

Current Researches in Health Sciences

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Health Sciences

Editors

**Prof. Dr. Nizami Duran
Prof. Dr. Halit Demir**

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web: www.ivpe.me

Tel. +382 41 234 709

e-mail: office@ivpe.me

Ivpe Cetinje, Montenegro

PREFACE

The book “Current Researches in Health Sciences” is serving an academic forum for both academics and researchers working in such fields. Health sciences research is an interdisciplinary by nature. So it covers several fields such as dentistry, veterinary medicine, medical sciences. In this book, the academics working in different fields share their results with the scientific community. Thus more researchers will be aware of these studies and have some new ideas for their future studies. The selected articles have been reviewed and approved for publication by referees. It is hoped that the book will be of interest and of value to academics and researchers.

We would like to take this opportunity to thank all our colleagues and writers for their efforts.

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REFEREE BOARD

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CHAPTER I
**ACTIVITIES FOR TEACHING ILLUSTRATED CHILDREN'S
BOOKS THROUGH CREATIVE DRAMA METHOD: THE
FRIENDS OF THE PAINTERS**

Ayşegül Oğuz Namdar & Esra Meryem Seven**
Aslıhan Alyıldız Uğurlu****

Introduction

Books, which affect individuals' perspectives on events, enable them to express themselves better and play an important role in the development of thought, provide a lot of information about life, the world, science and art and contribute to the development of imagination at the same time. Children who meet with books at a young age can benefit from these advantages and can make more progress in terms of both language development and cognitive development. It was revealed by Şirin (2000) that children's books prepared in line with the suitability principle for children affect the moral development, social development and personality development in addition to these areas of development. It was stated by Mendoza and Reese (2001) that works of children's literature can serve development in aesthetic, psychosocial and educational aspects. In order to support the development of all these areas, it is very important to choose quality books that are suitable for children's levels. One of the important elements to be considered in the selection of books for preschool and primary school children is that the books are supported with meaningful and quality images appropriate for the content.

Children combine their prior knowledge with the information contained in the pictures and texts of the book and use appropriate processes to recognize basic concepts, synthesize and summarize information, make inferences, and predict the next stage of the story (Paris & Paris, 2003; as cited in Mantzicopoulos & Patrick, 2011). In addition to reading, another method that can be used in terms of achieving these is creative drama.

Creative drama, which is a field of art in itself, is also an alternative teaching method. With the techniques used as a teaching method and applications with rich content, it provides permanent learning while creating a learning environment for the participants by experiencing and having fun. It offers a variety of ambiance for the participant to express himself/herself. With its aspect of being an art field, it has a very close relationship with the branches of art within the scope of the process.

* (Assoc. Prof. Dr.); Recep Tayyip Erdogan University, aysegul.oguz@erdogan.edu.tr

** (Graduate Student); Recep Tayyip Erdogan University, esramseven@gmail.com

*** (Dr.RA); Recep Tayyip Erdogan University, aslihan.alyildizugurlu@erdogan.edu.tr

Almost every field of art, especially painting, music and poetry, enriches the process and offers a rich environment where participants can express their creativity.

Some of the definitions of the creative drama in the literature are as follows: San (1990) describes the concept of creative drama as “individuals' interpretation and impersonation of an experience, an event and sometimes an abstract concept or a behavior in a group work through game-like processes by using improvisation, role playing, theater or drama techniques.” The concept is expressed by Adıgüzel (2013) as “impersonating a goal or a thought by using techniques such as improvisation, role playing based on the life experiences of the members of a group.”

There are many benefits of using creative drama as a method in education for the participant. According to Bell (2008), participants learn to express their own feelings through communication in the play and role playing processes. It has been stated by McNaughton (2004) that including drama in education can help the individual to develop his/her communication and decision-making skills and gain sensitivity towards friends. Sağlam (2004) also emphasizes that drama significantly contributes to the development of skills such as realization of one's own potential, self-expression, creativity, and empathy and can make individuals happy. It is stated by Üstündağ (1994) that the drama offers the opportunity to work together and share responsibilities for the individual to realize himself.

Creative drama processes include exercises such as movement and rhythm, pantomime, improvisation, character studies, and speech (McCaslin, 1990). There are three consecutive stages in a lesson plan structured with the creative drama method. These stages are preparation/warm-up, impersonation and evaluation/discussion. “Preparation/warm-up studies constitute the stage in which the body is put into action, the senses are used simultaneously and intensively, internally oriented work is carried out, which is performed in order to establish group dynamics such as gaining trust and adaptation, whose rules are identified and determined more by the leader in comparison to the other stages” (Adıgüzel, 2006). Free walks, games and some exercises are often used at this stage. The main purpose of this stage is to prepare the participant dynamically and mentally for the impersonation stage. The impersonation stage is the stage where many techniques are used, mainly theater techniques such as role playing, improvisation, role changing. The evaluation/discussion stage is the stage where opinions are shared on how the participants have felt in the process, what they have learned and what they have become aware of. Whether the gains have been achieved is evaluated at this stage.

Creative drama, which has an important role in terms of achieving educational goals, supports the learning process in many areas. This method, which overlaps with the philosophy of student-centered education approach, provides rich experiences to students in terms of enabling them to impersonate the events and situations in the texts, which shows that this method can be employed when teaching children's books. Therefore, within the scope of the study, it was aimed to prepare lesson plans that were structured with the creative drama method based on the children's books included in the "The Friends of the Painters" series.

Method

Within the scope of this study teaching of the books included in the series "The Friends of the Painters" were thoroughly examined and activities based on creative drama method were developed. The books titled Gauguin / My Friend Paul, Degas / My Friend Edgar, Monet / My Friend Claude and Van Gogh / My Friend Vincent were included in the study.

The plans, which were structured in accordance with the fourth grade level of primary school, were prepared to cover two lesson hours, and a total of eight hours of implementation was planned for four books. The gains that were desired to be achieved for each session were determined, and the methods and techniques and tools and equipment that would enable to achieve these gains were listed. Within the scope of the process, two activities were developed for three stages of creative drama for each book, and these activities were explained in detail. Information regarding the books covered in the study is given in Table 1:

Table 1. Information about the books

The Title of the Book	Original Title of the Book	Year of Publication	Date of Translation	Translator
Gauguin / My Friend Paul	Els Amics Dels Pintors-Gauguin	2013	2018	Berna Yılmazcan
Degas / My friend Edgar	Els Amics Dels Pintors-Degas	2013	2018	Berna Yılmazcan
Van Gogh / My friend Vincent	Els Amics Dels Pintors-Van Gogh	2013	2019	Berna Yılmazcan
Monet / My Friend Claude	Els Amics Dels Pintors-Monet	2013	2020	Berna Yılmazcan

“The Friends of the Painters” series consists of four books written by Anna Obiols and illustrated by Subi-Joan Subirana. The Turkish translations of the books, whose original language is Spanish, were made by Berna Yılmazcan and published by Binbir Çiçek Books. Information about the life of four painters, including Vincent Van Gogh, Edgar Degas, Paul Gauguin and Claude Monet, and the art they perform are told through stories created from the works of the painters. There are four books in the series that describe the painters separately. Each book includes a child protagonist whom the painter is friends with. The pages of the book are prepared based on the works of the painters, and the story characters are positioned on these works. In the biography section at the end of the books, the life of the artist is explained, and brief information about him is presented. In the “Artist's Style” section, the art movement by which the artist was influenced was mentioned and how the artist applied this trend was explained. Each of the books suitable for four years and above consists of thirty-five pages. Below is general information about the books in the series:

Gauguin, My Friend Paul: A painter named Paul Gauguin moves to the island where a child from Tahiti named Jotepha lives. Jotepha soon becomes friends and spends time with him. In the book, while Jotepha explains what he does with Gauguin, information about Gauguin's paintings and life is also provided.

Van Gogh, My Friend Vincent: Paula, who lives in Arles, becomes friends with Van Gogh, who has just moved there. With him, she goes for a walk in the countryside and spends time in the garden. In the book, Paula describes what she does with Van Gogh and gives information about his paintings.

Degas, My Friend Edgar: Ballerina Marie is a girl who has been doing ballet since she was a little girl. She becomes friends with a painter named Edgar Degas, who draws their paintings at the ballet school. Marie describes Degas and his works in this book.

Monet, My Friend Claude: A boy named Philippe goes to visit his aunt, where he meets a painter named Claude Monet. In this book, Philippe describes what he does with his new friend and the paintings he drew.

Lesson Plans Structured With Creative Drama Method

Session 1 Name: An Artist at the Ballet School: Degas

Gains:

- The student makes the movements he sees by using his/her body.
- Makes predictions based on visual and written materials.
- Builds empathy.

- Offers solutions suggestions to problems.
- Recognizes the major works of Edgar Degas.

Methods and Techniques: Drama/conscience alley, group impersonation, six thinking hats, collective/group picture

Tools and Equipment: Anna Obiols & Subi's "Degas, My Friend Edgar" book, drawing paper, crayons, printings of Degas' paintings, background cardboard in six colors (white, red, black, yellow, green, blue)

Process

A. Warm-up/Preparation

Activity 1: Students are dispersed in the classroom. The teacher asks which dance types they know, and after getting their answers, she/he asks each student to choose a dance type and perform that dance. While the students are dancing, the teacher says, "Stay as you are", and the students wait in the position they are in at that moment. The teacher touches the children and asks them to tell what dance they have been doing and then she/he asks them to continue their dance. (As each student will perform a different type of dance, a certain piece of music is not used in order not to limit their dance types.)

Activity 2: Students form a circle. The teacher asks the students to think about the dances they have performed in the previous activity. A student comes to the center of the circle and tries the dance type that one of his friends performed during the previous activity. The one who first guesses the student who performed the dance comes to the center of the circle and performs the dance type of another student. Standing students also try to do the new dance. The process continues in this way (Dancers cannot say that the dance type belongs to themselves).

B. Impersonation:

Activity 3 (Conscience Alley): The students sit in a circle. The teacher asks, "Have you heard of a painter named Edgar Degas?" If the students respond that they have heard, then the teacher asks what they know about him. Then she/he shows the book titled 'Degas, My Friend Edgar', asking students to make predictions about the content of the book and the artist based on the cover of the book. After the responses she/he receives, she/he tells that the paintings in the book are copies of the artist's paintings and asks them to examine the paintings while she is reading the book aloud. She/he then shows the original paintings of the artist and compares them with those in the book.

The teacher asks the students, "Ballerina Marie said that Degas was constantly watching them and drawing their pictures. Although they were uncomfortable in the beginning, they became friends later, and this

situation did not bother them much anymore. What would you feel if you were in Marie's shoes? ” and receives answers from the students.

Then she/he says, “If ballerinas were not friends with Degas and were uncomfortable with him, what should Degas have done? Should he have stopped drawing because the ballerinas were uncomfortable? Painting was everything to him, his profession. What would he do if he quit painting? ” and asks students to think about these questions. The class is divided into 2 groups by giving students odd and even numbers. The first group is lined up on one side of the class, side by side. The other group stands side by side, facing the 1st group. A distance between the two groups is left in such a way that a person can easily walk. A student from the class assumes the role of a painter. The first group makes suggestions for the painter to give up painting, and the second group recommends him to continue drawing paintings. The painter slowly moves through the alley between the two groups. Individuals in the groups speak out their suggestions. When the painter returns to the starting point again, she/he makes a decision and explains his/her decision to his friends.

Activity 4 (Group Impersonation): The class is divided into 4 groups by numbering the students with 1-2-3-4. The teacher tells the groups that Degas will continue to paint, but he does not want to disturb the ballerinas, and he is wondering how he can draw their paintings without them noticing and asks the groups to impersonate how Degas will observe the ballerinas and not show that he is drawing their pictures. Groups are given time to prepare, then the groups perform their impersonation.

C. Evaluation/Discussion:

Activity 5 (Six Thinking Hats): The teacher reads aloud Edgar Degas's biography and 'Artist's Style' sections at the end of the book. She/he asks students to analyze the printings of Degas' paintings and interpret his style. She/he asks them about what is in Degas's drawing style. She/he writes what the students remember and the information in the book on the board.

- Degas is known for his ballerina and race horses paintings.
- He was also an engraver, sculptor and photographer.
- Like his colleagues, he was not interested in capturing nature and light, he would rather depict artificially illuminated interiors.
- His similar aspect to his colleagues was to reflect concrete memories and to study the movement with utmost care.
- After softening the pastel paint with steam, he applied it to the canvas with brushes and his fingers.

Information similar to the above statements are written on the board in a way that students can see. Students are divided into 6 groups by means of counting. Each group represents one group of six thinking hats

technique. The teacher gives the groups a background cardboard of different colors to represent the hats. According to the nature of the cardboard color, the students evaluate Degas's painting style and write a slogan on the cardboard (White: neutral, red: emotional, black: pessimistic, yellow: optimistic, green: creative, blue: evaluative).

Activity 6 (Collective/Group Picture): The teacher distributes drawing papers and crayons to 6 groups and asks them to portray the classroom environment they depict in a similar way to Degas's paintings. When the groups complete their drawings, the pictures are displayed in the classroom.

Session 2 Name: Gauguin's Journey

Gains:

- The student dramatizes a short story.
- Improvises.
- Suggests different solutions to the problem.
- Paints in a similar way to the artist's style.
- Keeps a diary by putting himself/herself in someone else's shoes.
- Recognizes the major works of Paul Gauguin.

Methods and Techniques: Drama / narration, improvisation, role-play alley, collective / group picture, writing in role

Tools and Equipment: Anna Obiols & Subi's "Gauguin, My Friend Paul" book, drawing paper, crayons, printings of Gauguin's paintings

Process

A. Warm-up/Preparation

Activity 1 (Narration): The teacher tells the students a story, and while the students are listening to the story, they try to impersonate what the character is doing. The teacher also impersonates with the students.

Story Told: One day, the painter named Paul Gauguin decided to move. He collected his belongings and prepared his luggage. He put the paints necessary for painting in his backpack, put the backpack on his shoulder, and set out taking his canvases with him.. He walked, walked, and walked. He was having difficulty carrying his belongings because they were heavy. In the distance, he saw the train station, and he stayed put. What was that? The train he was going to take was about to leave, so he started running quickly. He ran, ran, and ran. He caught the train and threw himself in just as the doors were closing. He immediately got into a wagon and took a deep breath as he was able to catch the train. Throughout his journey, he watched out of the window and dreamed of his destination.

Activity 2:

The teacher continues the story: *“Gauguin finished his journey and arrived in Tahiti Island, where he was moving. Gauguin, who did not know anyone, went around house by house and searched for a place to stay.”* The teacher assigns the students numbers as 1-2-3. Students 1 and 2 hold hands face to face in the classroom. These people are the hosts. Students with number 3 become Paul Gauguin. The teacher calls out “Gauguin”, students who are Gauguin get into the arms of their hosts. One student can enter each home. When the teacher calls out “Gauguin” again, the guests change places, but they cannot go to the houses adjacent to the house they are in. When the teacher calls out “the host,” the hosts change places, students in the role of Gauguin remain steady. When the teacher calls out “Tahiti”, the whole class changes places and creates new houses. The student left out in the games tries to find a new place during the relocation.

B. Impersonation:

Activity 3 (Improvisation): Students form a circle. The teacher asks, "Are you wondering what Gauguin is doing on the island of Tahiti?" and says, "So let's read the book and see what he has done." She/he reads aloud the book "Gauguin, My Friend Paul". When the book is finished, she/he takes the opinions of the students about Gauguin, shows the original paintings of Gauguin to the children, and gives them the opportunity to examine them. She/he then creates 4 groups by means of counting numbers. She/he asks the groups to discuss what the people there thought about Gauguin when he arrived in Tahiti, where he was a stranger. She/he explains, "Gauguin does not speak the same language as they do, has a different appearance, and is constantly painting the environment; the local people discuss among themselves how to behave towards him." Each group represents a group of indigenous people, improvising according to the characteristics of their groups.

Group 1: They improvise about who Gauguin is.

Group 2: They improvise about how to meet Gauguin.

Group 3: Because they don't speak the same language as Gauguin, they improvise about ways to communicate without speaking.

Group 4: They examine the paintings drawn by Gauguin, and improvise about what he pays attention to when painting.

At the end of the impersonations, "Thus, the people of the island complete their process of meeting with Gauguin and become friends with him," says the teacher.

Activity 4 (Role-play Alley): The teacher tells the children that Gauguin loves drawing what he sees, he drew the island inhabitants a lot,

and opening the twenty-fifth page of the book, she/he explains the situation. “*While Jotepha's brother and friend Vaitua were sitting on the beach, Gauguin wanted to paint them. Jotepha's brother was very happy to have his painting made, but her friend did not like the idea very much. How can Jotepha's brother convince him?* ” the teacher asks. The class is divided into two groups, one group must have one more member than the other. Groups are lined up in a row in parallel. One group takes the role of the brother who wants his painting to be made, and the other group takes the role of the friend who does not want his painting to be made. The students in the front row begin to discuss the issue, and those in the role of the brother try to convince the ones impersonating the friend. When the teacher says change, the ones in the front row move to the last row, and now the new people in the front row continue from the sentence the others left. According to the course of the process, the activity is concluded either in convincing the friend or not.

C. Evaluation/Discussion

Activity 5 (Collective/Group Picture): Students are divided into four groups. The teacher reads out the sections of 'Biography' and 'Artist's Style' at the end of the book and gives each group drawing papers, crayons and printouts of Paul Gauguin's paintings (For awareness purposes, the names of the paintings should be written on the edges of the paintings). She/he asks the students to study the paintings and draw a picture of a beach similar to Gauguin's paintings. The pictures completed are exhibited in the classroom.

Activity 6 (Writing in Role): The teacher reminds the students of the performances in which they impersonated how the people of the island met Gauguin. Then she/he asks them to guess what Gauguin might have thought about the island people and to write notes in the diary named 'Noa Noa' placed at the end of the book as if they were Gauguin.

Session 3 Name: In Pursuit of the Small Boat

Gains:

- The student uses his/her body in accordance with the directive.
- Makes predictions based on written and visual material.
- Uses his/her body in a way to evoke entities and protagonists.
- Expresses his/her feelings and thoughts through written, oral and visual materials.
- Realizes the similarities between works of art.
- Recognizes the major works of Claude Monet.
- Paints in a similar way to the artist's style.

Methods and Techniques: Drama / thought tracking, station, frozen image, collective/group picture

Tools and Equipment: Anna Obiols & Subi's "Monet, My Friend Claude" book, drawing paper, crayons, printouts of Monet's paintings, small flowers made of cardboard of different colors

Process

A. Warm-up/Preparation

Activity 1: The students sit on the floor in a circle, the teacher tells the students to close their eyes and imagine themselves in a garden full of flowers. Meanwhile, the teacher scatters small flowers made of cardboard of different colors on the floor of the classroom and turns on the music (Dmitri Shostakovich-Waltz No.2). She/he tells the students, "You are in a garden full of flowers, and you hear a very nice piece of music. Now open your eyes and dance in the garden." Students dance freely on the flowers. The teacher stops the music and gives instructions such as "Collect red flowers, collect blue flowers". The students collect the flowers and the music is turned on again. The process is repeated several times.

Activity 2: The teacher shows the students Monet's painting titled "Poppy Field" and tells that the red flowers they have just collected come from this garden. He asks each student to get a flower seed. The students bend on the floor in a dispersed way and become seeds. They act according to the teacher's instructions.

Instruction: It is raining, you are getting wet, the sun is shining, you are slowly growing, the wind is blowing and you swing, you are about to break off the ground, the sun is shining again, you grow a little more and you open your leaves, the season is changing, you are slowly beginning to fade.

B. Impersonation:

Activity 3 (Thought Tracking): The teacher shows the book 'Monet, My Friend Claude', and, based on the cover of the book, asks for predictions about the content of the book and the artist. After the responses she/he receives, she/he tells that the paintings in the book are copies of the artist's paintings and asks them to examine the paintings while she is reading the book aloud. After reading the twenty-eighth and twenty-ninth pages, she/he stops and asks the children to think about where the boat might have gone. *"That night, I fell asleep believing that I could never find my boat anymore. 'I have to build another boat,' I said sadly. In the morning, the first lights of the sun was illuminating a boat that was floating in the water. "* Then, she/he asks the students to give the form of the boat in the water to their bodies. Touching the children randomly, she/he asks, "Where would you go if you were that little boat? What would you do on

the river?" At the end of the process, the teacher reads out the last page of the book.

Activity 4 (Station): The teacher tells the students that Monet turned a boat into a workshop, sailed away to the river and drew open-air paintings, and that one day Monet noticed that the boat was taking in water from some part when he was sailing away on the boat. The students are divided into 3 groups by means of counting numbers and the groups move to the pre-assigned areas for them. "The inside of the boat is full of paintings he made, Monet is wondering what to do desperately," says the teacher, and the students find solutions to what Monet could do in that situation.

Group 1: They draw a picture.

Group 2: They write a story.

Group 3: They prepare impersonation.

Groups change places in ten-minute periods. Finally, the prepared solutions are shared with the class.

C. Evaluation/Discussion:

Activity 5 (Frozen Image): Students are divided into four groups. They randomly choose one of Monet's paintings, and the teacher asks the students to impersonate the painting in the form of a frozen image by using their body in groups.

Activity 6 (Collective / Group Picture): The teacher reads out Monet's biography and "Artist's Style" sections at the end of the book. She/he assigns each group one of the places mentioned in the biography (the boat he turned into a studio in Paris, the big pink house in the town of Giverny, the orchard, the pond with the Japanese Bridge). Students examine Monet's paintings and guess which of these paintings were made where their groups are located and they draw a picture similar to that picture.

Session 4 Name: Living Paintings

Gains:

- The student acts in accordance with the instructions.
- Improvises.
- Expresses his/her feelings and thoughts in accordance with his/her role.
- Makes comparisons while listening.
- Writes a letter.
- Makes inferences about written and visual materials.
- Recognizes the major works of Van Gogh.

Methods and Techniques: Drama / imaginary painting, simultaneous improvisation, gossip ring, collective/group painting, writing in the role

Tools and Equipment: Anna Obiols & Subi's "Van Gogh, My Friend Vincent" book, drawing paper, crayons, gouache, printouts of Van Gogh's paintings, video titled 'Van Gogh's paintings: Masterpieces come to life', envelopes in the size in which the book could fit, Theo's Letter

Process

A. Warm-up/Preparation

Activity 1: The teacher asks the students, "Think about the paintings of the painters, how would it be like if the drawings in the paintings moved?" After the answers, she/he plays the video titled 'Van Gogh Living Pictures' (YouTube: The video titled 'Van Gogh's paintings: Masterpieces come to life' on Matt Bookman's channel). The teacher says that the class is a painting and asks the students to act like they were a drawing in this painting. Students can become an element such as a tree, a cloud, people, cars etc. as they wish. Students stand still and form the painting. The teacher says that the artist who drew them was Van Gogh, and they come to life when she/he says "Van Gogh", and they remain still when she/he says "Painting". During the process, the music piece titled 'Ludovico Einaudi- Experience' included in the video is played.

Activity 2 (Imaginary Picture): Students stand dispersed around in the classroom. The teacher tells the students that they are the painter now and asks the students to imagine that there is an imaginary canvas in front of them and to paint with imaginary paints. Students paint by imagining as if they were painting on a real canvas. During the process, music piece called 'Francine Leblanc- Rue de Rivoli' is played.

B. Impersonation:

Activity 3 (Simultaneous Improvisation): The students sit in a circle. The teacher shows a large envelope to the students and asks them to guess what is inside. After the guesswork, she/he opens the envelope and takes out the letter and the book 'Van Gogh, My Friend Vincent'. She/he reads out the letter: *"Dear students, I am sending this letter to you from far away. My name is Theo, I'm the brother of the painter Vincent Van Gogh. My brother is a very successful painter and loves children very much. So, I am sending you a book that gives information about him. If you read the book and examine my brother's paintings, you will make me very happy. So tell me, who wants to read the book first?"* One of the students reads the book by showing it to the class. After the book is finished, the teacher shows the original drawings that came out of the envelope next to the pages of the book. She/he lets them examine the paintings. The teacher asks the students

to form groups of 2 and reads out the following part from the biography on the last page:

“Thanks to the letters they wrote to each other with his brother Theo, we have information about his work and his life. Theo was the only person who had been with him throughout his life. He provided Vincent with financial support and introduced him to painters because he was an art dealer.” She/he then asks one of the group members to be Van Gogh and the other to be Theo and chat about the paintings. While all groups are chatting, the teacher selects a group randomly, other groups watch the improvisation of that group, the process continues with different groups.

Activity 4 (Gossip Ring): The class is divided into 4 groups by numbering as 1-2-3-4. The teacher reads out his biography. She/he emphasizes that Van Gogh could not get along with the painter named Gauguin, whom he lived together. She/he says that all four groups represent a group from the circle of these two friends:

Group 1: Art Merchants (People buying paintings)

Group 2: Neighbors

Group 3: Painter Friends

Group 4: The Staff of the Restaurant They Regularly Went To

The teacher starts the story: *“One day Van Gogh and Paul Gauguin have a big fight, they are so angry that Gauguin leaves Arles. The townspeople wonder about the cause of the fight, and rumors grow when the rumor that Van Gogh cut his ear spreads.”* Groups gossip in order according to their roles, one group can be influenced by the gossip of the other group and make additions. The townspeople try to clarify this fight. They cannot solve the cause of the incident, but at the end of the process, the teacher shows Van Gogh's self-portrait with his ear cut, shedding light on the rumors about his ear being cut.

C. Evaluation/Discussion:

Activity 5 (Collective / Group Picture): The teacher reads out the “Artist's Style” section at the end of the book and gives each group drawing papers, crayons, gouache and printouts of Van Gogh's paintings (For raising awareness, the names of the paintings should be written on the edges of the pictures). The teacher asks the students to examine the paintings and discuss how Van Gogh made the pictures that seemed so alive (how can Van Gogh's paintings look as if they were moving? What colors did Van Gogh use in his paintings? How does Van Gogh use brush strokes?), then she/he asks them to make a picture that looks as if they were alive, like Van Gogh's paintings.

Activity 6 (Writing in the Role): The teacher asks the students what they think of Van Gogh. She/he explains that Van Gogh's paintings were found strange and different in the period he lived due to his painting style, and that this situation also affected Van Gogh psychologically. She/he then asks the students to write a letter from Van Gogh to his brother Theo, considering these facts.

Conclusion, Discussion and Recommendations

Within the scope of the study, in which sample lesson plans for the teaching of four books in the “The Friends of the Painters” series with the creative drama method were prepared in detail, sessions plans were created for each book, and activities for the warm-up/preparation, impersonation and evaluation/discussion stages were developed in line with the determined gains. When the literature was examined, it was determined that there are many studies carried out regarding the creative drama method, and it was found that the studies were generally carried out in order to investigate the effect of this method at different grade levels and lessons, and that there was no study directly related to the subject covered in this study. In the study carried out by Susar-Kırmızı (2008) in order to determine the effect of creative drama method on the attitudes of primary school fourth grade students towards reading and their reading comprehension strategies, it was concluded that the method was effective in developing reading comprehension strategies while no significant difference was found between the attitude post-test scores of the experimental and control groups. The findings obtained in the study conducted by Kahyaoğlu, Yavuzer and Aydede (2010) to investigate the effect of creative drama method on the fifth grade science course showed that this method had a positive effect on students' success in the course. In the study carried out by Aykaç and Adıgüzel (2011), the effect of teaching the fourth grade social studies course with creative drama method was investigated and after the implementation process, it was found that there was a significant difference between the post-test scores of the experimental and control groups in favor of the experimental group with whom creative drama method was used. In the study conducted by Aktepe and Bulut (2014) in order to determine the effect of the method on mathematics achievement of third grade students in primary school, it was revealed that the method had a positive effect on mathematics achievement, and that there was a significant difference between the achievement post-test scores in favor of the experimental group. As a result of the study conducted by Eti and Aktaş-Arnas (2016) that investigated the effect of story-based creative drama activities on expressive language development of four-year-old children, the method was found to be effective on the variables handled. When the results of the studies were examined, it was seen that creative drama method generally had a positive

effect on the variables whose effects were investigated. The results obtained brought along suggestions that the method should be used in the lessons. While taking into consideration these suggestions, which emphasize the importance of the method, it should not be overlooked that the method should be applied effectively. For this reason, studies in which information and application examples on how to plan courses taught with creative drama method are included are important. Studies conducted for this purpose in the literature are rather limited compared to the studies investigating the effect of the method. Within the scope of the study conducted by Duatepe and Akkuş (2006), creative drama-based lesson plans were prepared for the sixth grade mathematics lesson sub-learning area of sets. In the study carried out by Şahin and Yağbasan (2012), examples of lesson plans related to the physics course structured with creative drama method were included. Within the scope of the study carried out by Küçükkaragöz and Av-Hartuç (2015), lesson plans were prepared for the teaching of primary school second grade life science subjects with the drama method. Increase in the number of these studies, which are important in terms of shedding light on the field, will enable the method to be applied more effectively. Therefore, the following suggestions have been developed within the scope of the study carried out:

- Special emphasis should be given to preparing plans for the application of creative drama method.
- In particular, studies including examples of how to use this method in the teaching of children's books should be made widespread.
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CHAPTER II
**LOGISTIC REGRESSION ANALYSIS AND AN OVERVIEW OF
THE USE IN TURKEY**

*Canan Demir**

1. Introduction

One of the most used statistical methods in examining the relationships between variables is regression analysis. Regression analysis is a method that aims to mathematically reveal the effect levels and effect types of explanatory variables that are supposed to affect dependent variables. The most well-known among these methods are simple and multiple linear regression methods. As it is known, it is a numerical data type obtained by dependent variable measurement in simple and multiple linear regression. In addition, it is often encountered when the dependent variable is of a categorical/qualitative data type. When the dependent variable is of two or more categorical qualitative data types, logistic regression method is used (Alpar, 2013).

The main purpose of logistic regression analysis is to establish an acceptable model that can describe the relationship between a dependent (outcome) and independent (explanatory) variables in a way that has the best match with the least variable (Atasoy, 2001). The dependent variable; binary logistic regression method in case of a two-category qualitative variable, multinomial logistic regression method in case of more than two-category qualitative variable, ordinal logistic regression methods in case of more than two categories sortable qualitative variable are utilized (Alpar, 2013).

In logistic regression, an estimation is made based on some variable values as in linear regression analysis. However, there are three significant differences between these two methods. While the dependent variable to be estimated in the linear regression analysis is continuous, the dependent variable takes a discrete value in the Logistic Regression Analysis. In linear regression analysis, the value of the dependent variable is estimated, and in Logistic Regression Analysis, the probability of realization of one of the values that the dependent variable can take is estimated. In the linear regression analysis, it is required to have multiple normal distributions of the independent variable, whereas, in Logistic Regression Analysis, such a condition is not sought (Elhan, 1997).

The aim of this study is to give a general literature review on logistic regression analysis and usage in Turkey.

* (Dr.); Van Yuzuncu Yil University, Vocational School of Health Care, Van, Turkey, e-mail: canandemir@yyu.edu.tr

2. Method

One of the alternative methods developed for estimation, which defines the relationship between the dependent and independent variables, is Logistic Regression Analysis (Gok and Ozdemir, 2011). This analysis has come to the forefront with its ease of use as well as its easy interpretation and is a method that has been widely used in many fields. Logistic regression analysis is preferred when the dependent variable consists of categorical data such as "successful-unsuccessful", "less-medium-high", and "positive-negative" (Cakin and Ozdemir 2019).

In logistic regression analysis, the value of the response variable is not estimated. The probability of realization of one of the values that the response variable can take is estimated. Logistic regression is mathematically based on probability, odds, and the logarithm of odds.

Odds: Odds is the ratio of success or probability ("p") to failure or probability of not being seen ("1 - p").

Odds Ratio (OR): It is the ratio of two odds to each other. It is a summary measure of the relationship between the two variables.

2.1 Logistic Model

Logistic function for the estimation of parameters in a logistic regression model that includes two categories such as dependent variable successful and unsuccessful;

$$\pi(x) = \frac{e^{(\beta_0 + \beta_1 X)}}{1 + e^{(\beta_0 + \beta_1 X)}} = \frac{1}{1 + e^{-(\beta_0 + \beta_1 X)}} \quad (1)$$

The nonlinear logistic regression function given in the above equation can be linearized when logit transform is applied. Logit transformation is done by taking the natural logarithm of the odds of an event.

Odds of an event is indicated by $\frac{p}{1-p}$. This ratio can be between 0 and $+\infty$. When the natural logarithm of the Odds' is taken, the logit transformation is made and the logits can be between $-\infty$ and $+\infty$.

$$\text{logit}_{\pi(x)} = g(x) = \ln\left(\frac{\pi(x)}{1-\pi(x)}\right) = \beta_0 + \beta_1 X \quad (2)$$

As seen in the equation, logit transformation creates a linear function of β parameters (Hosmer & Lemeshow, 1989). This is the only assumption of the logistic regression model.

The logit transformation applied to expand the boundaries of the outcome variable has the following properties:

1. When p is between 0 and 1, logit (p) can take values on the real numbers line.

2. Logit (p) increases as p increases.
3. When $p < 0.5$ logit (p) becomes < 0 , and when $p > 0.5$ logit (p) becomes > 0 (Hosmer & Lemeshow, 2000).

The maximum likelihood estimation method is used to estimate coefficients in logistic regression models. The likelihood function for a two-state univariate logistic regression model is shown below.

$$L_i(x, \beta) = [P(y_i = 1|x)]^{y_i} [1 - P(y_i = 1|x)]^{1-y_i} \quad i=1,2..n \quad (3)$$

Since the observation values are assumed to be independent of each other, the composite likelihood function equation of these observations is as follows;

$$L(\beta; x_1, x_2, \dots, x_n) = \prod_{i=1}^n f(x_i; \beta) \quad (4)$$

Then, the logarithm is taken on both sides to calculate mathematically easily. The log-likelihood function is shown as follows (Celik, 2019);

$$L(\beta) = \ln[L(\beta)] = \sum_{i=1}^n [y_i \ln(\pi(x_i))] + [(1 - y_i) \ln(1 - \pi(x_i))] \quad (5)$$

With the log-likelihood function, the observed and predicted values are compared with each other. The values observed and the values estimated by using the likelihood functions can be expressed as follows.

$$D = -2 \ln[\text{Possibility of current model} / \text{Possibility of saturated model}]$$

D statistics (Likelihood Ratio or Deviance statistics) play an important role in deciding on the goodness of fit, and this expression corresponds to the sum of squares in linear regression (Hosmer & Lemeshow, 2000).

3. Literature Review

In a study by Altunoz, he has used logistics regression and discriminant analysis, which are multivariate statistical methods, to estimate the financial failure of banks in Turkey. For this purpose, it was tried to estimate the probability of banks to fail financially one and two years in advance by using financial ratios belonging to 36 private banks. As a result of the study, both models predicted the failures experienced in the Turkish banking system at a high rate a year in advance (Altunoz, 2015).

In their studies, Akbulut and Rencber used logistic regression analysis to determine the factors affecting the activities of enterprises operating in the cement sector and the impact levels of these factors. 65.8% of the total effectiveness of the enterprises is explained with the variables of Equity Multiplier, Receivable Turnover Rate, Net Profit Margin, and Debt Rate. In their studies, it has been demonstrated that these factors determine the efficiency of businesses at a high rate (Akbulut & Rencber, 2015).

Ural et al. aimed to estimate the financial failure risk three years in advance with the help of the logistics model, taking into account the data of the food, drink, and tobacco companies traded in Istanbul Stock Exchange between 2005 and 2012. The predictive power of the model that aims to predict financial failure 1 year in advance is 91%, the predictive power of the model that aims to predict 2 years in advance is 91% and the predictive power of the model that aims to predict 3 years in advance is 74.5%. These results revealed that the logistic regression model can be used as an important tool in operational failures (Ural et al., 2015).

In his study, Akin Arikan investigated whether the items included in the PISA 2012 mathematics study ethics questionnaire show whether the Differential Item Functioning (DIF) varies according to gender. He also looked at whether the items showing DIF vary according to the methods. SIBTEST, one of the DMF determination methods, Ordinal Logistic Regression, and Item Response Theory based IRT-Likelihood Ratio (IRT-LR) have been used. The sample of the study consists of 1000 (500 females, 500 males) individuals randomly selected from 3217 individuals who respond to mathematics work ethic attitude items through the SPSS program. When the results obtained from the techniques are compared; According to the Poly-SIBTEST method, it was observed that item 3 showed a high level of non-uniform DIF in favor of males, and the item 3 according to the IRT-LR method was moderate and according to the OLR method, Item 3 showed a high level of non-uniform DMF (Akin Arikan, 2015).

Avçilar and Yakut aimed to classify the party preferences of voters who can vote in local elections. The data required for the research were obtained by using the face-to-face survey method and sampling method from 500 voters residing in Osmaniye. Artificial neural networks, multiple logistics, and multiple discriminant analyzes were used to classify the party preferences of voters. Analyses were performed using MATLAB 12, STATA 11.2, and SPSS 18.0 package programs. As a result of the analysis, it has been determined that artificial neural networks perform classification with higher accuracy compared to multiple logistics and multiple discriminant analyzes (Avçilar & Yakut, 2015).

Telli, in his study, compared factors affecting the profitability of commercial banks operating in Turkey during the crisis in 1999, 2000, 2001, and 2008 using logistic regression analysis. Asset profitability (ROA), equity profitability (ROE), and net interest margin (NIM) as dependent variables, capital adequacy ratio group, asset quality ratios, liquidity ratios, and income and expense ratios were used as independent variables. As a result of the findings obtained, the income-expense group ratios for the dependent variables of asset profitability and equity profitability came to the fore in the relevant years. For the net interest

margin dependent variable, although any ratio group did not stand out for 1999, 2000, and 2008, income and expense group ratios and capital adequacy group ratios stood out for 2001 (Telli, 2016).

In their studies, Yerdelen Kaygin and his colleagues tried to estimate the financial success and failure of the enterprises by making use of the annual balance sheet and income table data of 143 manufacturing industry companies that were traded on the Istanbul Stock Market between 2010 and 2013. In the research, CHAID and C 5.0 methods, which are decision tree algorithms, and Logistic Regression Analysis were used. Based on 2013, models for predicting one year, two years, and three years in advance were developed and the predictive power of the models was compared. In the CHAID Model, 30 were classified correctly and 6 were classified incorrectly for 2012. The success rate of the model was calculated as 83.33%. In C 5.0 Model, 32 were classified correctly and 4 were classified incorrectly for 2012. The success rate of the model was determined as 88.89%. In the Logistic Regression Model, 34 were classified correctly and 2 were incorrectly classified for 2012. The success rate of the model was calculated as 94.44%. In other words, when comparing the classification accuracy of models, the predictive power of the models, the Logistic Regression model was found to be more successful (Yerdelen Kaygin et al., 2016).

In their studies, Sagbas and Ballı used the built-in GPS, accelerometer, and gyroscope sensors of smartphones to predict human movements. For this purpose, the data obtained from the accelerometer, gyroscope, and GPS sensors were collected while the person was walking, running, cycling, climbing up and down stairs, using an elevator, and in a stationary state. The data are labeled at 5-second intervals. This process was performed 250 times for each action and a total of 1750 patterns were obtained. With the data obtained, human movements were determined and the performances of Logistic Regression and kNN (k Nearest Neighbor) algorithms were compared. Logistic Regression yielded better results than kNN method with a 94.5% accuracy rate (Sagbas & Ballı, 2016).

In his study, Ari tried to determine the relationship between individuals' level of hope and their thoughts about the future with the multinomial logistic regression model. To this end, it was benefited from the 2014-Life Satisfaction Survey data applied by TUIK to 7984 individuals. In the first stage of the application, the Chi-Square independence test was applied to many variables that are thought to be related to the level of hope included in the questionnaire and worked with 4 variables by removing the insignificant variables from the data set. The validity of the model examined in the study was found to be significant by calculating it with a likelihood estimator. Odds ratios of the variables that make up the model were obtained and 3 categories were compared according to the coefficient

odds ratios according to the selected reference category. Coefficient odds values for future comparison, economic, social rights, and public services provision for comparison 1, future comparison, economic and public services presentation for comparison 2, and future comparison for comparison 3 have been found to be significant (Ari, 2016).

In their study, Koybasi and colleagues examined in which direction and to what extent the variables that affect teachers' classification of trust levels in school. The sample of the study consists of 300 teachers working in primary schools in the central district of Sivas. An organizational trust scale with 21 items was used to obtain organizational trust data. Organizational trust level (low/high) as a dependent variable, logistic regression analysis was applied to variables such as gender, education service, school year, branch, and organizational cynicism as independent variables. According to the findings, organizational cynicism is the strongest predictive variable in determining the low level of organizational trust (Koybasi et al., 2016).

