 or more days.	Accuracy; ±20% of nominal value of each concentration level except LLOQ and ULOQ (±25%) Precision; <20% in all concentration levels except LLOQ and ULOQ (<%25).
Carry Over	Analysis of blank samples after the highest calibration standard.	<20% of the analyte response at the LLOQ and <5% of the IS response at the LLOQ	Usually NA	Usually NA
Dilution Integrity	At least 5 replicates per dilution factor in the same matrix from the same species used for preparation of the QCs	Accuracy; ±15% of nominal concentration and Precision; <15%	NA	NA
Dilution Linearity and Hook Effect	NA	NA	3 runs x at least 3 different dilution factors and undiluted spiked matrix with analyte at concentration above ULOQ.	Concentration of each dilution; ±20% of nominal concentration after correction for dilution Precision; <20% for all dilutions of final concentrations.

Stability	A minimum of three stability QCs should be prepared and analysed per concentration level/ storage condition/ timepoint.	The mean concentration of each QC level; ±15% of nominal value of each concentration level	A minimum of three stability QCs should be prepared and analysed per concentration level/ storage condition/ timepoint.	The mean concentration of each QC level; ±20% of nominal value of each concentration level
Reinjection Reproducibility	Replicate measurements of the QCs included in the assessment of precision and accuracy.	Obtained in accuracy and precision	NA	NA
Incurred Sample Analysis	Study samples; • <1000 – 10% • >1000 – 10% of first samples + 5% of exceeded samples	Percent difference; ≤ 20% for at least 2/3 of the repeats.	Study samples; • <1000 – 10% • >1000 – 10% of first samples + 5% of exceeded samples	Percent difference; ≤ 30% for at least 2/3 of the repeats.

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Chapter 19

A JOURNEY FROM PAST TO PRESENT ABOUT HAND HYGIENE

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1. INTRODUCTION

Hand hygiene is the first step in protecting against infectious diseases. Millions of patients are infected every year from health worker-borne infections. In this case, it causes extra diseases, increased resistance of microorganisms, long-term hospital stays and additional costs on the health system. Many infections and their effects can be prevented by hand hygiene within the scope of preventive health services (www.sysmex.com.tr).

Hand hygiene; it is defined as washing hands with water and soap to protect the deep and removing temporary microorganisms from the hands, or rubbing hands using alcohol-based hand antiseptic without water (Engdaw, et al., 2019). The aim is to remove all temporary bacteria found on the hands and some of the persistent bacteria from the hands.

Hand washing is important for the health of the person first and fore in daily life, while it also becomes important for the health of other people in the working environment. In many business sectors, especially in the health sector, disruptions in the hand washing of employees can quickly lead to serious problems that threaten the society. In health institutions, in addition to reducing occupational risks by applying employees' hand cleaning "adequately and correctly", control of hospital infections can be achieved with the most effective and inexpensive method (Dokuzoğuz, 2003). Washing hands prevents the spread of infections and antimicrobial-resistant pathogens (Gürel and Taşçı, 2020).

2. HAND HYGIENE

Ignaz Philipp Semmelweis (1818-1865), who emphasized the vital importance of the hand washing habits mentioned frequently today, has been involved in the medical literature since he was a pioneer of antiseptic procedures after his education at the University of Vienna. Semmelweis, also known as the "savior of mothers", discovered the relationship between the insidy of lohusay fever (albasis) and hand washing habits in maternity clinics.

Lohusaism fever was common in hospitals in the mid-19th century and was fatal among 10% to 35%. Semmelweis immediately launched a strict hand washing practice at the Vienna General Hospital in 1847; In this application, before the examination of pregnant women, the hands were sterilized with water containing chlorinated lime. The results were stunning: the mortality rate had dropped from 18.27 percent in April to 0.19 percent at the end of the year. But despite his books showing that handwashing reduced the mortality rate to below 1 percent, Semmelweis's observations contradicted the established scientific and medical views of the time, and his ideas were rejected by the medical community at the

time. “The purpose of my teachings is to end the fears in obstetric hospitals and to save the wives of husbands, the mothers of children” (Ignaz Semmelweis).

With Ignaz Philipp Semmelweis unable to provide any acceptable clear scientific explanation for his findings, some doctors find Semmelweis’ suggestion of washing hands for patient health humiliating and disturbing. Semmelwis, who began having nervous breakdowns in 1865 as a result of the increasingly intense reaction of his colleagues, is being confined to a mental institution by a friend. Four days after his hospital visit, Semmelweis, 47, who was beaten by guards, died of a wound in his right hand thought to be caused by gangrene after a beating.

Semmelweis’ practice is confirmed by Louis Pasteur after his death with germ theory, and his practices are widely accepted over several years. Joseph Lister, who influenced Pasteur’s research around the same time, is a name (www.britannica.com).

The World Health Organization has declared May 5 as World Hand Hygiene Day since 2009 to draw attention to the importance of hand hygiene, especially in health care. This year, awareness of hand hygiene has also increased as it coincides with the COVID-19 pandemic. Hand cleaning is the most effective method of preventing infectious diseases. There are 150 types of bacteria in the hands. Since we take our hands to our mouths, noses and eyes about 25 times an hour, hands have a very important place in the passage of diseases. The number of cases of diarrhea with hand washing only decreases by 50% and the number of respiratory infections decreases by 25%.

3. HAND HYGIENE IN INFECTIOUS DISEASES

Infection; it is the reproduction of germs, viruses, parasites, etc. that cause disease in the organism by entering the body. The immune system can prevent the occurrence of most infections. But diseases occur and develop if they have difficulty with rapidly multiplying infections. Common symptoms include fever, nausea, vomiting and muscle pain. Your infection; There are four types: viral, bacterial, fungal and parasitic.

The sum of each of the steps that make up the process of infection formation is called the chain of infection. In order for an infectious disease to occur, they must coexerce. The elements that make up the chain of infection are:

Infection Microbe: It is pathogenic microorganism(s), which is called bacteria, viruses, fungi, parasites. The higher the number of microorganisms, the more likely they are to get sick. The ability of microorganisms to form diseases is called viruleans. Some microorganisms are very virulant.