Akin Arikan et al. aimed to reveal the similarities and differences of MIMIC, SIBTEST, Logistic Regression, and Mantel-Haenszel methods used to determine the Differential Item Functioning (DIF) in their studies. Besides, the consistency of the results obtained with expert opinions and methods was examined. The research was carried out by selecting 300, 600, 1000, 1200, and 2000 sub-samples from the dataset with approximately 340000 students. When looking at common items containing DIF according to the samples for four different methods; 2nd item in the sample of 300 people; in the sample of 600 people, 13th item; in the sample of 1000 people, it was observed that there was no common DIF containing item, in the sample of 1200 people, the 19th item; in the sample of 2000, the items 2, 3, and 4 contained DIF. In this respect, it can be said that in the large sample, the methods give more compatible results. In the study, the results of the analysis were determined to be consistent with each other (Akin Arikan et al., 2016).

In their studies, Mutluer and Buyukkidik used the data of Program for International Student Assessment known as PISA survey and recurring every three years since 2000. Of students in mathematics literacy using the 2012 data in Turkey research, using the selected math classes eagerly waiting, interest in mathematics, mathematics self-detection / rapid learning, perseverance, giving up quickly, making reading about mathematics, enjoying mathematics, and parents' educational status as variables, they tried to determine what level of accuracy it can classify using logistic regression technique. As a result of the analysis of the data, it was observed that the mother education level, father education level, enjoying mathematics, math self-perception / fast learning, perseverance, and giving up quickly have significant effects on the classification of

mathematical literacy. Whether or not reading about mathematics and the level of mathematics interest does not have a significant effect on mathematical literacy. In the obtained logistic regression model, the correct classification rates of the observations were reported to be 85.2%. According to the research findings, 94.9% of the students who succeeded in mathematics literacy, and 54.9% of the unsuccessful students were estimated correctly (Mutluer & Buyukkidik, 2017).

Aytekin and Tunali studies dealt with the factors affecting the life satisfaction of Anadolu University TSTP (Teaching Staff Training Program) research assistants. In the academic satisfaction scale, which was used to measure the satisfaction of TSTP research assistants, the dimensionality reduction was made by factor analysis, and eight factors were obtained. The effects of factors obtained and demographic variables on the life satisfaction of TSTP research assistants were analyzed by binary logistic regression analysis. When the binary logistic regression analysis results were examined, it was observed that job-related factors such as attitude towards work, the perceived reputation of the institution, salary and additional earnings, job security and age variable were effective on the life satisfaction of TSTP research assistants (Aytekin & Tunali, 2017).

In the studies of Sapmaz and Yercan, it aimed to determine the factors affecting the consumption of market-branded food products for consumers living in İzmir Province and to compare these two methods. The main data of the study was obtained as a result of the survey conducted with 650 consumers living in central districts of Izmir Province. The dependent variable in the model is that “consumers consume market branded food products or not”. Since the dependent variable is a two-class variable, logistic regression and its alternative artificial neural network methods were used in the study. According to the results of both methods, while the most effective variable in consumers participating in the research in consuming market-branded food products is income variable, the frequency of shopping and behavioral factors during shopping were observed as other effective variables. When the application results of the two alternative methods used in the study were evaluated, the predictive power of the model obtained using the artificial neural network method (77.23%) was found higher than the model obtained by the logistic regression method (76.15%) (Sapmaz&Yercan, 2017).

Eksi et al. examined the effect of spouse selection strategies, perfectionist attitudes, and value preferences on the emotional relationship preferences of unmarried individuals. The sample of the study is composed of 174 women (81.7%) and 39 men (18.3%). When the participants were asked whether they had an emotional relationship, 67 (31.4%) answered yes, 146 (68.6%) answered no. Participants were given the Peer Selection Strategies Inventory, Multidimensional Perfectionism Scale, and Schwartz

Value Preference List. In the research, logistic regression analysis was used to analyze the data obtained from the samples. As a result of the research, it was found that the partner selection strategies; family institution and trust which are physical property variables, and the openness and self-transcendence which are value preferences, have an impact on whether the participants are in an emotional relationship (Eksi et al., 2017).

In his study, Altioek Gurel used logistic regression analysis to determine the factors affecting the work-life balance of 221 female academicians working in three major state universities. In the study, participants stated that 72.4% could not establish a work-life balance, 27.6% stated that they established work-life balance. As a result of the analysis; on the presence of work-life balance from high to low risk, respectively; the number of children, the presence of spouse support, marital status, the presence of a dependent person, family support, the presence of the assistant person and age were found to be statistically significant (Altioek Gurel, 2018).

Ugurlu et al. examined the variables that cause absenteeism in students in their studies and revealed the relationship between these variables and some demographic features through logistic regression analysis. The study group of the research consists of 548 students studying at Cumhuriyet University. In the research, the “Absenteeism Tendency Scale” (ATS) was used as a data collection tool. According to the findings, it was revealed that students' GPA, weekly course hours, weekly absenteeism levels were significant on their absenteeism tendencies. While the general averages of the students decrease their tendency to absenteeism, the increase in the grade averages shows that the students' interest in their lessons increases. A one-unit increase in one-week lesson hours also increases the tendency towards attendance. It has been observed that the increase of the weekly class hours of students at school decreases their absenteeism behaviors and enables the students to attend their classes more (Ugurlu et al., 2018).

In the studies of Sata and Cakan, they compared the classification success of the most commonly used decision trees (CHAID analysis) and logistic regression (binary LR) methods among the data mining methods. In the study, “Current Physical Attitude Scale for Secondary School Students” was used as a data collection tool. The population of the study consists of a total of 726 students, 358 girls and 368 boys studying in high schools affiliated to Ağrı Provincial Directorate of Education in the 2014-2015 academic year. As a result of both CHAID analysis and logistic regression analysis, it was determined that three of the independent variables considered in the study had a significant effect on the dependent variable. When the classification performances of CHAID analysis and logistic regression analysis are analyzed, it is determined that logistic regression analysis has a slightly better classification rate at a negligible

level. However, independent variables found significant in the CHAID analysis are presented in more detail in the final output (Sata & Cakan, 2018).

Karci and Bayram Arli examined the statistical effectiveness of variables that affect material deprivation by logistic regression analysis. In the established model, it was determined that the education level, employment status, age, and health status of the household responsible, as well as the number of rooms of the dwelling, the material deprivation of the heating system, are significantly effective. Regions of Turkey were added to the model as independent variables and it was seen that there was a significant difference. It is determined that the risk of material deprivation is the highest in the Southeastern Anatolia Region compared to other regions (Karci&Bayram Arli, 2018).

In the study by Korucuk, he examined the effect of green logistics applications on competitiveness and hospital performance with logistic regression analysis. For this purpose, employees of 31 public-private-university hospitals operating in Ankara were interviewed. The results obtained were evaluated using the SPSS statistical program. Accordingly, green logistics applications; it was determined that green supply, green packaging, and green reverse logistics activities had a positive effect on competitiveness. In this context, it was determined that green procurement and green reverse logistics activities had a positive effect on hospital performance, which is one of the green logistics practices that affect competitiveness. However, it has been determined that green logistics practices do not have any positive effect on the specified competitiveness and hospital performance in relation to green production and material management, green transportation, and green storage activities (Korucuk, 2018).

In their study, Koyuncu et al. compared the results of the International Student Assessment Program (PISA) conducted in 2012 with the data obtained from the student questionnaire and the results of Differential Item Functioning (DIF) analysis using classical test and item response theory methods. The study group of the research is 990 students selected by a random sampling method among Turkish students who do not have any loss data for 9 items related to mathematics study discipline. In terms of success in mathematics, 45% of students are in the lower group and 55% in the upper group. Mantel-Haenszel, logistic regression, and likelihood ratio methods were used as data analysis methods. In the analysis, JMETRIK, DIFAS, Zumbo SPSS Syntax, IRTLRFID, and IRTPRO software were used. According to the results of the research, according to all methods and software, it was determined that there was a DIF in the item 'completing the assignment on time'. Although the results of all

methods and software are partially similar, they also contain differences (Koyuncu et al., 2018).

In their study, Can et al. investigated the factors affecting students' course success with logistic regression analysis, one of the data mining techniques. Based on the questionnaire applied to 5820 university students, two different course success variables were defined. The relationship between the answers given to the Likert scale questions affecting the course success variables and the course success was examined. According to the logistic regression results obtained; It is seen that students' willingness to participate in the lesson, increasing their professional development, and gaining different perspectives give better results in measuring students' success. (Can et al., 2018).

Hanimoglu examined the effect of social skill level, gender, and class level on internet addiction level in primary school second-grade students. The research was carried out on 558 students in, Çukurova and Seyhan districts of Adana, attending 6th, 7th and 8th grade, 283 (50.3%) girls, and 275 (49.7%) boys. In the study, personal information form, Internet Addiction Scale, and Matson Evaluation of Social Skills in Children were used. Internet addiction level (internet dependent, not internet dependent) dependent variable; the logistic regression analysis was applied by taking “positive social behavior level (sub-dimension)”, “negative social behavior level (sub-dimension)” and “gender” and “class level” as independent variables. As a result, according to the results obtained from this study, it was found that students with low social skills, boys according to girls and students in the higher class are in the risk group for Internet addiction (Hanimoglu, 2019).

4. Conclusion

The use of the logistics model dates back to the years of 1845. Logistics analysis, which is mostly used in the examination of socio-economic issues, first appeared in studies aiming to explain the population increase in the society with a mathematical expression (Cokluk, 2010). Logistic regression models are widely used in biology, medicine, economy, agriculture, veterinary, and transportation fields in recent years (Bircan, 2004).

In this study, 23 articles from Turkey using logistic regression modeling, published between years 2015-2020 in Google Academy, were used to examine. In these articles, it is seen that logistic regression is used frequently in the fields of engineering, social, economics, and education.

In scientific studies, modeling the relationship between dependent and independent variables is very common. One of the most common examples of modeling is the logistic regression method. In this method, where the

maximum likelihood function is used for coefficient estimation, the fact that the assumptions about the variables are low, the coefficients are easy to interpret, and the package programs are numerous, have made it widely used.

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CHAPTER III
**ANALYSIS OF BRAIN WAVES CHANGE IN MATHEMATICAL
CORRECT AND WRONG ANSWER**

*Mehmet Cihan Yavaş**

Introduction

The electrical activity occurring in the brain can be measured with electrodes placed on the scalp and the observed signal is called electroencephalography (EEG). Generally, there are 10-20 EEG system electrode positioning locations determined internationally. Electrodes are placed in these reference places. The brain creates different signals during some different tasks. For example; such as writing a letter, counting, calculating, raising hands (Kuremoto, 2017: 65-78). Brain imaging studies are carried out to understand the changes in the brain of some of the talents transferred to children, arithmetic, and culturally (Dehaene, 2007: 384–398). There is a need for more complex analytical brain views related to how brain activity changes during the mathematical and arithmetic processes of the brain and the connectivity status between different regions of the brain (Peters, 2018: 265-279). It is difficult to directly analyze people's activities and thinking. Although this situation varies from person to person, it is possible only by analyzing the electrical activities in the brain, which is the unit of thinking through computers. EEG is a good device that measures neural activity. Electrical changes in mental and mathematical processes are determined by EEG. The alpha wave is often associated with creativity, relaxation, and visualization processes, with frequencies in the range of 8-13 Hz. The beta wave is in the 13-30 Hz frequency band and is associated with attention, thinking and concentration processes. Delta wave, on the other hand, is in the 0.5- 4 Hz frequency band and is characterized by more sleep (İsmail, 2016: 1005-1011).

Classification of the electroencephalogram (EEG) signal is important in mental decoding for brain-computer interfaces (BCI). Power spectral intensities of different mental tasks (imagination, resting state, mental arithmetic, etc.) are analyzed using single channel EEG signals. Power spectral density analysis is used to reveal EEG features, especially in the frequency analysis method of mental and motor tasks (Özmen, 2018: 1-8).

The hypothesis of this study is to analyze whether there is any change in the electrical activity of alpha, beta and delta waves formed by the brain during two different mathematical tasks directed to 20 healthy subjects. In other words, how the change in brain EEG electrical activity of two

*(Asst. Prof.Dr.); Kırşehir Ahi Evran University Medical Faculty Dept. of Biophysics, Kırşehir, Turkey mcihanyavas@ahievran.edu.tr

different responses given to the same mathematical operation was investigated. In our study, domain measurements, frequency analyzes and changes in power spectral densities will be analyzed for each subject. Thus, it was aimed to determine the brain activity that occurred during the correct and wrong answers given especially to a mathematics question.

Methods

This study was performed on 20 healthy subjects with age range 21.15 ± 2.10 . The decision of the Clinical Research Ethics Committee of Kırşehir Ahi Evran University Faculty of Medicine was taken, and at the same time, the consent of the volunteers was taken and work started after the consent forms were filled.

In the study, alpha, beta and delta waves of the subjects were recorded by Biopac MP36 (BIOPAC System Inc., Goleta, CA, USA) device with bipolar recording (black; ground electrode, two electrodes (red and white) - EL503).



Figure 1. Experiment design- recording and procedure

In the study, two different methods were followed as getting the correct and wrong answers. Each method continued for 5 questions and 10 seconds. Mathematical correct questions; a) $9 \times 2 = 18$, b) $35/7 = 5$, c) $108 + 3 = 111$, d) $57 - 8 = 49$. Mathematical wrong operations; a) $9 \times 2 = 16$, b) $35/7 = 8$, c) $108 + 3 = 1111$, d) $57 - 8 = 41$. Frequency and power analysis of the brain alpha, beta and delta waves were performed in mental calculations with the recording made. The experiment procedure is shown in figure 1.

Statistical data

Statistical analysis of the study was done with SPSS program (V21. Armonk, NY: IBM Corp., USA). The normality hypothesis was tested by the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Descriptive statistics of the variables are given as Mean±Standart deviation, Median (25th percentile - 75th percentile). Paired t test and Wilcoxon matched-pair test were used for univariate analysis of dependent variables.

Results

In the study, 2 electrodes and an electrode ground electrode were positioned to the left head region of the subjects. With the help of a computer, mathematical operations were followed with power point and recording was done with a computer. After standard conditions were met in the study, the records were analyzed. Time-based domain measurements for EEG alpha, beta, and delta waves were recorded, and the mean of mathematically correct answers for 20 subjects is given in table 1, and the mean of false responses is given in table 2. These domain parameters consist of mean, minimum, maximum, standard deviation, skew and kurtosis. In addition, frequency (Fast Fourier Transform=FFT) and power spectral densities (PSD) were analyzed by Biopac software and presented in Figure 2a, 2b and 2c.

When the respondents' responses to mathematical operations were examined, an increase in the amplitudes of wrong groups the mean, min, max, skew and kurtosis parameters of the alpha wave according to the correct response was observed. When the responses of the participants to the mathematical operations were examined, a decrease in the average value of the beta wave and a significant change in the other parameters were not observed in the amplitude of the wrong group. When the responses of the participants to the mathematical operations were examined, it was seen that there was a decrease in all the parameters in the wrong group compared to the correct responding group.

Table 1. Domain results for alpha, beta and delta wave during mathematical correct measurement

	Mean (μV)	Min (μV)	Max (μV)	Std dev (μV)	Skew (μV)	Kurtosis (μV)
Alpha	-0,0016	- 9,0574	8,9656	2,6536	-0,0062	3,2842
Beta	0,0018	- 13,3737	14,9254	3,9588	0,0457	3,1537
Delta	-0,0028	- 13,8712	12,3775	3,5992	-0,1184	4,4452

Table 2. Domain results for alpha, beta and delta wave during mathematical wrong measurement

	Mean (μV)	Min (μV)	Max (μV)	Std dev (μV)	Skew (μV)	Kurtosis (μV)
Alpha	0,0001	- 9,3246	9,2775	2,6394	0,0030	3,4159
Beta	0,0010	- 14,2226	15,5573	3,9051	0,0466	3,2122
Delta	-0,0087	- 10,3655	9,6011	3,3074	-0,1036	3,5148

Correct and wrong answers given during mathematical tasks of alpha wave are examined. There was no statistically significant difference between FFT max value, FFT max F and PSD max F and PSD max power for both groups in the study findings ($p>0.05$).

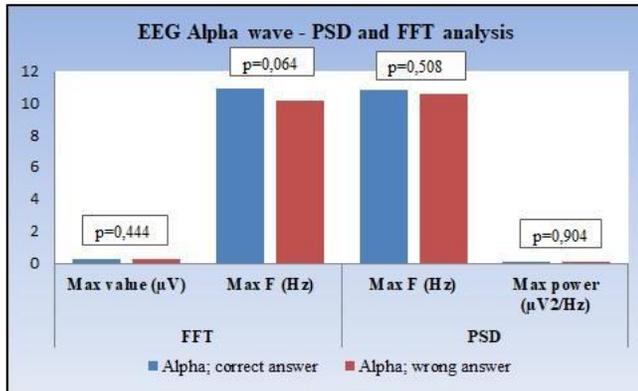


Figure 2a. Fast fourier transform and power spectral density for alpha wave

Correct and wrong answers given during mathematical tasks of beta wave are examined. There was no statistically significant difference between FFT max value, FFT max F and PSD max F and PSD max power for both groups in the study findings ($p>0.05$).

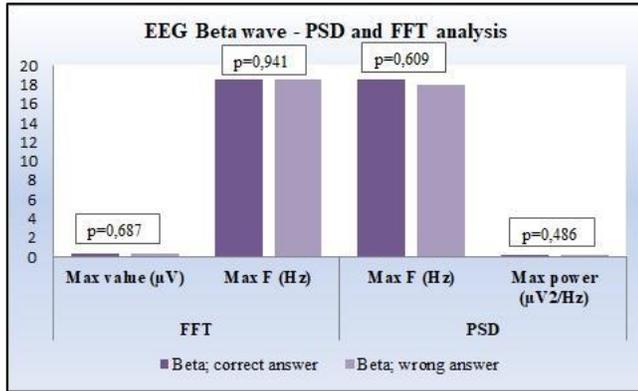


Figure 2b. Fast fourier transform and power spectral density for beta wave

Correct and wrong answers given during mathematical tasks of delta wave were examined. In the two groups comparison, a statistically insignificant difference was found between FFT max value, FFT max F and PSD maximum power parameters ($p > 0.05$). A statistically significant difference was found between PSD max F parameters ($p < 0.05$).

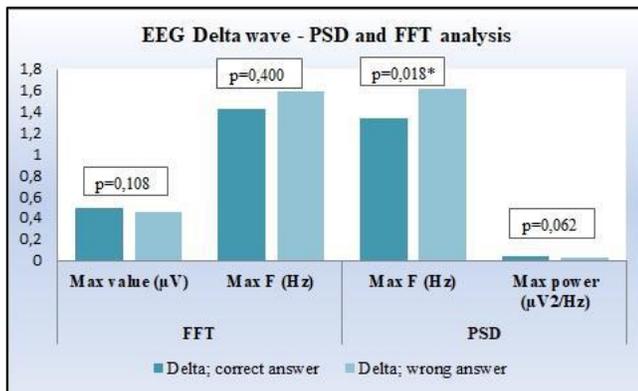


Figure 2c. Fast fourier transform and power spectral density for delta wave

Conclusion

Researchers studied EEG activations in task processes during four different mental performances. Absolute and relative power were calculated for alpha, beta and delta waves. It was found that EEG caused changes in four different task processes including mathematical calculation. A significant difference was detected between tasks in the delta and beta bands in the frontal lobe. When absolute and relative powers are compared between rest and tasks, there is a significant change in alpha

and delta bands. There was a decrease in alpha power and an increase in delta power (Fernández, 1995: 175-182). Two different arithmetic tasks were applied on eighteen healthy medical students. Using the international 10/20 system electrodes, they were asked to respond within 10 seconds against a screen. In comparisons with the control group, 4 subbands of the delta band were analyzed, and EEG delta activity showed a widespread increase in all subbands during the performance of both arithmetic tasks (Dimitriadis, 2010: 11-15). For mathematical calculations, different mathematical methods are also analyzed using non-invasive brain scanning methods for the analysis of EEG signals (Strmiska, 2019: 1-4).

Although our study is similar to the previous scientific study reports, the following results have been found. In our study, standard conditions were provided to prevent the formation of excitement and artifacts. Appropriate filters were used with the Biopac software. In our study, it was asked to give correct and wrong answers for 5 different mathematical tasks on 20 healthy medical students with bipolar method (2 electrodes) in a non-invasive way. We asked each subject to respond correctly to the operation that required 5 numerical calculations, respectively. In addition to this, we requested that the wrong answer prepared for the same numerical operations be provided. In this way, electrical activity in the brain was recorded during the instant numerical tasks. In our study, domain parameters and frequency analyzes and power spectral density analyzes were performed for EEG alpha, beta and delta waves for both tasks.

In our study, the mean, min, max, standard deviation, skew and kurtosis parameters recorded for alpha, beta and delta waves were compared and it can be seen that they may cause a change in the electrical amplitude values in the brain. In addition, fast fourier transform (FFT) (FFT max frequency and FFT maximum value) and power spectral density (PSD) (PSD maximum frequency and PSD maximum power) were also detected in our study. Statistical comparison was made between two tasks for alpha, beta and delta wave. As a result of the statistical analysis, no significant change was noticed ($p > 0.05$). Only a significant change in PSD max value was found for delta wave ($p < 0.05$).

In this study, our aim is to determine what kind of change the two different responses given to the same numerical calculation on the electrical activity of the brain EEG alpha, beta and delta waves. For this reason, our study analyzed the state of EEG activity, especially in case of a wrong answer to a correct answer. Although there are some increases and decreases in the amplitudes of the brain waves, a significant statistical change has not been determined. It is seen that new studies are needed to support our study, and more detailed studies and more detailed methods are needed regarding how the neural activity that the brain can change if the brain responds incorrectly to a correct answer.

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CHAPTER IV
**THE DENTAL CARE FOR PATIENTS ON STEROID
MEDICATION**

Tahir Belice & Suna Toksavul***

Introduction

There is a variety of adverse effects due to the steroids as follows: Immune suppression, hypertension, increased fatty tissue, hyperglycemia, osteoporosis, avascular osteonecrosis, myopathy, peptic ulcers, Cushing syndrome, edema, psychosis, infections, acne, weight gain, poor wound healing and so on. (Hargitai&Sherman 2001:1, Ata-Ali J et al. 2011:2). Glucocorticoids, as anti-inflammatory agents in medicine, are the most frequently prescribed classes of drugs. In the USA, they are prescribed up to 1 million patients over a year (Sewerynek and Stuss 2012:3). Corticosteroids, including cortisol, 11-deoxycortisol, and corticosterone, are steroid hormones that are produced by the adrenal gland, especially in case of a stress trigger happens (Prigent et al. 2004:4). After a physiologic stress, corticosteroids help in maintaining the vascular tone by a permissive effect for the rest of the stress hormones, including epinephrine and norepinephrine. The commercially available corticosteroids are lipophilic, and they bind to the glucocorticoid receptor in the cytosol of a cell to prevent translocation of NFκB from the cytosol into the nucleus (Yamamoto et al. 2001:5). As a result, an inhibition of the expression in pro-inflammatory genes cause a blunted inflammatory response (Stahn et al. 2007:6). With an experimental study in rats, particular glial cells were activated in the subnucleus caudalis of the trigeminal primary afferent fibers complex of rats innervating orofacial areas project following steroid administration (Endo et al. 2014:7). In several studies, there is a possible relationship between steroid administration and trigeminal nociception, and this could be a mechanism of pain signaling from a peripheral afferent nerve fiber, which causes the teeth to be sensitive to stimuli (Shoji et al. 2016:8). The steroid damage the pulpal tissue with narrowing and obliterating the pulp chambers and root canals(Kamatani 1998:9, Alliot et al. 2005: 10). The pulp stone formation as a result of steroid usage can also cause dental pain (Symons 1194:11). Because of this steroid-induced pain, dentists do not stop or change of steroid therapy instead of they extract teeth (Symons 1194:11). So steroids are regarded as a double-edged sword

* (MD, Internal Medicine Specialist, Ph.D. in Health of Elderly); University of Health Sciences, Izmir Bozyaka Education and Research Hospital, Izmir-TURKEY, e-mail: drtahirelf@hotmail.com

** (Prof Dr, MD, Ph.D. in Health of Elderly), Ege University Faculty of Dentistry, Izmir-TURKEY, e-mail: sunatoksavul@yahoo.com

to the patients. Patients with Cushing's can lose their teeth due to bone loss. It is not the same as both osteoporosis and any of the osteoporosis drugs lead to lose more teeth. Due to Cushing's disease, patients are often diagnosed with periodontal disease and its sequelae of tooth loss because of two reasons as follows: Inhibition of white blood cells and bone formation as a result of excessive cortisol production in the body (Chrousos 1198:12).

With this chapter, we aim at revealing the mechanisms of periodontal diseases that are related to the steroids, osteoporosis, and regulation of the nuclear factor-kappa B (NF- κ B), which also another reason for tooth loss.

Osteoporosis, Steroid and Periodontal Diseases

Due to the blockage of immunity to some extent by decreased white cell migration, based on osteoporosis in the alveolar bone as a result of hypercortisolism, periodontal diseases aggravate and finally cause malnutrition, which is also another reason of the immunosuppression (Marya 2015:13, Jacoangeli 2002:14). Interestingly enough, in women who are diagnosed with osteoporosis, the prevalence of tooth loss is higher than men (Rizzoli et al:15). Because of the silent course of osteoporosis, it is not easy to be detected until a fracture occurs mostly at the spine, hip, forearm and proximal humerus of the human skeleton (Mohammad et al. 1996:16). For about decades ago, both osteoporosis and tooth loss were considered as a physiological process associated with aging. But, today, they are accepted as multifactorial chronic systemic diseases (Malhotra and Mithal 2008:17). The associations of osteoporosis and density of the jawbone minerals with periodontal disease and tooth loss were investigated and documented well-enough by many researchers (Guiglia et al. 2013:18, Jeffcoat 2005:19, Persson et al. 2002:20, Geurs et al. 2003:21). A tooth stability could be achieved only by a supportive bony framework, and an osteoporotic alveolar bone can predispose to the progression of periodontal disease (Poole and Compston 2006:22).

Moreover, with another study, tooth loss and periodontal diseases were positively correlated with the low density of bone mineral in spinal regions (Bando et al. 1998:23). A history of the jawbone fracture would increase the risk of tooth loss as well as osteoporosis in older people, which is also another potential risk factor of periodontal diseases (Xie and Ainamo 1999:24). The cytokines are positively associated with the systemic bone loss. The level of cytokines, including the Interleukin-1 and 6, increase during the aging process. Furthermore, the periodontal infections increase the local cytokines, which in turn speeds up the local osteoclasts' activities. So the jawbone's structure is changed by the osteoclastic action as follows: Increased alveolar porosity, microarchitectural deterioration of trabeculae, and finally decreased cortical thickness (Wactawski 2001:25). All of these

changes make the periodontal tissue and, eventually, the tooth vulnerable to the infectious pathogens.

The mouth breathing is a negative factor on alveolar mucosa, which aggravates the gingival inflammation by dehydration. Accumulation of dental plaque decreases the salivation that makes conditions favorable for cariogenic microflora, including *Streptococcus mutans* and *Streptococcus sobrinus*. As a result, the process of irreversible demineralization starts to damage the enamel (Feather 2004:26, Feather 1999:27). The chronic usage of the corticosteroids can result in an alteration of alveolar bone due to the decreased osteoblasts and amount of osteoid matrix (Glickman et al. 1951:28, Applebaum and Seelig 1955:29). Therefore the height of alveolar bone and fibrous transformation of the periodontal space decrease and lead to the contraction of the dentin pulp chamber, concluding a change in the blood circulation and distribution of nutrition in dentin-pulp tissue complex (Goldsmith and Ross 1956:30, Yoshiho et al. 2007:31). However, the pulp calcification occurs after a necrosis start in the outer pulp layer of patients who are on chronic use of corticosteroids (Yoshiho et al. 2007:31, Obersztyn et al. 1968:33). The dentin layers are shaped and developed even though the fluctuations of steroid hormones and modified mature odontoblasts play an essential role in the formation of patients' teeth while they are on the effect of chronic steroid therapy. So they are at risk of gingivitis and tooth decay, as well (Boskabady et al. 2012:34, Daudt et al. 2011:35).

Nuclear Factor-Kappa B and Periodontal Inflammation

NF- κ B is actively crucial in inflammatory or immune responses. As a potent repressor of NF- κ B activity, steroids can antagonize the action of NF- κ B (Hudson et al. 2018:36). NF- κ B is one of the most critical pro-inflammatory transcription factors, and the anti-inflammatory activity of steroids in leukocytes is partly due to inhibition of signaling by NF- κ B. Interestingly, activation of the Toll-receptor family, which recognizes pathogen endotoxins, also leads to activation of NF κ B (Yang et al. 1998:37). Osteoprotegerin, which is a potent inhibitor of osteoclast formation, inhibits RANK by binding to RANKL and osteoprotegerin, is tightly involved in regulating NF- κ B activation. This protein is also a dendritic cell survival factor that is included in the regulation of the T cell-dependent immune response (Baudhuin et al. 2007:38). The odontoblasts activate NF- κ B and p38MAP kinase by the TLR2 interaction which results in a down-regulation of dentine formation as well as a production of pro-inflammatory molecules with immature dendritic cell accumulation into the odontoblast layer that is next to the carious dentine (Carrouel et al. 2013:39, Farges et al. 2009:40). There are two different mechanisms which explain the down-regulation of NF- κ B by glucocorticoids as follows: The

first one is the interaction of the ligand-bound GR with the p65 component of NF- κ B whereas the second mechanism is the transcriptional up-regulation of the I κ B α gene by glucocorticoids (Ray et al. 1994:41, Mukaida et al. 1994:42, Scheinman et al. 1995:43, Brostjan et al. 1996:44).

Furthermore, by way of genes expression mostly in cell survival, proliferation, and differentiation, NF κ B drives the attention of scientists since an invention in these metabolic ways of inflammation will be a leading effect for the development of new drugs for many pathological conditions (Wissink et al. 1997:45). In sum, particular attention is given to odontoblast, which has a role of defense against pathogens and also being the first cell that reacts to an infection in the tooth. So the cellular and molecular mechanisms of the odontoblasts need to be explored much more than that have done so far since it is critical for the development of therapeutic strategies for tooth decays.

Conclusion

The underlying mechanism of tooth decay due to the osteoporosis and chronic steroid therapy might be explored by the new studies which could focus on the NF κ B pathways. Especially in the elderly, the promising therapeutic targets could prevent and cure tooth decay in patients who are under the influence of chronic steroids.

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CHAPTER V
**RADIOGRAPHIC METHODS IN DENTAL AGE ESTIMATION
OF CHILDREN**

*Ahmet Aras**

Identification, which is a very important subject in anthropology and forensic sciences, can be done using fingerprints, palm and footprints, DNA identification and radiographic overlaps (vertebrae, cranial structures, pelvis, bone trabeculae) (1). One of the most important issues in identity determination is knowing the age of the person. When there are no reliable credentials in individuals, there is need of knowing whether the ability to take criminal and legal responsibilities, to perceive the legal meaning and consequences of the crime and to guide their behavior. Evaluation of tooth and bone development is among the most commonly used methods for age determination. In particular, orthodontists and pedodontists are concerned with dental age for diagnosis and planning of treatment (2, 3).

Using teeth for age determination of living individuals and corpses is one of the most reliable methods (4). Teeth in their remains provide valuable information to researchers because of their harsh structural properties, resistance to mechanical, chemical and physical effects and time, and preservation of their morphological structure for a long time (5, 6). In addition, teeth are less affected by environmental and genetic factors compared to other organs of the body. Because of these features, teeth have been a useful material in age determination in forensic medicine and anthropology (7). Besides to forensic medicine and anthropology, teeth are also used for the age determination of unaccompanied asylum-seeking children, which have increased in recent years (8).

Methods developed for age determination from teeth can be classified as histological, biochemical, morphological and radiographic methods. The methods based on the histological and biochemical properties of the teeth are difficult to apply because they require the use of complicated laboratory equipment and the extraction of the tooth, and also because of the time spent (9). Radiographic techniques have advantages such as not harming the integrity of the material compared to histological techniques, providing the opportunity for examination in living individuals, being fast and simple methods and being more economical when compared to DNA techniques (7, 10). Also, many studies have reported that radiographs can be used safely in age determination (7, 11, 12). In children, the error rate

* (Asst. Prof. Dr.); Harran University, Şanlıurfa, Turkey. E-mail: ahmetaras63@gmail.com

of radiological age estimation techniques performed by evaluating the tooth application time and/or mineralization stages is much lower (13).

Historically; in 1941, Schour and Massler showed human dentition from birth to 35 years of age with illustrated schemes that still remain valid today (14). The radiological techniques used by Nolla(12) in 1952, Moorrees (15) in 1963 and Demirjian and Goldstein (16) in 1976 was the most widely used radiological techniques for estimating age from the teeth. The techniques developed by Mörnstaad et al. (17) and Cameriere et al. (18) in subsequent years include radiomorphometric investigations. It is more common to use due to the easier application and standardization of radiomorphometric examinations.

Atlas of Schour&Massler

The aim of the researchers to show the dentition in such a detailed way was to provide dentists with the information they can use in dental practice every day. Transforming this information into an illustrated scheme in the form of an atlas is not only an atlas that specialists (especially pedodontists) can benefit from, but also it can be used in forensic cases for age estimation. This study includes comparative diagrams of all upper and lower jaw radiographs of dentition development stages expected at each age throughout life of a child. The authors suggested that, by comparing the radiographs of the child whose age estimation is to be compared with these diagrams, age can be determined with a certain error rate (14). This method has been criticized for the fact that the gender difference cannot be evaluated due to the small sample size, the number of patients, and the low number of samples. This atlas, which was updated by the American Dental Association in the following years, was last published in 1982 (19).

Technique of Nolla

In 1952, the author focused on dental development stages in his study on radiographs of 1656 male and 1746 female patients. Each permanent tooth was divided into 10 phases from the beginning of its development to its completion, and each stage was scored between 0 and 10. In the next stage, by collecting the points given to the seven teeth in the mandible and the seven teeth in the maxilla, the total tooth development score was obtained. A table of values with a total of 140 points was created by Nolla for girls and boys. And the dental age of the child is determined from the equivalent of the total tooth development by score obtained in the radiographic evaluation in the table of values (12).

The advantage of this system is that the dental development stages can be determined practically as a result of comparing the 10 phases defined in the drawings with the radiographs and not require any equipment and equipment other than the X-ray device (20). However, it is difficult to

define the intermediate stages in the root formation of the teeth, so, it increases the error rate. In the study of Bolonos et al. (13), by using only three teeth with the Nolla standards in 2000, they found the error prediction rate as ± 1.4 under the age of 10 and ± 2 over the age of 10 in the 95% confidence interval.

Moorrees, Fanning & Hunt

Moorrees et al. (15) expanded Fanning's data to include the age of 17-18 in their research to examine tooth development to identify skeletal remains and facilitated the age estimation by scoring the dental development in 14 randomly selected sequential stages (Figure 1) (15, 21). In the study, which aims to reveal the 14 developmental stages for each of the 8 incisors in the upper and lower jaw, 48 boys, 51 girls around the age of 10, and 136 boys and 110 girls around the age of 18 were used. Some researchers have obtained reliable results with an accuracy of ± 2 years when they apply this technique for age estimation in their populations (22). The disadvantage of this technique is that it is difficult to determine the stage of root development since the total length is unknown when evaluating root development (1/4 or 1/2 or 3/4).

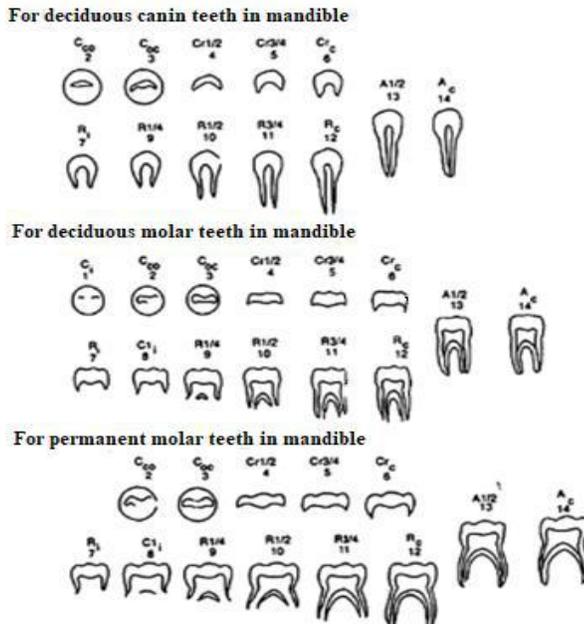


Fig 1. Demonstration of tooth development in 14 stages in Moorrees system (15)

Demirjian, Goldstein & Tanner technique

Demirjian, Goldstein and Tanner reported a technique based on the radiological evaluation of seven teeth in the left mandible in eight stages in Canadian children of French origin in 1973 (23). The researchers were inspired by Moorrees, Fanning, and Hunt's classification of dental development and used panoramic images of 1446 boys and 1482 girls between the ages of 2 and 20 in their research (15). Eight stages of tooth development are illustrated from A to H, and each stage of mineralization is clearly defined (Figure 2) (23).

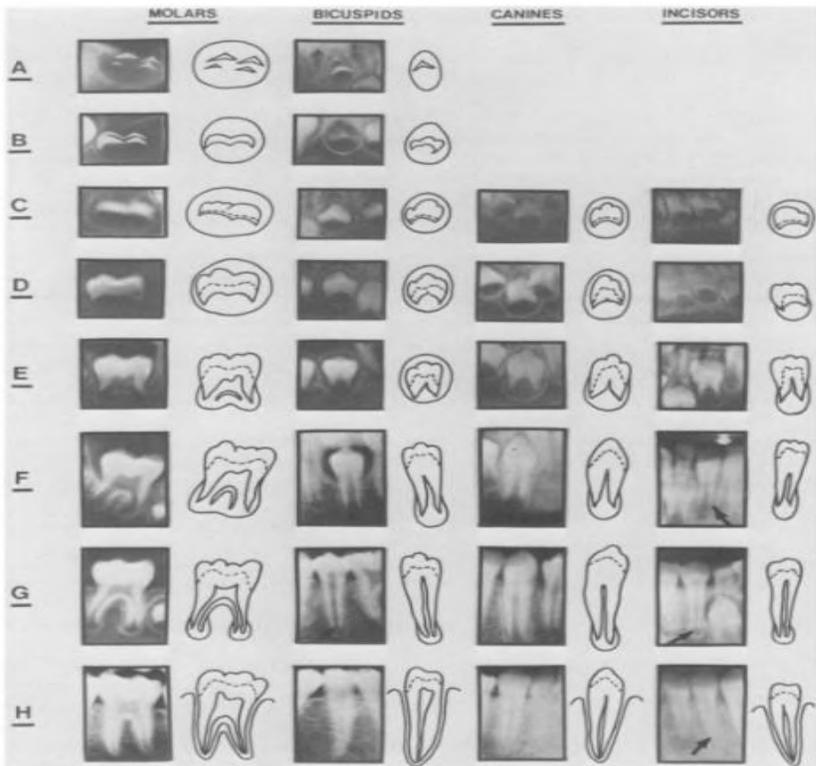


Fig 2. Demonstration of Demirjian classification stages in 8 levels (23)

In 1976, Demirjian and Goldstein increased the number of samples, and the system was modified using graphs of 2407 boys and 2349 girls between the ages of 2.5-17. In the following years, various researches have been carried out on the expansion of the system's usage area and improvement of its reliability(16). The scoring system used by Tanner, Whitehouse and Healy to estimate the age with bone development has been further developed. Numerical values are given for each phase of the seven teeth used in the technique and these are published in tables (Table 1) (24).

Male	Tooth Number (FDI)						
Stage	31	32	33	34	35	36	37
0					0.0		0.0
A				0.0	1.5		1.7
B				4.0	2.7		3.1
C	0.0	0.0	0.0	6.3	5.2	0.0	5.4
D	4.3	2.8	4.0	9.4	8.0	5.3	8.6
E	6.3	5.4	7.8	13.2	10.8	7.5	11.4
F	8.2	7.7	10.1	14.9	12.0	10.3	12.4
G	11.2	10.5	11.4	15.5	12.5	13.9	12.8
H	15.1	13.2	12.0	16.1	13.2	16.8	13.6

Table 1. According to Demirjian and Goldstein, dental development table in males(16)

By looking at the numerical values of these teeth in the related graph, the total development score is obtained for all teeth. For example, if a male panoramic graph is found to be in phase E for tooth 36, the value '7.5' is taken from this table for that tooth. By doing this for each tooth evaluated, the total development score is calculated on seven teeth. In the next stage, the age estimation is made based on the total dental development score obtained through the regression curves of the tooth development scores in boys and girls, through the conversion tables containing values between 0-100 (Table 2) (25).

Age	Score	Age	Score	Age	Score	Age	Score	Age	Score	Age	Score
4.0	23.5	6.0	41.7	8.0	71.3	10.0	88.7	12.0	95.7	14.0	98.6
4.1	24.7	6.1	43.0	8.1	73.0	10.1	89.0	12.1	96.0	14.1	98.7
4.2	25.3	6.2	44.1	8.2	74.5	10.2	89.5	12.2	96.2	14.2	98.8
4.3	26.1	6.3	45.2	8.3	76.0	10.3	90.0	12.3	96.4	14.3	98.9
4.4	27.0	6.4	46.1	8.4	77.1	10.4	90.4	12.4	96.6	14.4	99.0
4.5	28.0	6.5	47.4	8.5	78.2	10.5	90.8	12.5	96.7	14.5	99.1
4.6	29.2	6.6	48.7	8.6	79.3	10.6	91.5	12.6	96.9	14.6	99.2
4.7	29.9	6.7	49.9	8.7	80.1	10.7	91.9	12.7	97.0	14.7	99.2
4.8	30.8	6.8	51.0	8.8	81.0	10.8	92.2	12.8	97.1	14.8	99.3
4.9	31.4	6.9	52.1	8.9	81.8	10.9	92.6	12.9	97.3	14.9	99.3
5.0	32.1	7.0	53.0	9.0	82.7	11.0	92.9	13.0	97.5	15.0	99.4
5.1	33.2	7.1	54.9	9.1	83.4	11.1	93.2	13.1	97.6	15.1	99.4
5.2	34.3	7.2	56.2	9.2	84.0	11.2	93.6	13.2	97.8	15.2	99.5
5.3	35.4	7.3	57.8	9.3	84.7	11.3	94.0	13.3	97.9	15.3	99.6
5.4	35.9	7.4	59.3	9.4	85.2	11.4	94.2	13.4	98.0	15.4	99.7
5.5	36.8	7.5	61.1	9.5	85.9	11.5	94.5	13.5	98.1	15.5	99.8
5.6	37.9	7.6	63.3	9.6	86.6	11.6	94.8	13.6	98.2	15.6	99.8
5.7	38.9	7.7	65.5	9.7	87.0	11.7	95.0	13.7	98.3	15.7	99.9
5.8	39.8	7.8	67.5	9.8	87.6	11.8	95.2	13.8	98.4	15.8	99.9
5.9	40.7	7.9	69.4	9.9	88.2	11.9	95.5	13.9	98.5	15.9	100

Table 2. Table to convert dental development scores in males to dental age (16)

Willems method

Willems et al. (11) modified this method after a survey which revealed that Demirjian method overestimated age in the Belgian Caucasian population. The adapted method later became known as Willems method.

Mörnstaad, Staaf & Walender

In 1994, Mörnstaad et al. (17) developed an age estimation method based on the degree of development of Northern Swedish and Finnish children. Panoramic radiographs of 270 boys and 271 girls between the ages of 6-14 were examined and the variables determined in each x-ray film were measured digitally (Figure 3). Researchers found a correlation between the variables determined for tooth development and age, created a multiple regression model, and showed that a series of dental measurements can be used in the age estimation. As a result of the study, $R^2 = 0.78$ was calculated and the standard error was found as ± 2 age in the 95% confidence interval. The advantage of this method is that the measurements are based on precise and objective criteria. On the other hand, the age estimation reliability in other societies is low because it has been developed in a specific population.

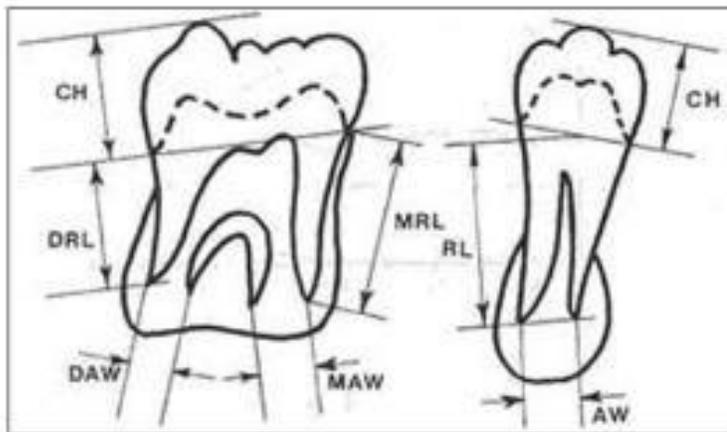


Fig 3. Measurement points defined in single and double-sided teeth (17)

(*CH: Crown Height, DRL: Distal Root Length in molars, MRL: Mesial Root Length in molars, RL: Root Length in single root teeth, DAW: Distal Apex Width in molars, MAW: Mesial Apex Width in molars, AW: Apex Width in unidirectional teeth*)

Recent developments in dental age estimation with radiological methods

The developments in digital imaging techniques (3D imaging, higher resolution, possibility to work with more materials) in recent years have increased the reliability of the existing studies and have enabled the development of new techniques (3, 26). AIQanhtani et al. (27) used radiographic materials from intrauterine to 22 years of age, by creating a practical dental prediction atlas containing drawings showing tooth

development and eruption for all ages. In their study, aiming the age estimation from the third molar tooth, Bassed et al. (28) found that computed tomography images are more useful because of display the development stages of the teeth better than conventional X-rays. In a study, it was tested whether a new stage classification based on CT sections visibility of the periodontal ligament in lower third molars in a Chinese population can be used for the 18- and 21-year thresholds and an effective method for age estimation was reported (29). Yang et al. (30) investigated the association between chronological age and the pulp/tooth volume ratio of specific teeth using CBCT scans enhanced with Materialise-Mimics Research software in children and young adult population from Eastern China. In a study, magnetic resonance imaging was used for dental and skeletal age estimation and claim that this imaging technique was useful for age estimation (31). In their study evaluating pulp chamber volume using Micro-CT, Agematsu and colleagues (32) reported that age and gender can be determined using pulp volume.

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CHAPTER VI
**WHITE SPOT LESIONS IN FIXED ORTHODONTIC
TREATMENT, CONTEMPORARY PREVENTION AND
TREATMENT APPROACHES**

Ela Eusmanaga & Sanaz Sadry***

Introduction and Aim

Fixed orthodontic appliances create serious retention areas for accumulation of bacterial plaque. Acidic products of accumulated bacteria in these areas are responsible for sub-surface demineralization of the enamel and formation of white spot lesions. These lesions cause problems such as poor aesthetics and patient dissatisfaction, after orthodontic treatment. Therefore, prevention of white spot lesions and early stage treatment, are very important in terms of achieving the desired fixed orthodontic treatment success. The purpose of this article is to investigate the prevention, diagnosis and treatment of white spot lesions caused by orthodontic treatment. It discusses available treatment options to intervene, especially before creating a structural defect.

1. Formation of Enamel Caries

Caries is a pathological condition in which demineralization increases due to the disturbance of the demineralization-remineralization balance between the tooth and surrounding tissues due to the acid produced by the cariogenic microorganisms localized on the tooth surface as a result of their microbial activities, by fermenting carbohydrates (1).

2. White Spot Lesions (WSL)

2.1 Definition of White Spot Lesions

White spot lesions (WSL) are described as "the first visible sign of caries lesions detected by the naked eye" on the enamel. Tufekci E et al. also defined WSL as "sub-surface porosity due to caries demineralization". (2) They are the earliest stage of tooth decay. There is mineral loss on the outer surface of the tooth without structural defect in the demineralized area yet. (3) At this stage, it is possible to stop and treat the caries.

2.2 Etiology of White Lesions

Tooth decay is traditionally a multifactorial disease involving host factors such as saliva, tooth tissue, microflora, and the interaction of diet and dental plaque. WSL are lesions that appear on the enamel due to the effect of carbohydrate-rich diet, bacterial activity, and insufficient saliva,

* (Dt.); İstanbul Aydın Üniversitesi, Ortodonti Anabilim Dalı. İstanbul, Turkey E-mail: elaosmanaga@gmail.com

as well as disruption of the demineralization and remineralization balance. (4)

Remineralization and demineralization balance can be impaired according to the state of the oral region, bacterial retention on the enamel surface, the standard oral care of the individual and the natural resistance of the tooth. (5) Enamel crystal dissolution begins at the sub-surface enamel, creating pores between enamel rods. After this dissolution, surface brightness and roughness loss is observed in the affected area. Since porous enamel reflects light better than solid enamel, there is a difference in enamel inner reflection and enamel opacity. (6)

In general, it is accepted that fixed orthodontic appliances create retentive areas for plaque accumulation which make oral care more difficult. Which cause an increase in colonization of bacteria such as *S. Mutans* and *Lactobacillus*. It was reported that during the orthodontic treatment, the level of *S. Mutans* increased up to 5 times, and this microbial level showed a significant decrease only during the 6-15 week period following removal of the appliances. (7)

2.3. Classification

White colored lesions in enamel can be classified as fluorosis, opacities and white spot lesions. (8) Various criteria have been determined to distinguish fluorosis and opacities. (9) Fluorosis are formations of uncertain boundaries, white-yellowish in color, spread over normal enamel and have a symmetrical distribution in the mouth. Non-fluoride enamel opacities have a more prominent shape, and are well distinguished structures and randomly distributed in the mouth. Important factors in the evaluation of WSL are the size and density of the lesion. In literature, white spot lesions were classified for the first time from an orthodontic perspective by Curzon and Spector (1977) (10) visually by size.



The classification is made from 0 to 3 according to the width of opacity formed on the enamel surface as following:

- Class 0: No opacity or less than 1 mm²,
- Class 1: Cases where opacity covers 1/3 of the surface of the tooth,
- Class 2: These are cases where opacity covers between 1/3 and 2/3 of the surface of the tooth.

Class 3: These are cases where opacity covers more than 2/3 of the surface of the tooth.

2.4 The Relation between White Spot Lesions and Fixed Orthodontic Treatment

Brackets and bands applied in fixed orthodontic treatment create a retention area in the mouth, causing plaque buildup. In addition, they prevent saliva and muscle functions, which are responsible for cleaning the accumulated plaque by natural mechanism. (10) For this reason, dental plaque volume of patients undergoing orthodontic treatment increases faster than others. Also the pH of the formed plaque is lower. (11) Therefore, the risk of caries increases in orthodontics patients (12). In studies investigating the incidence of white spot lesions in patients undergoing orthodontic treatment, lesion formation with 2-96% different results was observed. (13-15) For example, while at least one white spot lesion was observed in 50% of the patients treated, Hadler-Olsen et al. found this rate as 11% in the untreated control group. (16) In a recent study, Richter et al. (17) found the rate of occurrence of at least one white spot lesion in patients at 72.9%, and observed cavitation at 2.3% in these lesions. Boersma et al. (18) found that in 97% of the patients they followed 30% of the teeth developed white spot lesions. The white spot lesion is most common in the upper lateral incisors (19). Due to the short crown length of the upper side incisor, the distance between the bracket and the edge of the gingiva is reduced resulting in a difficult area to clean. Removal of the plaque in this area is more difficult than other teeth. (13, 14) When a regional examination is performed on the frequency of white spot lesion formation, the anterior region of the upper jaw and the canine and premolar region of the lower jaw are more affected. (13) In Turkey, Akın et al. (20) reported that 65% of patients developed white spot lesions after fixed orthodontics. However, more clinical studies in this topic are needed.

3. Evaluation of White Spot Lesions

3.1 Macroscopic Methods

Macroscopic methods are based on changes in the optical properties of the enamel after losing minerals. The reason why a demineralized lesion in the enamel is white is due to the increased backscatter of the light. (2) As enamel loses minerals, it becomes rougher. Some of the minerals are replaced by water, which leads to an increase in the refractive index between the solid and demineralized enamel. A light photon travels a much shorter distance before scattering in carious enamel. Most of the photons scatter inside the lesion, rather than penetrating it into the dentin. Since the number of backscattered photons is greater, it results in a clinical appearance of a white spot on the enamel.

Clinical Examination: Clinicians are trained to perform a visual examination to evaluate demineralization before, during and after orthodontic treatment. Advantages: simple, cheap and clinically valid. Disadvantages: validity can be questioned, bias risk and clinician related errors may occur.

Photographic Examination: Evaluation with photographic technique is common by clinicians. It is used for archiving purposes, comprehensive examination and motivating patients. Advantages: Fast and standardized method, permanent record, masking patient details, no bias risk and minimal clinician variability. The images can be digitized with the help of computer and evaluated by independent experts according to the severity, width and color of the lesion. Disadvantages: The possibility that camera records differently than the naked eye, the reflection of the flash shows more opacity, can make standardization difficult considering the wetness and lighting conditions. (15).

Optical Non-fluorescent Methods: It was first described by Ten Bosch et al. (21). They used 100 W white light and measured backscatter with a densitometer using the Optical Caries Monitor (OCM). Advantages: It is an Easy and convenient method to evaluate the tooth without causing any damage. Disadvantages: The sensitivity of the technique varies depending on the degree in which the tooth is dry or wet, in addition, the cost of the device limits the use. (22)

Optical Fluorescent Methods: Demineralization causes more scattering of light, so it absorbs less fluorescence and looks darker. There are different techniques for producing fluorescence in enamel.

Fluorescent Dye Uptake: After dye uptake, the sample is examined under the appropriate light source. The disadvantages of this procedure is that the slight variation of the procedures also leads to wide change in dye uptake. In addition, this technique is used for the detection and removal of carious dentin.

Ultraviolet: Some studies have been done using the Ultraviolet light for early detection of carious enamel, but the wavelength is harmful to the eyes and skin.

Laser: Bjelkhagen et al. used an argon laser to distinguish the luminescence of the carious and intact enamel. (23) Another method uses DIAGNODent device, it produces a reading, which is considered to be an indication of bacterial activity, rather than mineral loss. (24) Advantages; It does not contain X-ray, it can detect early caries in fissures, it does not damage the tissue due to its low laser power and it is painless and repeatable. (25) The disadvantages are; the size of the equipment that provides the light source, expensive, insufficient to make differential

diagnosis in advanced dentin caries and secondary caries, and making inaccurate measurements in the presence of plaque or calculus.

Light (Quantitative Light-Induced Fluorescence or QLF): It is a smaller and portable system for intraoral use with a new light source and filter system. It provides early detection of demineralized lesions and detects changes in mineral loss and size over time. (26)

3.2 Microscopic Methods

Caries Models: It is done in extraction cases, where a band or bracket is placed on a tooth planned to be extracted. The disadvantage of this method is that only extracted teeth can participate and the length of the experiment is limited.

The In Situ Caries Model: In this method, some of the enamel is placed in a specially designed holder and attached to the arc wire or to a removable appliance worn by the patient. It is evaluated after being placed for a certain time. (27) This technique has a number of advantages: First, it follows the natural caries process. The symmetry of the same tooth can be taken into the control group and does not affect orthodontic treatment, and it can be used during the treatment period. But its main disadvantage is that it is very time consuming.

4. Treatment Approach to White Spot Lesions

The best method is to prevent WSL before they occur. Therefore, prevention methods take the first place.

4.1 Prevention Methods of White Spot Lesions

4.1.1 Assure oral hygiene: After placing brackets, the resting pH drops slightly in the mouth, but good oral hygiene keeps the pH above the critical point of 4.5. Saliva fluoride prevents lesions by increasing remineralization and inhibiting demineralization. (28) When oral hygiene is poor, the plaque collects around the orthodontic appliances, and the remaining pH may fall below the fluoride effect of pH 4.5. At this low pH value, the lost minerals will not be stored again. On the contrary, decalcification will occur. (29) Before starting orthodontic treatment in patients who do not provide good oral hygiene, the consequences of poor hygiene should be explained. When patients realize that it can result in ending their treatment, their motivation increases. It is recommended to brush at least twice a day and after each meal. In patients with motivation problems, ultrasonic or electric toothbrushes can be used to remove dental plaque. (30)

4.1.2 Use of Fluorinated Agents: Fluorine ion in saliva and plaque plays a role in the prevention of demineralization and the formation of remineralization. Fluorine enters the crystalline structure in the hard tissue of the tooth and replaces the hydroxyapatite crystal to form the

fluoroapatite crystal. (31) The newly formed fluoroapatite crystal is more resistant to acid attacks than hydroxyapatite. (32) The application forms:

4.1.2.1. Community Fluoride Support: The fluoridation of drinking water and salt is an effective and inexpensive method that does not require the adaptation of people in terms of protecting public health, recommended by the World Health Organization (WHO). In Turkey, fluoride cannot be added to drinking water or salt yet. In the studies conducted, the caries protective and safe drinking water fluoride concentration is around 1 ppm. It is reported that fluorosis table appears at higher concentrations. (33)

4.1.2.2 Fluorinated Toothpaste, Gels and Mouthwashes: In order for remineralization to occur in orthodontic patients, the fluoride concentration in toothpastes should not be below 0.1%. The pastes typically consist of sodium fluoride, monofloraphosphate, Stannous fluoride or their combinations. (34) According to the reviews made in recent years, the most effective program for the prevention of demineralization has not been prepared. (15, 35, 36) Despite this, daily use of 0.05% fluoride was recommended in patients undergoing fixed orthodontic treatment. (15, 37) In an in-vitro study conducted by Linton (38), it was found that the solution containing 50 ppm fluoride was more suitable for remineralization than the solution containing 225 ppm fluoride. However, Wilmot did not find an advantage of 50 ppm (low concentration fluoride) over fluoride-free mouthwash and toothpaste measures. In other words, the dose-response relationship and the benefits of the frequency of administration of concentrated fluoride-containing agents have not yet been fully stated. (39)

4.1.2.3 Fluorinated Elastomeric Modules and Ligatures: In vivo, studies pointed that elastic ligatures releasing fluoride are successful in reducing demineralization. (40, 41) Banks et al. (40) compared Stannous-fluoride-releasing elastic ligatures to conventional elastic ligatures in an in vivo study. As a result of the study, it was observed that the elastic ligature releasing fluoride decreased demineralization by 49% for each tooth. However, although ligatures initially release high levels of fluoride, the amount of release during the treatment does not remain constant and shows a rapid decrease over time. Therefore, it is not possible for elastic ligatures to prevent caries during orthodontic treatment. (15)

4.1.2.4 Fluoride-releasing Sealent, Primer and Adhesive: As the fixed orthodontic treatment time increases, the risk of demineralization in the enamel increases as well. For this reason, it is important to release fluoride around the bracket of the bonding system. Resin sealants are of two types; chemical and light-curing. Initially chemical-curing resin sealants were used. But their disadvantages have arisen over time. (42, 43) Some studies suggest that light-curing sealants are better polymerized and better in preventing demineralization because they completely cover the

enamel surface. (43) Another method is the application of varnishes with a high content of fluoride. It has been reported that demineralization is reduced by 38-50% in patients with fixed orthodontics who have varnished applied during treatment. However, varnish application also has disadvantages. The effectiveness of fluoride is limited with clinical visits. The fact that the applied varnish may cause temporary discolorations on the teeth and gums causes aesthetic problems. (44) In addition to varnishes, the Glass ionomer cement can be used to prevent demineralization. By adding resin into this cement, its adhesion increases and is called resin modified glass ionomer cement (RMCIS). When the brackets are cemented with RMCIS, an increase in plaque accumulation is seen around the bracket. (8) RMCIS releases fluoride like conventional glass ionomer cements, but it has higher binding strength.

In in-vitro studies, fluoride releasing Transbond Plus SEP has been reported to have less binding strength than etch-rhinse adhesive systems. However, in some studies, it has been observed that Transbond Plus SEP is clinically accepted and even has more binding power than etch-rhinse adhesive systems. (45-49)

4.1.3 Use of Casein Phosphopeptides Amorphous Calcium Phosphate

Recent studies showed that milk and dairy products have anti-caries properties. Harper et al. (50) compared the anti-cariogenic properties of four different cheese types. They found that cheese, which has the most protective properties against caries, contains the highest amount of CPP-ACP. It has an anti-caries effect as it stabilizes the calcium and phosphate required in the human mouth (51). Since CPP-ACP reduces the incidence of dental caries, it supports the beneficial effects of topical fluoride. CPP-ACP does not cause fluorosis. Therefore, it can be used to reduce the need for fluoride, and the incidence of fluorosis. (51) CPP-ACP is added to sugar-free gum, mouthwash, pastille, pat, sport drinks and restorative materials. (52) Before applying orthodontic appliances, it is recommended to prophylactically use agents containing CPP-ACP. Although it has been suggested that agents containing CPP-ACP will affect the binding strength of the brackets, studies haven't reported such an effect in clinical practice. (53)

4.1.4 Usage of Antimicrobial Agents: Antimicrobial agents are used to remove microorganisms in the demineralization phase. Chlorhexidine and benzalkonium chloride are the most preferred agents for this purpose. (54) These agents reduce the number of *S. mutans*. There are studies showing that the caries formation decreases significantly with the decrease of the number of bacteria in the environment. (54) Chlorhexidine is the clinically most accepted antimicrobial agent and is generally available as a

0.2% mouthwash or a 36% varnish. In a study using chlorhexidine mouthwash, this agent was found to cause a significant reduction in all bacteriological parameters. But when used regularly, it causes discoloration on teeth. Therefore, 0.2% chlorhexidine mouthwashes should be used at certain intervals in addition to other protective methods. (55) Korbmacher et al. (56) stated that the caries inhibitory effect of fluoride can be enhanced by the combination of antibacterial agents. It is mentioned that stannous fluoride prevents the bacteria adhesion to the enamel surface. (57, 58) stannous fluoride ions blocks the pathways for sucrose in bacteria. Thus, they prevent the acid production that can occur by fermentation. Therefore, fluoride and antibacterial toothpastes are more effective in preventing caries around the brackets than only fluoride-containing toothpastes. (59) Another method is the use of antibacterial materials in combination with adhesive systems. Bishara et al. (60) stated that the binding strength of the brackets did not decrease significantly in the samples of chlorhexidine applied mixed with the primer before bracketing or in the chlorhexidine samples applied after bracketing. As a result of another in vitro study by Bishara et al. (61), the bond strength formed by the 2.5% cetylpridinium chloride-containing adhesive was not found significantly different from the control group.

4.1.5 Xylitol: It is known that xylitol has an anti-caries effect by limiting the carious lesion. This is based on being a non-fermentable sugar and inhibiting the proliferation and growth of *Streptococcus mutans* (62). It has been reported that chewing of xylitol gum for three minutes five times a day is effective in preventing acid attacks in the mouth (63). However, since it is not desired to chew gum in patients undergoing orthodontic treatment, it is recommended to use xylitol lozenges. It should be remembered that xylitol affects the digestive system and overdose should be avoided (64).

4.2 Treatment of White Spot Lesions

4.2.1 Oral Hygiene: Oral hygiene improvement and motivation programs are given in many countries, starting primarily from schools. There are many studies reporting that active enamel caries lesions can be stopped and treated by improving oral hygiene and using fluoride toothpastes. (65-69) The regulation of diet depends on the frequent consumption of fermentable carbohydrates, as well as saliva flow rate, plaque formation and the use of anti-cariogenic agents. (70) While the biofilm layer keeps forming continuously on the surface of the teeth, at the same time, the demineralization-remineralization balance continues dynamically within this formation. (71) When the dental plaque is not removed regularly and mechanically, the balance shifts to the demineralization side, resulting in the formation of initial lesions. Tooth brushing and flossing is the most effective method to modify the content

of dental plaque and change the process of the initial enamel caries. (72) This method ensures the ideal oral conditions, heals damaged crystals by ensuring remineralization. (73) Dietary carbohydrates are a local risk factor for dental caries. Hard and fibrillated structures of some foods have an anti-caries effect. Some of them help with mechanical cleaning, some by their taste and smell increase the amount of saliva flow. (74)

4.2.2 Topical Fluoride Application: Applying topical fluoride on the WSL is considered the first step of treatment for many orthodontists. Theoretically, it is thought that applying high concentrations of fluoride will be more useful in treatment, but this process may actually cause undesired aesthetic problems. High concentration of fluoride applied directly after the treatment provides remineralization on the surface only. But untreated parts remain in the deeper layers of the lesion and continue to cause aesthetic problems. (75) Therefore low calcium and fluoride penetration from saliva should be allowed after treatment. (8) In his study, Linton found that mouthwash containing 50 ppm fluorine is much more effective than 250 ppm fluorine mouthwash. Applying acid etching before fluoride application can facilitate remineralization (38).

4.2.3 Casein phosphopeptide-amorph Calcium Phosphate (CPP-ACP) Application: In 1980, Reynolds found that the casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) obtained from milk affected caries development. CCP-ACP agents have been produced to carry calcium and phosphate, such as foam, mouthwash, topical paste, chewing gum, sugar-free lozenge forms. Reynolds et al. (1998), in their study on humans using a solution containing 1% CPP-ACP they determined an increase in both calcium levels by 144%, and phosphate level by 160% of in oral environment. Which decreased the mineral loss caused by frequent sugary solution consumption by $51 \pm 19\%$. As a result, they have demonstrated the anti-caries feature of CPP-ACP. (51) Reynolds found that CPP-ACP increased the ratio of calcium (Ca) and inorganic phosphate (Pi) in plaque by 118% and 57%. Which increases the pH of the medium. Thus, it decreases demineralization and increases remineralization. In his study in 1995, Reynolds found that a solution containing 1% CPP-ACP treated 55% more white spot lesions than a normal water-only solution. (51)

4.2.4 Dental Bleaching: The purpose of this procedure is to camouflage small and medium white point lesions by whitening the periphery tissue. Whitening can be applied to the remaining lesions after the natural remineralization process occurs. Whitening after orthodontic treatment may be beneficial in some cases. In patients who are uncomfortable with the yellowish discoloration of their teeth, bleaching is applied with different doses of hydrogen peroxide gel whitening systems, using transparent trays overnight or at dental offices. (76)

4.2.5 Microabrasion: Clinicians generally consider to restore surface defects or discolorations when bleaching is not a solution. Microabrasion process is known as the most harmless method that creates a permanent solution as an alternative to restorative methods. (77) It is a method based on the removal of enamel surface by applying different mixtures which contains acidic and abrasive components. (78) Studies showed that with a one-minute application, 12 μ m in the first process and 26 μ m in the following processes are removed from the enamel.(79) Since the outer surface of the enamel is richer in fluoride and more resistant to external factors, less enamel is removed in the first step. The most commonly used form is to apply 18% hydrochloric acid (HCl) and medium grain pumice in the form of a gel to polish with a rotary tool. When HCl is mixed with pumice, its abrasive feature increases. Although the microabrasion process removes some enamel from the surface, it leaves a smoother enamel texture behind. During this process, since the calcium and phosphate minerals are plugged into the interprismatic space, the enamel surface after the process looks both different than normal enamel and becomes more resistant to external factors (80).

4.2.6 Usage of Chlorhexidine Gluconate: Chlorhexidine Gluconate, which has a broad spectrum effect, is a cationic antimicrobial agent. The positively charged chlorhexidine molecule is attached to the oral mucosa, microorganism or phosphate, carboxyl or sulfate groups of the pellicae by electrostatic forces. As a result of its antimicrobial effect, it also reduces the metabolic activity of the dental plaque. (81) Chlorhexidine gluconate is applied as mouthwash (0.12-0.2%), toothpaste or varnish. It has been reported that mouthwash containing chlorhexidine gluconate (0.2%) cannot prevent demineralization of enamel and dentin in situ. (82) In vivo study comparing the anti-cariogenic efficacy of chlorhexidine and fluoride-containing toothpastes, it was determined that chlorhexidine and fluoride-containing paste were more effective than pastes containing only one of these agents. (83) It has been found that the use of chlorhexidine and fluoride-containing varnishes is more successful in remineralization of early enamel lesions. (84, 85) However, among the local adverse effects of this agent, discoloration of teeth, tongue and restorations have been reported, in addition to desquamation and changes in taste. (86)

4.2.7 Resin Infiltration Technique: The study, which made the first definition of the term "infiltration of carious lesions" was made by Robinson et al using organic resins in 1976. The researchers found that after applying "resorcinol-formaldehyde" resin to white spot lesions the pore volume in the carious tissue was reduced by up to 60%. (87) However, this substance was excluded because it is toxic to the oral tissues. For this method, an ideal resin material should be hydrophilic, have low viscosity and high surface tension value, can be polymerized, stay stable in the

mouth environment and should be considered aesthetically pleasing. Various dental adhesives were then marketed, exhibiting some accepted properties for infiltration. (88) It has been reported that the resin with higher penetration coefficients is TEG-DMA. (89) As a result of these studies, a resin infiltration technique that provides the transition between noninvasive and minimally invasive treatments of WSL has emerged. This technique was introduced to the market as ICON (DMG America Company) after the collaboration of Charite Berlin University and Kiel University. (90, 91) The first step of ICON application presented as a kit is to apply 15% HCl acid for 2 minutes on the early enamel lesion to remove the hyper-mineralized thick superficial layer of the lesion. In the second step, 99% ethanol is applied for 30 s and the lesion is sufficiently dried. At the last step, TEG-DMA, is applied for 3 minutes, then it is polymerized for 40 s with a light source with a minimum wavelength of 450 nm. The goal of this technique is to allow the resin to penetrate the porous enamel tissue by following the capillary configuration. In this way, microporosities that act as diffusion pathways are closed to acids in the mouth environment and the progression of the lesion is prevented. This is done without removing healthy dental tissue. In this way, the progression of caries is prevented and survival rate of the tooth is increased. (92) The resin infiltration technique is suitable for active smooth surface lesions that do not progress to dentine, and without visible cavitation. Although this treatment method was originally developed only for early caries lesions, it was stated that it can be used to mask white lesions formed for different reasons. (93) In addition, the resin infiltration technique has other advantages such as ensuring high patient satisfaction as well as having no risk of postoperative sensitivity and pulpitis, and less likely to form secondary caries compared to conventional restorative treatments. (87)

Senestraro et al reported that there was a significant decrease in the size and clinical appearance of white spot lesions treated with this method compared to the untreated control group in their study. (94) In a study similar to this, Feng and Chu reported that they were successful in camouflaging the appearance of initial carious lesions in their treatment on 8 patients and that no color change was detected in the 12-month control. (95)

5. Conclusion: Decalcifications of enamel around fixed orthodontic appliances is a common complication during and after orthodontic treatment. White spot lesions are managed mainly by educating and motivating the patient to assure good oral hygiene. In addition, prophylaxis with topical fluoride can be carried out. Other materials and methods including antiseptics, CPP-ACP, probiotics, sealants, tooth bleaching, microabrasion, and resin infiltration have also been recommended. Nevertheless, good oral hygiene is the most important factor in fixed orthodontic patients to prevent formation of white spot lesions.

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CHAPTER VII
**CERAMIC DENTAL IMPLANTS and PROSTHODONTIC
APPLICATIONS**

*Mahmut Sertaç Özdoğan**

Introduction

The use of ceramic materials in dentistry is becoming more and more common. Ceramic implants in implant dentistry are of curiosity and desirability. The molecular structure of ceramics is closer to the human body and increases the importance of these materials.

Dental Implant

Development of Ceramic Implant

Inadequately compensated by a dental prosthesis, edentulousness does not only mean oral dysfunction and loss of alveolar bone but also lowers self-esteem due to aesthetic concerns. In recent years, dental implants have been compensated for by increasing the number of patients. Dental implants are biomaterials used in the treatment of tooth losses by placing them in or on the jaw bone (Adell, 1981).

There are several limitations to the use of titanium for dental implants. Due to its color, it causes aesthetic problems, causes hypersensitivity when compared with ceramic materials (Muller, 2006), low ion release (Elias, 2008), allergen effect and plaque colonization (Evrard, 2010; Chaturvedi, 2013), while the negative aspects of titanium.

Ceramic materials are frequently used in dentistry. It is used as veneer material in super metallic structures, as core material and superstructure in full ceramic restorations.

Ceramic implants have been applied in the dental field for a while and the first aluminum oxide was used in the field. Although osseointegration was good, it did not have sufficient mechanical properties (Oliva, 2010).

Sandhaus carried out the first applications of aluminum oxide ceramic implants (CBS = Crystal Bone Screw) (Odontostomatol, 1968). however, it reported that 25% success after five years of GIS cases (Strub, 1987). Cerasand (Incermed, Lausanne, Switzerland), another ceramic implant, was applied by Sandhaus in 1987, but there are no patient follow-ups. Today, these two ceramic implants are not applied.

* (Asst. Prof. Dr.); Ankara Yıldırım Beyazıt University Faculty of Dentistry, Prosthodontics Department, Ankara, Turkey. E-mail: msertacozdogan@gmail.com

Schulte and Heimke (Schulte, 1976) introduced the Tübingen aluminum oxide implant (Frialit I; Friadent, Mannheim, Germany) as an anterior region implant in 1976.

As a result of the examinations, it has been reported that this substance adapts to the bone and soft tissue. (Anneroth, 1990; Büsing, 1981; Zetterqvist, 1991). Approximately 10 years later, D Hoedt (d_Hoedt B, 1986) recorded 924 Tübingen implants in 631 patients. Since 1982, when it was routinely applied, a success rate of 92.5 percent has been determined.

D Hoedt and Schulte (d_Hoedt B, 1989) in the follow-up reports from 1982 to 1987, only two of 448 treatments failed; 396 treatments with 85% confidence limit reported 85-92% success.

Today, new generation ceramic materials with zirconia content have been developed.

Zirconia has more positive mechanical properties than aluminum oxide (high bending strength (900-1200Mpa), hardness (1200Vickers) and Weibull module (Andersson, 1999; Andersson, 2001) (Andersson, 1993). Has poor plaque adhesion (Andersson, 2001; Sorensen, 200; Anneroth, 1990).

Ceramic Implant Design

Ceramic implant materials have different designs and it is difficult to produce these designs with traditional ceramic forming methods. Therefore, the final shape of the implants is given by the processing process, but the ceramics being brittle and hard, complicate the process, and also loss of material and additional costs (Miyazaki, 2009). In terms of strength, the factors such as the small volume of the application area and the versatility of the loads on the implant make the design difficult. To overcome this challenge, implant designers often use screw-type implants. Screw type implants increase the primary stabilization (initial adhesion) in the bone tissue by the grooves on them. Dental implant systems consist of two parts: root screw and abutment. There is also an internal screw that secures this system and connects the parts together. Although the two-piece implants provide ease of application in practice, the internal screw breaks as a result of repeated loads due to micro-mobility. In addition, in the following years, patients avoiding advanced surgical procedures, insufficient bone augmentation methods and increasing costs significantly in vertical bone level insufficiencies have started short large diameter one piece implant designs.

Use of Dental Implants

The concept of tooth extraction and dental implant technology and the treatment of tooth deficiencies was first proposed in the 1960s (Gotfredsen, 2008).

A more common and accepted alternative has been the artificial replacement of missing teeth with dental implants (Sahoo, 2013). Dental implants can be built as fixed bridges to support intra-oral prostheses and thus remove problems such as removal and cleaning of removable prostheses (Allen, 2003).

Implants are functionally more effective than removable prostheses. Patients with partial or complete tooth deficiency prefer implant treatment (Allen, 2003). Today, the use of implants is of great importance in the prosthetic treatment of a toothless patient. (D. Mericske-Stern, 2000). Implant diversity has also increased with the development of design and manufacturing technologies. Therefore, users now attach importance to aesthetics as well as functionality. Thus, the studies performed by researchers in the field of medicine and engineering in implant design are increasing. Endosseous implants have reported success rates of over 90%, so dental implants have become commonplace to replace missing teeth and stabilize prostheses (Foley, 2010).

In the dental implant area, there are many designs. Basically, the implants consist of a monoblock-one-piece and two-piece structure. The one-piece implant shown in Figure 1; abutment (restoration area, neck area, root area (osseointegration area) and apical (end) parts.

The abutment is built into the implant design of one-piece implants, therefore there is no micro-gap between the implant and the abutment. Titanium one-piece implants have provided positive results in terms of overall survival and an average improvement in crest bone thickness (Meirelles, 2008). The resulting excellent attachment of soft tissue can be aligned with the one-piece character of the implant used, thereby preventing subgingival microcavities (Roehling, 2015). Generally, one-piece implants are preferred in cases with a minimally invasive transmucosal flapless placement and it limits the requirement of hard tissue grafting procedures (Rajput, 2013). However, in the following years, in patients with vertical bone insufficiencies, avoiding advanced surgical procedures, inadequate bone augmentation methods and increasing costs significantly brought short-diameter one-piece implant designs to the agenda. While two-piece and one-piece dental implants are identical in technology and cost of manufacture, significant differences occur in clinical usage. The major advantage of double-piece implants is that it allows the use of angled abutments for anterior tooth deficiencies with deep tissue undercuts. However, in cases where the interdental spaces are less than 4mm in these implants, it is not possible to produce thin implants with a biomechanical strength that can meet occlusal forces in single tooth deficiencies and can withstand incoming forces. Studies and trials have shown that the majority of two-piece dental implants smaller than 3' mm result in a fixture, abutment or abutment fixing screw fractures (Gleiznys,

2012). The thinnest implants required for dental implant applications can be produced in one piece. These are usually 2 mm, 2.5 mm or 3 mm in diameter. Also, the forces applied in orthodontics are present in implants manufactured by smaller machining methods to form anchors. (Çini, 2015).

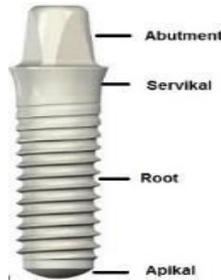


Figure 1: An exemplary ZrO₂ dental implant and its parts Straumann(2019).(<https://www.straumann.com/en/dentalprofessionals/products-and-solutions/dental-implants/ceramic-implants.html>)

One-piece implants offer some positive properties during use. Basically, it simplifies the number of instruments needed for positioning, reduces the number of operations, and increases the mechanical strength of the implant in the neck. On the other hand, without additional constituent elements, the implant itself prevents the formation of bacterial colonies or accumulation of dirt in the joint areas as in conventional implants (Hunter, 2007).

Increasing the region between the bone tissue and the implant interface to shorten the time of osseointegration may contribute positively to the healing process. The consistency of the dental implant is determined by the surface properties. Biocompatibility and roughness of the implant surface increase the success of osseointegration. Increased roughness values increases the implant surface area and is important in increasing its interaction. It has been observed that increased roughness can also improve cell migration and implant adhesion and improve the osseointegration process (Goyal, 2012). In addition, the surface treatment may be categorized by mechanical, chemical and physical processes Increasing the region between the bone tissue and the implant interface to shorten the time of osseointegration may contribute positively to the healing process. To overcome this challenge, implant designers often use screw-type implants. Screw type implants increase the primary stabilization (initial adhesion) in the bone tissue by the grooves on them. Screw design provides implant-

bone anchorage. Patient satisfaction was achieved with fixed prostheses made with bone anchor implants (Adell, 1990).

The long-term success of osseointegrated implants in the early years of dental implantology was more focused on the surgical process of dental implantology. In the following years, success has shifted from a surgical effect to the appropriate implant placement to be determined according to the prosthetic and aesthetic needs of each particular case (Silverstein,1998). Therefore, studies in this field can be accomplished by bringing together different disciplines such as dentistry, materials engineering and bioengineering.

Maintaining the health of soft tissues is as critical to the long-term success of implants as osseointegration (Humphrey,2006). Prevention of peri-implantitis is an important issue for the clinician and the patient and regular checks are needed. Oral bacteria attached to the implant surfaces may disrupt of the biological occlusion surrounding the dental implant (Bauman, 1993). The interface between the mucosa and the implant is critical to protect the intra-bone part of the implant from the oral environment.

By applying surface treatments to modern ceramic implants, osseointegration has been further improved (Patil, 2015).

After the implants are placed in the toothless area, control assessments and radiographs are required to ensure the long life of these restorations and this requires the implant team to perform implant maintenance procedures (Cohen, 2003).

Over time, the emphasis on long-term success of the implant has focused on achieving osseointegration from the surgical stage of treatment, and more recently, on the long-term health maintenance of peri-implant hard and soft tissues. (Silverstein, 2006; Kurtzman, 2007).

The invasion of bacterial plaques was recognized as one of the main factors influencing the success rate of dental implants.

Zirconia

Tetragonal zirconia polycrystals (TZP),in particular, demonstrate exceptional mechanical properties, biocompatible and aesthetic value.