Source of Infection (reservoir): It is the environment in which microorganisms live and multiply naturally. Human, animal or inanimate objects (water, soil, etc.).

Portal of Exit: These are the places where microorganisms leave the source of infection. In humans; respiratory system, discharge system, digestive system and skin integrity are disturbed. Example: they come out of these doors through phlegm, saliva, blood, urine, feces, selar, vagen secretion, tears, etc.

Modes of Transmission (spreading ways, exit from source): Microorganisms are transported from place to place in various ways. Ways of transmission: there may be contaminated water and nutrients, or in contaminated items; example, scissors, thas, hats, clothes, etc. Microorganisms can also be transmitted by air, dust particles, a portable or vector.

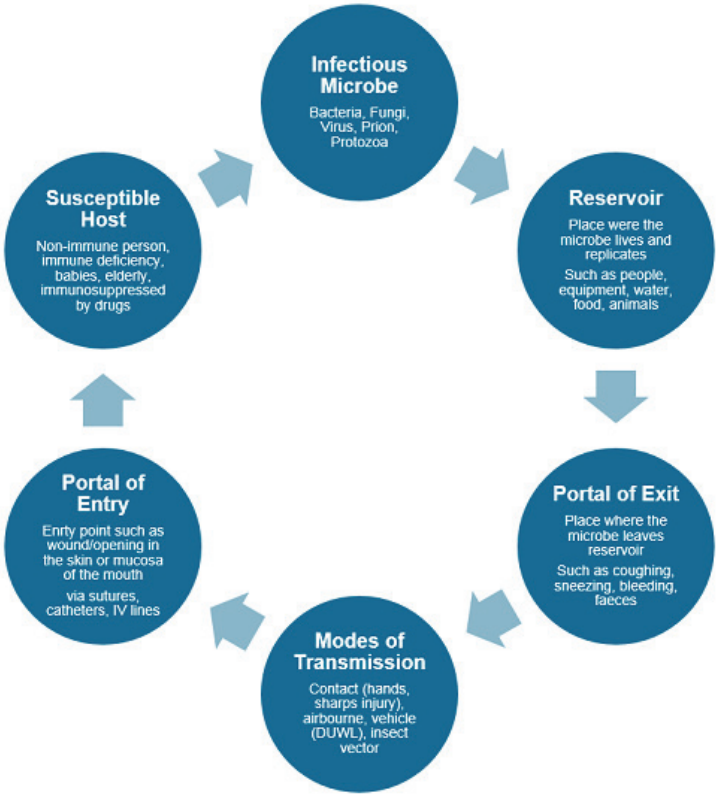
Vector: Some animals of flies, lice, ticks, mice, etc., carry some diseases, they are called vectors. For example, the malaria microbial completes its development in the female mosquito called anopel, making a disease by going into man with the fly stinging the human. In order for the disease to occur in human beings, the malaria microbial must complete the stage of development by staying in the mosquito's body for a while. That's why it doesn't get from person to person.

Carrier: If a human or animal carries a microorganism in its body and can infect other people without any symptoms of disease, these persons are called porter. The most common are Hepatitis A virus (HAV) carriers. These people should never work in the food industry.

Portal of Entry: They are places where microorganisms enter the body. Usually the entrance door and the exit door are the same: mouth, nose, ear, eye, blood vessels, damaged skin, etc. (It cannot enter microorganisms from intact skin).

Susceptible Host: The person (or animal, plant) that harbors the microorganism on or inside its body. Especially the body resistance is decreased; People with chronic diseases (such as chronic kidney disease, COPD, cancer, etc.), malnutrition, excessive fatigue, alcohol and cigarette addiction, and inadequate immune system, are suitable hosts for microorganisms**.**

Figure1: *Chain of Infection (https://learnpac.co.uk/lessons/chain-of-infectio)*



Contact with patients' blood and other bodily fluids can cause infectious diseases. In addition, droplets hang in the air in the environment where the patient breathes; And you breathe this air. The diseases that may occur due to these reasons and their symptoms are:

Hepatitis B: Fever, exhaustity, loss of appetite, nausea, muscle and joint pain.

Hepatitis C: Feeling tired, high fever, nausea, loss of appetite, abdominal pain.

HIV: Fever, redness of the skin, headache, wounds to the mouth and genital organs. Doctors, nurses and cleaners are at risk.

Tuberculosis (Tuberculosis): Fever, cough, chest pain, loss of appetite, weight loss and night sweats.

Swine flu: Fever, sore throat, cold, body aore.

Chickenpox: Runny nose, fever, weakness, redness of the skin.

Rubella: Headache, runny nose, inflamed eyes, rash on the skin.

Regular washing of hands and use of protective equipment by health personnel are among the measures to be taken against the spread of infectious disease inside and outside the health institution. Proper hand washing has an important role to play in reducing the insidy of both societal and hospital infections. The standard rules prepared by national and international infection protection and control organizations in this area confirm that hand washing alone is the most important procedure for the prevention of infections. Breaking the chain we call the transmission ring in the fight against infections, not being a part of the chain is the most important issue. Attention to hand hygiene ensures that the infection end on the transmission path in the 4th link of the chain (www.webhatti.com)

There are three types of hand washing; social hand washing, hygienic hand washing, surgical hand washing. To draw attention to this issue, October 15th has been declared "Global Hand Washing Day" since 2008.