1. Mechanical Properties

Ytria (3YTZP) added zirconia is one of the ceramics used in the field of CAD / CAM in dentistry. Prepared from pre-molded molds, it reaches its final form at temperatures around 1450C. Currently produced from pre-

sintered 3Y TZP blanks using soft machining and sintering (Fig. 2)

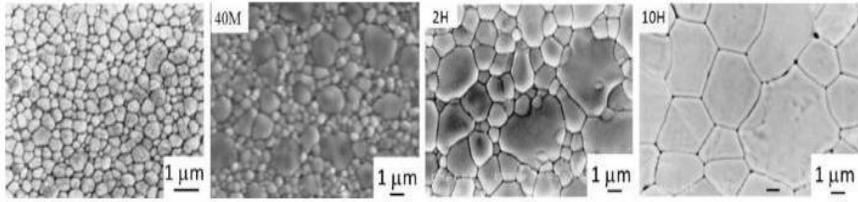


Figure 2: Microstructure of 3Y-TZP a) after sintering at 1450°C; after heat treatment at 1650°C: b)40 min; c) 2h; d)10h. (Turón-Vinas, 2018)

When 3Y-TZP is sintered at higher temperatures and/or longer times the t-c phase partition becomes more significant. The higher cubic phase quantity and its larger grain size make it more evident. Likewise, if 3Y-TZP is heat-treated at temperatures greater than the sintering point for various periods, the microstructure becomes rougher (Fig.2b – d) and the transformability of t – m becomes significantly enhanced, as can be seen from the high amount of monoclinic mediaround process in dental imprints and along the flanks of the indentation crack (Turón-Vinas, 2018). Aboushahba et al. (Aboushahba, 2018) reported a study that evaluated the surface hardness of various zirconia crowns. The veneered copings displayed significantly higher wear values than crowns with complete anatomical zirconia. The normal enamel antagonists showed the same findings. About hardness, complete anatomical zirconia showed better mechanical surface properties compared to veneered zirconia, whether polished or aged. (Aboushahba, 2018)

2. Biological Properties

Biological compatibility of zirconia with bone and soft tissue is very important. Shin et al. found out that zirconia posts alone, and posts with various types of dental cements are biocompatible with minimal cytotoxicity (Shin, 2016).

Zirconia alone or cemented blocks demonstrated reasonable biocompatibility with dental cement. Hempel (Hempel, 2010) studied SAOS-2 cells in a sample and was cultivated on either sandblasted or sandblasted / etched zirconia, compared to sandblasted / etched titanium. Ito et al. also investigated the answer of osteoblast-like cells to zirconia with different surface topography and found that TZP blasting and acid-etching improved proliferation, Alkaline phosphatase (ALP) activity and osteocalcin gene expression. Osseointegration is vital to dental implant's long-term success and relies on the tissue-implant interface response. Mechanical properties and biocompatibility make zirconia an efficient

material for dental implants, although processing of the surface is still a concern.

In their analysis of the actions of osteoblastic cells grown on titanium and engineered zirconia surfaces, Depprich et al. (Depprich, 2008) reported that primary bovine osteoblasts are capable of binding, proliferating and differentiating in vitro on modified zirconia surfaces, indicating that when used in patients, the ceramic substrate may also have beneficial effects on biocompatibility and osseointegration. Zirconia enhances cell growth during the first days of development but it does not enhance binding and strength of adhesion. (Depprich, 2008)

3. Osseointegration

As a result of their chemical and mechanical properties, ceramic biomaterials are commonly used in orthopedics and dentistry. If we find zirconium oxide as a surface material, the effects of the studies are optimistic. In a research Sollazzo et al. (Sollazzo, 2008) found that the ratio of bone-implant interaction in implants treated with ZOC was higher and statistically significant than in untreated controls (Fig3).

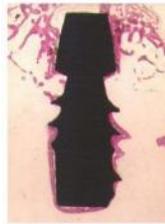


Figure 3: Control implant. The newly formed bone around the implant surface. (Sollazzo, 2008)

In another study on the osseointegration ability of titanium vs. zirconia, Kohal et al. (Kohal, 2004) stated that no implant was compromised during the investigation time and that the mineralized bone-to-implant contact was 72.9 % (SD:14 %) for the titanium implants after 9 months of healing and 5 months of loading and 67.4 % (SD:17 %) for the zirconia implants.

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Wenz et al. (Wenz, 2008) reported in a systematic review that osseointegration of Y-TZP implants might be comparable with that of titanium implants. Surface and microstructure changes have the potential to improve initial bone healing and torque removal tolerance, but there are few current evidences and they do not require implants that are commercially available.

Zirconia dental implant clinical applications

In the evaluation of zirconia implants, aesthetics, biocompatibility, osseointegration success should be taken into consideration in evaluating the success in clinic applications. Failures due to their chemical or mechanical structure can be seen. These problems may occur during implant surgery or under functional forces in the patient's mouth. Identifying these problems will lead to better osseointegration, gum compliance, and long-term clinic use. It will be necessary to evaluate the creation of designs that differ from the conventional grooved screw implant design.

In studies where one-piece zirconia implant was evaluated, a good functional and aesthetic result was achieved with a success rate of 92% to 95% at 2.5 to 5 years of follow-up, and therefore it was concluded that it was an alternative to titanium (Pirker, 2009; Oliva, 2010; Borgonovo, 2012; Borgonovo, 2013).

Also, in another study, 96.5% survival rate was reported after 3 years (Brüll, 2014).

Osman et al. (Osman, 2015) evaluated the implants placed in the tooth extraction cavity and the implants placed in the healed bone after immediate loading and more failures were detected in the implants placed in the tooth extraction area.

In the study of one-piece implants, Andreiotelli and Kohal (Andreiotelli & Kohal, 2009) stated that fracture strength will be negatively affected when performing a preparation on a zircon implant in vitro.

The way of construction in zirconia abutments affects its mechanical properties. Abrasion with diamond burs using a high-speed dental handpiece at the chairside may affect its strength. There are not enough studies on this subject (Nakamura, 2010).

Osman et al. evaluated overdenture cases where 34 of 79 patients were in the titanium (control) and 35 were in the zirconia (test) group. With the loss of patris, crack repair and matrix replacement, cases were maintained. There were no statistical difference between titanium and zircon groups (Osman, 2015).

In a study by Gahlert et al. (Gahlert,2012), that applied 170 zirconia implants to 79 patients in the last 3 years before 2007 and they had found 10% fracture rate.

Also, manufacturers of the Z-Look3 implant system reported a 0.3% fracture in their implants placed in extreme positions in 2008. (Z-Newsletter, 2008).

Regarding the 2-piece zirconia implant, Bankoglu Gungor et al stated that a two-piece zirconia implant in the anterior maxillary region was successful in the short-term evaluation, and the radiographic and clinical results were successful and patients were satisfied with the final result (Bankoğlu Güngör,2014).

Kohal et al (Kohal, 2004) reported in their study that they compared two-piece zirconia and titanium implants and found similar peri-implant soft tissue changes between two types of implants.

Result

With the ceramic implants, tissue compatibility and osseointegration level, the idea is to create a new study area for today's dental implant approaches. Developing different ceramic materials will open new horizons for ceramic implants.

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CHAPTER VIII
**PARENTAL SATISFACTION AND ORAL HEALTH
RELATED QUALITY OF LIFE FOR SILVER DIAMINE
FLUORIDE APPLICATION ON DENTIN CARIES**

Ruhan Öner* & Aslı Patır Münevveroğlu**

Introduction

Early childhood caries (ECC) is one of the most prevalent chronic disease in childhood aged 2-5 years with varying degree of severity (1). ECC is common among children in both developing and industrialized countries (2). Dental treatment of young children is a big challenge for pediatric dentists. Pharmacological behaviour management techniques, including sedation and general anesthesia are used for the treatment of dental caries in young children because of their young age and uncooperative behaviours (3). This technique is very expensive for patients management and requires surgical equipment, special dental equipment and advanced skills of clinicians (4). Therefore, to reduce the burden of dental caries and to avoid consequences of untreated decay, it is important to identify an effective, low-cost dental treatment method in young children.

In 2014, SDF was cleared by the Food and Drug Administration in the United States of America as a desensitizing agent (5). The University of California, San Francisco School of Dentistry (UCSF) in 2016, SDF is indicated as a preventive or therapeutic agent who cannot tolerate standard care for medical and behavioral reasons and for patients with extreme caries risk, without access to dental care and cannot be treated conventionally (6).

Silver diamine fluoride (SDF) is an ammonia solution containing fluoride and silver ions (7, 8). SDF has been used to prevent new caries formation and manage ECC in children of low-income nations (9). The recommended frequency of application to arrest caries is every six months to once a year (10, 11). Systemic reviews of clinical studies have found that 38% SDF solution (44,800 ppm fluoride) is effective in arresting ECC (12, 13). Laboratory studies have found that 38% SDF could slow enamel and dentin demineralisation and inhibit the growth of common cariogenic bacteria (7, 8). Moreover, SDF preserve collagen from degradation in

* (Dr.. Öğr. Üyesi); İstanbul Medipol Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği Anabilim Dalı İstanbul-Türkiye, email: ruhan.oner@hotmail.com

** (Dr Öğr. Üyesi); İstanbul Medipol Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği Anabilim Dalı İstanbul-Türkiye apatir@medipol.edu.tr

demineralized dentin and increase the hardness of treated dentin caries lesions (14).

There are several advantages in using SDF for caries treatment. It kills cariogenic bacteria and provides instant caries arrest. As result, it reduces pain and infection in children. Furthermore, it is safe, inexpensive and the treatment procedure is very simple and non-invasive. Thus, it is an effective material to use to arrest caries progression in very young children who are less cooperative until definitive restorative care can be completed. In addition, SDF can be used in community health clinics where there is limited access to dental care (15). However, SDF has some adverse effects such as metallic taste, staining the caries lesion and mucosal irritation (16,17)

Oral Health-Related Quality of Life (OHRQoL) has been defined as a multidimensional concept which includes a subjective evaluation of the individual's oral health, functional well-being, emotional well-being and sense of self. The Early Childhood Oral Health Impact Scale (ECOHIS) is the most commonly used tool which was proposed to assess the impact of dental health problems and treatment experiences on the quality of life of preschool children (2). Recent Chinese version of the ECOHIS demonstrated good reliability and validity (18). ECOHIS is a standardized instrument that has been used in literature before to measure OHRQoL. This questionnaire consists of 13 oral health-related behaviors and asks parents various questions related to the child's behavior, physical abilities, growth/development, bodily pain/discomfort, temperament and moods, and how well the child gets along with others. ECOHIS consists of two subscales: CIS (Childhood Impact Scores) and FIS (Family Impact Scores). CIS show the impact of dental caries on the child and FIS demonstrate the impact of dental caries on the family (19).

Few published studies evaluated the parental satisfaction and OHRQoL of children after the utilization of SDF and this study is the first study investigating the SDF on the OHRQoL of preschool children in Turkey. The purpose of this study was to assess the changes in parental satisfaction and OHRQoL of preschool children after SDF application on early childhood caries lesions. In addition, we investigated the associations between oral health outcomes and patients' sociodemographic characteristics and oral health-related behaviors.

Materials and methods

This study was approved by Ethics Committee of the Faculty of Dentistry, University of İstanbul Medipol. The participants for the study were recruited from pedodontics department of İstanbul Medipol University Dentistry Faculty between February 2019 and February 2020, when they came to the clinic for a new patient. Inclusion criteria were

preschool children aged 4-5 years who had at least one dentine caries and had never previously received SDF treatment. Exclusion criteria included children who were uncooperative, refused examination or had major systemic illness. The purpose and procedures of this study were explained to the parents of the children involved and their written consent was obtained before the investigation. We provided no compensation for participation. Parents were also provided information about SDF, follow-up instructions, oral hygiene instructions, and diet education.

A total of 124 parents (mother: 72, father: 52) and 124 children (63 girl and 37 boy) wanted to get involved in this study. Sample size calculation was carried out using open-epi online program with 80% power. Baseline examinations of the children were performed by a dentist using an disposable dental mirrors and intra-oral LED light. The decayed, missing and filled teeth index (dmft) was used for recording the caries status. Caries was diagnosed at the cavitation level following the criteria of the WHO (20). Dentin caries lesions was assessed by visual inspection and a sharp probe. Cavities with smooth and hard surfaces which could not be penetrated by the probe were classified as arrested caries. The Oral hygiene status was measured using the visible plaque index (VPI). Five surfaces in each posterior tooth (occlusal, buccal, lingual, mesial and distal) and four surfaces in each anterior teeth were assessed. The surfaces of six index teeth (55, 51, 63, 71, 75 and 83) were examined.

At baseline, parents were asked to complete a questionnaire. After SDF treatment (6-month follow-up), parents were asked to complete a follow-up questionnaire which contained the same questions of the of the baseline questionnaire. The validated Chinese version of the ECOHIS (C-ECOHIS) was used to assess the OHRQoL of preschool children. It contained 13 items corresponding to two sections: 1) child impact section (nine items) consisting of four descriptive domains (symptoms- one item; function- four items; psychological- two items; self image/social interaction- two items) and 2) parent impact section consisting of two domains (parent distress- two items and family function- two items). Questionnaire with missing response in any of the questions were excluded from statistical analysis. The total scores ranged from 0 to 52, with higher scores indicating greater degrees of oral impact on the quality of life of the child.

The teeth were isolated from saliva with cotton rolls and 38% SDF (Saforide, Toyo Seiyaku Kasei Co. Ltd., Japan) was applied directly to the lesion with microbrush and it was allowed to absorb for up to 2 minutes. Parents were instructed that the child should not eat or drink for 1 hour after application.

Statistical analysis

Data were analyzed using IBM SPSS Statistics 22 (IBM SPSS, Türkiye). Shapiro-Wilk normality test was used to evaluate convenience between parameters and normal distribution of the mean changes of the C-ECOHIS scores. Intra-group comparison of the scores was assessed by using Wilcoxon test. The McNemar-Bowker test was used to compare the data of the C-ECOHIS before and after SDF treatment.

Results

At the beginning of the study, 124 children with 1120 tooth surfaces with active dentin caries received treatment. Their mean (SD) age was 40 ± 5 months. The demographic and clinical characteristics of the children are displayed in Table 1.

Table 1: Demographic characteristics of children with dental caries and their oral health status (n= 124).

Parent and child characteristics	Frequency	Percentage
Sex		
Male	45	37%
Female	79	63%
Relationship of respondent to the child		
Mother	96	78%
Father	28	21%
Mother's education level		
Up to junior secondary school	39	31%
Secondary school	55	44%
Post secondary school/University	30	24%
Father's education level		
Up to junior secondary school	31	25%
Secondary school	62	50%
Post secondary school/University	31	25%
Tooth type included		
Upper anterior teeth	98	79%
No	26	21%

Among these, 79 children (63%) were girls and 45 children were boys (37%). All the children were treated under OS. Their mean (SD) dmft score was 5.2 ± 3.4 . Most (64.3%) of the children had 3-8 decayed teeth. Totally 548 active dentine caries teeth were treated with SDF at baseline. A majority (79%) of teeth had dental caries on their upper anterior teeth. Consequently, more anterior teeth were treated with SDF than posterior teeth. Their VPI score was 0.48 (0.19). A majority of the respondents (78%) were mothers. Most (58%) of questionnaires were answered by the child's mother. A majority of the fathers (62%) and around half of the mothers (55%) had completed secondary education.

The frequency of ECOHIS responses (%) at baseline and 6-month follow up examinations are displayed in Table 2.

Table 2: Frequency of ECOHIS response (%) at baseline and follow-up examination (n=124)

Items	Baseline					6-month follow-up					p-value
	Never	Hardly ever	Occasionally	Often	Very often	Never	Hardly ever	Occasionally	Often	Very often	
Child impact section^a											
Symptom											
a)Had pain in the teeth, mouth or jaw?	29	17,7	16,9	18,5	17,7	25	25,8	20,2	15,3	13,7	0,000*
Function											
b)had difficulty drinking beverages?	34,7	37,1	9,7	10,5	8,1	36,3	39,5	6,5	10,5	7,3	0,061
c)had difficulty eating some foods?	16,9	14,5	21,8	25,8	21	19,4	18,5	26,6	18,5	16,9	0,000*
d)had difficulty pronouncing any words?	31,5	33,1	21,8	8,9	4,8	32,3	33,9	20,2	10,5	3,2	0,172
e)missed school?	15,3	9,7	27,4	25,8	21,8	9,7	8,1	30,6	29	22,6	0,000*
Child psychology											
f)had trouble sleeping?	34,7	25	18,5	14,5	7,3	36,3	27,4	14,5	15,3	6,5	0,046*
g)been irritable or frustrated?	32,3	23,4	16,1	18,5	9,7	33,9	25	15,3	17,7	8,1	0,027*
Social interaction											
h)avoided smiling or laughing	22,6	12,1	25	26,6	13,7	21	11,3	25,8	28,2	13,7	0,072
i)avoided talking	35,5	29	21,8	8,1	5,6	35,5	28,2	20,2	9,7	6,5	0,172

Parent impact section^b											
Parent distress											
j)been upset?	13,7	9,7	25,8	31,5	19,4	11,3	8,9	29,8	30,6	19,4	0,046*
k)felt guilty?	11,3	8,9	28,2	32,3	19,4	11,3	8,1	29	31,5	20,2	0,368
Family function											
l)had to takehours or days off work?	33,9	24,2	30,6	4,8	6,5	27,4	29	34,7	4,8	4	0,003*
m)affected the family's economic situation	35,5	25	27,4	4,8	7,3	25,8	29,8	32,3	6,5	5,6	0,000*

Mc Nemar-Bowker Test **p<0.05*

At baseline, ‘pain in the teeth or mouth’, ‘had difficulty eating’, ‘avoiding smiling or laughing’ and ‘missing preschool’ were the most frequently reported items in the child impacts section. Regarding the family impact section, ‘being upset’ and ‘feeling guilty’ were the most frequently reported items. When baseline responds compared with after SDF treatment responds, there was statistically significant results in child impact section, ‘pain in the teeth, mouth or jaw’ (p:0.000; p<0.05), ‘difficulty eating some food’ (p:0.000; p<0.05), ‘missing school’ (p:0.000; p<0.05), ‘being trouble sleeping’ (p:0.046; p<0.05) and ‘being irritable or frustrated’ (p: 0.027; p<0.05) items. In parent impact section, ‘being upset’ (p: 0.046; p<0.05), ‘having hours or days off work’ (p:0.003; p<0.05), ‘affecting the family’s economic situation’ (p: 0.000; p<0.05) items. For other items, no differences were found between the distribution of ECOHIS responses before and after SDF treatment (p>0.05).

At baseline, about 71% of the parents reported that their child never or hardly ever had difficulty drinking beverages. No statistically significant change was found between baseline and post-SDF treatment. About 75% of the children never or hardly ever had difficulty drinking beverages. Furthermore, about 64% of the parents reported that their child never or hardly ever had difficulty pronouncing any words and avoided smiling or laughing. No statistically significant change was found between baseline and post-SDF treatment. About 65% of the parents reported that their child never or hardly ever had difficulty pronouncing any words and avoided smiling or laughing. As for the FIS, around 78% of the parents reported having parental distress, ‘being upset’ or ‘feeling guilty’ and there was no much change after 6-month SDF treatment (Table 2).

After six months, the overall caries arrest rate at tooth level was 56.1% (312 arrested teeth/ 236 active caries teeth). No significant differences were found in the changes of C-EHOHIS scores between children with arrested caries and those with active caries (p>0.05). We detected higher arrest rates for anterior primary teeth (77%) (ie., incisors and canines) as compared to posterior teeth (23%) after six-month SDF treatment.

The mean C-ECOHis scores at baseline and 6-month follow-up are shown in Table 3.

Table 3: Total and individual domain of the ECOHIS score before and after SDF therapy (n=12)

	Baseline	6-month Follow-up	p
	Mean±SD (median)	Mean±SD (median)	
Total score	21,9± 16 (21)	21,8± 15,3 (20,5)	0,924
Child impact	14,7± 11,4 (13)	14,4± 11 (13)	0,900
Symptoms	1,8± 1,5 (2)	1,7± 1,4 (1)	0,621
Function	6,9± 4,9 (6)	6,7± 4,7 (7)	0,889
Child psychology	2,8± 2,6 (2)	2,7± 2,6 (2)	0,659
Social interaction	3,2± 2,5 (3)	3,3± 2,5 (3)	0,761
Parent impact	7,2± 4,6 (8)	7,4± 4,4 (8)	0,604
Parent distress	4,7± 2,5 (6)	4,8± 2,4 (6)	0,867
Family function	2,5± 2,4 (2)	2,7± 2,1 (2)	0,378

Wilcoxon signed Ranks Test

The mean (SD) of the overall C-ECOHIS scores at baseline and follow-up, which are 21,9±16 and 21,8±15,3 respectively. The mean changes of the C-ECOHIS score were not normally distributed (Shapiro-Wilk test). The results of the Wilcoxon Signed Ranks Test indicated that no statistically significant changes in the mean total C-ECOHIS, CIS and FIS scores were found between baseline and after six-month SDF treatment ($p>0.05$).

Discussion

Early childhood caries is the most common chronic disease in childhood (21, 22). ECC is one or more decayed, missing or filled tooth of primary dentition in a child 71 months of age or younger. ECC can have severe effects on different aspects of children’s lives, such as physical development, emotional growth, quality of life, and learning at school. Severe destruction of the primary incisors frequently occurs due to ECC. Dental pain, infection, dysfunctional eating and sleeping patterns were described as the most common reasons for seeking dental care (23). Pharmacological behaviour management techniques including sedation and general anesthesia are used for the treatment of dental caries in children with ECC, because of their young age and uncooperative behaviours. Studies have reported that ECC can effect the family’s economic status seriously (24). This technique is very expensive for patients management and requires surgical equipment, special dental equipment and advanced skills of clinicians (25). Overall, ECC has been found to negatively impact on OHRQoL (26).

In our study, we detected higher arrest rates for anterior primary teeth (77%) (ie., incisors and canines) as compared to posterior teeth (23%) after six-month SDF treatment but some active caries lesions had still developed. This finding is consistent with several other studies (27, 28). This might be explained, posterior teeth have pits and fissures as well as deeper curves in comparison to anterior teeth, which may lead to more plaque retention in the posterior teeth. This also may be due to, SDF treatment in the posterior teeth may be obstructed by plaque that still remained although the children had already brushed their teeth. Caries lesions may have become active again because the children might have eaten and drunk immediately after SDF treatment. Dentists should remind to children not to eat, drink, or rinse their mouth for 1 hour after application.

Silver diamine fluoride (SDF) therapy is an important prevention-centred caries management strategy during critical early childhood periods (29). Several clinical studies have reported positive clinical outcomes of SDF on dental caries prevention and arrest (30, 31). Despite the high effectiveness of SDF therapy in treating caries in children, the black stain on the arrested caries lesions may cause patient and parental dissatisfaction, and affect OHRQoL (2). This study is the first study investigating the impacts of non-invasive treatment with SDF on the OHRQoL of preschool children in Turkey.

We used the Early Childhood Oral Health Impact Scale (ECOHIS) to assess the impact of SDF treatment on the quality of life in children and their families. The ECOHIS consists of child and family impact sections, with a total of 13 items. By using the input of health professionals and parents in the development process, we were able to identify items that were considered to be important by individuals closely involved in ensuring children's health and well being (32). In this study, we found that SDF treatment did not affect the oral health-related quality of life (OHRQL) of children and their families.

Regarding the OHRQoL of the preschool children in this study, it was noted that the mean ECOHIS score at baseline was lower than those in studies conducted in other countries (2, 33, 34). This might be because, children who seek dental treatments probably have poor oral health and they have a lot of oral health problems. Furthermore, Turkish parents may perceive less impact from their child's dental problems compared to the parents in other countries. Besides, parents who are already aware of that children's poor oral health could not pay their child dental treatment cost because of their poor economic situation. In this study, our results indicated that OHRQoL of the preschool children had been unchanged and stabilized after SDF treatment.

The advantages and disadvantages of SDF application have been elucidated in previous research (35, 36). The black stain of the dentine surface after SDF application, was detected on arrested teeth. The black stain may be eliminated by the application of potassium iodide (KI) after SDF application (37); however further studies are needed to investigate the clinical effectiveness of KI. Some side effects such as dental fluorosis, chronic and acute toxic after the application of SDF has been debated (38, 39). In the present study, besides the black stain, no adverse effects were observed during the 6-month follow-up.

Russo et al. (40) reported possible toxicity to the pulp of teeth after SDF solution application. In the present study, there was no patients who show symptoms of irreversible pulpitis after application of SDF solution. Yamaga et al. (41) have predicted reversible lesions in oral mucosa through inadvertent contact with SDF solution. This mucosa lesions was detected in two patients in this study, with the appearance of a small, mildly painful white lesions in the mucosa, which dissappeared at 48 hours without treatment.

To our knowledge, only few studies has investigated the relationship between oral health-related quality of life (OHRQoL) and SDF treatment of ECC. In this study, we investigated association between SDF treatment and OHRQoL. OHRQoL aid clinicians to appropriately select and treat patients with SDF, particularly those patients for whom conventional surgical management is difficult (due to special needs, behavior, young age, or lack of access to care), not indicated, or delayed (due to long GA waitlist times or administrative barriers). We found no significant differences between baseline and after six-month SDF treatment in the children, parents and overall impact section's C-ECOHIS scores.

Conclusion

Within the limitations of this study, it is concluded that SDF therapy has no significant effect on the OHQRoL level of preschool children. However, SDF is an important minimal-invasive treatment alternative and it is effective in arresting dentin caries lesions. Further studies are needed to investigate the clinical effectiveness of SDF.

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CHAPTER IX
TOXICITY OF RESIN CEMENTS

Server Mutluay Ünal & Bozan Serhat Izol***

A wide variety of using ways of chemical substances have already made them an indispensable part of our lives. These chemical substances are taken directly into human body (food additives, medicines, cosmetics, etc.), or they are firstly accumulated in water, air and soil in the nature and then transported indirectly to human body. Therefore, biological activities of chemical substances and their effects on our health have become frequently the main topic of studies (Eren & Bektaş, 2006; Van Meerbek et al., 2005).

The use of adhesive biomaterials in restorative dentistry has been developing rapidly. It is desirable that the dental adhesives used in the vicinity of pulp and gum are highly biocompatible for clinical success. Thanks to this biocompatibility, dental adhesives can bind on dental

structures for a long time without damaging the tissues. Biocompatibility can be described as the ability of a material to form an appropriate host tissue response after its distinctive application. The biocompatible, that is, a biomaterial 'easily compatible with body', is a material that does not interfere with the normal changes of the surrounding tissues and does not cause unwanted reactions in the tissue (Burke, 1995; Pospiech, 2002; Barclay et al., 2002).

Once the resin cements have been used, non-polymerized residual monomers can be released. Studies reported that these monomers produce toxic effects on oral epithelial cells and cause apoptosis in cell culture. Studies also reported that these monomers produce inhibitory effects on growth of gingival fibroblasts, DNA self-renewal, and RNA synthesis (Eren & Bektaş, 2006; Van Meerbek et al., 2005; Burke, 1995).

Many studies report that the unreacted residual monomers (Bis-GMA, TEGDMA, UDMA, HEMA etc.) released from resin-based restorative materials can produce allergic, cytotoxic, genotoxic, mutagenic and carcinogenic effects. Therefore, it is very important to know the molecules

* (Asst. Prof. Dr.); Afyonkarahisar Health Sciences University, Faculty of Dentistry, Department of Prosthodontics, Afyonkarahisar, Turkey
servermutluay@hotmail.com

** (Asst. Prof. Dr.); Bingol University, Faculty of Dentistry, Department of Periodontology, Bingol, Turkey
bozanserhatizol@gmail.com

and their quantities that can be released from adhesive materials into oral environment (Burke, 1995; Pospiech, 2002; Barclay et al., 2002).

BIS-GMA is a hydrophobic, almost colorless and viscous liquid. Arossi et al. reported that an increase in BIS-GMA monomer reduced the color change in aesthetic material (Arossi et al., 2010). Di or tri methacrylate can be added to reduce the viscosity of BIS-GMA. The resin thus obtained is called triethyleneglycol dimethacrylate (TEG-DMA). TEGDMA is a comonomer with lower molecular weight known as viscosity control. In recent years, urethane dimethacrylate (UDMA) and bis-ethylene glycol dimethacrylate (Bis-EMA) are added to the resin matrix to provide better mechanical properties. UDMA is hydrophobic, and its molecular weight is close to BIS-GMA, but it has lower viscosity. It is resistant to color change and provides good adhesion. Bis-EMA monomer is similar to Bis-GMA in terms of its molecular structure, except that it does not have a hydroxyl group. This difference makes Bis-EMA less viscous. Hydroxymethacrylate (HEMA) is a hydrophilic monomer. HEMA prepares the surface for adhesive bonding by moistening the dentin (Burke, 1995; Pospiech, 2002; Barclay et al., 2002).

In recent years, urethane dimethacrylate (UDMA) and bis-ethylene glycol dimethacrylate (Bis-EMA) are added to the resin matrix to provide better mechanical properties. In addition, the resin must be diluted so that filler particles can be placed in organic matrix. Therefore, the comonomers of lower molecular weight known as viscosity control agents are mixed with, for example, triethylene glycol dimethacrylate (TEGDMA) (Hanks et al., 1996; Van Landuyt et al., 2007; Cao T et al., 2005).

Today, many new materials that can be used in dental restoration have been developed. Initial studies on these materials have shown that they produce side effects on biological tissues and release some substances (Wataha 2001; Hanks et al., 1991; Camps et al., 1997; Sengun et al., 2006). The use of oxirane (epoxy) monomers in dental resins has necessitated testing of their genetic safety. Since oxiranes are reactive molecules, they can produce undesirable biological effects on living organisms. All materials should be investigated for possible biological responses before being supplied to the market and applied on patients. These investigations should include the components released from these materials, and their local and systemic relationships with various tissues (Issa et al., 2004; Sengun et al., 2008; Lai et al., 2004).

The controls of these materials should be mandatory using with reliable and cytotoxicity evaluation tests in accordance with clinical conditions. Studies evaluating the cytotoxicity of resins, include researches that have used pulp fibroblasts, 3T3, gingival fibroblasts, immortalized cells

obtained from cattle dental papillae, and odontoblast-like cells (MDPC-23) obtained from lab mice (Lee et al., 2006). Non-specific tests are performed by bringing cells or bacteria into contact with the material. The purpose of these tests is to control the biological responses that occur in relation to various cells and materials. In animal tests; a mammalian organism is used to test the complex interactions between the material and the biological medium. Experimental animals such as mice, rats, dogs, cats, sheep, rabbits or monkeys are used in these tests (Lee et al., 2006; Schweikl et al., 2006; Eckhardt et al., 2009).

There are very few *in vivo* studies on the biological risks of resin-based materials. A lot of information about the harmful effects of the components of resin-based materials have been obtained from *in vitro* studies (Eckhardt et al., 2010; Schweikl et al., 2007).

Lee et al. reported that non-polymerized surfaces and fibroblast cells that directly interact with methacrylate monomers have no chance of survival (Lee et al., 2006). Cimpan et al. reported that resin liquid killed U-937 monoblast cell through apoptosis and necrosis (Camps et al., 1997).

Schweikl et al. reported that high cross-linked acrylic resins have high durability and low water absorption (Schweikl et al., 2007)

The resin must be diluted so that filler particles can be placed in organic matrix. Therefore, the co-monomers of lower molecular weight known as viscosity control agents are mixed with for example (TEGDMA) (Küçükeşmen & Erdoğan 2009; Lefebvre et al., 1999).

Despite it is non-cytotoxic, TEGDMA inhibits cell growth. A study on white albino mice reported that TEGDMA degrades the metabolic activities of cells, reduces the ratio of nucleoside triphosphate, and increases the rate of nucleoside diphosphate (Sengun et al., 2006; Issa et al., 2004)

Yank et al. examined the TC50 values of HEMA, Bis-GMA, TEGDMA and UDMA, the components of dentin bonding agents, on mouse fibroblasts. These components are listed as Bis-GMA > UDMA > TEGDMA > HEMA according to their toxicity. They also found that HEMA and Bis-GMA have a synergistic effect when applied together. However, components with low molecular weight (HEMA, 4-META, TEGDMA) show a solvent effect for more viscous resins and cause them to penetrate more quickly in the cell. Studies indicated that the cytotoxic values of different components are different. Due to some limitations in *in vitro* experiments, these values cannot be used directly for humans. However, it should not be forgotten that biological risks increase as the amount of dissolution increases (Yang et al., 2017)

Issa et al. evaluated and compared the mitochondrial dehydrogenase (MTT) and lactate dehydrogenase (LDH) activities of five resin composite monomers (HEMA, HPMA, DMAEMA, TEGDMA and Bis-GMA) in terms of cytotoxic effect, and listed them as BisGMA> TEGDMA> DMAEMA> HPMA> HEMA, according to cytotoxic effect (Issa et al., 2004).

Van Landuyt et al. compared the resin monomer contents of dentin bonding agents in terms of cytotoxic effects and reported that the strongest cytotoxic effect was observed in Bis-GMA followed by UDMA, TEGDMA and HEMA, respectively (Van Landuyt et al., 2007)

Sengün et al. used Chinese hamsters in a study of TEGDMA. They observed that TEGDMA cleared the V79 genome, which is also found in DNA. TEGDMA changes the structure of proteins found in chromosomes. However, no serious effects of BISGMA were found. If the resin monomer is directly interact with the DNA; DNA undergoes mutation (Sengun et al. 2006) Because DNA will tend to response to foreign material. Thus mitotic division will begin in the DNA. This division will not be enough for the monomer, and the DNA will multiply uncontrollably; so that the cells called cancer will form. UDMA and TEGDMA create mutation in chromosomes and genes by 51% and 56%, respectively (Sengun et al. 2006; Sengun et al. 2007)

Bisphenol-A (BISGMA) is also a monomer of the resin structure. Cao et al. reported that the released Bisphenol-A should not be exceeded 1.5% of the resin (Cao et al., 2005).

Bakopoulou et al. reported that resin monomers can delay the odontogenic differentiation of apical stem cells, thus may impair the physiological growth and the repair and development processes of the cells (Bakopoulou et al., 2012).

Torun et al. reported that resin monomers can disrupt the structure of pulp cells and alter their functions (Torun et al., 2017).

The bonding agents have toxic effects on DNA. In a study on toxicity and DNA, researchers used fruit flies called *Drosophila melanogaster*, applied bonding agent directly on the mucosa, and then observed that the different arrangement of wings, which is typical feature of these flies, has changed. As a result, the study reported that the wings lose their characteristic heterozygote phenotype (Küçükeşmen & Erdoğan 2009; Moilanen et al., 2015; Akay et al., 2017).

Conclusion

This review study describes toxicity of the resin cements used in dentistry. Before using materials in clinics, their harmful effects on tissues must be examined using in vitro tests and their toxicity must be assessed

according to the results obtained in subsequent animal studies. This is unsettling when considering possible severe damages of the monomers. As the technology progress, similar to other sectors, new materials are used in the dental sector. Of course, the biocompatibility of these materials is very important. Dental sector is an important place due to materials used in this sector. Despite the materials used are chemical, the area where they are used is a biological environment. This also suggests their toxicity. We must know the chemical structure of every material we use, and also put these materials into application area knowing their effects on human beings. We must use our materials, knowing that each application we will make with them has feedback regarding human life.

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CHAPTER X
**ER: YAG LASER FOR DEBRIDEMENT OF ROOT CANAL
SYSTEMS: USE OF PHOTON-INDUCED PHOTOACOUSTIC
STREAMING: A REVIEW**

*Zuhal Görüş**

Introduction

Introduction of laser in dentistry, in the 1960s, led to a continuous research in the various applications of lasers in dental practice. CO₂ and Nd: YAG lasers are the first entering lasers use in the dental practice, to roughen, clean, disinfect and shape the root canal system. There are scenarios, on the one hand there are hard lasers, which offer both hard tissue and soft tissue applications, but have limitations due to high costs and a potential for thermal injury to tooth pulp, whereas, on the other hand in cold or soft lasers, based on the semiconductor diode devices, which are compact, low-cost devices used predominantly for applications, are broadly termed as low-level laser therapy or ‘biostimulation’. On account of the ease, efficiency, specificity, comfort, and cost over the conventional modalities, lasers are indicated for a wide variety of procedures in dental practice. However, these lasers have not been effective in removing the smear layer. Thus, erbium-doped aluminum nitride garnet (Er: YAG) and erbium, chromium: yttriumscandium-gallium-garnet (Er, Cr: YSGG) systems known as hard tissue lasers have been used clinically (1-4). This review, study describes photon-initiated photoacoustic streaming (PIPS) used in dentistry.

Using Er: YAG Laser With PIPS

The erbium ‘family’ of lasers has two distinct wavelengths, Er, Cr: YSGG (yttrium scandium gallium garnet) lasers and Er: YAG (yttrium aluminum garnet) lasers. The erbium wavelengths have a high affinity for hydroxyapatite and the highest absorption of water in any dental laser wavelengths [5,6]. Consequently, it is the laser of choice for treatment of dental hard tissues. In addition to hard tissue procedures, erbium lasers can also be used for soft tissue ablation, because the dental soft tissue also contains a high percentage of water. It is known that the absorption of water in the erbium lasers is higher than that of all lasers. They have a high affinity for collagen and hydroxyapatite (7-11)

* (Asst. Prof. Dt.); Harran University Faculty of Dentistry, Şanlıurfa, Turkey. E-mail: dtzuhal24@hotmail.com

Lasers are a more recent suggestion for root canal disinfection and debridement. The erbium, diode, and Nd:YAG lasers have been evaluated in the pretreatment of post space preparation (12-14).

However, the heat produced by the near infrared lasers can change dentin morphology by melting, carbonization, or recrystallization and thus affect the bond strength of intracanal resin cements. Erbium lasers can be used for the activation of irrigants in the canal (laser-activated irrigation, LAI) as a result of the formation of vapor bubbles and cavitations in liquid (15,16).

Er:YAG laser has been used at very low and short pulses (20 mJ, 50 μ s) causing a pure photo-acoustic effect in the canal without thermal effect and vaporization, a protocol called photon-initiated photoacoustic streaming (PIPS). LAI has been claimed to enhance the elimination of smear layer and dentine debris from root canal walls. However, there is still no sufficient data on the interface of self-adhesive cements and dentine after laser root canal pretreatment (17,18).

The first studies on the ER: YAG laser in dentistry were reported by Hibst and Keller in 1989 (19). Er: YAG lasers are used for cavitation preparation and soft tissue treatment. Er: YAG lasers are composed of YAG main crystals doped with Erbium (Er 3+) ions. The erbium ions emit laser radiation at a wavelength of 2940 nm. Er:YAG laser has been used at very low and short pulses. (20 mj, 50 Hz) causing a pure photo-acoustic effect in the canal without thermal effect and vaporization, a protocol called photon-initiated photoacoustic streaming (PIPS) (19,20).

The pips are connected to a cone shaped handpiece made of fiber tip. There are varieties in different lengths and diameters. The diameter of the fiber used in the channel ranges from 200 to 400 microns. Coronal application of laser beam is considered as the optimal technique. After the root canal is expanded by conventional methods, the laser tip is placed in the pulp chamber using optical fiber. Pips tip is used at sub-ablative power level in lasers of wavelength 2940 (21-23).

The fiber tip is used with various irrigation agents such as sodium hypochlorite (NAOCL), ethylenediamine tetraacetic acid (EDTA). Unlike traditional laser methods, the PIPS tip removes the deep and distant effects from the apex. The PIPS tip transmits the activity of irrigation agents with the photoacoustic effect until the apex (24-26).

A new tip design consisting of a 600 micron diameter, 9 millimeter long tapered end is used for this technique. The final 3 millimeters of coating is stripped from the end to allow for greater lateral emission of energy compared to the frontal tip. This mode of energy emission allows for

improved lateral diffusion of the low energy, enhanced photoacoustic waves (27).

Using extremely short bursts of peak power, laser energy is directed down into the canal and the action actively pumps the tissue debris out of the canals while cleaning, disinfecting and sterilizing each main canal, lateral canals, dentinal tubules and canal anastomoses to the apex (1,2,3). This movement of irrigant is achieved without the need to place the radial and stripped laser tip. The tip is held stationary in the coronal aspect of the access preparation only. With the irrigant occupying the entire root canal system, the shock wave created by PIPS travels in all directions during activation and effectively debrides and removes organic tissue remnants. Through this laser-activated turbulent flow phenomenon, clinicians following the PIPS protocol are not required to place the tip into each canal, thus eliminating the need to enlarge and remove more tooth structure to deliver standard needle irrigation to the smaller and more delicate apical anatomy, commonly seen in the apical one third. The results are canal convenience forms that are more conservative, minimally invasive and biomimetic preventing the unnecessary removal of tooth structure (1,5,6,8,9,10).