3.1. Social Hand Washing :

The correct technique applied to clean hands consists of the following stages:

1. Accessories such as rings and watches are removed before hand washing.

2. Hands are soaked under running water.

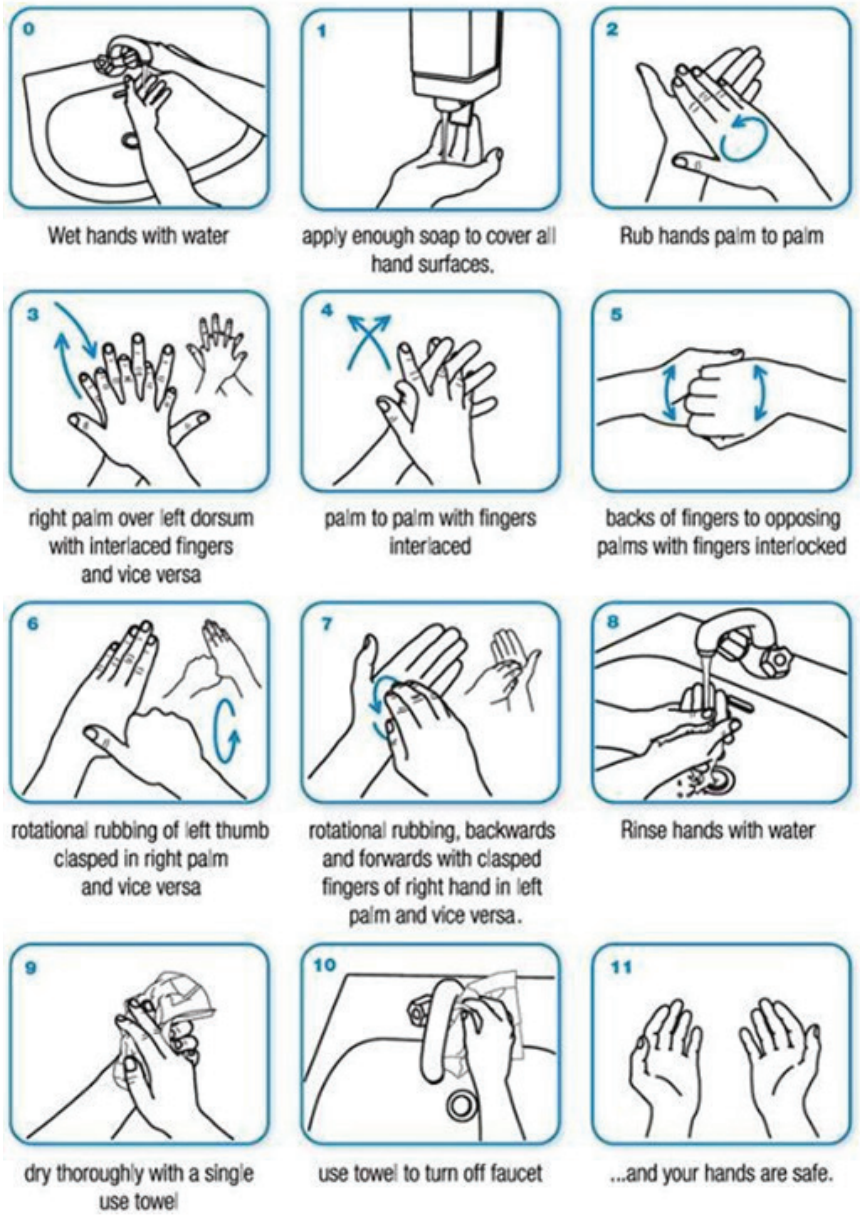
3. The wrists, palms, back and finger areas of the hands and the edges and ends of the nails are foamed with soap and rubbed vigoruly for at least 20 seconds.

4. Hands are thoroughly rinsed under water.

5. Hands are dry with paper towels starting from the wrists.

6. The faucet is turned off with the same paper towel.

Figure 2: *How Should We Wash Our Hands? (T.R. Ministry of Health)*



There is no need to use soap containing antibacterials when cleaning hands, normal soap is enough. Washing hands with water and soap in sufficient time, with the right technique (with soap and water touching all over the hand) is important to increase protection (covid19.saglik.gov.tr).

3.2. Hygienic (using antiseptic) Hand Washing:

Hand cleaning using antiseptics when hands cannot be washed and when there is visible or invisible contamination. The aim is to remove all temporary and partially permanent flora available. Alcohol-containing hand antiseptics are available in various formulations such as liquid, foam, and gel. Alcohols are more effective than soap / antimicrobial soap in healthcare workers' hand hygiene. It shows the highest effect at 60-90% concentration. The application time varies between 20 seconds and one minute depending on the purpose. The amount should be enough to wet the whole hand. In other words, at least 3-5 mL should be used (Çiçek, et al., 2014).

3.2.1. Usage of Antiseptic Solution:

1. Take a sufficient amount of at least 3 mL of hand sanitizer in your hand.
2. Alcoholic antiseptic hand rub your hands.
3. Rub the back of your left hand with your right hand and the back of your right hand with your left hand.
4. Rub the palms clean between the fingers.
5. Clean the backs of the fingers by rubbing them into the palm of the other hand.
6. Rub both hands, the thumb of your other hand.
7. Do not clean the nail tips by rubbing them on the palm.
8. Without drying your hand with a towel, rub it for 20-30 seconds until it dries on its own, do not rinse with water again.

Hand disinfection should not completely replace hand washing. When there is visible contamination on the hands, they should be washed with soap and water.

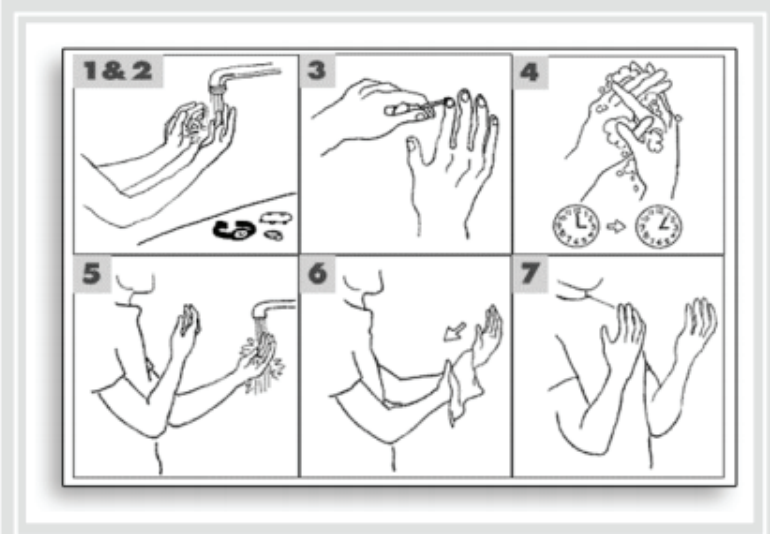
Factors Affectıng The Selectıon of Hand Hygıene Products: People who choose products for hand hygiene should consider the antimicrobial effectiveness of the product to be selected and whether the users will accept the product or not. The smell, content and color of the product affect its acceptability. Since hand washing-scrubbing will be applied approximately 30 times during a shift, the skin irritation and dryness feature of the product to be used should be taken into account. For example; The skin-drying effect of alcohols is the most common cause of incompatibility reported by healthcare professionals. With moisturizers added to alcohol, this unwanted effect was prevented and it was shown in many studies that it was accepted by healthcare professionals. The time taken for alcohols to

dry is also one of the factors cited for incompatibility. Considering that alcohols are flammable and volatile, storage and storage should be done under appropriate conditions (Boyce, 2002). Today, alcohol-based hand antiseptics are the most effective antiseptics used for this purpose on microorganisms. The World Health Organization recommends alcohol-based hand sanitizer for the following reasons: It is fast-acting, has a broad spectrum, has excellent microbicidal properties, does not develop resistance, can be used in areas where there is no sink, clean water and towels, increases compliance with hand hygiene during application, less than hand washing It can be done in time and reduce the cost.

3.3. Surgical Hand Washing

The concept of washing hands with an antiseptic agent emerged at the end of the 1800s when Lister encouraged them to wash hands with carbolic acid before surgical procedures, and has continued to be effective in antisepsis practices of doctors and nurses / midwives until today (Lundy, 2009). The aim of hand washing is to reduce the number of bacteria as much as possible before surgery (www.hider.org.tr). In surgical hand washing, soaps containing 4% chlorhexidine or 7.5% povidone iodine are generally preferred (Liu, 2016). Povidone iodine and chlorhexidine show similar efficacy in reducing the number of bacteria. While both agents reduce the bacterial count by 70-80% in the first use, this rate increases up to 99% as a result of repeated application. Although it is stated that it causes more allergic reactions in operating rooms, traditionally soaps containing povidone iodine are preferred more than chlorhexidine. In addition to these solutions, triclosan, chlorhexidine gluconate, iodophor or plain soaps can be used for antisepsis in operating rooms (Widmer,2013).

Figure 3: *Surgial Hand Washing (www.engenderhealth.org)*



The procedure steps that should be applied by healthcare personnel for surgical hand washing are as follows:

1. Rings, watches, bracelets and other hand jewelery should be removed.

2. Surgical mask should be worn during the washing process.

3. If there is visible contamination, hands and arms should be washed with soap in a circular motion from bottom to top.

4. Under running water, under the nails should be cleaned with nail cleaner.

5. Hands and arms should be rinsed in running water.

6. It should be applied from the approved antimicrobial product according to the company instructions.

7. Apply to wet hands and hands with a soft, non-abrasive sponge.

8. Keeping hands upright, all skin surfaces should be washed with a sponge for 3-5 minutes according to the company instructions. There is no difference in efficacy for washing longer than five minutes.

9. To save water, if possible, the faucet should be turned off when not in use.

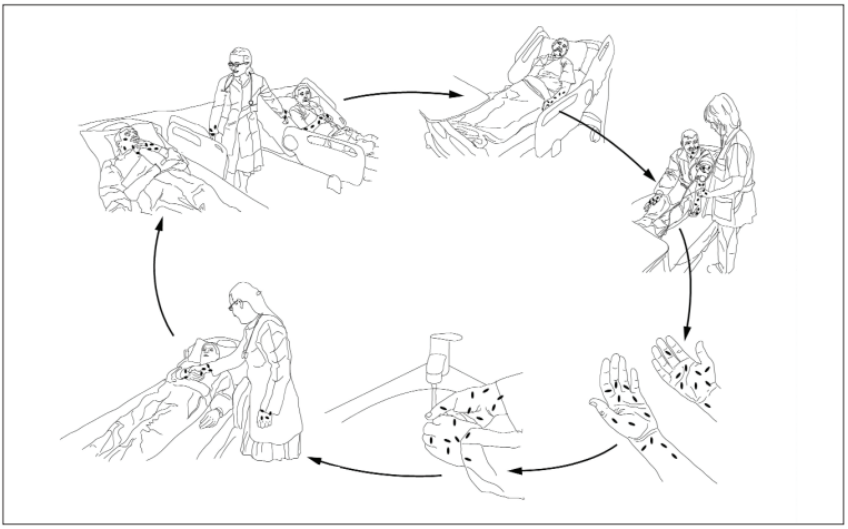
10. Splashing water on clothes should be avoided.

11. The used sponges should be disposed of properly.

12. Rinse hands and arms from fingertips towards elbows under running water, keeping hands up.

13. Before wearing sterile gowns and gloves, hands and arms should be dried with a sterile towel.

Figure 4: *Carrying Pathogenic Microorganisms Through the Hands of Healthcare Professionals (Pittet, et al., 2006)*



Hand washing habits; 52% for women and 48% for men. Hand washing habits according to age; 22% among young people, 38% between 35-54 years old, 38% among those over 55 years old. Handwashing habits disappear in very crowded places, at unsuitable times, in places without a toilet, in toilets without soap.

3.4. Situations Requiring Washing of Hands:

Critical situations in daily and health activity

- Before using the toilet, after going out
- Before putting on gloves, after removing gloves
- Before starting work, after finishing work and during job changes
- Before touching cooked foods
- After handling spoiled food and garbage
- As I start preparing food
- Before and after eating
- After touching dirty materials
- After handling raw and dirty foods
- After changing clothes
- After touching the hair, mouth and nose
- After cleaning

- After nasal cleaning
- After sneezing, coughing
- After touching the wounds and pimples
- After smoking
- After touching body wastes such as feces, urine, blood
- Money, newspaper, pen, etc. after touching things
- After handling all the animals
- After getting on public transport, going to a public place including markets and places of worship
- After touching the garbage in the house
- Before and after infected wound contact
- Before after blood or body fluid contact
- Before dressing after
- After care for the infected person

3.5. Nail Care and Hygiene

Many microbes can easily settle and reproduce under the nail tips. For this reason, cleaning the nail base is very important. The fingernails and toenails should be cut short and round once a week. In addition, the personalization of the tools used in nail cutting and care plays an important role in the prevention of some infectious diseases. Nail polishes, nail polishes and nail polish removers should not be used whenever possible. Because, these substances cause dryness of the nails and splitting and breaking of the nail in layers. Hands and nails should be prevented from drying by lubricating them with a good hand lotion (docs.google.com).