It is still unclear how the mechanism of ablation today and how it develops. The first studies focused on the dehydration of hard tissues of the teeth. Studies involving water absorption show that only half of the water can be diffuse and that the diffuse rate is slow enough to prolong for several days from hours to hours. Thermal analyzes indicate that the target tissue should be heated to at least 200-300 degrees Celsius before the diffusible water is removed from the environment. In order to separate the water which is more tightly attached to the hard tissues of the tooth, it is necessary to reach 800 degrees. Therefore dehydration does not result in laser ablation (16-20).

A common feature of all erbium lasers is that they make a 'pat' sound when the teeth come into contact with hard tissues (5,9,19). This pulsation is actually a very rapid shock wave that occurs when the laser energy explodes and disperses. This sound is called 'photo-acoustic effect'. The tone and resonance of this sound wave vary according to the presence of the decaying tooth. This photo-acoustical effect is a characteristic feature of short pulsatile wear and high energy density (9,10,15,27).

Advantages of using pips:

a. Laser energy is directed down into the canal and the action actively pumps the tissue debris out of the canals while cleaning, disinfecting and sterilizing each main canal, lateral canals, dentinal tubules and canal anastomoses to the apex (6-8).

b. The results are canal convenience forms that are more conservative, minimally invasive and biomimetic preventing the unnecessary removal of tooth structure. Eliminating the need to enlarge and remove more tooth structure to deliver standard needle irrigation to the smaller and more delicate apical anatomy, commonly seen in the apical one third (6,7,14,23)

c. The laser tip is placed into the coronal access opening of the pulp chamber only and is kept stationary without advancing it into the orifice of the canal. So time is saved to and dentin tubules completely open (14,23).

Clinical Protocol

Laser settings

An 2940 nm Er: YAG laser equipped with a tapered and stripped 600 micron tip (LightWalker AT, FOTONA, Ljubljana-Slovenia), is placed at the coronal orifice (not inserted into the canal), left stationary and activated for 30-second cycles (20 mJ, 15 Hz, 50 microseconds) during the irrigation between each instrumentation used (18,25).

PIPS technique for debriding and decontamination of the endodontic system

During the canal preparation, the PIPS™ technique is used between each shaping file step to produce an improved streaming of fluids into the endodontic system. Because of the enhanced streaming activity of PIPS™ and its ability to move irrigants threedimensionally without needing to enlarge the canal size, an improved debridement and decontamination of the endodontic system is possible together with a minimally invasive canal preparation. In the authors' experience, an apex preparation of #20-25 in the apical third is currently performed for vital teeth. For necrotic or retreated teeth, the apical preparation is closely related to the previous condition of the tooth anatomy (6,7,11,12,13).

Discussion

Laser application is a commonly used method in shaping and disinfection of the root canal system. Lasers are used with different wavelengths and protocols. Nd: YAG and Diode lasers kill bacteria by creating a thermal effect. These lasers are not very effective and cause thermal damage (1-3).

When Er: YAG lasers are used with the conventional tip, there is a risk of apex and perforation at the root surface. Unlike conventional laser applications, the PIPS tip does not need to be placed in the channel; it should be in pulp only. This approach avoids the use of more complex instruments to reach the root or to reach the canal during irrigation. This

method kills both bacteria and opens the dentin tubules by lifting the smear layer (15,18,19).

Takeda et al. Reported that; when the Er: YAG laser use in the root canal, dentinal tubules are opened and the it can also three-dimensionally remove the smear layer and debride in and around the lateral canals (4).

The presence of debris and smear layer in root canal walls affects the bonding strength in the negative direction. The traditional technique used mechanical instrument ultrasonics along with chemical attempt to shape, clean and decontaminate the root canal system (10,11). These techniques still could not remove all the debris and infective microorganisms. Therefore, appropriate to search for new materials, techniques and technologies that can improve the cleaning and decontamination. Ultrasonic activation is not effective in removing smear and debris, especially in angle and irregular root canals. For this reason, the effectiveness of removing the smear layer of laser application with PIPS was investigated in the literature (13-15).

Görüş et al. reported that the smear layer was removed by using a PIPS and that the bonding strength was increased (14). Vangala et al. reported that when the Pips was used, the strength of the bonding was affected positively (24).

Peters et al. reported that PIPS can not completely destroy bacteria in infected root canal but it is more effective than conventional methods (3). Jaramillo et al. reported that PIPS was effective in eradicating *E. faecalis* and in inhibiting new bacterial growth (9).

Some authors reported that using the PIPS Lightwalker Er:YAG technique to enhance chemical debridement after the coronal flare, once during the cleaning and shaping phase and just prior to obturation (2,3,9,10,11).

Ordinola et al. evaluated the effect of PIPS using 6% NaOCl for the removal of an in vitro biofilm and showed an improved cleaning of the infected dentin on PIPS groups when compared to the PUI group. The extraordinary result from this study was the fact PIPS tip was placed 22 mm away from the target area, while sonic, ultrasonic, and passive irrigation were made at the exact target area (12).

Conclusion

Recent studies have reported how the use of an Er:YAG laser, equipped with the newly designed radial and stripped tip, used along with 17% EDTA solution, using a very low pulse duration (50 microseconds) and low energy (20 mJ) resulted in effective debris and smear layer removal with minimal or no thermal damage to the organic dentinal structure.

Although laser irradiation may be considered as a useful device for root canal disinfection, its potential risks is better to be assessed.

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CHAPTER XI
RECENT ADVANCES IN CATARACT TREATMENT

*Ahmet Elbeyli**

Cataract is the loss of transparency of the lens. With age, the possibility of cataracts increases. It is one of the primary causes of treatable blindness. Although many reasons have been suggested in the etiology, the mechanism has not been fully elucidated. It usually occurs in older individuals, but can also be seen in newborns. When cataracts are seen in young and middle ages, it should be considered that some systemic diseases (diabetes mellitus, atopic dermatitis etc.) may accompany(1-3)

Loss of transparency of the lens reduces the light reaching the retina from the outside and this causes a decrease in vision. Cataract patients apply to the doctor with complaints of decreased vision. Currently, the only option for cataract treatment is to remove the contents of the lens surgically and place an artificial intraocular lens. Since the eye of each patient may differ anatomically, the refractive power of the artificial lens to be placed in the eye must be calculated before surgery. As a result of these measurements, the diopter of artificial lens calculated according to the needs of the patient.

The diagnosis of cataract is made by performing an eye slit lamp examination in an outpatient clinic. No additional examinations are required. (4,5).

Anatomy and physiology of the lens: Lens consists of four layers in that order from outside to inside: capsule, lens epithelium, cortex and nucleus.

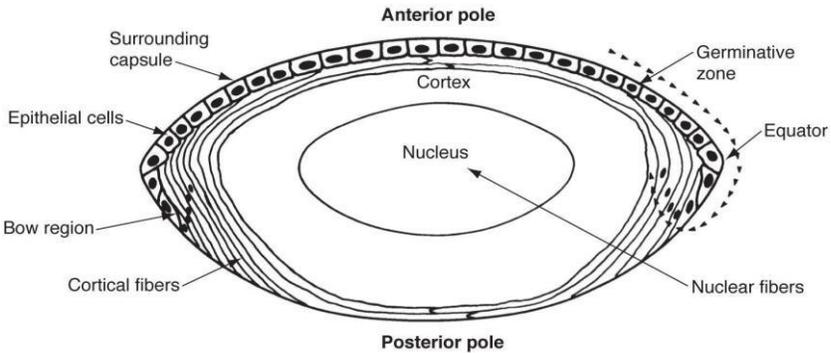


Figure: Anderson RE, ed. Biochemistry of the Eye. American Academy of Ophthalmology; 1983;6:112, San Francisco(Ref.:6)

* (Asst. Prof. Dr); Mustafa Kemal University, E-mail:ahmetelbeyli@yahoo.com

The structure of the lens consists of 66% water, 33% protein, 1% electrolytes, lipid, carbohydrate complex. Since the lens has an avascular structure, its nutrition and energy production also occurs through aqueous humor. Protein production continues in the lens for a lifetime and the lens volume is kept constant by decreasing the water content. With aging, the water ratio of the lens decreases and the protein ratio increases. There are two types of protein in the lens: water-soluble and water-insoluble proteins. With age, the amount of protein that is insoluble in water increases and elasticity of the lens decreases. As a result of all these changes the lens loses its transparency(7,8). Risk factors for the development of cataract include smoking, UV exposure, diabetes, long-term steroid use (systemic, inhaled, topical), trauma, high myopia. In addition, exogenous estrogen intake, alcohol consumption and high body mass index may increase the risk of cataracts(9,10).

There are studies showing that the risk of vitamin C and E intake is decreased when the role of diet in the prevention of cataract development is considered. As a result of studies showing that the risk of developing cataract decreases in vitamin C and E intake, it is thought that diet plays a role in the prevention of cataract formation. There are also studies suggesting that diets containing lutein and zeaxanthin are also beneficial(11).

Indications for surgery: Surgery is the only treatment option for patients with decreased vision. The level of vision that will lead the life of each patient may be different. The patient is examined on the visual chart and the level of vision is determined. It is roughly expressed in decimal, percent, etc. If the general approach is 5/10 and below, surgery is recommended and this level can be revised according to the vision needs of the patients. Another determinant for the surgery decision is whether the cataract causes another problem in the eye. If cataracts are not treated on time, the lens may swell and cause glaucoma by closing the iridocorneal angle, where intraocular fluid (aqueous humor) is transferred out of the eye. In addition, if the cataract is not operated for a long time, the operation may be difficult and the possibility of complications increases as a result of the lens hardening. The timing of surgery should be done taking into account all the situations mentioned(4,5,12,13).

Complications of cataract surgery: The success rate in cataract surgeries is high. However, there are also rare and serious complications. Complications and prevalances of cataract surgery are listed in the table below.

Prevalence intraoperative complications

Posterior capsule rupture with or without vitreous loss 0·5–5·2%

Intraoperative iris floppy syndrome or iris prolapse 0·5–2·0%

Iris or ciliary body injury 0·6–1·2%

Lens materials dropped into vitreous 0.002–0.2%

Suprachoroidal effusion with or without haemorrhage 0–0.4%

Early postoperative complications

Transient elevated intraocular pressure 0.3–18.1%

Corneal oedema 0.1–5.4%

Toxic anterior segment syndrome 0.1–2.1%

Intraocular lens decentration or dislocation 0.1–1.7%

Retained lens materials 0.5–1.7%

Wound leak or rupture 0.02–1.1%

Hyphema 0.02–0.1%

Endophthalmitis 0.006–0.04%

Late postoperative complications

Posterior capsule opacification 0.3–28.4%

Clinical cystoid macular oedema 1.2–11.0%

Pseudophakic bullous keratopathy 0.3–5.4%

Anterior Capsule fibrosis and phimosis 0.47–3.3%

Chronic uveitis 1.1–1.8% Retinal tear or detachment 0.1–1.3%

Endophthalmitis 0.017–0.05%

Table: Prevalence of complications of cataract surgery(13).

The most serious postoperative complication after surgery is endophthalmitis. Endophthalmitis is a serious intraocular inflammatory disorder resulting from infection of the intraocular tissues. The frequency of endophthalmitis following cataract surgery varies between 0.08% and 0.68%(14,15). It usually occurs within 3-5 days after surgery. In the Endophthalmitis Vitrectomy Study (EVS), 94.3% of patients reported blurred vision, 82.1% reported red eye, 74% reported pain, and 34.5% reported a swollen lid(16). When endophthalmitis is detected, the patient should be hospitalized and intravitreal (intraocular) antibiotic treatment should be given. If there is no clinical improvement, vitrectomy should be planned(13-16).

During cataract surgery (intraoperative) posterior capsule rupture may develop. This complication may be due to manipulation of hand tools or suddenly moving the patient's head or eyes during surgery. In this case, the artificial lens may not be placed inside the capsule, so different alternatives

must be evaluated for lens stabilization. Posterior capsule rupture may cause retinal detachment due to vitreous loss and vitreous traction. Posterior capsule rupture may cause the lens content to fall into the vitreous, in which case lens particles should be removed by applying vitrectomy to the patient(17,18). In the postoperative period, posterior capsule opacification may develop within weeks or months. Posterior capsule opacification may reduce vision, and this causes patients to worry. Posterior capsule opacification (PCO) can be easily opened with an Nd:YAG laser in an outpatient environment(19).

Treatment Options

Cataract surgery is one of the most common surgeries performed worldwide. In the last 20 years, the elderly population of the world has increased from 425 million to 677 million. The rising elderly population probably caused an increase in the number of cataract surgeries. An average of 32 million cataract surgeries will be performed annually in the last 20 years(20). The purpose of cataract surgeries is to take the capsule content and insert artificial lenses into the capsule. The capsule should remain in place, otherwise further techniques should be applied for artificial lens stabilization(21).

Three main methods have been used in cataract surgery;

Extracapsular cataract extraction: The lens capsule is left in its place and the lens content is taken out of the eye as a whole. Recently, it is not done except for compulsory situations. If advanced cataract is present, the lens may need to be removed as a whole. In this case, the corneal incision should be made in such a way that the lens can be removed. At the end of the surgery, the artificial lens is placed into the capsular bag(22, 23).

Intracapsular cataract extraction: The removal of the lens as a whole with the capsule. It is necessary to make an incision at the size where the lens can be extracted from the cornea. Today, intracapsular cataract extraction is considered if there is a zonule weakness. Since there is no capsule at the end of the surgery, the artificial lens should be stabilized with another technique(24). These techniques may include scleral fixation, anterior or posterior iris fixation or anterior chamber lens implantation. Although sclera fixation is technically more difficult, it can be done in a very short time in experienced hands. Scleral fixation can be performed with or without sutures. Sutureless methods and its modifications have gained importance recently. Nowadays, many scleral fixation methods have been defined(25). The fixation of the iris requires special artificial lenses and may cause glaucoma by discharging the iris pigment during lens implantation and in the postoperative period(26,27). The iris fixation method also requires experience. The anterior chamber lenses can easily be placed in the anterior chamber, but over time it can damage the corneal

endothelium and impair corneal health. It can also damage iridocorneal angle elements and cause glaucoma(28).

Phacoemulsification (Phaco): Phacoemulsification is the most widely used method of cataract surgery today. In this technique, lens content is separated into fragments with the help of an ultrasonic probe and this content is aspirated from the same probe simultaneously. The capsule is left in place and the intraocular lens is implanted in the capsular bag. In this method, since the lens is fragmented, a smaller corneal incision is made. Since the corneal incision is smaller, there is less risk of infection and postoperative astigmatism. Also, phaco has a shorter recovery time than other techniques.

In the phaco technique, a 1.8-2.75 mm incision in the cornea is sufficient(29,30). Then, anterior chamber is created and capsulorhexis is performed. Capsulorhexis is usually done manually by means of a forceps. In cataract surgery, this stage requires manual skills and experience. Capsulorhexis can be called the most important stage of surgery. A problem at this stage can complicate surgery and cause complications. Capsulorhexis should be smooth, curvilinear and continuous, and approximately 5-5.5 mm in diameter. Performing a capsulorhexis suitable for all these conditions requires attention and experience(31,32). In recent years, femtosecond laser method has been developed to reduce the human factor effect in cataract surgeries.

Femtosecond laser technology was approved by the U.S. Food and Drug Administration (FDA) for use in cataract surgery in 2010(33). Current femtosecond laser systems use neodymium: glass 1053 nm near-infrared wavelength light(34).

Femtosecond lasers used for years in refractive surgery have been recently adopted by many surgeons. Corneal incisions, capsulorhexis, initial fragmentation of the lens, and incisions that alleviate astigmatism can be done with a femtosecond laser. Its potential advantages are safer, smoother and continuous incision, decreased phacoemulsification energy and possible better refractive results due to more central capsulorhexis placement. Disadvantages include significantly higher costs, longer surgery time, and technical difficulties in complicated cases(35).

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CHAPTER XII
**THE EVALUATION OF KNOWLEDGE AND CONSUMPTION
STATUS ON PROBIOTIC NUTRITIONS OF INDIVIDUALS
PRACTICING SPORTS**

Serim Tuna Koç & Hülya Demir***

Introduction

In recent years, investigation of the effects of nutrition on our health and finding answers to the questions about this issue has become an important topic throughout the world. Many health problems and diseases have been correlated with the structural deficiency of nutritional elements in our body due to inadequate and unbalanced nutrition. It is known that some nutrients increase the quality of health and prevent the occurrence of some of the common cancer types (Rada and Petr, 2002). In addition, it is considered that the regulation of the human intestinal flora with diet may lead to some improvements in health. Today, many people change their living conditions and eating habits in order to have a healthier life and be protected from diseases. In healthy individuals, the colon microbiota acts as a prophylactic by directly preventing the adhesion of pathogenic microorganisms, and indirectly by preventing the colonization of the pathogens via the production of chemically modified fatty acids. Due to this barrier effect, endogenous gastrointestinal bacteria may have the feature of preventing the inoculation and development of enteric pathogens (Ceylan and Halic, 2012). There is a state of balance in the host and intestinal microbial populations in the body, and the reasons disrupting this balance cause diseases. The prediction that this protection and maintenance of this balance will decrease the health problems will be replaced by positive experiences gained by positive results. The gastrointestinal tract microflora plays a key role in the health and nutrition of the host and the intestines are called as the second brain. The regulation of microflora has an important place in the probiotics and prebiotics. There are studies showing that probiotics increase the stool volume and fecal nitrogen excretion in humans and stimulate the growth of Bifidobacteria (Nichols, 2007). The benefits of probiotic consumption have been known for many years, but the main goal focuses on the prevention of the diseases such as colon cancer, diabetes, and obesity at the onset. Probiotics have an important place among these functional nutrients. The beneficial effects of probiotics in health promotion have been revealed in many studies. In contrast, the number of studies conducted to determine the probiotic

* Yeditepe University, İstanbul-Turkey

** (Asst. Prof. Dr.); Yeditepe University, İstanbul-Turkey, e-mail:hdemir40@gmail.com

consumption and knowledge level of individuals is limited. Within this scope, determination of the knowledge and consumption status of people regarding the beneficial effects of probiotics has become an important issue.

This research is planned to determine the probiotic knowledge levels of adults caring about their health and going to gym, and to evaluate their consumption status.

Material and Method

The research was planned and conducted to determine the knowledge level and consumption status regarding probiotic nutrients of 104 people between the ages of 18 and 55, who went to two private sports centers in Tekirdağ between February-April 2019. The ethics committee approval was taken from Yeditepe University Ethics Committee Presidency of Clinic Researches (CREC No: 1083). *Data Assessment:* SPSS 20.0 data analysis program was used for statistical analyses and statistical analyses were performed regarding the evaluation of the findings of the research. While evaluating the data, descriptive statistical methods (frequency) and chi-square test were used to compare the qualitative data. Significance was assessed at $p < 0.05$ level.

Limitations of the Study: The research had some limitations. The fact that the sample covered by the research consisted of male and female individuals between the ages of 18-55 who went to private gyms in Tekirdağ province created a limitation in terms of generalizability of the research results.

Results

When the gender distribution of the participants was examined, it was seen that 51.9% of them were male and 48.1% of them were female. According to the age groups, 44.11% of the participants were in 18-25 age group, 24.50% in 26-30 age group, 11.53% in 31-40 age group, 15.68% in 41-50 age group, and 5.88% were over 50 years old. It was seen that 11.5% of the participants were high school and below, 78.5% from high school and upper.

When it is examined, 47.6% of male and 52.4% of female specified that they knew the term “probiotic food”, while 77.8% of male and 22.2% of female did not know. There was a significant relationship between gender and the knowledge about the term “probiotic” ($p < 0.05$).

Question 1. "Relationship between whether to know the term probiotic and the level of education". When the answers given to the question regarding whether the participant in the study knew the term “probiotic” before or not within the framework of the education level, it

was determined that the level of knowledge about the probiotics increased in parallel with the educational status. There was a significant relationship between gender and the educational status about the term “probiotic” ($p < 0.05$).

Question 2. "Where did you hear the term probiotic?"

The participants were asked to hear about the term of probiotic from which information source and 33.65% stated that it was specialist (doctor, dietitian), 26.92% recommendation of friend, acquaintance, family etc., 30.76% advertisements, 4.80% pharmacy and points of sales, and 4.80% internet.

Question 3. "Probiotic food consumption and reasons for consumption." When the probiotic food consumption levels of the participants were analyzed, 79.80% specified that they consumed probiotic foods and 20.20% that they did not consume probiotic foods. When the reasons for consuming probiotic food are examined, the rate of those who find it beneficial for intestinal health was 29.8%, the rate of those who think it is protective against cancer was 14.4%, and the rate of those who find it delicious was 10.2%. The rate of those who think that probiotics strengthen the immune system was 23.9%, the rate of those who find it beneficial for oral health was 9.2%, the rate of those who stated that the effects of the advertisements on the consumption of probiotics was 7.2%, and the rate of those whose friends specified that it has an effect on the use of probiotics was 5.2% .

Question 4. "If you are consuming probiotic foods, do you read the packaging labels?" The participants were asked whether or not they read package label of probiotic food, 58.7% of them gave “yes” answer to the question, 28.8% said “no” and 2 people did not answer this question. No significant correlation was found between the answers given to the question “Do you read the packaging labels of probiotic foods” ($p > 0.334$).

Question 5. "Do you use powder or capsules as a probiotic supplement?" When the distribution of powder and capsule use of the participants as probiotic supplements was examined, 12.5% stated that they use them, while 81.7% stated that they did not. Of the participants, 7.8% of male and 19.1% of female answered as yes. No significant correlation was found between gender and the use of powder or capsules as a probiotic supplement ($p > 0.099$).

Question 6. "Do you know which microorganisms are present in probiotic supplements?" The question of “Do you know which microorganisms are present in the probiotic supplement” stated that 15.4% of the participants knew, while 45.2% stated that they did not know .

Question 7. "Which of the following take place among the microorganisms used in probiotics?" When the knowledge level of probiotic bacteria species was examined, *Lactobacillus* species were

known by 29.80%, *Bifidobacterium* species by 26.92%, and *Streptococcus* species by 17.30%. The rate of recognizing yeasts was 49.0% and the rate of molds was 17.30%. The rate of those who do not recognize any of them was 4.80%.

Question 8. "Do you have any idea about the shelf life of probiotic foods?" It states that 48.5% of the participants had an idea about the shelf life of probiotic foods, while 51.0% reported that they had no idea. It was found that there was no significant correlation between gender and the shelf life of probiotic foods ($p > 0.282$).

Question 9. "Where do you store the probiotic foods?" 76.9 of the participants answered the question where do you store the probiotic foods? as in the refrigerator, 8.7% as consume at once, and 3.8% as at room temperature.

Question 10. "Reasons for not consuming probiotic foods." When the reasons for why the participants do not consume probiotic nutrients are examined, 30% of the participants stated that they did not know what it was, while 18.0% stated that they did not find it natural, 16% did not need it, and 16% stated that they found it expensive, while 20.0% said it was not delicious. It was specified that 66.7% of male and 33.5% of female stated that they did not consume these products because they did not know what it was. In

Question 11. "Relationship between educational status and reasons for not consuming probiotic foods (n=51)." The correlation between the reasons of the participants who not consume probiotic foods and their educational status was evaluated. Among the reasons why the participants did not consume, the answer that they did not know the probiotic foods was on the first rank with the rate of 29.41%.

Question 12. "Do you recommend probiotic food consumption to your friends?" Of the participants answered the question, 87.5% said yes, however 6.7% said no. This question was not answered by 5.8% of the participants.

Question 13. "What do you think the consumption of probiotic food help reducing which one of your following complaints?" When examining the distribution of complaints which probiotic food were thought to be good for in those consuming such food, it was found that it was good for constipation at 28.4%, diarrhea at 13.1%, flatulence and swelling at 25.7%, and allergy at 4.5%. It has been stated that it is beneficial for intestinal diseases at a rate of 17.1%, high cholesterol at a rate of 1.4%, gastric ulcer at a rate of 6.8%, blood pressure at a rate of 2.3%, and other diseases at a rate of 0.9%.

Question 14. "How long have you been consuming probiotic foods?" The answers given to the question stated that 2.9% of the participants used them for days, 3.8% for weeks, 6.7% for months, and 60.6% for years. It was specified that 25.96% of the participants did not consume them at all.

Question 15. "How often do you consume the following probiotic dairy products?" When examining the frequency of probiotic food consumption in the question, it was found that the rate of those consuming yoghurt once a day was 44.2%, the rate of those it 2-3 times a day was 16.3%, the rate of those consuming it once a week was 12.5%, the rate of those consuming it 2-3 times a week was 18.3%, the rate of those consuming it once every 15 days was 1%, the rate of those consuming it once a month was 2.9%, and the rate of those stating not to consume it was 4.80%. When examining the frequency of probiotic food consumption in the question, it was found that the rate of those consuming milk once a week was 17.30%, the rate of those consuming it 2-3 times a week was 15.4%, the rate of those consuming it once every 15 days was 5.8%, the rate of those consuming it once a month was 24.0%, and the rate of those stating not to consume it was 1.9%.

When examining the frequency of probiotic food consumption in the question, it was found that the rate of those consuming kefir once a day was 6.7%, the rate of those it 2-3 times a day was 2.9%, the rate of those consuming it once a week was 7.7%, the rate of those consuming it 2-3 times a week was 9.6%, the rate of those consuming it once every 15 days was 7.7%, the rate of those consuming it once a month was 12.5%, and the rate of those stating not to consume it was 52.88%.

When the consumption frequency of probiotic food is examined in the question, the rate of those who consume kumiss once a month is 1.0% and the rate of those who state that they do not consume is 99.03%.

Question 16. "How much probiotic products do you consume at a time?" 21.15% of the participants stated that they consumed 1/2 water glass, 62.50% of them consumed more than 1 water glass, and 13.46% left this question unanswered.

Question 17. "What is the criterion or criteria that you pay attention while receiving probiotic foods?" When the purchase criteria of the participants for probiotics were examined, price was effective at a rate of 11.6%, the brand at a rate of 31.6%, the food label at a rate of 18.7%, the nutrient content at a rate of 36.8%, and the appearance at a rate of 1.3% .

Question 17. "Have you taken a nutritional class?" While 35.57% of participants answered yes to the question of whether they took a nutritional course, 63.46% said no, and 1 person did not answer this question.

Question 18. "Answer the statements below by marking only one of the options "I strongly agree", "I agree", "I don't know", "I disagree", or "I strongly disagree."

Table 1. Answer the statements below by marking only one of the options "I strongly agree", "I agree", "I don't know", "I disagree", or "I strongly disagree".

	Stroly Agree	Agree	Do not know	Disagree	Strongly disagree
Probiotics contain live microorganisms that are beneficial for one's health when taken in sufficient quantities.	55 (52.9%)	32 (30.8%)	17 (16.3)		
Probiotic foods help to lose weight.	16 (15.4%)	42 (40.4%)	36 (34.6%)	10 (9.6%)	
Probiotic foods affect the mental health of people in a positive way.	19 (18.3%)	38 (36.5%)	42 (40.4%)	5 (4.8%)	
Probiotic foods help to prevent cancer.	16 (15.4%)	43 (41.3%)	43 (41.3%)	1 (1%)	1 (1%)
Probiotics help to decrease cholesterol.	12 (11.5%)	40 (38.5%)	51 (49.0%)	1 (1%)	
Microorganisms in probiotic foods always remain as living.	13 (12.5%)	30 (28.8%)	45 (43.3%)	11 (10.6%)	5 (4.8%)
Adequate use of probiotics contributes to the regulation of the digestive system.	46 (44.2%)	37 (35.6%)	20 (19.2%)	1 (1%)	
Probiotic foods strengthen the immune system.	43 (41.3%)	39 (37.5%)	34 (32.7%)	3 (2.9%)	
Probiotic foods contain high number of microorganisms.	28 (26.9%)	39 (37.5%)	37 (35.7%)	3 (2.9%)	
Probiotic foods help to prevent the development of pathogenic microorganisms.	25 (24.0%)	39 (37.5%)	32 (30.8%)	8 (7.7%)	

Probiotics have positive effects on various allergic diseases.	12 (11.5%)	20 (19.2%)	66 (63.5%)	5 (4.8%)	
Probiotics have therapeutic effects on diarrhea.	20 (19.2%)	36 (34.6%)	46 (44.2%)	2 (1.9%)	
Probiotics are effective in the prevention of infections that develop after surgery.	11 (10.6%)	19 (18.3%)	68 (65.1%)	6 (5.8%)	
Probiotics have an anti-hypertensive effect.	10 (9.6%)	21 (20.2%)	65 (62.2%)	8 (7.7%)	
Probiotics are effective in regulating the intestinal microflora.	44 (42.3%)	38 (36.5%)	19 (18.2%)	2 (1.9%)	1 (1.0%)
Probiotic foods are generally obtained by natural techniques.	16 (15.4%)	44 (42.3%)	34 (32.7%)	9 (8.7%)	1 (1.0%)
Probiotic foods may cause diarrhea.	2 (1.9%)	18 (17.3%)	56 (53.8%)	25 (24.0%)	3 (2.9%)
Probiotic foods are appetizing.	1 (1.0%)	20 (19.2%)	49 (47.1%)	32 (30.8%)	2 (1.9%)
Probiotic foods reduce the need for vitamins and minerals.	6 (5.8%)	16 (15.4%)	53 (51.0%)	23 (22.1%)	6 (5.8%)
Home-fermented yoghurt and kefir help to regulate the digestive system.	37 (35.6%)	36 (34.6%)	26 (25.0%)	5 (4.8%)	
Natural probiotics are more effective than commercial probiotics.	27 (26.0%)	32 (30.8%)	37 (35.6%)	8 (7.7%)	
Probiotic foods should not be used without an expert advice.	9 (8.7%)	17 (16.3%)	34 (32.7%)	38 (36.5%)	5 (4.8%)

Considering the predictions of the participants in Table 1, regarding the expression “Probiotics contain live microorganisms that are beneficial for one's health when taken in sufficient quantities”, the rate of the participants given the answer “strongly agree” is 52.9%, given the answer “agree” is 30.8%, and the ratio of those who answered as “do not know” is 16.3%.

Discussion

Probiotics is a food group with an increasing consumption rate due to its positive effects on gastrointestinal system and immune system and its significance in the protection of human health. In a study of Dokur *et al.* (2006) conducted on 496 students at Faculty of Medicine of Marmara University regarding measuring the knowledge level about probiotics; while 83.7% of the students stated that they heard the term probiotic before, 49.6% reported that they did not know about the content of the probiotics. In the study, 47.6% of male and 52.4% of female specified that they knew the term “probiotic food”, while 77.8% of male and 22.2% of female did not know. When the same question was evaluated within the framework of the educational status, it was found that the level of knowledge of the individuals about probiotics increased in parallel with the increase in educational level. In a similar study conducted on this matter, it was reported that the knowledge of the term “probiotics” of the people with a post-graduate degree constitutes a higher average score when compared to the other educational groups (Al-Muammar *et al.*, 2013). In the study of Agathou and Beales (2013), it was found that the use of probiotic is more common in individuals continuing their education after the age of 18. Yücecan (2002) conducted a study on 450 individuals in Ankara, Istanbul and Samsun in order to determine the causes of consumption of the individuals consuming probiotics and prebiotic dairy products, and to evaluate their effects on their health. According to the results of the research, it has been shown that milk products containing probiotics and prebiotics are preferred by individuals with higher education levels (high school: 36.7, university graduate 38.9%). Similar results were achieved in the present study. It was found that the level of knowledge of the individuals about probiotics increased in parallel with the increase in educational level. There was a significant relationship between gender and the educational status about the term “probiotic” ($p < 0.05$). In a study conducted by Yurttas and Yilmaz (2017) to determine knowledge and consumption status

of health school students concerning probiotic products, it was found that among information sources of the participants knowing the concept of probiotic, school was 35.5%, taking place on the top, which was followed by television (27.6%). In the study conducted by Balkış (2011) with the aim of determining the nutritional habits, probiotic milk products consumption frequencies, and the information of high school students, among the sources where probiotic foods were heard, advertisements were specified by 30.8% of the female students and friend, relative, family etc. by 43.8% of the male students. In the study, in the first rank of the sources from where the term probiotics is heard, the answer “expert” (doctor, dietitian) was given by 33.65%, “advertisement” by 30.76%, and “advice of friends, relatives, family etc.” by 26.92%. In other studies, on probiotic food consumption, it is seen that the advertisements have a great effect in the first rank. Other reasons for consumption is specified as health problems, advice of an expert or friends, and other reasons (Schultz *et al.*, 2011; Derin and Keskin, 2013, Özdemir *et al.*, 2009). When the reasons for why the participants do not consume probiotic nutrients are examined, 30% stated that they did not know what it was, while 18.0% stated that they did not find it natural, 16% did not need it, and 16% stated that they found it expensive, while 20.0% said it was not delicious. It was specified that 66.7% of male and 33.5% of female stated that they did not consume these products because they did not know what it was. In the studies conducted on this matter, the first reason for not consuming probiotics is not knowing what it is. In the study arranged by Yabancı and Şimşek (2007) on the university students in order to determine the status of probiotic food consumption, concerning the reasons for not consuming probiotic products by the students, it was found that they did not consume them because of not knowing and 19.5% did not find these products natural. In another study conducted by Balkış (2011), it was concluded that 31.5% of female students and 41.3% of male students did not consume probiotic foods because they did not know what a probiotic food is. A similar result was obtained when the correlation between the educational status and the reasons for not consuming probiotic foods was evaluated. Among the reasons why the participants did not consume, the answer that they did not know the probiotic foods was on the first rank with the rate of 29.41%.

In a study conducted by Balkış (2011) with the purpose of determining the nutritional habits, probiotic dairy consumption rates, and knowledge of high school students, it was found that 64.3% of those who consumed probiotics recommended such foods to their surrounding while 35.7% did not. In the study conducted by Yabancı and Şimşek (2007), it was found that 75.6% of students using probiotic products recommended these products to their surrounding while 24.4% did not. In the present study, similar results to previous studies were found. Of the participants answered the question “Do you recommend probiotic food consumption to your surrounding?”, 87.5% said yes, however 6.7% said no. In a study conducted by Derin and Keskin (2013), 80% of the participants answered yes to the question “did you benefit from the probiotic product you consume?”, and 70.2% stated that they benefited from probiotics. When the status of benefiting from such food among those consuming such food in the present study, it was determined that the rate of “yes” answer was 79.80%. In the study, when the reasons for consuming probiotic food of the participants were examined, the rate of those who find it beneficial for digestive system was 29.8%, the rate of those who think it is protective against cancer was 14.4%, and the rate of those who find it delicious was 10.2%. The rate of those who think that probiotics strengthen the immune system was 23.9%, the rate of those who find it beneficial for oral health was 9.2%, the rate of those who stated that the effects of the advertisements on the consumption of probiotics was 7.2%, and the rate of those whose friends specified that it has an effect on the use of probiotics was 5.2% (Herbison, 2011). In the research of Aydın *et al.* (2010), 47.6% of the students stated that probiotic products benefit the regulation of gastrointestinal system. In a study conducted to monitor and measure the changes in knowledge levels of consumers about functional food products in USA in 2007, it was determined that 80.0% of Americans used these products for heart health, 77.0% for whole health, 76.0 for physical energy and endurance, 71.0% for immune system, 70.0% for the feeling of fullness, and 65.0% for reducing the development risk of certain diseases. In the study, when the reasons for consuming probiotic food of the participants were examined, the rate of those who find it beneficial for intestinal health was 29.8%, the rate of those who think it is protective against cancer was 14.4%, and the rate of those who find it delicious was 10.2%. The rate of those who think that probiotics strengthen the immune system was 23.9%, the rate of those who find it beneficial for oral

health was 9.2%, the rate of those who stated that the effects of the advertisements on the consumption of probiotics was 7.2%, and the rate of those whose friends specified that it has an effect on the use of probiotics was 5.2% . When it is examined, the participants were asked whether they read the package labels of the probiotic foods and 58.7% stated as yes, while 28.8% said no, and 2 people did not answer this question. No significant correlation was found between the answers given to the question “Do you read the packaging labels of probiotic foods” ($p>0.334$). In a study conducted by Balkış (2011), it was found that 67.3% of female and 59.4% of male the read package labels of probiotic products. The percentage of those who did not read the labels was 32.7% for female and 40.6% for male. In the research conducted by Yabancı and Şimşek (2007), it was determined that 61.6% of the students consuming probiotic food read the package labels of such products and 48.6% of male and 70.6% of female read the package labels of the probiotic products consumed. In the current study, the leading criteria for purchasing probiotic food is the content of the food substance by 36.8%. It was determined that 31.6% of the participants paid attention to the brand while purchasing probiotic foods. In the study, when the distribution of powder and capsule use of the participants as probiotic supplements was examined, 12.5% stated that they use them, while 81.7% stated that they did not. Of the participants, 7.8% of male and 19.1% of female answered as yes. It has been observed that the majority of those who consume probiotics benefit from the probiotic foods, not the supplements. The rate of those who use probiotic supplements know the probiotic microorganism species inside the supplement was 15.4%. No significant correlation was found between gender and the use of powder or capsules as a probiotic supplement ($p >0.099$). In a similar study conducted on this matter, it was determined that the leading criteria considered by the participants while purchasing probiotic foods was the content of the food substance with the rate of 72.8% and the second criteria is the brand with the rate of 46%. In the same study, the rate of knowing the type of probiotic microorganism in the supplement by the participants using probiotic supplements was found to be 57.1%. In the study conducted by Zeren (2015) to determine knowledge levels and consumption status of 117 bank employees in the age range of 18-50 years about probiotic food, it was determined that concerning the status of recognizing probiotic bacteria species, the participants recognized mostly yeast (65.9%) and they recognized mold at the

rate of 47.6%. In a similar study conducted by Öztürk (2018), among the known probiotic bacterial species, the answer “yeast” was taken with a rate of 62.1%, and “molds” with a rate of 37.4%. Among the known probiotic bacterial species in the study, after the leading answer “yeast” was taken with the rate of 33.8% and then the answer “*Lactobacillus* species” with a rate of 20.5%. In the present study, the highest consumed product in the study is yoghurt with the rate of 44.2%. While milk takes the second rank among the consumed products, kumiss is at the last rank with a rate of 1% . In similar studies conducted, it has been stated that the most consumed product as probiotic is yoghurt with a rate of 46.10% (Koçak and Kalkan, 2014; Betz, 2011). In a study conducted on the nutritional frequency of the probiotics, it was found that probiotic foods were frequently consumed once a day (Yabancı and Şimşek, 2007). In another study, when the consuming habits of the students consuming probiotic foods were examined, it was reported that 39.2% of female consumed once a day and 28.6% of male consumed rarely (Yurttaş and Yılmaz, 2017). In the current study, it was found that the participants consumed yoghurt once a day with a rate of 44.2%. The probiotic-supplemented milk consumption rate of the participants was determined as once a day with the rate of 26.9%. Kefir consumption was found to be once a month with a rate of 12.5% at most. In the study conducted by Sevilmiş (2008), it was determined that the frequency of consuming functional products was 14.8% of the participants every day, 29.5% of them twice a week, and 19.7% of them once a week. In general, it was determined that 64% of functional food consumers consumed functional products at least once a week. In a study conducted on this matter, when nutritional course was given to the students, it was determined that there were positive changes in the nutrients consumed in 72.8% of them (Zeren, 2015).

Suggestions

Today, unhealthy nutrition is increasing every passing day and thus, it shortens the life cycle, and decreases the quality of life. In the medical field, it is thought that dietitians and nutritionists should raise the awareness of the people regarding the probiotic products that can be recommended to all age groups due to their therapeutic effects as well as their protective effects. Although the studies showing the positive effects of probiotics on health are increasing day by day, the rate of those consuming probiotics is found to be

20.20%. Although most of the participants in the study are graduated from university, even post-graduate and doctorate programs, 30% have stated that they do not know what it is. In this sense, it is important to raise awareness of the public in this regard, and even to teach the public at a young age in the language they will understand about the awareness of healthy nutrition by introducing nutritional lessons at kindergarten and primary school levels. Scientific terms are used on the product packagings of probiotic foods. However, it will be useful to provide information about the health problems on the label of the product in order to develop a connection between the content and health in the minds of the consumers. It will be useful to provide information about health problems on the label of the product. The probiotic consumption rate is found to be high in the people with a high education level. Individuals with lower education levels can be trained on the benefits of probiotics, and it can be aimed to increase the consumption rates by knowing the probiotics in general.

As a result, consumers should be informed about the probiotic products and they should also be notified in a way that everyone can understand and use them. Labels should be prepared in such a way that functional information rather than the microorganism names is provided.