3.6. Hand Washing Rate of Countries

According to the report of Kamuran Samur from Euronews, Gallup International created hygiene maps of 63 countries in its 2015 survey. According to the research, the country with the highest rate of people who wash their hands with soap after toilet is Saudi Arabia. Turkey ranks the ranks 6th among 63 countries. The survey results, the Balkans and Turkey shows that high compared to other European countries the hand washing habit.

Bosnia and Herzegovina is at the top of the list with 96% in the list of countries that wash their hands with soap the most after toilet use. Bosnia and Herzegovina is watching at a rate of 94% in Turkey and Kosovo 85’l%. Among 63 countries, the country with the lowest rate of hand washing after

the toilet is China with 23%, Japan with 30% and South Korea with 39%. In the Netherlands, this rate is seen to be 50%. It is noteworthy that the Dutch habit of washing their hands after toilet is less than other European countries. Other European countries where the habit of washing hands after toilet is weak are Italy with 57%, and Spain, France and Russia with just over 60%.

4. CONCLUSION

Why is hand washing so important? Studies show that poorly washed hands are effective in the spread of infectious diseases and the occurrence of food poisoning. As a result of the investigation of the causes of hospital infections, food-borne disease epidemics and food poisoning, one of the most important reasons is the inadequate hand cleaning of the staff. For this reason, acquiring the right hand washing habits in accordance with the rules will be effective in controlling infectious diseases and preventing food poisoning as well as increasing the quality of life in the society.

We must not forget that the virus is transmitted by respiratory droplets, and in addition, these droplets can survive for hours and even days on different surfaces contaminated with them. Our top priority in all infectious diseases should be hygiene. We need to pay maximum attention to both our personal hygiene and the hygiene of the areas we live in. The most important step in protecting ourselves and the people around us is personal hygiene. Washing hands is the basis of this step. You should wash your hands for at least 20 seconds by rubbing them thoroughly, rinse them thoroughly, dry with a clean towel that you use only, and this process should be repeated frequently during the day. In the absence of water and soap, you can use alcohol-based cleaners. The important thing is not to touch the mouth, nose, eyes and face without cleaning your hands. In the rules we pay attention in personal hygiene, we should behave considering both our own health and the health of the people around us.

Against Covid-19, which has recently become a global epidemic, experts also recommend washing hands, one of the most effective protection methods of modern medicine. One of the effective protection methods known to Covid-19, which spreads to 220 countries or regions, is hand hygiene. Experts state that washing hands many times during the day for at least 20 seconds and keeping them constantly clean is the most effective method against coronavirus.

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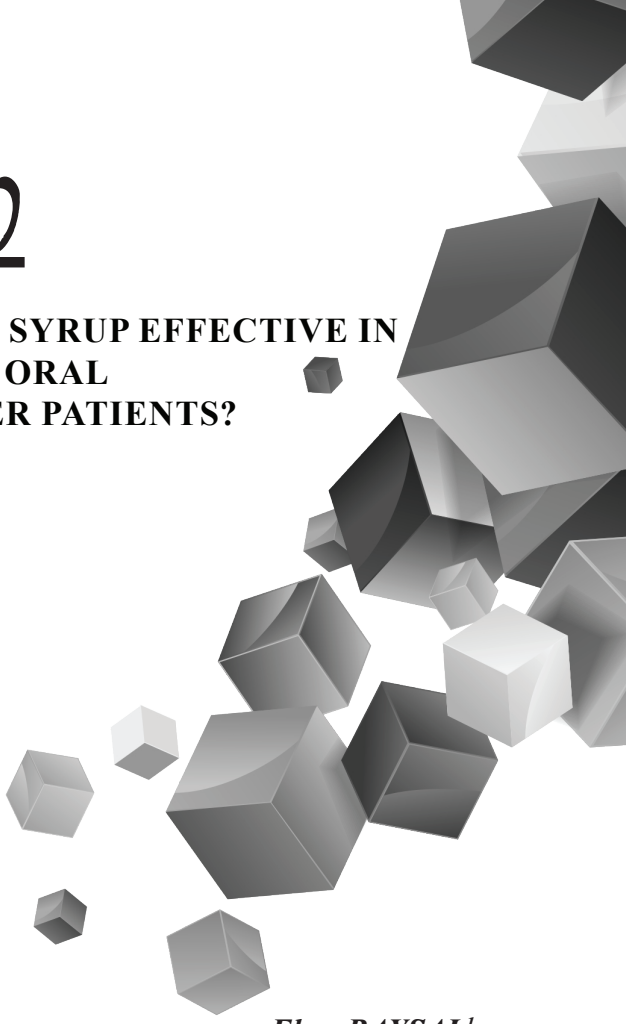
Chapter 20

IS BLACK MULBERRY SYRUP EFFECTIVE IN THE PREVENTION OF ORAL MUCOSITIS IN CANCER PATIENTS?



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INTRODUCTION

Mucositis is a common toxicity of chemo-radiotherapy characterized by the ulceration of oral mucosa causing pain and dysphagia ((Raber-Durlacher, 1999; Bensinger et al., 2008; Sonis, 2009)). Oral mucosal epithelial cells regenerate every 7-14 days (Naidu et al., 2004). Chemotherapy (CT) agents may prevent the maturation of epithelial cells by changing its regeneration cycle and causing cell death. Therefore, oral mucositis (OM) generally begins 5 to 7 days following the initiation of CT and lasts approximately 7 to 10 days (D'Hondt Lionel et al., 2006). According to the results of several studies in the literature, the incidence of OM ranges from 80% to 97% depending upon the type of radiotherapy received and the concomitant use of chemotherapy (Raber-Durlacher, 1999; Bensinger et al., 2008; Sonis, 2009) and it is 35-100% in patients undergoing hematopoietic stem cell transplantation (Carulli et al., 2013) while it is 5-15% in patients receiving standard dose chemotherapy (Rubenstein et al., 2004). OM adversely affects a patient's oral functions as speaking, eating and swallowing. Therefore, it may develop life-threatening infections and lead to increase in total parenteral nutrition (TPN) and opioid analgesic use as well as in the duration of patient's hospital stay and their health care costs and thus, patients' quality of life decreases (Owlia et al., 2012; Carulli et al., 2013; Batlle et al., 2014; Wang et al., 2015) Although there are several methods that have been used for the prevention and treatment of OM, there is no accepted standard approach (Stone et al., 2005). Developed by the specialists in the Multinational Association for Support and Care in Cancer (MASCC) and the International Association of Oral Oncology (ISOO) for the prevention and treatment of OM, oral care protocols in the clinical practices guideline are suggested to be used (level of evidence II) (Lalla et al., 2014). The guideline includes different recommendations for the prevention of OM in the patients undergoing radiotherapy and/or receiving standard dose or high dose chemotherapy as well as those undergoing chemotherapy with radiotherapy. According to the guideline, recombinant Human Keratinocyte Growth Factor-1 (KGF-1/palifermin) (level of evidence II), low-level laser therapy (level of evidence II), cryotherapy, and benzydamine mouthwash are recommended for the prevention of oral mucositis (level of evidence I). Cancer patients often use complementary and alternative medicine (CAM) along with medical treatment in the prevention and treatment of OM (Yurdakul & Esenay, 2019). CAM usage is reported to be 10.9–87.6% around the World (Italia et al., 2014). The recent studies carried out on cancer patients have reported that black mulberry (Morus nigra) syrup prevents the occurrence of tenderness and dysphagia in the gums, delays and reduces the appearance of severity of OM (Çubukçu & Çınar, 2012; Alışarlı, 2017; Demir Doğan et al., 2017; Harman et al., 2019). Because of the

components in its content, Black mulberry shows a strong antifungal and antimicrobial activity. Black mulberry is used in the treatment of tonsillar, oral and dental wounds, particularly in the treatment of candida infections, known as thrush in children (Yiğit et al, 2007). It is estimated that OM related complications and health care costs may be reduced once the health professionals use evidence-based CAM practices and guide patients. Therefore, this literature review was conducted aiming to determine the efficacy of black mulberry syrup in the prevention of OM in the cancer patients.

MATERIALS AND METHODS

ELIGIBILITY CRITERIA

This study included experimental clinical trials regarding the use of black mulberry for oral mucositis prevention in cancer patients of any age, receiving chemotherapy and/or radiotherapy treatment. Eligibility criteria: Only full-text clinical studies published either in Turkish or in English were selected for this literature review. Studies on animals and irrelevant studies were excluded.

STUDY SELECTION

In this literature review, in accordance with the eligibility criteria established by the researchers, four research studies published between September 2009 and December 2019.

Online searches were performed using “Ebscohost”, “Pubmed”, “Science Direct”, “Scopus”, “ProQuest”, Google Scholar” and Turkey National Thesis Center. Search strategies were created according to the following MeSH terms: black mulberry-Morus nigra L.”, “oral mucositisstomatitis” and “cancer patients”.

The necessary information about the studies including the titles, abstracts and sections of the researches were first assessed by the researchers in the initial stage of the search in accordance with the eligibility criteria. The researches that fail to meet the eligibility criteria and duplicate researches were excluded from the study. Only full-text researches that meet the eligibility criteria were included in the study. A standard form was developed for each of the studies decided to be included in the literature review, which includes information about the author, the year and country of the study, as well as the information about the sample and intervention, the OM assessment scale, endpoints and main results. In line with this form, the studies were read and evaluated independently by two researchers. Finally, the studies evaluated by both researchers were combined in a single form.

RESULTS

Of the 188 studies found, 182 were excluded because they did not meet the inclusion criteria. The reasons for excluding these researches are as follows: (i) they were not related to the prevention of OM in cancer patients, (ii) they were not full text researches, (iii) there was no clinical research and (iv) the language of publication was not English or Turkish. As a result, only six full-text researches meeting the above stated criteria were included in this study (Figure 1). The studies on the use of black mulberry syrup in the prevention of OM in cancer patients are summarized in Table 1. Only two of the studies included in the literature review was a randomized controlled clinical trial and the other four were non-randomized controlled trials. The study samples of the studies are adults (n= 4) and pediatric patients (n= 2), undergoing chemotherapy for various types of cancer, adult patients receiving RT for head and neck cancer (HNC) (n= 1), and adult patients receiving autologous or allogeneic stem cell transplantation (n= 1). When the clinical trials were analyzed, it was determined that black mulberry syrup was compared with standard oral care, calcium and phosphate solution, chlorhexidine gluconate, Hyaluronic acid (Gelclair), chewing gum, benzydamine hydrochloride and sodium bicarbonate mouthwash.

It was also found that while four of the studies used the WHO Oral Toxicity Scale in order to assess the severity of OM in the patients and the other two studies used two different scales; one used the National Cancer Institute Common Terminology Criteria for Adverse Event version 4 (NCI-CTCAE), and the other used Children's International Mucositis Assessment Scale (ChIMES). In all of the studies in the literature review, it was determined that black mulberry syrup is effective in preventing OM in patients.