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CHAPTER XIII
**A RESEARCH ON DETERMINATION OF AFLATOXIN M1
LEVEL IN RAW MILK BY RAPID METHODS: FOOD SAFETY
STRATEGIES AND FIELD APPLICATION
RECOMMENDATIONS**

Ufuk Eren-Vapur & Tulay Ozcan***

I. Introduction

Recently there have been many research on the hazard analysis of critical control points (HACCP) and good manufacturing practices (GMP) to develop foods safety and to protect public health (Kumar et al., 2017). Aflatoxins are the great concern worldwide due to the health issues. Mycotoxins are produced by filamentous fungi in food and feed and naturally appear such as toxic biologically active secondary metabolites (Bryden, 2007; Tomasevi et al., 2015).

Aflatoxins, which are toxic metabolites produced mainly by *Aspergillus flavus* and *A. parasiticus* and rarely by *A. nomius*, *A. tamaris*, and *A. pseudotamaris* strains. Since they are hepatotoxic, teratogenic and genotoxic compounds, when temperatures are between 24°C and 35°C and moisture content exceeds 7% (Williams et al., 2004; Ostry et al., 2017).

Aflatoxins B1, B2, G1, and G2, is the most prevalent and potently carcinogen in foods and feeds that naturally contaminated with mycotoxin. In this crossing cereals and silage are the major source of contamination, especially in tropical and warm regions (Gourama et al., 1995; Flores-Flores et al., 2015; Granados-Chinchilla, 2016). The level of toxicity associated with aflatoxin varies with the types present, with the order of toxicity being Aflatoxin B1>Aflatoxin TG1>Aflatoxin TB2>Aflatoxin TG2 (Jaimez et al., 2000). Therefore, contamination feeds with mycotoxins is a problem for the animal industry, with a significant impact on the animal welfare and human health (Oliveira et al., 2014; Iqbal et al., 2015).

Aflatoxin M1 (AFM) is considered a potential carcinogen for animals and humans (Cavaliere et al., 2006; Prandini et al., 2009). Lactating animals are exposed to the aflatoxins by consumption of molds contaminated feeds during growth, harvest and/or storage of feeds and pasture (Tsakiris et al.,

* (Dr. Öğr. Üyesi); Nisantasi University, Faculty of Art and Design, Gastronomy and Culinary Arts, Sariyer, Istanbul-TURKEY, e-mail: ufuk.erenvapur@nisantasi.edu.tr

** (Prof.Dr); Bursa Uludag University, Faculty of Agriculture, Department of Food Engineering, Gorukle, Bursa-TURKEY, email: tulayozcan@uludag.edu.tr

2013; Freire and Sant'ana, 2018). When consumed AFB1 with contaminated feed by the lactating animal is absorbed in the gastrointestinal tract and metabolized in the liver into aflatoxin M1 (AFM1), which is expressed in the milk. The AFM1 is the main hydroxylated derivative of AFB1 formed in liver by means of P450 cytochrome enzymes and secreted into milk through the mammary gland of dairy cows (Tsakiris et al., 2013; Jager et al., 2016).

About 0.30-6.20% of AFB1 in animal feeds is converted to AFM1, and the AFM1 derivative can be determined in milk within 12-24 h after the first intake of AFB1, and decreases to an undetectable level 72 hours after last ingestion of AFB1 (Creppy, 2002; Ayar et al. 2007; Tsakiris et al., 2013).

Geographical distribution, environmental and climate conditions, development level of the country are effect the contamination of milk with AFM1. Therefore, it is important to determine AFM1 levels in raw milk in different locations to protect consumers from its harmful effects (Ghazani, 2009; Prandini et al., 2009; Rahimi et al., 2010; Bertocchi et al., 2014; Hajmohammadi et al., 2020). It should be kept in mind that the interactions between water activity and temperature have main effect on *Aspergillus* spp. and its aflatoxin production (Sanchis and Magan, 2004).

Mycotoxins cannot be inactivated by conventional heating and cooking procedurs in contaminated foods. AFM1, shows high heat resistance and ability to bind to casein even after thermal processing of milk (Campagnollo et al., 2016). Aflatoxins decompose at temperatures of 237-306°C. Most studies indicate that pasteurization, sterilization, evaporation, concentration, or drying processes as do not cause an appreciable change in the concentration of AFM1. The AFM1 is not destroyed by pasteurization of milk, and can be transferred into yogurt, milk powder and other dairy products (JECFA, 2001; Creppy, 2002; Kumar et al., 2017).

Aflatoxins are causing acute or chronic liver disease depending on the doses used, but they are also show immunosuppressive, hepatotoxic, mutagenic, teratogenic, and carcinogenic effects (Williams et al., 2004; Flores-Flores et al., 2015). AFM1 are classified in possibly carcinogenic Group 1 toxin for humans by International Agency for Research on Cancer (IARC, 2002)

Milk and dairy products are the main foods in human diet especially for infants and children and presence of AFM1 in milk may have harmful health effects for consumers. The frequency of occurrence of AFM1 in commercially products its probable carcinogenic effect with high intake of these products that led to an increased concern about the establishment of measures to control AFM1 contamination (Prandini et al., 2009; Škrbić et al., 2014). For this reason, AFM1 level in milk and dairy products are

regulated in large number of countries. The European Union identified at 50 ng/L the limit of AFM1 in milk whereas regulations for United States and Asian countries' is 500 ng/L. By the way, the consumption of significantly higher amounts of concentrated feeds in high yielding cows, might results in high percentages as 6.2% (EFSA, 2004; EC, 2010). AFM1 level which are allowed in milk is 50 ng/L in Turkey (Turkish Food Codex, 2011)

In dairy operations and farms, detection of AFM1 is important for food safety, quality/healthy separation of milk, animal welfare and milk yields. If this problem is not solved at the point where the raw plow is supplied, serious economic losses will be experienced in the enterprises. Since AFM1 in milk is directly responsible milk establishment in the case of milk, it is important for afloroxin M1 determination methods to determine problematic points not only for milk intake phase but also for related enterprise.

In 2013, 7,938 million tons, while cow's milk in Turkey is 9,560 million tons in 2019. The amount of cow's milk produced within 6 years has increased by about 16.9 % (TUIK, 2013; 2019). Milk and the sector are rapidly developing food sector, and in the last 6 years 45 to 50% of the industry has been processed. This value was 10% in 1990's and now it is 50%. These collected milk passes through many controls include aflatoxin analysis before and after the factory acceptance.

The number of firms that perform AFM1 analysis in routine is low. In general, companies with high milk processing capacity perform this analysis in their own businesses. As with antibiotics, AFM1 analysis should be done every day at every acceptance stage. The biggest reason why aflatoxin M1 analyzes cannot be performed in this way is the costs per analysis or the current method used requires much effort and length.

To protect the food safety and consumer health, the detection and quantification of aflatoxin in food and feed is a very important aspect for the eliminate these toxins from foods. Commonly, absorption and emission spectra are used detection and identification of aflatoxins. The main methods for aflatoxin detection are thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography mass spectroscopy (LCMS), and enzyme linked immune-sorbent assay (ELISA) techniques. Enzyme-linked ELISA can be used based on estimation of AFB1-lysine (metabolite of AFB1 toxin) concentration in the blood. It is the rapid and simple method for detection of AFM1 in milk with sensitive, high precision, and optimal recovery than the other methods (Sarimehmetoglu et al., 2003; Cavailere et al., 2006; Rosi et al., 2007; Sulyok et al., 2015).

Most of AFM1 techniques employ solid phase column cleanup of extracts and immunoaffinity procedures to remove interferences to

improve the measurement of mycotoxins. They give results within hours or days. Competition within the food and feed industry forces them to reduce cost, employ cheaper labor and deliver goods rapidly. The flow-through assay is rapid, easy-to-use and is suitable for testing mycotoxins in the field. The method does not require any equipment and most any individual can perform this assay (Zheng et al., 2006)

In Turkish cuisine, people of all ages consume dairy products such as milk, yoghurt and cheese in daily life. Therefore, the presence of AFM1 should be regularly monitored in milk, dairy products and also feeds. Pasture feeding are encouraged by official authorities to reduce mycotoxin and AFM1 occurrence due to the previous results and appropriate climatic and geographical conditions in Turkey (Sarica et al., 2015). In addition, the presence of AFM1 in milk is a biocontrol mechanism that will demonstrate safety from feed to food.

The objective of this research was to determine the presence of AFM1 in raw milk using ELISA and rapid test method in southeast region of Turkey. In the study, raw milk samples were taken from milk collection centers in Kahramanmaraş, Osmaniye and Gaziantep provinces in December.

II. Material and Methods

Milk samples were taken from milk collection tanks in milk collection centers (100 mL, n=2). One of the samples was assigned with competitive ELISA method (Leszczyńska et al., 2001) on the same day and the other was analyzed by biosensor rapid test method (<http://www.mayasan.com/tr/portfolio-item/aflasensor-kit041>) Randomly selected five samples were evaluated by HPLC (Gdnçalves et al., 2017) to control results. Here, ELISA and rapid test method were compared and discussed. As a kit, flash sensor kits of Unisensor Company were used and the results with Unisensor Company's sensor reader were evaluated at ppt level. As a result of the study, at the first step AFM1 level was controlled and qualitatively indicated the standard of milk and these results were compared with the ELISA method in operation. The results are applied to speed up the improvement researches that can be done in the enterprises and usability of the quick test method selected in the area. The standard deviations of the methods within themselves may vary, as today aflatoxin analysis that needs to be determined at the ppt level. However, the important point here is that in the acceptance stage, before the milk production, raw milk quality controls that are made before the standardization of the raw milk in terms of AFM1 levels are determined correctly. In the study, only the milk of that region was examined from the perspective of AFM1, but also the methods of determination of AFM1 in a different way and the corrective and preventive action steps for the solution of the problem were defined.

Rapid Tests Method and Reaction Mechanism /Aflasensor Test Method)

(Unisensor

Aflasensor aflatoxin is a test based on the principle that the antibody molecule specific to the M1 molecule moves on a special membrane. Aflasensor is a test containing AFM1 molecules specific and highly sensitive antibodies. Two components are required for testing. First, reagent containers containing antibodies bound to a certain amount of gold particles are required. The second one is the test strips made from the membrane set with special holding lines. The red control line at the top should be visible for the test to be valid. During the first incubation, the specific antibodies present in the reagent vessel in minutes are bound by analyze (AFM1 molecules) in the milk. Then, when the bars are immersed in the mixture, the milk advances vertically upwards on the bar and passes through the holding lines. If the sample does not contain AFM1, the color development on the lower test line will occur. If the sample contains AFM1, there will be no color in the test strip, and the color will be faint or not at all according to the amount of AFM1 (<https://unisensor.be/en/catalog/readsensor-2-app088~13b79584-6840803c-66f2-39f2119654dd>)

The result of the analysis up to this point is evaluated visually or not as a result, and the accuracy of the result cannot be made very sure. In the next last step, the qualitative evaluation of the test line density on the test strip was done with Readsensur (Figure 1). (<http://www.mayasan.com/tr/portfolio-item/aflasensor-kit041/>)

Elisa Method

The aflatoxin levels in the milk also are detected using the ELISA test (Romer Aqraquant Aflatoxin M1 Sensitive 25/500-Singapore) according to the procedure at 450 nm absorbance against an air blank was measured spectrophotometrically on a reader. In the ELISA method, for example, there is the stage of getting ready for analysis. 5 mL of fresh milk sample is taken, put into the test tube and kept at +4°C for 30 minutes. The sample is centrifuged at 3000 g for 10 min. After centrifugation, 0.4 mL of serum under the oil layer accumulated on the test tube is taken and placed in a new test tube (without reaching the bottom of the tube). 0.1 mL of 100% methanol is put into this 2nd test tube so a mixture is obtained. This mixture is ready for ELISA analysis.



Figure 1. Detection of Aflatoxin M1 in 10 Minutes Using an Incubator and Test Kits/Evaluation of the Result at the ppt Level Using a Reader

The strip is put on the microwell until it is desired to be analyzed. Each well is for 1 standard and 1 sample. 200 μL are put into each well in the strip (a). Then 100 μL of each standard and sample are put into the wells (b). Next, the antibody coated strip is put into the microtiter indicated in the red circle indicated in figure (c). 100 μL are taken from mixtures in green edged strip and transferred to 2 strips respectively. These second strips are the strips indicated by the red circle. Then it is waited for 60 minutes at room temperature. Then the washing process is started. For this, each well is washed with the washing solution prepared in the pest. This wash is repeated 4 times (d). Then the microwell is turned over on the absorbant pad or paper towel placed on the bench and the wells are emptied completely (e). After washing, 100 μl are put into each well from the "substrate" bottle with blue cap. The mikrowells in the dark at room temperature for 20 min. It is suspended (f). After the second incubation period, 100 μL are taken from the "stop solution" bottle and placed in each microwell. Then, the reader is read at a wavelength of 450 nm in the reader. The obtained absorbance values are calculated using the exell calculation table provided from the supplier (Figure 2).

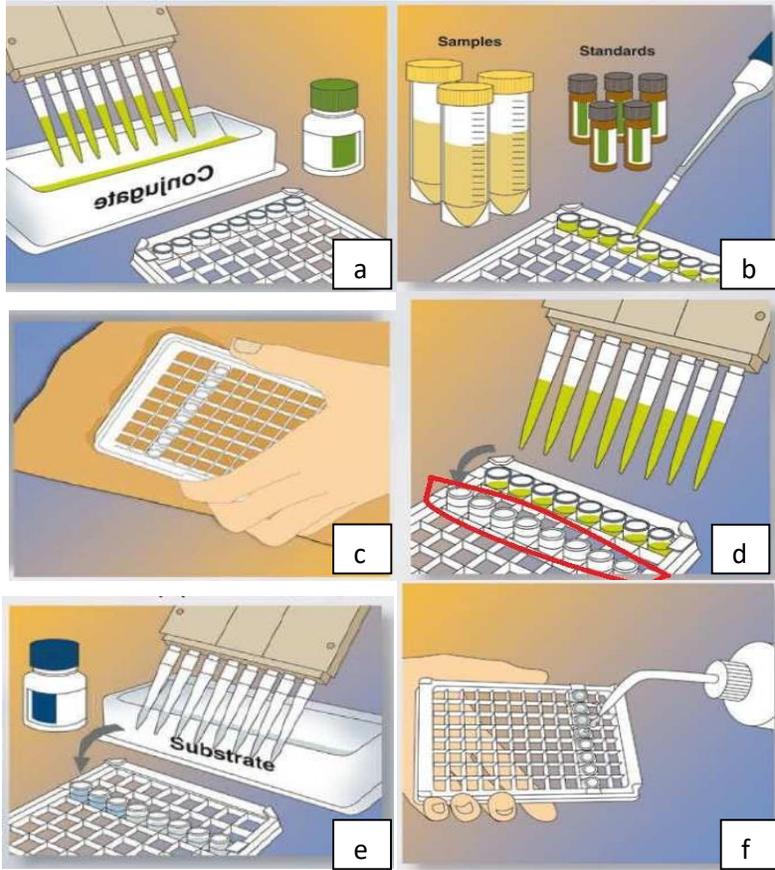


Figure 2. Elisa Method (<https://www.bioser.com/p/390/agraqant-aflatoxin-m1-sensitive-25-500-ppt/>)

III. Results

Generally, studies done in Turkey's East, Southeast and Central Anatolia Aflatoxin seems to be the problem. In the study of raw milk in Adana province, 53 samples of 176 samples (30.11%) were found to have AFM₁ levels above the EU limits of 50 ppt (Golge, 2014).

In a study by Isleyici et al. (2012), milk samples were examined by ELISA technique in order to determine the presence of AFM₁ in UHT milk (25 whole milk and 25 semi skimmed milk) consumed in Van province. AFM₁ is found between 22.57 and 76.58 ng/L, average 42.78 ± 14.81 ng/L in 23 samples (92%) and above 80 ng/L in 2 samples in (8%) for 25 UHT sterilized whole milk samples. In 25 UHT sterilized semi-skimmed milk samples, AFM₁ is found between 7.61 and 58.78 ng/L, average 38.73 ± 10.98 in 21 samples (84%), it is found above 80 ng/L in

4 samples (16%). It is found out that, 9 (36%) of UHT sterilized whole milk samples and 7 (28%) of UHT sterilized semi-skimmed milk samples aren't compatible with the limits (50 ng/L) for milk in Turkish Food Codex in terms of AFM1 levels. The detection of high levels of AFM1 in UHT sterilized cow milk samples exceed the legal limits, shows that consuming these products poses a serious public health risk.

Tamamogulları and Kanici (2014) examined AFM1 in cheese and yogurt in Şanlıurfa. They detected the AFM1 in raw milk (n=38), UHT milk (n=12), white pickled cheese (n=50), and yogurt (n=50) using ELISA method. Their results showed that, 21 (55%) raw milk, 3 (25%) UHT milk, 10 (20%) white pickled cheese, and 10 (20%) yogurt samples were contaminated with AFM1 over the acceptable levels (≥ 50 ng/kg), ranging from 0.82 to 130.89 ng/kg. None of the white pickled cheese samples contained AFM1 levels above the Turkish legal limit (250 ng/kg) and AFM1 levels determined in white pickled cheese were not considered to give public health hazard. However, the AFM1 levels in raw and UHT milk and yogurt samples indicate an increased human health risk in Turkey related to high aflatoxin levels. Therefore, milk and dairy products should be followed by the Turkish public health authorities continuously to detect AFM1 contamination to save safe food for now and future.

Children who consume high amounts of milk and their products and whose body weights are less than adults have higher sensitivity to AFM1. Therefore, there is more concern about the health of children. So, AFM1 should be considered as a risk and the subject should be taken seriously (Scaglioni et al., 2014).

Considering all these evaluations, in this study, as shown in Table 1, when an evaluation of raw milk in compliance with the legal standards of 50 ppt was made, 22 samples of 40 samples were determined as intra-standard. It was determined that the test kits are in compliance with the ELISA method. Although there is no one-to-one numerical compatibility, the applicability of the test kits has been seen in the field.

The results above the legal limit are given in Table 2. The number of samples that are 50 ppt and above is 16, and the test kits together with the elisa method gave compatible results. Results of only 2 samples out of 40 samples were incompatible. These results are shown in Table 3. The result found in the test kit as 70 ppt was 40.33 ppt with the ELISA method. In the second incompatible sample, the value found in the test kit as 58 ppt was determined as 49.30 ppt by ELISA method. The Aflatoxin M1 value, which was determined as 49.30 ppt, can be evaluated as exceeding the residue limit in the sense of 50 ppt value AFM1.

Table 1: <50 ppt Results Showing Compliance within the Standard

Sample Number	Unisensor /Aflasensor Test Method	ELISA Test Method	Sample Number	Unisensor /Aflasensor Test Method	ELISA Test Method
1	<20	34.94	12	36	36.94
2	<20	25.66	13	<20	17.84
3	<20	21.13	14	<20	21.38
4	<20	41.23	15	49	38.26
5	<20	10.16	16	<20	33.61
6	<20	40.02	17	39	15.08
7	31	40.64	18	<20	28.36
8	<20	4.91	19	<20	27.95
9	<20	39.37	20	<20	21.44
10	<20	40.52	21	<20	21.44
11	<20	20.49	22	<20	34.91

Table 2: > 50 ppt Results Showing Non-standard Compatibility

Sample Number	Unisensor /Aflasensor Test Method	ELISA Test Method	Sample Number	Unisensor /Aflasensor Test Method	ELISA Test Method
1	52	54.97	9	51	48.98
2	54	65.01	10	98	79.39
3	>150	101.72	11	82	59.18
4	>150	113.21	12	>150	76.43
5	>150	85.87	13	150	67.51
6	99	64.30	14	150	67.51
7	64	58.73	15	>150	58.36
8	65	65.39	16	112	71.74

In the AFM₁ analysis performed by applying both ELISA and the test kit, the reliability of both methods was confirmed in HPLC with five raw milk samples. The results of this can be seen in Table 4. Apart from this, in 40 samples, only two methods did not give any compatible results (Table 3). In the total of 40 raw milk samples examined, 55% AFM₁ was detected under the legal limit, while 45% raw milk sample was found above the

legal limits AFM₁. All of these raw milk samples were taken in raw milk collection centers.

Table 3: Incompatible Results of Samples (ppt)

Sample Number	Unisensor/Aflasensor Test Method	ELISA Test Method
1	70	40.33
2	58	49.30

In this study, most particularly it is considered Turkey's southeastern point of the aflatoxin problem was selected and will work with 40 different raw cow's milk. In this study, not only the situation of AFM₁ in that region but also the methods of aflatoxin determination were compared and the practical application of the results could be interpreted and application proposals were presented to the sector employees. It should be seen that the use of data in accelerated test kits both in operation and milk collection centers is practical and appropriate. The necessary measures can be taken for the farms by making the checks on the milk coming to the establishment before the milk is accepted to the enterprise.

Table 4: HPLC Results of Some Selected Samples (ppt)

Sample Number	Unisensor /Aflasensor Test Method	ELISA Test Method	HPLC Method
1	<20	21.13	Undetectable
2	<20	10.16	Undetectable
3	54	65.01	92.00
4	70	83.00	55.00
5	>150	101.72	180.00

Sarica et. al. (2015) studied on AFM₁ with total of 70 samples consisting of 24 UHT pasteurized milk, 27 white cheese and 19 yogurt. 83% of the milk, 92.6% of the cheese and 89.5% of the yogurt samples were contaminated with AFM₁. The levels of AFM₁ in the samples ranged from 7.30 to 107.20 ng/kg. Only 5 yoghurt samples and none of the milk or cheese samples exceeded the safety limits established by the

Turkish Food Codex of 50 ng/kg for milk and yoghurt and 250 ng/kg for cheese. The survey results showed the necessity of regular monitoring for the occurrence of AfM₁ in dairy products.

The ELISA method was used in determining the level of M₁ in the UHT milk samples collected from the central region of Turkey. A total of 129 commercial full-fat UHT milk samples were examined and the average AFM₁ value was found at 108.17 ng/L. It was determined that 75 milk samples (58.1%) were contaminated with AFM₁, 68 samples (53%) were below the limit allowed by the European Union and the remaining 61 samples (47%) were above the limit (Unusan, 2006).

Studies have been reported on the occurrence of AFM₁ in milk and dairy products in many countries with differences are presumably attributable to variation in feeding systems. Many researcher reported that higher AFM₁ levels during cold and humid seasons as compared to hot seasons, due to the stored feeds (e.g., dry hay, corn, concentrates, and silages) with a higher AFB₁ (Bertocchi et al., 2014; Škrbić et al., 2014; Gonçalves et al., 2017).

Conclusion

The occurrence of aflatoxin in feed used for the cows diet is a very striking factor that effects AFM₁ level in milk and dairy products. It is not correct to say that the farmer is literally responsible in the presence of aflatoxin in milk. The grower takes the feed and gives the animal, and the important point here is that the feed given is not moldy. The feed that the animal eats must be brought under control from the planting of the feed plant to its consumption. For this reason, this problem cannot be solved only by controlling milk producers' milk. AFM₁ is a serious food safety problem that may arise from time to time when we cannot control the source of feed, its processes and storage conditions.

Considering from the perspective of the farmer, this incident does not only mean the detection of AFM₁ problem milk. When the necessary safety conditions are provided, it will ensure that precautions are taken beforehand for the breeder, the result of which goes up to the offspring. Although it is thought that there is no problem in animal feeds, the control of the AFM₁ of the feeds will be ensured during the feeding of the animals. When raw milk control points control existing feeds in their hands, feeds that cause problems directly with mold or AFM₁ can be detected and supply can be taken under control.

Aflatoxin news, which has been on the agenda in some periods and published in the media as a carcinogenic substance, continues to have a wide impact on the society. In the face of this danger, businesses take some precautions. AFM₁ control mechanisms should be operated well in order

not to be late in the measures taken to eliminate the problem. This hazard is usually determined when milk arrives at the factory and a work plan is followed based on the samples taken at milk collection centers. However, the controls should start from raw milk purchase points. In this application, it can be seen that with biosensors that give results within 10-15 minutes, the milk is within the standard, that is, suitable or non-standard and does not comply with the sellers.

Since aflatoxin analysis is an analysis method that requires results at the ppt level, even the applied analysis methods must be very sensitive in itself. When different aflatoxin analysis methods are compared, the difference between them may occur normally in this sense. Even if the analysis is carried out with the HPLC method, there may also appear points where different results are obtained from the same milk in different laboratories. Here, there are factors such as the preparation of the column and the solutions used, such as the preparation. However, detection of AFM1 takes place over a long period of time. In this sense, when comparing aflatoxin methods, an evaluation should be made by looking at whether aflatoxins gives in-standard or non-standard results according to the legal limits. In almost all samples taken, the evaluation of the methods should be made taking into account the fact that the result gives analysis results below 50 ppt and above 50 ppt and the final decision should be made accordingly. While comparing the aflatoxin analysis methods, it is necessary to avoid getting caught in the compatibility search point and to verify suspicious results.

In such a serious safety problem, which has been proven 100% proven to cause cancer and various organ damage in humans, the consumer should be made conscious and definitely how risky the consumption of street milk should be explained. The training given to the farmer in cooperation with the public and private sector from the field to harvest and consumption is also important here. The problem of aflatoxin in milk is not only an issue of public health and food safety, but also an important and critical biosafety point that causes serious economic losses.

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CHAPTER XIV
BURDEN OF COMMUNICABLE DISEASES IN TURKEY

*Arzu Yiğit**

1. Introduction

The main purpose of health systems is to increase the health status of the society. Health systems are developing health policies to prevent and control diseases, to protect and improve health, to improve the quality of life, and to prolong life. Among these policies, combating communicable diseases is the most important health policy. There are many demands for governments to increase health care costs and invest in health systems.

The range of communicable diseases occurring throughout the world is considerable (Webber, 2016). Life expectancy is generally limited to uncontrolled diseases. Non-communicable diseases started to cause big problems in industrialized countries (Boutayeb, 2006:192). Treatment of diseases is the main activity of health systems around the world, and the overwhelming portion of most financial instruments and healthcare professionals are dedicated to this. Although they can be much more beneficial to health in the long term, preventive measures are still widely neglected (Krickeberg, Trong and Hanh, 2019:2) and very low financial resources are allocated. There is an urgent need to develop effective preventive strategies to stop the growth trend of communicable diseases (Boutayeb, 2006:197).

Communicable diseases have always been a major concern to human health, particularly in the developing countries (Saxena, 2018) and these diseases are still the major killers in the world (Webber, 2016). Communicable diseases kill more than 14 million people every year, especially in developing countries. In these countries, about 46% of all deaths are caused by infectious diseases, and 90% of these deaths are linked to acute diarrhea and respiratory infections of children, AIDS, tuberculosis, malaria, and measles (Heymann, 2015: xxi). Considering to success of vaccination programs for polio and a great deal childhood diseases, diseases such as malaria, tuberculosis, HIV/AIDS have still not been brought under control in most African countries (Boutayeb, 2010a:

* (Asst. Prof. Dr.); Süleyman Demirel University, Department of Health Management, Isparta, Turkey. E-mail: arzuyigit@sdu.edu.tr, ORCID ID: orcid.org/0000-0002-5777-3405

540). AIDS, which has killed millions of people to date, is one of the most devastating infectious diseases in human history.

A communicable disease is an illness that is transmitted from a person, animal, or inanimate source to another person either directly, with the assistance of an intermediate host or by a vector. These diseases present in an epidemic, endemic, and pandemic form (Webber, 2016). Some are transmitted by biting insects, while others are caused by ingesting contaminated food or water. Diseases and pathologies have devastating consequences for the individual, family unit and society, including impact on nations or all geographic regions (Preedy and Watson, 2010).

Communicable diseases are an important public health problem, especially in low-income countries. In five out of six WHO regions, the burden of non-communicable disease is greater than that of communicable diseases. In Africa, the burden of communicable diseases is higher than those of non-communicable diseases and injuries in other regions (Boutayeb, 2010b: 1176). The effect of health care on health status also varies according to the development level of the countries. While health services still have a significant impact on improving health status in underdeveloped and developing countries, healthcare services are not so much in developed countries. Because when it is examined the disease tissues of underdeveloped and developing countries, it is the disease burden of infectious diseases (Çelik, 2016:91). Despite the burden of communicable diseases has been steadily declining in industrialized countries over the past decade, it is still significantly increasing worldwide. However, there is a clear difference between industrialized low-income countries in terms of burden of disease, morbidity, and mortality (Santoro, Simone and Timen, 2015:5). In this context, we can say that Covid 19 affects the epidemic mostly in high-income countries.

Measles, diarrheal diseases, acute respiratory infections, malnutrition, and malaria are the most common reasons for morbidity, and mortality among refugees (Mikolajczyk, Maxwell and Eljedi, 2010:3403). In industrialized and high-income countries, chronic diseases such as cardiovascular diseases, cancer , and diabetes have the highest burden of disease. In non-industrial and low-income countries, infectious diseases still represent the biggest health problem. Low respiratory infections, HIV/AIDS, diarrheal diseases, malaria , and tuberculosis (TB) collectively make up about a third of all deaths (Santoro et al., 2015: 5–6).

The purpose of public health is for the health of humanity. Health is a basic human right , and everyone should benefit equally. It is one of the key elements for the sustainable development of a society. Health determinants include human behavior , and lifestyles, environmental , and biological factors, and health care (Li and Jiang 2019:2). Health

determinants such as poverty, lifestyle, income inequality, educational status, access to health care and demographic changes are an important factor in the burden of disease. The scientific literature provides indisputable evidence for health differences between populations based on race, ethnicity, and socioeconomic status. However, studies focusing on geographic health differences provide a more robust indicator of striking health inequalities in morbidity, mortality, and decreased quality of life (Taylor, 2019:1).

Burden of disease (BoD) studies are an important source of information and data for making detailed comparisons about communicable diseases, determining priorities, and evaluating the results of health programs by presenting data at the level of gender, age group, year, country, region, disease, and risk factors (Ministry of Health, 2019: 97). Measuring health loss is one of the most complex issues in health research. Non-fatal health loss can result in loss of human capital in young, and middle age (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). Global Burden of Disease (GBD) studies determine the disability-adjusted life years (DALYs) approach to compare diseases, injuries, and risk factors, and factors affecting health loss in a particular region, country, time, and age-gender group (Murray et al., 2012: 2063). The purpose of this research is to analyze the burden of communicable disease in Turkey.

2. Material and Methods

The purpose of this research is to analyze the burden of communicable disease in Turkey. The research is a descriptive research based on disability adjusted life years (DALY) approach. The data used in the research was taken from an online database of the Global Burden of Disease Collaborative Network (2018), which included the cross-sectional data set between 1990 and 2017. In the classification of communicable diseases, it is based on the list of diseases announced by GBD 2017.

In the GBD study, causes and their sequels were organized at hierarchical levels. Level 1 includes three broad groups of causes: communicable, maternal, neonatal, and nutritional disorders (CMNN), non-communicable disease, and injuries. DALY's (average rate per 100,000) data for level 1 communicable diseases, and causes of level 2, and level 3 disease related to this disease were analyzed in this study. GBD aims to propagate uncertainty throughout its estimation process, which results in uncertainty intervals (UIs) accompanying each estimate. Therefore, uncertainty intervals (UIs) values were used to evaluate the results of this study. In this study, Turkey's three main causes of disease burden in the evaluation group (group 1=communicable, maternal, perinatal, and nutritional disorders; group 2=non-communicable diseases;

group 3=injuries) were compared. Burden of communicable diseases was analyzed by grouping the countries according to the Socio-Demographic Index (SDI). SDI is a summary measure that defines where countries are located in the development spectrum. Expressed on a scale of 0 to 1, SDI is the combined average of per capita income ranking, average educational attainment, and fertility rates of all areas in the global disease burden study (IHME, 2020). This book section used data published at the country level from the global burden of disease cooperation network. This study does not contain any individual or personal data. Therefore, this study does not require any ethical permission.

Since the burden of communicable diseases was analyzed according to the variables such as gender, and age according to the DALY approach, brief information about this concept is given below.

In health care, the result is closely related to the two main concepts, mortality and morbidity. The results are measured by scales that evaluate the symptoms, and severity of symptoms associated with specific diseases (Razzouk, 2017: 37). In 1992, the World Bank commissioned the initial GBD study to provide a comprehensive assessment of the disease burden in 1990. Significant scientific, and policy interest in the methods, and findings of the 1990 GBD study led to its widespread use by all countries (Lopez et al., 2006).

DALY methodology provides a means to link information on disease causes (Mathers et al., 2001:4). DALYs are a summary metric of population health. DALYs are the sum of two components: years of life lost due to premature mortality (YLLs), and years lived with disability (YLDs) (Murray et al., 2012: 2199). People suffering from various diseases have a different amount of disease burden on populations. Over the past decade, this burden has been increasingly measured to compare internationally (Doney et al., 2010:716).

Measuring health loss is one of the most complex efforts in population health research. The GBD study provides a standardized approach to estimate incidence, prevalence, and YLL, YLDs by cause, age, gender, year, and location (James et al., 2018:1790–1791).

DALYs are used in two ways - to calculate the "global burden of disease" by estimating to what extent the disease causes loss of life and disability in geographically defined populations, and as a general measure for use in cost-effectiveness analysis. The measure combines the estimate of the time lost due to premature death; a weight that represents the social value of time at different ages; a weight for disability; and a discount rate (Mcpake, Kumaranayake and Normand, 2002:102). One of the important concepts in disease burden is health status. DALY provides important information on the determination and evaluation of the real health status

level of the society, revealing the differences between regions, especially on resource allocation. The number of people lost due to poor health in the world is quite high.

DALY is basically used for four different purposes. These objectives are listed below as items (Fox-Rushby, 2002: 12);

- Global burden of disease estimation
- Cost-effectiveness analysis
- Sectoral planning and prioritization
- Determining the direction of research and development in health care

3. Results

According to the research results, communicable diseases are one of the main causes of disability and death today. Infectious communicable accounted for 8,57% (17.8-19.5) of total global deaths in 2017 and caused 10.4 million deaths. It represents 27,88% (26.35-29.36) of the communicable diseases in the total DALYs in the worldwide. Comparison of Turkey burden of disease by major groups of causes are given in Table 1. Accordingly, while the burden of communicable diseases was 37, 93% of the total burden of disease in 1990, this rate decreased to 8, 94% in 2017. While the total burden of non-communicable diseases within the disease was 5, 73% in 1990, it increased significantly to 82, 41% in 2017. While the share of injuries in the total burden of disease was 8, 33 % in 1990, this rate remained at the same level as 8, 64% in 2017. Between 1990 and 2017, Group 1, Group 2 and Group 3 disease load decreased by 85.95%, -8.50%, -38.23% respectively. In Turkey, the total burden of disease decreased by -40.35% in 2017 compared to 1990. According to the findings obtained, the contribution of the three main groups of groups to the overall disease and injury burden varied considerably.

Table 1. Comparison of Turkey Burden of Disease by Major Groups of Causes (DALYs per 100.00)

Major groups of causes	1990		2017		Change (%)
	DALYs (95% UI)	%	DALYs(95% UI)	%	
Group I: Communicable, diseases	14.937(16.461-13.492)	37,9	2.099(2.443-1798)	8,9	-85,9
Group II: Non-communicable diseases	21.174 (23.715-18.919)	53,7	19.373(22.377-16.695)	82,4	-8,5
Group III: Injuries	3.281 (3.571-2977)	8,3	2.027 (2.252-1818)	8,6	-38,2
Total	39.392 (43.747-35388)	1,00	23.498 (27.072-20.311)	1,00	-40,6

Classification of infectious diseases according to level 2 and infectious disease loads are given in Table 2. The disease with the highest disease burden is maternal and neonatal disorders, while the disease with the lowest disease burden is Neglected tropical diseases and malaria.

Table 2. DALYs of Communicable Diseases by Disorders Categories in Turkey (2017)

Category Disorders	Rank	Value	DALYs*	
			Upper	Lower
A.1 HIV/AIDS and sexually transmitted infections	6	33	48	24
A.2 Respiratory infections and tuberculosis	2	462	544	391
A.3 Enteric infections	3	150	194	115
A.4 Neglected tropical diseases and malaria	7	12	18	9
A.5 Other infectious diseases	4	120	155	97
A.6 Maternal and neonatal disorders	1	1.120	1.358	919
A.7 Nutritional deficiencies	5	202	314	125
Total		2.099	2.443	1.798

*per 100,000 population

Classification of infectious diseases according to level 3 and diseases causing infectious diseases are given in Table 3. Diseases that cause infectious diseases and have the highest burden of disease; neonatal disorders, lower respiratory infections, diarrheal diseases, dietary iron deficiency, upper respiratory infections, vitamin a deficiency (HIV-1), iodine deficiency, tuberculosis, other unspecified infectious diseases, meningitis, otitis media.

Comparison of the leading causes of DALYs for communicable diseases in Turkey (1990-2017) is given in Table 4. Total infectious disease burden decreased by 103% in 2017 compared to 1990. Neonatal preterm birth, which is the primary cause of infectious disease in 1990, is in the first secret in 2017. However, the burden of disease decreased by 84.88% in 2017 compared to 1990. Lower respiratory infections, which ranked second in 1990, decreased 90.95% burden in 2017. Diarrheal diseases, which ranked third in 1990, fell 91.47%, ranking fifth in 2017. Other neonatal disorders, which took the fourth place in 990, dropped by 79.88% in 2017, ranking third.

Table 3. Leading causes of DALYs for communicable diseases in Turkey (2017)

Rank	Code	Diseases	DALYs*			Communicable Diseases (%)	Total DALYs
			Value	Upper	Lower		
1	A.6.2	Neonatal disorders	1.100	1.336	899	52,40	4,70
2	A.2.2	Lower respiratory infections	318	355	265	15,14	1,36
3	A.3.1	Diarrheal diseases	129	171	95	6,16	0,55
4	A.7.4	Dietary iron deficiency	111	178	65	5,30	0,47
5	A.2.3	Upper respiratory infections	92	146	53	4,39	0,39
6	A.7.3	Vitamin A deficiency (HIV-1)	38	74	17	1,79	0,16
7	A.7.2	Iodine deficiency	35	58	19	1,68	0,15
8	A.2.1	Tuberculosis	30	34	27	1,45	0,13
9	A.5.9	Other unspecified infectious diseases	26	31	19	1,23	0,11
10	A.5.1	Meningitis	26	31	19	1,23	0,11
11	A.2.4	Otitis media	22	34	13	1,03	0,09
12	A.6.1	Maternal disorders	20	24	17	0,95	0,08
13	A.5.8	Acute hepatitis	19	25	16	0,92	0,08
14	A.1.2	Sexually transmitted infections excluding HIV	19	34	11	0,92	0,08
15	A.5.6	Measles	19	43	7	0,91	0,08
16	A.3.2	Typhoid and paratyphoid	16	28	7	0,74	0,07
17	A.5.2	Encephalitis	14	16	12	0,67	0,06
18	A.1.1	HIV/AIDS	13	16	11	0,63	0,06
19	A.7.1	Protein-energy malnutrition	13	16	11	0,63	0,06
20	A.5.4	Whooping cough	10	32	1	0,48	0,04
21		Others	28	43	19	1,36	0,12
Total			2.099	2.726	1.603	100,00	8,94

*per 100,000 population.

Table 4. Comparison of the leading causes of DALYs for communicable diseases in Turkey (1990-2017)

Leading Causes	1990		2017		Percentage Change (%)
	Rank	DALYs (95% UI)	Rank	DALYs (95% UI)	
Neonatal preterm birth	1	4280 (3456-5257)	1	647 (524-798)	-84,88
Lower respiratory infections	2	3511 (2769-4343)	2	318 (265-356)	-90,95
Diarrheal diseases	3	1514 (1098-2099)	5	129 (95-171)	-91,47
Other neonatal disorders	4	906 (571-1425)	3	190 (141-251)	-79,08
Neonatal encephalopathy due to birth asphyxia and tr	5	882 (544-1506)	4	174 (134-225)	-80,31
Measles	6	565 (213-1218)	14	19 (7-43)	-96,61
Dietary iron deficiency	7	457 (282-690)	6	111 (65-178)	-75,66
Drug-susceptible tuberculosis	8	411 (350-482)	11	27 (24-31)	-93,34
H influenzae type B meningitis	9	337 (74-494)	21	11 (8-14)	-96,76
Other meningitis	10	224 (51-327)	23	9 (7-11)	-96,04
Neonatal sepsis and other neonatal infections	11	212 (70-375)	8	73 (34-101)	-65,36
Vitamin A deficiency	12	189 (107-313)	9	38 (17-75)	-80,13
Protein-energy malnutrition	13	131 (96-182)	18	13 (11-16)	-89,91
Whooping cough	14	124 (14-400)	22	10 (1-32)	-91,93
Hemolytic disease and other neonatal jaundice	15	107 (74-153)	15	16 (13-20)	-84,99
Tetanus	16	105 (36-260)	25	1 (1-1)	-99,32
Meningococcal meningitis	17	104 (23-160)	24	3 (2-4)	-96,85
Upper respiratory infections	18	103 (61-164)	7	92 (54-146)	-10,59
Typhoid fever	19	80 (40-144)	16	15 (7-27)	-80,77
Other unspecified infectious diseases	20	71 (48-101)	12	26 (19-31)	-63,90
Iodine deficiency	23	59 (33-97)	10	35 (19-58)	-40,02
Acute hepatitis B	25	40 (24-56)	20	12 (10-17)	-69,06
Otitis media	28	32 (19-57)	13	22 (13-35)	-33,15
Encephalitis	29	30 (20-62)	17	14 (12-16)	-52,66
HIV/AIDS others	60	0,7 (0-1,1)	19	13,2 (10,4-16,2)	1822,55
Others		463 (196-1145)		80 (48-133)	-82,68
Total		14937 (10265 1509)		2099 (1540-2804)	-103,85

*per 100,000 population.