Figure 1: Flow Chart

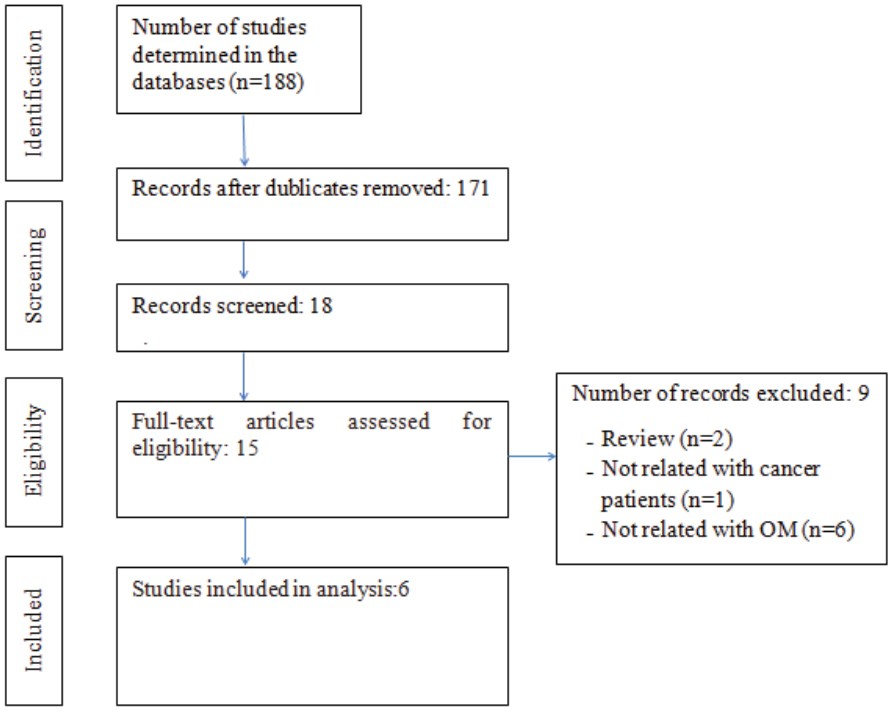

Table 1. Summary of Clinical Trials on Black Mulberry and Oral Mucositis

Author/Year/ Country	Sample (Intervention and Control Group)	Oral Mucositis Assessment Tool	Endpoints	Main Results
Çubukçu & Çınar, 2012, Non-randomised controlled trial, Turkey	90 adult patients receiving chemotherapy for various cancer diagnoses Group 1: 30 patients gargling with benzydamine hydrochloride (5 ml, 4 times a day) Group 2: 30 patients gargling with sodium bicarbonate (5 ml, 4 times a day) Group 3: 30 patients gargling with with black mulberry syrup 5 ml (the patients were required to gargle and swallow 4 times a day)	- World Health Organization Mucositis Assessment Scale	- OM incidence and grade - Oral pain assessment score (Visual Analog Scale) - Symptoms related to cancer treatment with the Rotterdam Symptom Checklist (physical discomfort, psychological discomfort, activity level and quality of life). - Oral problems (sore throat and dry mouth)	-Lower incidence and grade of OM in black mulberry group. Total OM incidence of patients: Group 1: %56.7 Group 2: %36.7 Group 3: %26.7 -Less sore throat and dry mouth were seen in the group 3. -Group 2 were found to experience more physical discomfort.

Doğan et al., 2017 Randomized controlled trial, Turkey	80 adult patients with head and neck cancer Group 1: 38 patients gargling with black mulberry molasses (took it in their mouths for 1-3 minutes and swallowed it 3 times a day: 15–20 min before RT, 15–20min following RT, and again, 6 h later) Group 2: 42 patients gargling with Nystatin (3 times a day)	- Common Terminology Criteria for Adverse Events, (CTCAE) version 4.0 - Oral Assessment Guide (OAG)	- OM incidence and grade - Oral pain assessment score - QoL	-Lower incidence and grades of OM in black mulberry group. Total OM incidence of patients: unknown Grade 3 OM incidence of patients: Group 1: %8.3 Group 2: %41.5 -Lower average pain assessment score in black mulberry group. -There were no differences between the quality of life scores.
Alışarlı, 2017, Non-randomised controlled trial, Turkey	69 pediatric patients receiving chemotherapy for various cancer diagnoses Group 1: Oral care with sodium bicarbonate and black mulberry lollipop (4 times a day, black mulberry lollipop made from syrup and frozen in 0° C) Group 2: Oral care with sodium bicarbonate (4 times a day)	- Children¨s International Mucositis Evaluation Scale (ChIMES)	- OM incidence and grade - Oral problems (i.e., swallowing, drinking, eating, mouth and throat pain)	-Lower incidence and grades of OM in black mulberry group. Total OM incidence of patients: Group 1: %28.6 Group 2: %61.8 -Less oral problems in black mulberry group.
Albayrak, 2019 Non-randomised controlled trial, Turkey	82 pediatric patients receiving chemotherapy for various cancer diagnoses Group 1: 36 patients with the black mulberry syrup (gargling and swallowing 4 times a day in addition to routine oral care) Group 2: 10 patients chewing gum in addition to routine oral care. Group 3: 36 patients with standart oral care.	- World Health Organization Mucositis Assessment Scale - Children¨s International Mucositis Evaluation Scale (ChIMES)	- OM grade and OM duration - OM pain	-Group 1 had a lower median score value on the Oral Mucositis Assessment Scale and the ChIMES. -Group 1 had lower pain and OM begins later than other groups.

Çullu, 2019 Randomised controlled trial, Turkey	106 adult patients receiving chemotherapy for digestive system cancer Group 1: 36 patients with the black mulberry syrup (gargling and swallowing 4 times a day in addition to routine oral care) Group 2: 36 patients with the Hyaluronic acid (Gelclair) (gargling and spitting out 4 times a day) Group 3: 34 patients with standart oral care.	- World Health Organization Mucositis Assessment Scale	- OM incidence and OM grade - Life Quality Scale in Patients with Oropharyngeal Mucositis (QMQoL)	-Lower incidence and OM grades in group 1. Total OM incidence of patients: (7^{th} day); Group 1: %13.9 Group 2: %27.8 Group 3: %50 (14^{th} day); Group 1: %0 Group 2: %5.6 Group 3: %8.8 (21^{th} day); Group 1: %13.9 Group 2: %27.8 Group 3: %47.1 - Less pain and higher score in QMQoL seen in the group 1.
Harman et al., 2019, Non-randomised controlled trial, Turkey	83 adult patients undergoing stem cell transplantation (autologous or allogenic) Group 1: 30 patients with chlorhexidine gluconate and benzydamine hydrochloride (gargling two minutes and spitting out 4 times a day) Group 2: 28 patients with the calcium and phosphate solution (gargling and spitting out 4 times a day) Group 3: 25 patients with the black mulberry syrup (gargling and swallowing 4 times a day)	- World Health Organization Mucositis Assessment Scale	- OM incidence and OM grade	-Lower incidence of OM in the group 1 and group 2. Total OM incidence of patients (14^{th} day): Group 1: %53.4 Group 2: %42.9 Group 3: %48 -Grade 2 and 3 OM was less common in the group 1 and group 2 but the difference was not statistically significant.