Gender and age specific YLLs, YLDs and DALYs (2017) related to communicable diseases are given in Table 5. YLL refers to the year of life lost due to premature death, and YLD refers to the years of life related to disability. DALY is a criterion that refers to the years lost by early deaths and diseases and injuries that did not result in death but lead to long-term loss of function. When the distribution of infectious diseases by gender is analyzed, YLL is higher in men than women, while YLD is higher in women than men. DALYs per 100,000 people are higher in males than females. When communicable diseases are analyzed by age group, it is under the age of 1 with the highest disease burden. The age group with the lowest disease burden is 5-64 years old (Table 5).

Comparison of global infectious disease burden's share in total disease burden by region (1990-2017) is given in Table 6. Accordingly, the burden of globally communicable diseases in the total disease burden in 1990 was 46.3%. It was determined that the lowest contagious disease burden belongs to High SDI countries with 6.8%. In 1990, the highest contagious disease burden was found to be low SDI countries with a rate of 72.1%. Turkey in 1990, the proportion of total disease burden of infectious disease burden was determined to be 37.9%. The infectious disease burden between 1990 and 2017 decreased globally. The burden of globally communicable diseases in total disease burden in 2017 was 27.9%. It was determined that the lowest contagious disease burden belongs to High SDI countries with 5.2%. It was determined that the highest infectious disease burden in 2017 belonged to Low SDI countries with 52.1%. Turkey, 2017, contagious disease burden share of the total load of 1990 decreased 76% compared to the snake and infectious disease burden in 2017 was 8.9% (Figure 1).

According to results obtained from research findings, both globally and in turkey, while reduced burden of communicable disease burden of non-communicable diseases is increasing (Figure 1). The health financing system adopted by countries has an important effect on the diagnosis, treatment and prevention of infectious diseases. For example, due to the high out-of-pocket expenses and the inability of individuals to access health services in lower SDI countries, the burden of communicable diseases increases.

Table 5. Gender- and Age-Specific YLLs, YLDs, and DALYs in Turkey (2017)

Category	YLLs*	%	YLDs*	(%)	DALYs*	(%)
Gender						
Male	1554(1832-1291)	71,1	632(869-450)	28,9	2186(2553-1852)	100,0
Female	1207(1442-1007)	60,0	805(1100-577)	40,0	2011(2375-1721)	100,0
Both	1.380(1629-1165)	65,7	718(962-517)	134,2	2.099(2443-1798)	100,0
Age Group (Year)						
<1 year	65721(81171-52635)	98,0	1284 (1813-915)	2,0	67004(82984-53550)	100,0
1-4	1320 (1885-900)	51,7	1232 (1975-810)	48,3	2552(3416-1902)	100,0
5-9	256 (319-199)	16,9	1258 (2084-773)	83,1	1514(2315-1020)	100,0
10-14	199 (247-154)	18,8	862 (1261-573)	81,2	1061(1456-767)	100,0
15-19	211 (258-156)	21,7	760 (1112-532)	78,3	971(1321-731)	100,0
20-24	221 (264-180)	24,2	694 (983-493)	75,8	915(1201-703)	100,0
25-29	217 (255-183)	24,0	687 (950-481)	76,1	903(1178-689)	100,0
30-34	209 (242-178)	24,8	635 (893-447)	75,2	844(1104-654)	100,0
35-39	216 (254-182)	26,2	609 (834-439)	73,8	825(1048-648)	100,0
40-44	223 (263-188)	27,6	585 (794-422)	72,4	808(1019-636)	100,0
45-49	273 (321-231)	33,8	534 (722-391)	66,2	807(995-653)	100,0
50-54	393 (477-318)	40,8	570 (782-408)	59,2	963(1192-787)	100,0
60-64	784 (948-621)	64,7	428 (577-314)	35,3	1211(1433-1012)	100,0
65-69	1113 (1371-850)	73,3	405 (539-294)	26,7	1518(1803-1232)	100,0
70-74	1530 (1888-1209)	76,8	462 (654-322)	23,2	1992(2374-1642)	100,0
75-79	1961 (2392-1554)	82,1	428 (583-301)	17,9	2389(2857-1943)	100,0
80 plus	2520 (2889-2141)	84,7	454 (619-323)	15,3	2974(3361-2563)	100,0
All Ages	1.380 (1629-1165)	65,7	718 (962-517)	34,2	2.099(2443-1798)	100,0
Age-standardized	1866 (2234-1560)	71,2	755 (1013-543)	28,8	2621(3066-2224)	100,0

*per 100,000 population.

Figure 1. Comparison of Global Communicable Disease Burden's Share in Total Disease Burden by Region (1990-2017)

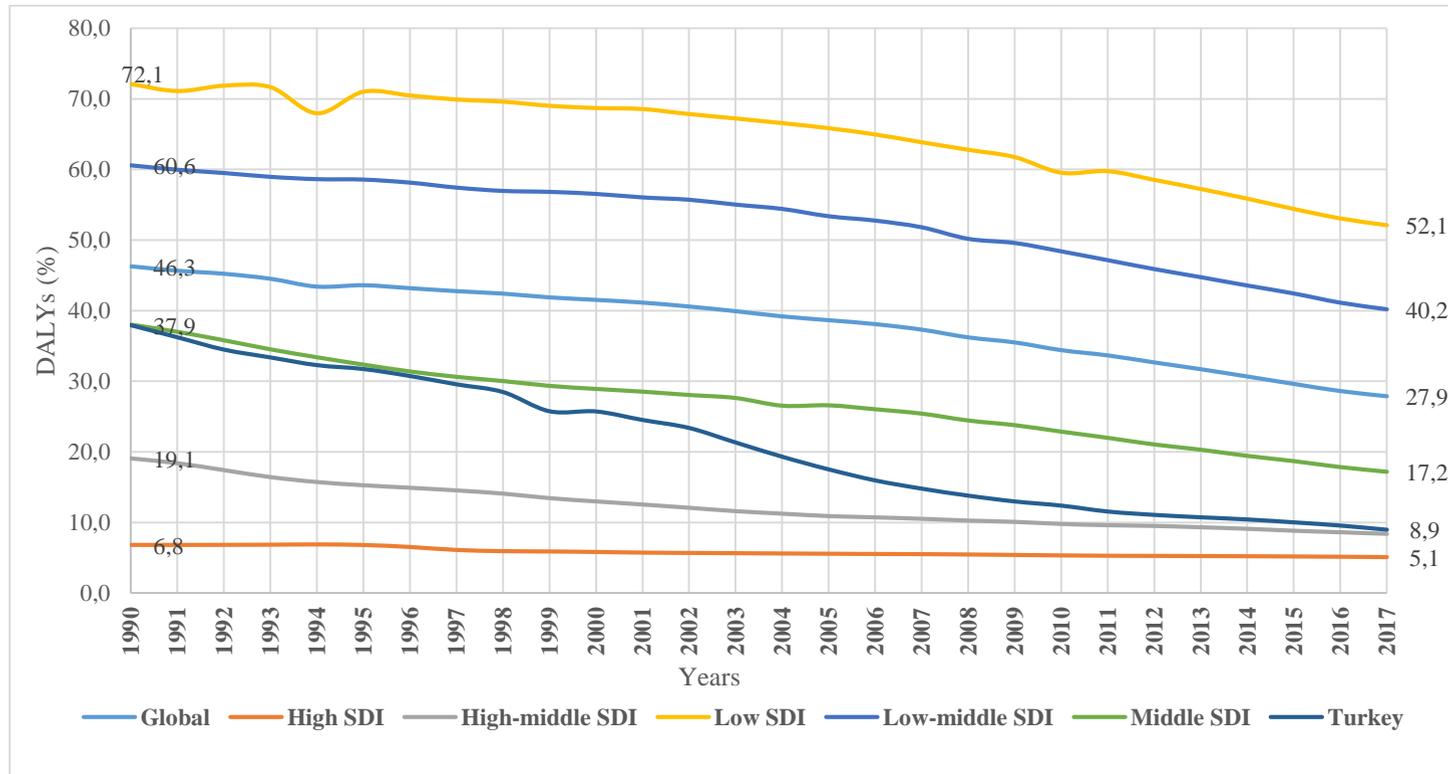


Table 6. Comparison of Global Communicable Disease Burden's Share in Total Disease Burden by Region (1990-2017)

Years	Global	High SDI	High-middle SDI	Middle SDI	Low-middle SDI	Low SDI	Turkey
1990	46,3 (48,2-44,5)	6,8 (7,3-6,4)	19,1 (19,9-18,3)	38,0 (39,5-36,6)	60,6 (62,6-58,8)	72,1 (74,7-69,8)	37,9 (40,9-35,0)
1991	45,7 (47,5-43,9)	6,8 (7,3-6,4)	18,4 (19,2-17,6)	37,0(38,5-35,6)	60,0(62-58,2)	71,1 (73,5-69)	36,2 (39,1-33,4)
1992	45,2 (47,0-43,4)	6,8 (7,3-6,4)	17,4 (18,2-16,6)	35,8 (37,2-34,4)	59,5 (61,5-57,7)	71,9 (74,1-69,7)	34,5 (37,3-31,8)
1993	44,5 (46,3-42,7)	6,9 (7,3-6,4)	16,4 (17,1-15,7)	34,5 (36,0-33,2)	59,0 (60,9-57,2)	71,7 (73,8-69,6)	33,4 (36,2-30,8)
1994	43,4 (45,2-41,7)	6,9 (7,4-6,4)	15,7 (16,4-15,1)	33,4 (34,7-32,1)	58,6 (60,6-56,8)	67,9 (69,8-66,2)	32,3 (35,1-29,6)
1995	43,6 (45,4-41,9)	6,8 (7,3-6,4)	15,3 (15,9-14,6)	32,3 (33,7-31,1)	58,6 (60,5-56,8)	71,0 (72,9-69,1)	31,7 (34,6-29,0)
1996	43,2 (44,9-41,4)	6,5 (7,0-6,1)	14,9 (15,5-14,3)	31,4 (32,7-30,1)	58,2 (60,1-56,3)	70,5 (72,4-68,6)	30,7 (33,5-28,1)
1997	42,8 (44,6-41,0)	6,1 (6,5-5,7)	14,5 (15,1-13,9)	30,6 (31,9-29,4)	57,4 (59,4-55,6)	69,9 (71,7-68,1)	29,6 (32,2-27,0)
1998	42,4 (44,2-40,7)	5,9 (6,4-5,5)	14,1 (14,7-13,5)	30,0 (31,3-28,8)	57,0 (58,9-55,2)	69,6 (71,4-67,8)	28,5 (31,1-26,1)
1999	41,9 (43,7-40,1)	5,9 (6,3-5,5)	13,4 (14,0-12,8)	29,3 (30,6-28,2)	56,8 (58,8-55,0)	69,0 (70,8-67,2)	25,7 (28,0-23,5)
2000	41,5 (43,3-39,8)	5,8 (6,2-5,4)	13,0(13,5-12,4)	28,9 (30,1-27,7)	56,6 (58,4-54,7)	68,7 (70,5-66,9)	25,7 (28,1-23,4)
2001	41,1 (42,9-39,4)	5,7 (6,1-5,3)	12,5 (13,0,1-12)	28,5 (29,8-27,3)	56,1 (58,0-54,2)	68,5 (70,3-66,8)	24,5 (27,0-22,1)
2002	40,6 (42,4-38,9)	5,7 (6,1-5,3)	12,1 (12,6-11,5)	28,0 (29,3-26,9)	55,7 (57,7-53,9)	67,8 (69,7-66,0)	23,4 (25,8-21,0)
2003	39,9 (41,7-38,2)	5,6 (6,1-5,2)	11,6 (12,1-11,1)	27,6 (28,9-26,5)	55,0 (57,0-53,2)	67,2 (69,1-65,4)	21,3 (23,4-19,1)
2004	39,2 (40,9-37,5)	5,6 (6,0-5,2)	11,2 (11,7-10,7)	26,5 (27,7-25,4)	54,4 (56,4-52,5)	66,5 (68,4-64,8)	19,3 (21,4-17,3)
2005	38,7 (40,4-36,9)	5,6 (6,0-5,2)	10,9 (11,4-10,4)	26,6 (27,8-25,4)	53,4 (55,4-51,5)	65,8 (67,7-64,0)	17,5 (19,6-15,6)
2006	38,1 (39,8-36,3)	5,5 (6,0-5,1)	10,7 (11,2-10,2)	26 ,0(27,3-24,9)	52,8 (54,8-50,9)	64,9 (66,9-63,1)	15,9 (17,9-14,2)
2007	37,3 (39,1-35,6)	5,5 (5,9-5,1)	10,5 (11,0-10,0)	25,4 (26,6-24,3)	51,8 (53,9-50,0)	63,8 (65,8-61,9)	14,8 (16,6-13,1)
2008	36,2 (38,0-34,5)	5,4 (5,9-5,0)	10,3 (10,8-9,8)	24,4 (25,6-23,3)	50,2 (52,2-48,3)	62,8 (64,7-60,9)	13,8 (15,4-12,2)
2009	35,5 (37,2-33,8)	5,4 (5,8-5,0)	10,1 (10,6-9,6)	23,8 (24,9-22,7)	49,6 (51,6-47,7)	61,7 (63,7-59,9)	13,0 (14,4-11,5)
2010	34,4 (36,1-32,7)	5,3 (5,8-5,0)	9,8 (10,3-9,3)	22,9 (24-21,8)	48,4 (50,4-46,5)	59,5 (61,5-57,8)	12,4 (13,8-11,0)
2011	33,7 (35,3-31,9)	5,3 (5,7-5,0)	9,6 (10,1-9,1)	22,0 (23,1-21,0)	47,2 (49,2-45,3)	59,8 (61,8-57,8)	11,5 (12,9-10,3)
2012	32,7 (34,4-31,0)	5,2 (5,7-5,0)	9,5 (10,0-9,0)	21,0 (22,1-20,1)	45,9 (48,0-44,0)	58,5 (60,5-56,6)	11,0 (12,4-9,9)
2013	31,7 (33,4-30,1)	5,2 (5,7-5,1)	9,3 (9,8-8,8)	20,3 (21,3-19,4)	44,8 (46,9-42,9)	57,2 (59,2-55,3)	10,7 (12,0-9,5)
2014	30,7 (32,3-29,1)	5,2 (5,6-5,1)	9,1 (9,6-8,6)	19,4 (20,4-18,5)	43,6 (45,7-41,7)	55,9 (57,9-53,9)	10,4 (11,7-9,2)
2015	29,6 (31,2-28,1)	5,2 (5,6-5,1)	8,8 (9,3-8,4)	18,7 (19,6-17,9)	42,4 (44,6-40,5)	54,4 (56,4-52,5)	10,0 (11,3-8,9)
2016	28,6 (30,1-27,1)	5,2 (5,6-5,1)	8,6 (9,1-8,2)	17,8 (18,7-17)	41,1 (43,3-39,2)	53,1 (55,1-51,0)	9,5 (10,8-8,4)
2017	27,9 (29,4-26,3)	5,2 (5,5-5,1)	8,4 (8,8-7,9)	17,2 (18-16,4)	40,2 (42,3-38,2)	52,1 (54,2-50,1)	8,9 (10,1-7,8)
Change (%)	-39,8 (-39--40,8)	-24,3 (-24,5--20,5)	-56 (-55,7--56,5)	-54,8 (-54,5--55,2)	-33,7 (-32,3--35,1)	-27,7 (-27,5--28,3)	-76,4 (-75,2--77,6)

4. Discussion and Conclusion

In Turkey, the measurement of health issues was adequately addressed sufficiently developed due to sub-discipline of health economics. However, this issue is an important agenda item in Western countries where we take the systems and applications as an example today (Tatar and Tatar, 1997:54). In Turkey, funded by the World Bank national burden of disease study was first launched in 2004. In this study, the burden of disease was calculated which takes into account the fatal or non-fatal effects of the diseases and injuries and important risk factors (Ünüvar et al., 2006). In Turkey, it is observed that there is still a serious problem in the service of the fight against infectious diseases. Looking at the source of these problems; the lack of health policies that meet the needs of the society in the best way, and produce adequate and efficient health services and professional health managers who are competent in this field in the field of health management are not adequately involved in the sector (Çelik, 2016:311).

Many communicable diseases can be prevented by chemoprophylaxis or vaccination. Regardless of the urgency of control measures, it should also be disclosed to the society at risk. It is critical for the population to report new cases, participate in vaccination campaigns, improve hygiene standards or successfully control such activities (Heymann, 2015). Infectious diseases remain the major killers of children in the developing world. Although notable success has been achieved in certain areas, for example, polio, communicable diseases still account for 7 out of the top 10 causes and are responsible for about 60 % of all child deaths. Overall, the 10 leading causes in low- and middle-income countries represent 80% of all child deaths in the worldwide (Lopez et al., 2006:69). Today, although health spending has increased rapidly all over the world, the resources allocated for health care provision are limited (Yiğit and Erdem, 2015: 211). Regardless of the development gap, all countries face a significant scarce resource in health as well as in other sectors. This problem brings with it the necessity to use the available resources effectively and efficiently (Tatar, 1995:66). DALY approach is used as an important tool in solving this problem.

Gross national income per capita is widely regarded as a key determinant of health outcomes (Sterck et al., 2018:14). Developed regions account for 11,6% of the worldwide burden from all causes of death and disability, and account for 90,2% of health expenditure worldwide. Communicable disorders explain 40,2% (2017) of DALYs worldwide (Murray and Lopez, 1997:1436). Not only those who use healthcare but also have those who do not use healthcare benefited in the treatment of infectious diseases. For example, when a person with an infectious disease

is treated, this disease is not transmitted to other people, so it prevents other people who do not use this service. If the person with the infectious disease is not treated, it will be transmitted to others and in this case will cause much more resources to be consumed.

Whichever method of financing and delivery adopts, the main goal should be equitable and efficient delivery of healthcare services in every country at an acceptable level of quality and access. In Turkey, the regulations associated with the delivery and financing of health services, especially after 2003, "Health Transformation" program has gained momentum with both the financing and the provision of services both radical changes have taken place (Tatar, 2011:103). Health transformation program with life expectancy at birth increased, maternal deaths, infant deaths, and the incidence of infectious diseases decreased. The role of the public in financial risk-sharing has increased, the burden of individuals has decreased, the rate of satisfaction with health services has increased, the rate of benefiting from health services has improved significantly and health service delivery has improved in quality (Yılmaz, 2018).

Communicable diseases are a major global public health problem worldwide. For example, HIV / AIDS is like a weapon of mass destruction that kills people in poor countries. In 2020, the Covid 19 pandemic, which was the number of cases in developed and developing countries all over the world also damaged the economies of all countries. The corona virus, which started in China and then spread to many parts of the world, affected the world economy very deeply. Following the measures taken to reduce the rate of spread of the coronavirus epidemic, global economic activity has been hit hard. Moreover, due to coronavirus travel restrictions and prohibitions, it affected tourism, transportation and other sectors very radically.

Like Ebola, Sars, Avian influenza, which affected the world in the 21st century, Korona spread to almost the whole world in a short time. According to the data of May 17 2020 (Worldmeters, 2020), 4,752,426 people were infected and 313,859 people died. In Turkey, 148.067 people suffering from this disease and the 4,096 people died. The top ten countries in the world as the number of deaths caused by coran virus are USA (90.178), UK (34.446), Italy (31.763) Spain (27.650), France (27.625), Brazil (15.662), Belgium (9.052), Germany (8.027), Iran (6.988) and Netherlands (5.680). In Turkey, 4,096 people died from the same disease.

In Turkey, the reduction of the total burden of disease in 1990 until 2017, the decline in communicable diseases has an important place. Communicable diseases have an enormous burden of disease in terms of morbidity, mortality and economic resources. Therefore, world health organization and international organizations should control the control of

infectious diseases at local, regional, national and international levels. The global struggle in the diagnosis, treatment and prevention studies of communicable diseases has become an imperative today. Therefore, necessary financial resources should be provided for the elimination of healthcare services and the elimination of these diseases in low-income countries.

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CHAPTER XV

NATURAL AND SYNTHETIC REMEDIES AS BREAST CANCER THERAPIES

Selda Goktas & Elif Burcu Bali**

Introduction

Breast cancer is the most prominent cancer type amongst women worldwide and many preclinical and clinical studies are underway to cure this deadly disease. Breast cancer patients are often treated with surgery, radiotherapy, and chemotherapy/immunotherapy drugs. Drugs that fight with breast cancer may develop resistance by the patient and another drug or combination of drugs need to be administered. Due to the costly treatment of breast cancer patients, there is an emerging need to find alternative therapies, both natural- and synthetic-based, to cure the disease. Therefore, this section summarizes the most favorable natural and synthetic approaches as promising replacement treatments for breast cancer patients.

1. Natural-Based Anti-Breast Cancer Approaches

1.1. Polyphenols

Polyphenols are naturally-derived organic compounds comprised of aromatic benzene rings bonded to hydroxyl groups. These compounds demonstrate anti-angiogenic, anti-metastatic, cytotoxic, and differentiation-inducing activities towards breast cancer cell lines. The anti-cancer activities of polyphenols are mediated through various mechanisms such as: **(i)** control over apoptosis, inflammation and signaling pathways, **(ii)** modulation of reactive oxygen species (ROS), **(iii)** regulation of estrogen receptor, **(iv)** modulation of aromatase activity, and **(v)** modulation of autophagy (Abdal Dayem, 2016). Some of the most popular anti-tumor polyphenolic agents investigated for their efficacy in the treatment of breast cancer are listed below:

* (Ph.D.); The University of Oklahoma, selda.goktas@gmail.com

* (Asst. Prof. Dr.); Gazi University Vocational School of Health Services., Ankara, Turkey.
E-mail: burcubali@gazi.edu.tr

Dietary Polyphenols

1.1.1.1. Resveratrol

A natural phytoestrogen resveratrol (RES) (3,4',5-trihydroxy-trans-stilbene) is used extensively as a dietary supplement and naturally found in red wine, grapes, peanuts, cocoa and berries (**Fig. 1**) (Andreani, 2017).

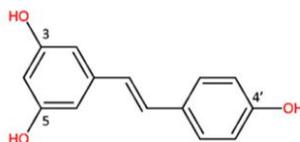


Figure 1. Molecular structure of resveratrol (Ferraz Da Costa, 2020).

In an *in vitro* study, RES oligomers cis- and trans-gnetin H were found to have strong potential as anti-cancer agents and reduced the biological activities of MDA-MB-231 cells by hindering the tissue degrading protein inhibitors TIMP-1 and TIMP-2 (Gao, 2015). Another study demonstrated that RES combined with grape seed proanthocyanidins synergistically reduced human breast cancer cell viability by inducing apoptosis while upregulating Bax expression and down-regulating Bcl-2 expression, as well as altering DNA methylation and histone modifications (Gao, 2018). Zhao et al. (2020) also reported that the RES loaded into peptide and sucrose liposomes (PSL) named as the complex: PSL@RES inhibited breast tumor growth in mice by inducing apoptosis through elevation of p53 and Bax expression and depression of Bcl-2 synthesis. The complex has been considered as an encouraging new tool for future clinical practice of RES. RES can also govern the biological functioning of microRNAs (miRNAs) in breast cancer. miRNAs, short non-coding RNAs, act as epigenetic regulators of cancer. Amiri et al. (2020) found that RES could block the proliferation of breast cancer cells by stimulating tumor-suppressive miRNAs, such as miR-503, miR-34a, and miR-424 through p53 pathway and suppressed heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1) that plays a key role in tumor initiation and progression (Amiri, 2020).

Previous preclinical studies confirmed RES intake exhibited promising anticancer effects while inducing apoptosis and promoting S-phase arrest of select cancer cells and preventing tumor growth (Garvin, 2006; Taylor, 2017). A 12-week placebo-controlled clinical trial with 5 or 50 mg dosage of RES twice daily revealed a decrease in *RASSF-1α* that was an indication of the chemopreventive action of RES (Berman, 2017). This finding encouraged researchers to conduct more clinical trials for RES as an anti-cancer phytochemical.

1.1.1.2. Curcumin

Curcumin (CUR), the yellow spice, is a polyphenol derived from turmeric (*Curcuma longa* L.) (Fig. 2) (El-Far, 2020).

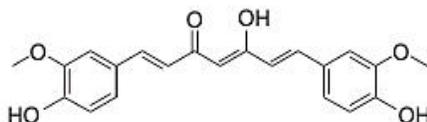


Figure 2. Molecular representation of curcumin.

It is an effective natural compound in several types of breast cancer and combination therapy approaches including at least two of the polyphenolic constituents and/or its combined effect with chemotherapeutic drugs or methods have also been adopted. For example, a combinational approach utilizing both quercetin and CUR showed the synergistic effect induced anticancer activity against breast cancer cells (Kikuchi, 2019). Mittal et al. (2020) also reported that CUR combined with electrical pulses treatment decreased the clonogenic character of breast cancer cells by inducing apoptosis, thus enhanced its anticancer effect on triple-negative breast cancer (TNBC). Results of this combinational therapy approach are expected to have clinical significance for overcoming the resistance of TNBC cells against chemotherapeutic agents and enhancing the overall survival rate of patients (Mittal, 2020). Another current study confirmed that treatment with CUR in combination with paclitaxel (PAX) was superior to the PAX in patients with metastatic and advanced breast cancer (Saghatelian, 2020). The combinational therapy approach also revealed that inclusion of CUR and/or vitamin D3 to PAX demonstrated synergistic anticancer action on breast cancer cells by promoting cell death and compared to the PAX monotherapy, combined therapy with CUR and vitamin D3 increased tumor response to PAX (Attia, 2020). Synergistic antitumor properties of CUR with quercetin and different CUR-polymer conjugates such as CUR-loaded solid lipid nanoparticles, chitosan-coated liposomes encapsulating CUR and dextran-CUR nanoparticles were also reported to increase its anticancer performance and to develop new strategies regarding breast cancer combination therapy (Mansourizadeh, 2020; Fathy Abd-Ellatef, 2020; Hasan, 2020; Curcio, 2020).

1.1.1.3. Quercetin

Quercetin (QU) is a dietary flavonoid with strong potential as an alternative therapy in breast cancer (Fig. 3).

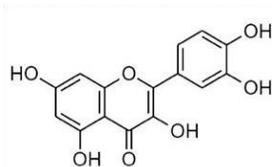


Figure 3. Molecular structure of quercetin (Abotaleb, 2019).

Recent review studies have reported the epigenetic modifying actions of QU against breast cancer. The review study of Selvakumar et al. (2020) clarified that QU increased epigenetically silenced BRCA1 expression in TNBC and its combination with CUR also increased BRCA1 expression synergistically and the BRCA1 promoter histone H3K9 acetylation was significantly enhanced with the combined treatment in a TNBC cell line. In another review study, it was reported that QU impacted the G1 phase and stimulated apoptosis and twist expression in estrogen-positive breast cancer cells and acted as anti-proliferative in MCF-7 cells by decreasing the phosphorylation of P38MAPK (Ezzati, 2020). Modification of miRNA expression via QU against breast cancer was also reported by Ahmed et al. (2020). In this review study, they concluded that QU up-regulated miR-146a expression and obstructed cell proliferation by stimulating apoptosis-related pathways and inhibited invasion of cancer cells (Ahmed, 2020). In a recently-published study, the effect of QU on the oncogenic protein kinases has been investigated. It was found that QU blocked migration and matrix invasion of TNBC cells by restricting protein kinases through oncogenic PI3K/AKT, MAPK, and STAT pathways. The result of this study have shown that treatments with QU create broad effects at a molecular level and the use of QU with tumor models formed by patient-derived cells enlighten its translational potential as a therapeutic compound (Shahi Thakuri, 2020).

1.1.1.4. Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (EGCG) from green tea (*Camellia sinensis*) is a polyphenolic compound that inhibits the activity of DNA methyltransferases (Fig. 4). The utilization of small compounds displaying DNA methyltransferase inhibitory effect is critical to sensitize cancer cells to cytotoxic agents, partially, by reverting the acquired epigenetic modifications related to the resistance to therapy. In a recent study, it has been found that the treatment with EGCG significantly decreased the cell viability of different triple-negative breast cancer cell lines (Assumpção, 2020). In a recent review study, it has been also reported that EGCG blocked breast cancer cell viability by stimulating apoptotic events and by modulating various molecular signaling pathways. Additionally, it delayed

the growth of MDA-MB-231 cells, by blocking the β -catenin signaling pathway. Therefore, it is considered as a new anti-cancer therapeutic of patients suffering from TNBC (Bimonte, 2020).

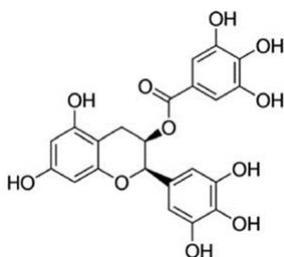


Figure 4. Structure of epigallocatechin-3-gallate (Gu, 2020)

1.1.1.5. Gallic Acid

Gallic acid (GA), 3,4,5- trihydroxy benzoic acid, is a natural polyphenolic constituent and commonly found in pomegranate, mangoes, grapeseeds, blueberry, etc. (Fig. 5) (Khorsandi, 2020; Gu, 2020).

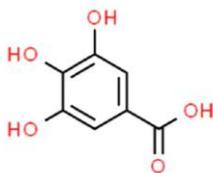


Figure 5. Structure of gallic acid (Khorsandi, 2020)

Natural compounds like GA are highly valuable novel agents against dysregulation of the EGF/EGFR-mediated matrix metalloproteinase-9 (MMP-9) expression in cancer cells. EGF/EGFR-mediated MMP-9 expression is an established hallmark of the invasion and metastasis of breast cancer. Chen et al. (2016) proved that when exposed to gallic acid, MMP-9 expression in EGF-treated MCF-7 cells was hampered (Chen, 2016). In a recent study, as an anticancer agent, the effects of GA as monotherapy or in combinational therapy approach including low-level laser irradiation on MDA-MB-231 cells have been reported. The results of this study have shown that preirradiation followed by GA treatment did not significantly alter normal cell viability but decreased the survival rates of cancer cells more than GA alone (Khorsandi, 2020). In another recent study, it has been confirmed that GA nanoparticles coating with alginate-chitosan demonstrated a significant level of cytotoxicity against breast T47D cancer cells; therefore, GA was considered to be a promising candidate for new anti-breast cancer agent (Arsianti, 2020).

1.1.1.6. Hesperidin

Hesperidin (HS), (30, 5, 9-dihydroxy-40-methoxy-7-orutinosyl flavanone), is a natural citrus fruit (such as orange peel) derivative flavonoid and known to have various beneficial health effects such as its scavenging power against cancer (**Fig. 6**).

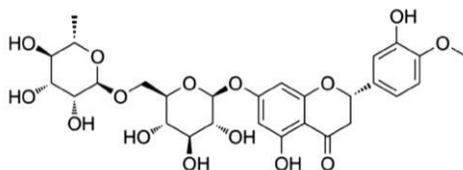


Figure 6. Chemical structure of hesperidin (Kongtawelert, 2020)

In an *in vitro* study conducted by Lee et al. (2010), it was found that HS significantly decreased the proliferation rates of MCF-7 cells. Additionally, Kongtawelert et al. (2020) have recently clarified how HS influences programmed death-ligand 1 (PD-L1) expression. PD-L1 is excessively expressed in the most destructive TNBC, helping the elimination of antitumor immunity, and thus maintaining the longevity of the tumor. In their study, HS suppressed the proliferative capacity of MDA-MB231 cells and also blocked the expression of PD-L1 in these aggressive cancer cells. Their findings have shown that HS might also have potential clinical implications (Kongtawelert, 2020).

1.1.1.7. Naringenin

Naringenin (NG), a flavanone that is abundantly present in fruits of the citrus family, is known to possess a considerable potential as a tumor-suppressive agent against breast cancer (**Fig. 7**).

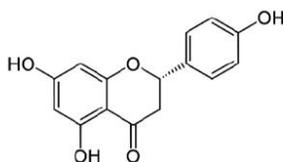


Figure 7. Structure of naringenin.

In an *in vitro* study, it was demonstrated that combined treatment of NG and tamoxifen (Tam) decreased cellular metabolic activity and cell number to a greater extent compared to monotherapies utilizing either component alone in MCF-7 cells by inducing apoptosis (Hatkevich, 2014).

Additionally, Zhang et al. (2016) proved that NG hampered TGF- β 1 trafficking by repressing protein kinase C activity, resulting in a reduction of TGF- β 1 expression by the breast cancer cells. They also suggested that NG could represent a remarkable therapeutic ingredient for dosing studies in the clinical trials of TGF- β 1 related diseases.

1.1.2. Other Common Polyphenols

Other common polyphenolic constituents that have therapeutic action in breast cancer treatment are kaempferol, fisetin, myricetin, galangin and casticin (vitexicarpin) (**Fig. 8**) (Abotaleb, 2019). The anti-cancer activity for these polyphenols varies in terms of the mechanism of action of the specific structure in the fight with the disease.

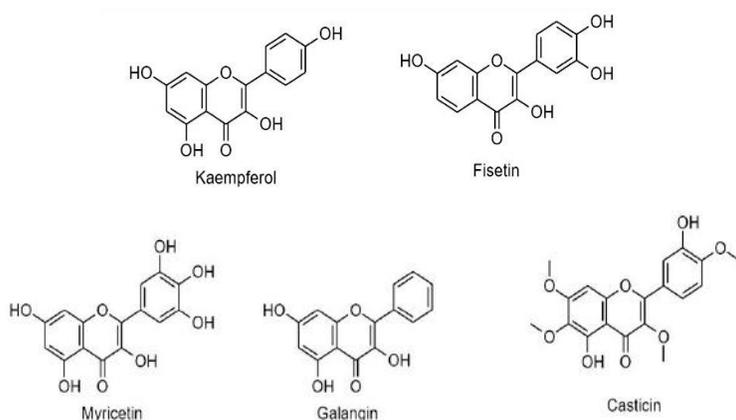


Figure 8. Structure of other common polyphenols (Abotaleb, 2019).

1.2. Lycopene

Lycopene (LY) is a red tomato-originated pigment in the human diet that assists to struggle with several cancer types like breast cancer (**Fig. 9**). The intake of LY-containing foods could decrease breast cancer risk (Przybylska, 2020). In review studies, it has been reported that the major anticancer mechanism of LY on MCF-7 cells is mediated through the blockage of the IG-1R (insulin-like growth factor-1) pathway. Moreover, LY consumption proved beneficial to breast cancer patients during radiotherapy with its protective effect on the radiation-exposed skin (Caseiro, 2020; Przybylska, 2020).

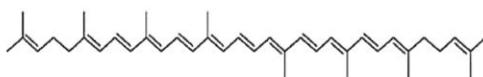


Figure 9. Structure of lycopene (Caseiro, 2020).

1.3. Propolis

Propolis (PR), known as an anticancer molecule, contains many bioactive compounds such as phenolic acids, flavonoids, diterpenoids, and triterpenoids (Gal, 2020). Xuan et al. (2014) demonstrated that Chinese PR could stimulate apoptosis by effecting intracellular ROS and mitochondrial membrane potential in hormone-independent and hormone-dependent breast cancer cells. Additionally, Gal et al. (2020) have recently proved that a diet including PR can decelerate the improvement of breast tumors growth with lower variation, gravity, and extent according to control. They have also found that 30% of rats with breast tumors have no tumor when treated with PR. In another recent study, it has been reported that ethanolic PR (EPR) extract decreased MCF-7 cell viability by triggering apoptosis through increased pro-apoptotic protein levels. They also detected that EPR reduced mitochondrial membrane potential and changed the expression levels of specific tumor suppressors and oncogenic (miR-21) miRNAs. This study claimed that Turkish propolis could have the potential to be a natural anticancer agent in the future (Misir, 2020).

1.4. Terpenes/Terpenoids

Terpenoids (terpenes) are plant-derived compounds that have a long history of use in pharmaceutical preparations. Discovery of paclitaxel (Taxol[®]), a diterpene from *Taxus brevifolia*, made a huge impact in the breast cancer drug industry and was the strongest motive to study terpenoids for clinical use. This phytopharmaceutical inhibits primary tumor progression and protects against angiogenesis and late-stage metastasis (Ateba, 2018). The triterpenoid (terpenoid with 6 five-carbon building blocks) CDDO-Me is also known for its efficiency in inhibiting COX-2 expression and blocking the normal growth mechanisms in breast cancer cells *in vivo* (Rabi, 2009).

1.5. Oligosaccharides

Chitosan oligosaccharides were reported for their suppressive action on the expression levels for MMP-9 in breast carcinoma cells as a dose-dependent response (Nam, 2009). In a study conducted both *in vitro* and *in vivo*, hyaluronan deca-saccharides, a sub-type of *Hyaluronan oligosaccharides*, were proved to show a remarkable anti-cancer activity while inhibiting the metastasis-promoting cellular signaling in breast cancer cells (Urakawa, 2012).

1.6. Herbal Mixtures

The phytochemical remedy may also be in the form of a mixture of several constituents, such as a 12 herb mixture called *Jaumkanghwa-tang (JEKHT)* (Hanjung Pharmaceuticals). One study investigating the anti-

cancer activity of JEKHT reported that when administered with tamoxifen (TAM), JEKHT overcame the resistance of endocrine-resistant LCC9 human breast cancer cells to TAM (De Oliveira Andrade, 2019). More herbal mixture candidates are yet to be discovered as a curative agent for breast cancer.

2. Synthetic-Based Therapies for Breast Cancer

2.1. Glucose Transporter (GLUT) Inhibitors

The inhibitors of GLUT1 (a GLUT family member), WZB117 and STF-31 were found to drastically attack the proliferative capacity and enhance the apoptotic activity of breast cancer cells (Barbosa, 2020). Since GLUT1 is known as the main glucose transporter in cancer cells, its inhibition has a profound effect most critically during the early stages of breast cancer development.

2.2. Synthetic ω -3 Epoxyfatty Acids

Select synthetic ω -3 epoxides of saturated fatty acids showed promising antiproliferative and proapoptotic agents in MDA-MB-231 cells and their multi-mode of action included irreversibly interrupting the cell cycle and the ATP production (Dyari, 2014).

2.3. Synthetic Analogs of Natural Phytochemicals

Synthesis of novel analogs of natural compounds has attracted the attention of cancer researchers since the extraction of the required amount of pharmaceutically active ingredients from the natural source is problematic. 5-Bis (4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidone (PAC), a synthetic analog of CUR, was tested in a breast cancer model of mice and it was shown that PAC proved to be superior to CUR in triggering apoptotic events specific to cancerous cells (Al-Hujaily, 2011). The same study also revealed that PAC reduced the secretion of immune suppressor cytokines IL-4 and IL-10. Park et al. (2010) reported that 7-O-butyl naringenin (BN), a chemically synthesized derivative of NG, hindered MCF-7 cell proliferation, resulting in cellular accumulation in the sub-G1 phase. They also found that BN activated the cellular processes leading to increased cytotoxicity in MCF-7 cells through inducing the production of ROS as well as other mechanisms of actions. Hence, they considered it to be a possible chemotherapeutic analog of NG as an anti-proliferative agent (Park, 2010).

2.4. Synthetic Derivatives of Carbazoles

Carbazoles are a group of polycyclic aromatic drugs that have proven antitumor activity on cancer cells (**Fig. 10**). For example, Clausine B (a naturally occurring carbazole) has been reported for its ability to exert

therapeutic action on MDA-MB-231 breast cancer cells (You, 2018). This has encouraged synthetic chemists to create synthetic derivatives of these carbazoles for the treatment of different types of cancer. An *in vitro* anticancer screening study conducted by You et al. (2018) claimed the discovery of a novel N-aryl- sulfonyl carbazole that proved to exhibit a cytotoxic effect in pancreatic cancer xenograft mice models. Similar synthesis strategies need to be undertaken for the discovery of carbazole-derived therapeutic agents for breast cancer.