DISCUSSION

When the literature was reviewed, it was observed that there were only 6 studies on the effectiveness black mulberry syrup on the prevention of OM in cancer patients and all of them were carried out in Turkey. Black mulberry is a fruit commonly used in various health problems in our country. Syrup originally made from the fruit is used as a mouthwash in mouth and throat related diseases. Compounds of Black Mulberry including Papyriflavonal A, curaridine, saphoraflavanone D and sophora isoflavonone A, show a strong antifungal and antimicrobial activity. In addition, its 2-arylbenzofuran compound also shows an antimicrobial effect on methicillin-resistant staphylococci (Yiğit et al, 2007). In all of the studies reviewed in this study, it was observed that black mulberry was compared with various oral care methods and that it was found to have positive effects in the prevention and severity of OM. Although there are a limited number of studies investigating the effectiveness of black mulberry syrup in the prevention of OM in cancer patients, it is known that cancer patients frequently use black mulberry syrup in our country in order to prevent OM. In the study conducted by Yurdakul and Esenay (2019), the authors aimed to find out the CAM methods used by the parents for the prevention and treatment of OM in pediatric cancer patients and they found that 41.5% of the participants used black mulberry syrup (Yurdakul & Esenay, 2019). OM causes mouth-throat pain, eating and swallowing difficulties and thus, adversely affects the patients' quality of life (Owlia et al., 2012; Carulli et al., 2013; Batlle et al., 2014; Wang et al., 2015). Only in the study carried by Doğan et al. (2017), it was found that the quality of life of the patients was assessed and the quality of life of the patients, who were treated with black mulberry syrup were found significantly higher (16). In some of the studies, mouth-throat pain and dysphagia were evaluated and black mulberry syrup was found to reduce such complaints (Çubukçu & Çınar, 2012; Alışarlı, 2017; Demir Doğan et al., 2017; Albayrak, 2019; Çullu, 2019). It is estimated that the antimicrobial and antifungal effect of black mulberry syrup is effective in preventing and reducing the severity of OM, as well as reducing dry mouth and mouth-throat pain. In Bayındır's (2018) doctoral dissertation conducted with COPD patients, it was found that the OM incidence was 70.0% in patients receiving black mulberry syrup and 95.0% in patients in the control group. It was found that the mean OM duration of the patients in the treatment group was 9.1 ± 2.5 days, while it was 12.1 ± 1.4 days for the patients in the control group. In addition, patients receiving black mulberry syrup were found to experience less pain and less eating difficulties (Bayındır, 2018). In this respect, it can be concluded that black mulberry syrup can also be effective in the prevention and secondary effects of OM in other patients not receiving cancer treatment, as well. In the studies reviewed in the literature, it was found that incidence of OM was different at 26.7%-48% in patients receiving black mulberry syrup, and the highest incidence of

OM was observed in the study of Harman et al. (2019) (17). The incidence of OM varies depending on the treatment protocol used, the type of disease, and some individual characteristics of the patients (Carulli et al., 2013). Harman et al. (2019) carried out their study with patients undergoing hematopoietic stem cell transplantation. Due to the use of high-doses chemotherapy agents during conditioning regimens, the incidence of OM is more common in this patient group than in patients receiving standard-dose CT (Stiff et al., 2006). In this sense, it is not surprising that the incidence of OM was found high is in Harman et al. Children and elderly individuals are considered to be at risk for developing OM. It is agreed that OM occurs more frequently in very young patients due to the high rate of epithelial division (Laheij & de Soet, 2014). Depending on the type of cancer and treatment regimen, 80% of children receiving chemotherapy have various degrees of OM (Cheng et al., 2004). In this sense, in the study carried out by Alışarlı et al. (2017), the incidence of OM was expected to be higher. In the study, black mulberry syrup was put into molds and frozen in the refrigerator and given to children in the form of lollipops (Alışarlı, 2017). It was estimated that in addition to the effect of black mulberry syrup, cold application of lollipops might have an positive effect on the incidence of OM. Ice reduces the exposure of the oral mucosa to toxic agents, causing vasoconstriction of the vessels in the oral mucosa (Lilleby et al., 2006; Mori et al., 2008). There have been previous studies indicating that the frequency, severity and duration of OM were significantly reduced as a result of the application of ice and cold water (Lilleby et al., 2006; Chen et al., 2017; Marchesi et al., 2017). In the study of Doğan et al. (2017), although the overall incidence of OM was not known, it was reported that 8.3% of the patients in the experimental group and 41.5% of the patients in the control group developed grade 3 Mucositis (16). Grade 3-4 mucositis is 37% in the patients receiving standard radiotherapy and 43% the in patients receiving chemotherapy along with radiotherapy (Trotti et al., 2003). In this respect, it can be stated that black mulberry is also effective in patients receiving chemotherapy and/with radiotherapy. This literature review has some limitations. The results cannot be generalized because of the limited number of studies on the effectiveness of black mulberry syrup in the prevention of OM in cancer patients and because these studies were carried out with different sample groups, using treatment protocols. Since black mulberry syrup is cost effective method and has no side effects, clinical trials should be continued to search for the effectiveness of black mulberry syrup on such diseases. It necessary to carry out evidence-based research to prevent the misuse of black mulberry syrup and other CAM methods by patients. In order to determine the effect of black mulberry syrup on the incidence and severity of OM, it is highly recommended that randomized controlled trials with homogenous sample groups should be carried out.

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