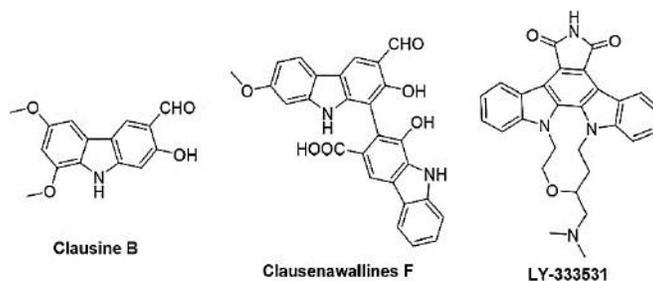


Figure 10. Schematic presentations for the carbazoles with anti-cancer activities (You, 2018).

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CHAPTER XVI
**THE PROTOCOL OF HYPERTONIC SODIUM CHLORIDE ON
PREVENTION OF DELIRIUM AFTER SURGERY**

Fadime Çınar & Fatma Eti Aslan***

1.INTRODUCTION

Post-operative delirium (POD) is defined as an acute neuropsychiatric syndrome characterized by a temporary and reversible brain functional disorder that can be seen after surgical intervention due to physical or physiopathological reasons (Godfrey *et al.*2020). Surgical intervention is a risk factor in the development of delirium, and risk factors for delirium due to surgical intervention may vary at different stages of the surgical process. Surgical intervention is a stress-filled stimulus that elicits an inflammatory response and as such is a form of trauma. Metabolic, immunological, neuroendocrine and inflammatory changes develop in response to overcoming this trauma in a stressed organism. The release of stress hormones and cytokines, inflammatory mediators, plays a role in the formation of these changes (Girard *et al.*2012). After the surgical intervention, the systemic inflammatory process is activated, cytokine and neurotransmitter release begins. Cytokines are polypeptide hormones secreted by glial and macrophages in the central nervous system. Generally, extracellular levels increase in stress, inflammation, tumor, trauma and infection. Cytokines initiate the activation of microglia, increasing the permeability of the blood brain barrier to neurotoxins and cognitive impairment (Belarbi *et al.* 2012).

In the literature, it is stated that anxiety, fluid electrolyte imbalances, hypoxia, infections, and the neuroendocrine and neuroinflammatory response given to the surgical treatment by the organism increase the serum levels of inflammatory cytokines and increase the levels of S-100B from specific proteins that are neurobiochemical markers. (Cerejeira *et al.*2010, Cortese & Burger 2017). Serum S-100B protein level plays an important role in determining cerebral affection and cognitive loss in delirium (Kunihara *et al.* 2006). The main cytokines released after surgery in delirium are; interleukin-1 (IL-1), tumor necrosis factor (TNF- α), IL-6 and IL-10. The first reaction is the release of IL-1 and TNF- α from activated macrophages and monocytes in damaged tissues. These factors cross the blood-brain barrier, causing cognitive impairment. The penetration of neurotoxic agents and

* Sabahattin Zaim University Faculty of Health Sciences, Health Management, Istanbul, Turkey. E-mail: fadime.cinar@hotmail.com

** Bahçeşehir University Faculty of Health Sciences, Nursing Istanbul, Turkey

inflammatory cytokines in the impaired blood-brain barrier can cause delirium (vanMunster *et al.* 2010, Cavallazzi, *et al.* 2012).

Delirium is mostly seen in surgical clinics after intensive care units in hospitalized patients (Mulkey *et al.*,2019; Ortega *et al.*2020). In the literature, it has been stated that the prevalence of delirium is 50% in elderly hospitalized patients, affecting 3-53% of patients undergoing major surgery and 83% of critical patients in intensive care unit (Cerejeira *et al.* 2010; Sanders *et al.* 2011; Cohen *et al.*,2019). Although there are many factors that cause delirium, it is observed more in some patient groups. These patient groups; elderly patients are those undergoing cardiac and hip surgery (Holroyd-Leduc *et al.* 2010; Martinez *et al.* 2015). Studies show that postoperative patients have a high relationship between high CRP (C-reactive protein) and interleukin 6 (IL-6) levels and delirium in patients undergoing hip prosthesis surgery (VanMunster *et al.* 2008; Capri *et al.* 2014). VanMunster *et al.* (2008) found that IL-6 and IL-8 levels were high in patients developing delirium in their studies with patients who were operated on for hip fracture. As a result, they report that there is a relationship between IL-6 and IL-8 level and delirium pathogenesis, which may be related to IL-6 hyperactive delirium behaviors (VanMunster *et al.* 2008).

Delirium; It has negative effects on patients, patient relatives, health system in terms of emotional, functional and financial aspects. These effects are; Increases the length of hospital stay, mortality and morbidity, and increased hospital costs (Morandi *et al.* 2009; Koster *et al.* 2011; Banerjee *et al.* 2010;Grealish *et al.*2019). Cognitive and functional losses, which have long-term effects of delirium, may continue in the period after discharge, causing patients to be hospitalized again (Koster *et al.* 2011). Because of its negative effects on health, the symptoms of delirium are not yet fully recognized and because it is mixed with different cognitive disorders, it is generally not noticed by health professionals (Fong *et al.* 2009; Banerjee *et al.* 2010).

Studies have reported that cytokines, one of the inflammatory markers, can cross the blood-brain barrier after surgery and cause brain damage and delirium (Xin *et al.* 2017, Mazandarani *et al.* 2012). Therefore, it is necessary to determine the diagnostic usage possibilities of inflammatory markers and their role in delirium etiology. Thus, preventive and diagnostic evidence-based studies can be conducted to reduce cytokine release. Evidence-based studies to prevent delirium development by decreasing the level of cytokines after surgery in literature reviews are insufficient. It is stated in the literature that administration of hypertonic sodium chloride infusion to the patient before surgery can reduce cytokine

release by preventing delirium and prevent delirium (Xin *et al.* 2017; Mazandarani *et al.* 2012). Hypertonic sodium chloride has an immunomodulatory effect by blunting neutrophil activation and reducing cytokine production and preventing cytokines that are effective in the development of delirium from crossing the blood barrier system (Mazandarani *et al.* 2012).

In this article, it was aimed to give information about the intervention protocol applied in the study of the effect of hypertonic sodium chloride solution on prevention of delirium in surgical patients over 65 years of age. In order for delirium to be seen at a high rate in surgical clinics and to reduce the negativities caused by it, it is necessary to diagnose with early biological markers and screening scales and apply preventive interventions. With these preventive applications, early diagnosis will be provided for delirium, morbidity and mortality rates will be reduced, and diagnosis-treatment costs will be positively affected. In addition, this randomized controlled clinical study, which requires a multidisciplinary team approach, is an indication that nurses can take part in these studies with their educational level, knowledge, skills, clinical experience, and the roles and responsibilities they have undertaken as a member of the team in predicting and preventing delirium patients.

2. METHOD

In this article, it was aimed to explain the protocol followed as a randomized controlled clinical trial to determine the effect of hypertonic sodium chloride (NaCl) application in the prevention of delirium seen in patients over 65 years old after surgery.

2.1. Type of the Research

This research is a randomized controlled clinical study from epidemiological studies and an interventional protocol that is tracked in this clinical study with application-oriented.

2.2. Place and Date of the Research

This research was conducted in University of Health Sciences Derince Training and Research Hospital's orthopaedics and cardiovascular surgery clinics between the dates September 2018 and May 2019. Orthopaedics and cardiovascular clinics and the patients in these clinics were selected due to the reason that in literature, delirium development rate was high especially following hip fracture, hip prosthesis and cardiovascular surgery (McPherson *et al.* 2013)

The patients that are operated with orthopaedic and cardiovascular diagnosis are hospitalized generally one day before the surgery since they are prepared as elective surgery, and next day surgical intervention are

applied. All orthopaedic patients that undergo a surgery generally are not taken into intensive care unit unless it is needed, and after the operation, nurses make their admission into clinics and routine postoperative follow-ups begin. In general, the patients those come to orthopaedic clinic after surgical intervention start to oral intake with fluids after 6-8 hour, and when they tolerate the fluid, immediately normal alimentation is started. After cardiovascular intervention, most of patients are taken into intensive care unit and after extubate, they can start to oral feeding with fluids in 6-8 hour. In orthopaedic, cardiovascular and intensive care clinics' routine practice, there are post-op drug administration protocols. Patient-controlled analgesia for pain treatment is used on most of the patients undergoes cardiovascular and orthopaedic surgery. This practice is also valid for intensive care patients, and it is followed up by anaesthesiologist and nurses. Usually on the morning of the second day after the surgery, patients are mobilized by standing up with doctors and nurses. Within patient care, by taking part of in all processes during the hospitalization of hospitalized patients for the purpose of planned surgical intervention, nurses has a guiding and key role in the patient's risk assessment in terms of delirium. Patients are often discharged from hospital in the third or fourth day after surgery. In this study, patients are evaluated in terms of delirium throughout one day before surgical intervention and average three days after intervention.

2.3. Population and Sample of the Research

This interventional protocol's population is composed of hospitalized patients, who accept voluntary participation and received written and oral approval, for cardiac surgery intervention to cardiovascular surgery clinic and for hip prosthesis surgery to orthopaedic clinic during the study in the institution, that research will be conduct. For the number of sample, power analysis of the study calculated in GPower 3.1. In the study of Xin *et al.* (2017) named as "Hypertonic saline for prevention of delirium in geriatric patients who underwent hip surgery", delirium prevalence difference between hypertonic saline applied group and isotonic applied group is given as 26,6%. According to results of this study, for 80% power number of samples must be at least 76, in two groups that each has 38. With considering the population representation ability of samples should be strong, in this study, number of samples was made with 100 patients, for each group has 50 patients.

In the time period that is study conduct, total 11 patient that were comply with criteria and accepted the participate the study were excluded from the study, since five of them had their operations cancelled, four of them was transferred to intensive care unit before operation due to respiratory problems and two of them is refused to undergo an operation.

Sample exclusion criteria are as follows;

- i. Those who are under 65,
- ii. Those who has score in Standard Mini-Mental State Examination (MMSE) less than 24,
- iii. Patients with a history of delirium before the surgical intervention,
- iv. Patients with neurologic and mental medical record,
- v. Those who are using tranquilliser and antidepressant,
- vi. Patients that are recently using glucocorticoid because of infection and chronic inflammatory
- vii. Those who have anti-inflammatory drug utilization at least three days before operation, who have communication obstacle and who have addiction of alcohol or drug are excluded.

In addition, at every stage of research, termination of the research and taking related precautions in case of developing acute coronary failure, renal failure and electrolyte abnormality are taken into account.

Randomisation of the sample: Before implementation, firstly, assigning of groups that will be applied HSC and SC was realised.

Group that was applied Hypertonic Sodium Chloride (NaCl) (HSC): A group of 50 patients who receive 150 ml of 3% + 150 ml of 3% = 300 ml of 6% of hypertonic sodium chloride, which is selected randomly with MedCalc 11.5.1. Packaged software from 100 patients (150 ml of 3% hypertonic sodium chloride in mediflex bag is product of Eczacıbaşı-Baxter Hospital Products Industry and Trade Inc. and is applied intravenously).

Group that was applied Sodium Chloride NaCl (Isotonic) (SC): A group of 50 patients who receive 500 ml of 0.9% sodium chloride (isotonic) (NaCl) and which is selected randomly with MedCalc 11.5.1. Packaged software from 100 patients (500 ml of 0.9% of isotonic polypharmacy is a product of Eczacıbaşı-Baxter Hospital Products Industry and Trade Inc. and is applied intravenously).

During the research, in order to reduce affection and ethical problems, MedCalc 11.5.1 packaged software is used for the HSC and SC groups (Kanik, 2009). With this program, two different interventions from 100

patient as A (6% of hypertonic sodium chloride NaCl) and as B (0,9% of sodium chloride NaCl) was randomly assigned. Through the program that is used, between 1 and 2 random 20 number was written and when it came 1, A intervention to first patient, B intervention to second patient was applied and when it came 2, B to first patient, A to second patient was applied. By this operation, arrangements were made so that there will be two patients in each column (Table 1).

Table 1: Assigning HSC and SC Groups Randomly

Random Number	1		1		1		2		2		2		1		1		2		2	
Patient No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Assign	A	B	A	B	A	B	B	A	B	A	B	A	A	B	A	B	B	A	B	A

2.4. Data Collection Tools

In this randomize clinic study, individual Features Form, Pre- Intra- and Postoperative Risk Assessment Form, The Nursing Delirium Screening Scale (Nu-DESC) and Mini-Mental State Examination (MMSE) was used as data collection tools. Required information for pre and postoperative risk assessment forms and also laboratory findings (blood tests) of patients was obtained by doctor and nurse inspection form that taking part in patient file. Furthermore, results about pre and postoperative serum cytokines level was recorded in pre and postoperative assessment forms. During 1-10 September 2018, with 10 patients in the University of Health Sciences Derince Training and Research Hospital’s orthopaedic clinic, pre-application about only using forms and comprehensibility of forms was made by researchers. Through pre-application, intelligibility of forms and expediency for the aim of the study was evaluated. In the line with the results, necessary arrangements about the forms that will be used were made.

2.5. Individual Features Form

Twelve questions took part in this form, which is developed by the researcher in order to determine of patients’ age, gender, marital status, educational background, medical diagnosis, comorbid chronic diseases, smoking and alcohol usage and continuous drug use status.

2.6. Pre- Intra- and Postoperative Risk Assessment Form

This form is actually three-step follow up forms that is developed by the researcher with the knowledge of literature and which consist of preoperational, intra-operational and post operational risk factors about post-op delirium (Oh *et al.*2015; Scholz *et al.* 2016; Gosselt *et al.* 2015; Raats *et al.* 2015; DeWitt, 2018). These forms are consist of the questions about presence of invasive procedure, visual and hearing impairment, risky drug usage status, presence of diseases that develops in hospitalisation time period, urinal catheter, bowel evacuation, level of mobilisation, circle of sleep, nutrition and pain status, duration of anaesthesia, duration of surgery, blood transfusion status and laboratory findings.

2.7. The Nursing Delirium Screening Scale (Nu-DESC)

This scale was used for pre and postoperative delirium screening. Gaudreau *et al.* (2005) develop this scale and it is for nurses to use. It consists of five articles that are orientation disorder, inappropriate behaviour, inappropriate communication, illusion-hallucination and psychomotor retardation. Application of it takes less than 2 minutes. Evaluation of articles is made is based on 0,1 and 2 points. In this interventional protocol, delirium screening with the scale was performed every day in 24 hour period throughout 3 days after surgery. Since delirium is usually seen second or third day after surgery (Chang *et al.*, 2008), patients were examined in total 1200 times in the sense of delirium diagnosis three times in a day at 8 hour intervals between one day before the surgery and three days after the surgery. Presence of delirium is determined with the guidance of the DSM-IV scales as gold standard by nurses of clinics that patients stay and two-neurology specialist. It was ensured that researchers that are making the tests were blind to each other.

2.8. Mini-Mental State Examination

It is a test that used for cognitive function screening. The short test which is developed by Folstein and friends in 1975 and Turkish legitimacy and that is the most frequently used test for dementia scanning (Folstein *et al.*1975). It consists of eleven questions and it is evaluated over 30 points. Completing of the test is takes almost 10 minutes. Between twenty-nine and thirty point normal, between 18-23 points mild dementia, 17 point and

below is compatible with severe dementia. Orientation, memory, attention, calculation, recall, language, motor functions and perception, visuo-spatial abilities are tested. It's easy and quick application is the greatest advantage of it.

In this study, all patients' first assessment is made within 24 hour following their hospitalization. In the first evaluation, Standardize Mini-Mental Test was applied and patients' data were recorded. For the uneducated patients, Standardize Mini-Mental Test for uneducated (MMSE) was used. Patients who had MMSE score below 23 in first assessment were excluded from the study. If the patients who have hearing impairment were using hearing aid, it was ensured that the device was used during the application of the test.

2.9. Data Collection Method

Data was collected as randomized controlled double blind. In data collection, MedCalc 11.5.1 packaged software is used for the randomization of samples. Patients that will be performed application and be collected their data were divided into two as group that will be applied hypertonic sodium chloride (experimental) and group that will be receive sodium chloride (control). In September 2018, implementation of research was begun and in April 2019, it is finalized when it reached the planned figure. Since continuity of intervention have to be provided through 24 hour due to the feature of the studied subject, the interventions of the study was maintained with anaesthesiologists, clinic doctors and nurses. Therefore, orthopaedic, cardiovascular surgery and intensive care clinic physicians and nurses were trained. Training scheme about postoperative delirium, delirium risks, diagnosis and prevention was prepared and it was realised by ensuring that all nurses in orthopaedics and cardiovascular surgery clinics participated into them. Research was performed as double blind. Hypertonic sodium chloride (HSC) and sodium chloride (SC) group patients' files were also used on the purpose of follow-ups from their admission in the hospital till their discharge.

2.10. Implementation

It was ensured that preoperative intervention assessment and blood samples were taken before passing the application of hypertonic sodium chloride.

Preoperative Assessment and Collecting Blood Samples: On the behalf of delirium, risk assessment, one day before the surgery, Delirium

Screening Scale (Nu-DESC), Preoperative Delirium Risk Factors Assessment Form and for cognitive function evaluation Mini-Mental State Examination (MMSE) was applied to both groups and recorded. In preoperational period, patients who had score in screening scale over 2 and MMSE point between 23-17 and below 17 were excluded from study.

For both hypertonic sodium chloride and sodium chloride groups, venous blood sample was taken into sterile EDTA tubes before surgery and it was centrifuged at 4°C by separating the plasma in the laboratory (30 minutes at 3000 rpm) in order to determine blood level of systemic cytokine cells that are produced by macrophages and provide peripheral inflammatory activation that caused the formation of delirium. Plasma was divided into polypropylene tubes and kept in a freezer on -80°C until number of samples is completed and analysed. Centrifuging of venous blood samples and placing them into freezer was made by clinic nurses when the researcher was absent.

Prophylactic HSC and SC Application: On behalf of the application of prophylactic HSC and SC, it was ensured to order it into the files of patients who were divided into two as experiment (hypertonic sodium chloride) and control (sodium chloride) by randomized controlled. Registering the solutions into patients' file was made by researcher and anaesthesiologist who carry on the study as second consultant. In addition to routine treatment protocol, with supervision of anaesthesiologist in their clinic and researcher, 500 ml of 0.9% sodium chloride solution was applied on right upper arm intravenously to patients who were in sodium chloride group, an hour before anaesthesia in the way that it will be completed in 60 minute time period (Mazandarani *et al.* 2012). In the same way, 150 ml of 3% + 150 ml of 3% = 300 ml of 6% of hypertonic sodium chloride was applied on right upper arm intravenously to patients who were in hypertonic sodium chloride group, an hour before anaesthesia in the way that it will be completed in 60 minute time period in addition to routine treatment protocol (Mazandarani *et al.* 2012). Before this application, patients were informed about the process and their written and oral consent was received. During all these processes, patients were observed by nurses and doctors. In the case of acute heart failure, renal failure and electrolyte abnormality on patients in both groups during the realization of the stages, the treatment was ended and related precautions were taken.

Intraoperative Assessment: Hypertonic sodium chloride and sodium chloride applied patients were transferred to operating room for surgical intervention. Patients in both groups were monitored during the surgical

intervention and same anaesthetic applications were to all patients. In terms of delirium risk factors, patients were followed up during the surgical operation and findings were recorded with “Intraoperative Delirium Risk Assessment Form”. In order to prevent delirium development due to blood pressure, pulse, oxygen saturation, electrocardiogram and deep sedation, bispectrality index and end-tidal CO² were monitored in both groups to ensure sedation control.

Postoperative Assessment and Taking Blood Samples: It is ensured that patients in both groups were transferred to their clinics and patients who need intensive care were transferred to intensive care units, and their treatments were made in accordance with the hospital routine protocol. Almost all patients who undergo cardiovascular surgery were taken into intensive care units. On the first day after surgery at 6 am, 5 ml venous blood samples were taken into sterile EDTA test tubes and it was centrifuged at 4°C by separating the plasma in the laboratory (30 minutes at 3000 rpm). Plasma was divided into polypropylene tubes and kept in a freezer on -80°C until number of samples is completed and analysed.

In order to determine postoperative delirium, between 1-3 days after surgery delirium screening was made with The Nursing Delirium Screening Scale three times a day between 08-16, 16-24 and 24-08 hours. Moreover, delirium risk was evaluated with “Postoperative Delirium Risk Assessment Form”. For the diagnosis of delirium according the symptoms that were arisen on these time intervals, consultation of neurologist was requested and registered. For those patients who were diagnosed delirium, clinic doctors and nurses informed in order to begin required interventions.

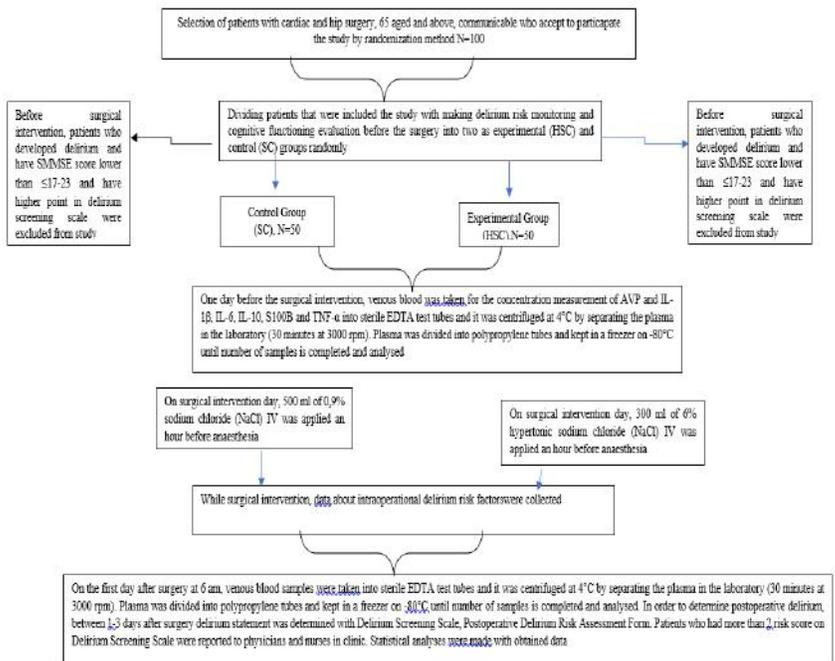
The Analysis of Blood Samples: In the blood samples of patients in both group, antidiuretic hormone level, IL-1 β , IL-6, IL-10, TNF- α and S100B serum concentration measurements were made. Furthermore, in accordance with clinics’ routine diagnosis and treatment protocol, apart from cytokine levels that are monitoring in blood samples taken from patients before and after surgery, other laboratory findings were taken from patients’ files and recorded in delirium risk screening forms. **Blood Samples of Patients:** It consist of values indicating complete blood cell count, Na, K, Mg, BUN/Creatinine ratio, albumin, Hb and glucose levels. While these examinations were made in central laboratory of the hospital, measurement of cytokine levels were made in a contracted laboratory out of hospital. Financing of external laboratory tests was covered by the researcher.

Measurement Method of Patients' Blood Samples: methods of measurement were made in accordance with the suggestions of producer company. Person who conducted the tests was completely blind to information about study.

- i. By using protein (Roche Elecsy 2010, Roche Diagnostics, Germany) kit, S-100B was studied in serum with immunoradiometric assay (sandwich method). S100B analyser automatically calculates based on calibration curve in every sample. Results are specified in terms of $\mu\text{g/L}$.
- ii. Measurement of TNF- α with DIAsource ELISA kit, results in terms of pg/ml ,
- iii. IL-6 with AviBion branded kit from (Vantaa, Finland)
- iv. IL-1 β with Human ELISA Kit (BMS224HS Duoset, United Kingdom)
- v. Level of IL-10 has been studied according to recommendations of manufacturers with commercial ELISA kits
- vi. For AVP, DEH / VP / AVP ELISA kit was used.

Reference values for cytokines and for blood samples studied in the laboratory of the hospital where the research was conducted.

Figure 1. Research Flow Chart



2.11. Ethical Aspect of the Research

After acceptance of thesis topic and title by Bahçeşehir University Graduate School of Health Sciences, permission was obtained from Kocaeli University Ethical Committees of Clinical Research and from the chief physician of University of Health Sciences Derince Training and Research Hospital (Ethical approval number: KİA2018/195) . Before data collection forms were filled, patients were informed about the study and their written and oral consent were received. While studying control and experiment groups, clinic physicians and nurses were not informed about hypertonic saline application in order to prevent confusing factors. Nurses only informed about follow-ups of patient in the clinic to obtain prior information before the study.

3. DISCUSSION

This research was conducted to evaluate the effects of hypertonic sodium chloride on preventing develop of delirium that is seen as a very common medical complication on geriatric patient after orthopaedics and cardiovascular surgery even though it can be preventable. In line with this purpose, while searching the modulator effect of hypertonic sodium chloride on neuro-inflammatory processes that activated after surgery and its relation with delirium, we confirmed that our hypothesis which was “H₁; Application of hypertonic sodium chloride has an effect on preventing postoperative delirium.

Although type of surgical intervention is different, especially after cardiovascular and orthopaedic surgery both incidence and prevalence of delirium on geriatric patients is higher than other patient groups. Moreover, it affects elderly patients and their relatives emotionally, functionally and financially. These effects also cause prolonged hospital stay and increased hospital cost, mortality and morbidity (Banerjee *et al.* 2010). Therefore, evidence-based studies are needed to prevent the delirium.

The basic principles in health care are preventing disease, predicting medical problems and complication beforehand and taking precautions. In health care which required multi-disciplinary approach, prediction-prevent attitude is expected from healthcare professionals. Within this expectation and responsibility, knowing role and responsibilities of nurses on preventing, care and treatment of postoperative delirium is important for value-based care. These role and responsibility of nurses assist early

diagnosis of delirium and contribute to the healing process. In the sense of preventing the development of delirium, finalized pharmacological interventions are not fully available yet; however, it is priority of healthcare professionals to conduct studies that will form a basis of evidence (Champell, 2009). As a healthcare professional, nurses' involvement in evidence-based pharmacological and non-pharmacological interventions enables them to contribute the patients' healing process in value-based care and it will increase the visibility of the profession. In terms of preventing delirium, finding chance to combine critical thinking and decision-making skills with health care process by nurses will positively affect the decreasing the diagnosis-treatment costs and the rate of morbidity and mortality associated with delirium development. Through this conducted research, it is considered that awareness of duty and responsibilities of nurses is increased with giving opportunity of using critical thinking and decision-making skills effectively to nurses that play key role in clinical studies.

It is known that cytokines that is one of the indicators of inflammatory can cause brain damage and delirium after surgical intervention by passing blood-brain barrier. In the researches it states that application of hypertonic sodium chloride solution to patient before surgery reduces the cytokines release and can prevent delirium (Xin *et al.* 2017; Mazandarani *et al.* 2012). Hypertonic sodium chloride can provide a decrease in the level postoperative cytokine by modulating local and systemic inflammatory response (Kim *et al.* 2013; Zeng *et al.* 2017).

In the sense of effectiveness of this research which is conducted for evaluating the effects application of hypertonic sodium chloride after surgery on cytokines and delirium development, experimental and control groups with the same characteristics were determined by selecting randomly. To patients that were determined as experimental group 6% of hypertonic sodium chloride and to patients who were control group 0,9% of sodium chloride were applied one hour before surgical intervention. For both of groups, data about risk factors that are important on pre- intra- postoperative delirium development were recorded with data collection forms that prepared with literature review. In order to determine before and after surgery serum cytokine levels of patients that were included to study, their blood was taken and analysed. During their hospital stay, delirium risk assessment was made and patients who had more than 2 risk scores were reported to physicians and nurses in clinic to diagnosis delirium. In every stage of the research, the recorded data were analysed.

4. CONCLUSION AND SUGGESTIONS

In this study, interventional application protocol to determine the effects of hypertonic sodium chloride in preventing postoperative delirium was analysed on patients over 65 who underwent orthopaedic and cardiovascular surgery.

- i. According to the results with this applied protocol, POD development incidence (6,6%) on HSC group decreased significantly compared to the SC group (40%).
- ii. It was out of expectation for study that from 50 patients who enrolled in the study with hypertonic sodium chloride application, POD development was seen only 3 of them. It is found that presence of the risk factors for pre-in-post delirium was higher than other patients since the average of these three people who developed delirium was 80 and above.
- iii. In general it was determined that the patients who developed delirium in both groups had chronic diseases, and among these diseases there were diabetes and hypertension most, there were continuous multiple drug use, too.
- iv. It was found that as a result of the application of hypertonic sodium chloride of inflammatory cytokines involved in the development of POD, development of delirium was prevented by decreasing the level of cytokines in blood of the experimental group after surgery. However, it was also found that level of cytokines was higher on control group that applied sodium chloride; therefore, incidence of delirium development was also high.
- v. With hypertonic sodium chloride application, severity of delirium was also reduced beside incidence of POD, and severe delirium was never developed in the group that was applied hypertonic sodium chloride. It was determined that hypertonic sodium chloride has a modulator effect on the neuro-inflammatory processes that become active after surgery, and it prevent the delirium development.
- vi. It was identified that recognition and preventing of the risk factors that effective in delirium development on pre-in-post operative periods is important to prevent and reduce of delirium that can be seen after surgery.
- vii. In this national level clinical study that requires a multidisciplinary team approach, the role of the nurse in the study and the importance of the success that they contributed to the study were determined.

Based on these results;

- i. Measurement of inflammatory cytokines guides preventive interventions on foreseeing, recognition and monitoring the process on delirium development. Therefore, it is suggested that measurement of serum cytokines after surgery as a supportive to delirium diagnosis tools may be beneficial at the level of protection.
- ii. Conducting interdisciplinary study that includes wider patient groups and nurses by supporting application protocol of this study and getting evidence-based results are suggested.
- iii. On the behalf of delirium diagnosis, it is also suggested that scales should be developed, nurses should be trained and these screening methods should be added to their daily routines.
- iv. It is believed that this study guides the researchers who will use randomized controlled clinical trial from epidemiological research as a method of research. For this purpose, testing this intervention protocol on studies that will be conducted on similar or different patient groups is recommended.

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ABSTRACT

Background: The basic principle in health care; to prevent diseases, to predict medical problems and complications and to take precautions. The forecasting-prevention approach in healthcare, which requires a multidisciplinary approach, is expected from health professionals. Within the scope of this expectation and responsibility, delirium cannot be prevented and its frequency is increasing. This ongoing situation-problem "How can we prevent delirium in surgical patients?" The answer to the question was the most important factor in our search. While seeking this answer, it is important in terms of seeing the roles and responsibilities of nurses in preventing delirium and this study also confirms the concept of "key role" attributed to nursing. **Aim:** This research was performed to determine the effect of hypertonic sodium chloride (NaCl) on the prevention of delirium in surgical patients. **Material and Method:** This randomized controlled double-blind study was conducted between September 2018 and April 2019 in a training and research hospital, orthopedics and cardiovascular surgery clinics. It was determined that hypertonic sodium chloride was effective in reducing the incidence of delirium and the severity of delirium in elderly patients undergoing orthopedic and cardiovascular surgery to prevent elevation of serum plasma cytokines after surgery. All patients who were hospitalized for surgery at these dates formed the universe of the research. All patients who were hospitalized for surgery at these dates formed the universe of the study. The study group consisted of 50 patients with ≥ 65 years of age and 6% (NaCl) of hypertonic sodium chloride. The control group consisted of 0.9% NaCl sodium chloride (isotonic). The total of 100 patients were research sample. Delirium Screening Scale (Nu-DESC), Pre-in-Post Surgery Risk Factor Evaluation Form, Minimental Condition Assessment Test were used as data collection tools. SPSS 25.0 statistical package program was used to evaluate the data. In this article, the results of the analysis were not included because it was aimed to provide information about the protocol of the research. **Results:** It was determined that all the methods and protocols used in this study had important results in making decisions about the diagnosis, prevention and treatment of delirium. **Conclusion;** It is believed that this study will guide researchers who will use randomized controlled clinical trials from epidemiological studies as a research method. For this purpose, it is recommended to test

this intervention protocol in studies to be performed in similar or different patient groups.

Key Words: Delirium After Surgery, Aging, Neuroinflation, Cytokines, Hypertonic Saline:

CHAPTER XVII
SURGICAL PROCEDURES IN COVID-19 PATIENTS

*Niran Çoban * & Sonay Göktas ** & Elif Gezginç****

Introduction

As of December 2019, it has been reported that a disease of unknown cause has emerged in Wuhan, Hubei Province, China. It is announced by the National Health Commission that the number of cases had risen to 80,151 as of March 2. The outbreak is known to have spread significantly in 19 countries and around the world by January 31, 2020 (He et al., 2020; Rothe et al., 2020).

The virus was first called as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). But it was later named as coronavirus disease 2019 (COVID-19) by World Health Organization (WHO). WHO has declared the "International Public Health Emergency" for the Coronavirus outbreak. It is stated that common symptoms of COVID-19 infection are high fever, fatigue and dry cough, while more severe symptoms include dyspnoea, diarrhoea and pneumonia (Lu et al., 2020; Wu & McGoogan, 2020).

The number of infected patients is increasing day by day. As of May 3, WHO reported that the number of cases worldwide has risen from 3 356 to 205. Healthcare professionals fight with COVID-19 in clinical areas. Many medical personnel who came into contact with infected patients during this fight are known to have tested positive for COVID-19. It is stated that healthcare professionals are psychologically exhausted due to the heavy workload, lack of specific drugs, lack of clear information on the management of the process, difficulty of the use of personal protective equipment and, from time to time, lack of it (Maunder et al., 2003; Bai et al. 2004; Chua et al., 2004; Lee et al., 2007).

Although there are experienced healthcare professionals for the surgical procedures of patients with hepatitis, hydatid cysts and HIV infection, there is no experience or knowledge about the surgical process of COVID-19 infected patients. In the treatment of patients who require an emergency surgical procedure or who are diagnosed with cancer, healthcare workers and other patients need to be protected (Kamer & Çolak, 2020). The

* (RN, MSc); University of Health Sciences, Hamidiye Faculty of Nursing, Department of Surgical Nursing, Istanbul, Turkey, e-mail: nirancoban@gmail.com

** (RN, PhD, Assoc Prof.); University of Health Sciences, Hamidiye Faculty of Nursing, Department of Surgical Nursing, Istanbul-Turkey, e-mail: sonay.goktas@sbu.edu.tr

*** (RN, PhD, Asst Prof.) University of Health Sciences, Hamidiye Faculty of Nursing, Department of Surgical Nursing, Istanbul-Turkey, e-mail: elif.gezginç@sbu.edu.tr

management of the preoperative, intraoperative and the postoperative phases of the patients diagnosed with COVID-19 should be defined in detail.

Although non-urgent surgical procedures have been delayed due to COVID-19, a comprehensive study of the management of the process in emergency surgical procedures was not found. It is thought that hospitals should prepare surgical guidelines for healthcare professionals during the COVID-19 outbreak. In our study, it was aimed to provide guidance to the healthcare professionals involved in the surgical process of COVID-19 infected patients.

I. Preoperative Phase

Today, Enhanced Recovery after Surgery (ERAS) protocols are implemented in order to maintain organ function and to ensure early recovery by reducing the stress response following surgery. The components of preoperative protocols include informing the patient, anaesthesia protocol, use of nasogastric catheter, preoperative bowel cleansing, selection of surgical incisions, urinary catheter, preoperative fasting, evaluation of nutritional status and, if necessary, nutritional support, preoperative optimization, medication before anaesthesia, thromboembolic prophylaxis, antimicrobial prophylaxis and preparation of the surgical site (Ljungqvist, 2014; Neville et al., 2014). However, utmost care is required during the implementation of the protocols specified in the preoperative period of the COVID-19 infected patient.

By following the standard procedures, patients can come to surgical services from the outpatient clinic or emergency department. In such cases, the initial assessment of patients is required to be conducted in a protected area previously determined according to the current hospital protocols (Kamer & Çolak, 2020). COVID-19 is known to be transmitted through droplets and contact. Therefore, in aerosol generating procedures such as manual ventilation, intubation, extubation, non-invasive ventilation and cardiopulmonary resuscitation, precautions should be taken to prevent transmission. In order to minimize aerosol production, changes should be made in airway management and, if possible, regional anaesthesia should be applied.

Healthcare professionals are in direct contact with patients. For this reason, they must ensure their own safety during the care process. Personal protective equipment (PPE) such as gowns, gloves, face shield and mask must be used by all healthcare professionals who will contact the patient. Donning and especially doffing of PPE are very important. Healthcare professionals should be careful in wearing and removing the equipment (Table I, II).

Table I: Steps of Donning Personal Protective Equipment

Gown	<ul style="list-style-type: none">• Neck and arms should be completely covered while wearing gowns.• The gown needs to be fastened in back of the waist and neck.
Mask	<ul style="list-style-type: none">• In the use of a mask, the ties or elastic bands of the mask should be fixed at the middle of the head.• The flexible bridge should be fixed on the nose and the mask should be worn to cover the lower part of the chin.
Goggles and/or Face shield	<ul style="list-style-type: none">• Goggles and/or face shield should be placed on the face precisely.
Gloves	<ul style="list-style-type: none">• Gloves should be worn to cover the wrist part of the isolation gown.

Source: Department of health & human services USA. Center for disease control and prevention. “CDC PPE Donning and Doffing Sequence”, Retrieved April 19, 2020, from <https://www.cdc.gov/hai/pdfs/ppe/ppe-sequence.pdf>.

Table II: Steps of Doffing Personal Protective Equipment

Gown and Gloves	<ul style="list-style-type: none">• The sleeves of the gown, and the gloves are areas that are directly contaminated. In case of contamination while removing gowns or gloves, it is recommended to wash hands immediately or use an alcohol-based disinfectant.• First of all, it is necessary to pull the gown from the front by using dirty gloves and to remove it from the body.• While removing the gown, it must be folded from the inside out.• In the meantime, it is recommended to remove the gloves and leave them inside the gown. Only the inside of the gown should be touched with bare hands.
	<ul style="list-style-type: none">• Goggles and face shield are contaminated and caution is required.• In case of contamination while removing goggles and face shield, it is recommended to

Goggles and/or Face shield	<p>wash hands immediately or use an alcohol-based disinfectant.</p> <ul style="list-style-type: none"> • Without touching the front of the goggles or face shield, the headband must be removed by lifting. • If it is a reusable material, it is recommended to leave it to the area of cleaning, and if it is not to be used again, it should be disposed of in the waste container.
Mask	<ul style="list-style-type: none"> • Mask is contaminated and caution is required. • In case of contamination while removing the mask, it is recommended to wash hands immediately or use an alcohol-based disinfectant. • The mask should be removed by pulling the tie or elastic bands on the back of the head. • The doffed mask must be thrown into the waste container. It is recommended to wash hands with water and soap or clean them with alcohol-based hand sanitizer immediately after removing all personal protective equipment.

Source: Department of health & human services USA. Center for disease control and prevention. “CDC PPE Donning and Doffing Sequence”, Retrieved April 19, 2020, from <https://www.cdc.gov/hai/pdfs/ppe/ppe-sequence.pdf>.

Preoperative management of COVID-19 infected surgical patient;

- Confirmed or suspected COVID-19 infected patients requiring surgical operation should be operated without delay.
- All interventions should be recorded regularly and the patient's consent for surgical intervention must be obtained.
- During the examination of the patient, healthcare professionals must wear PPE in accordance with the appropriate procedure.
- All disposable medical products, body fluids and faeces must be disposed of according to the standards of medical waste in the preoperative phase.
- Safe Surgery Checklist should be applied.
- The transfer of the patient should be done by clinical nurses wearing N95 masks, goggles / face shields, gowns and footwear that fully covers the foot.
- During the transfer to the operating room, the patient needs to wear a surgical mask.

- The transfer of the patient, from the starting point to the point of arrival, should be done by one personnel.
- During the transfer to the operating room, a path with the least contact should be determined and the patient should be transported to the operating room with the designated route. After the transfer, the paths and elevators used should be disinfected appropriately.
- If the patient is transferred from intensive care to the operating room, a transfer ventilator should be used.
- In case of replacement of ventilators, the endotracheal tube must be clamped with forceps and the gas flow must be stopped in order to prevent the spread of aerosol.
- Contamination should be confined to a room. For this reason, it is recommended to use the same rooms before and after surgery (Peng et al., 2003; Luo & Zhong, 2020; Yu et al., 2020).

II. Intraoperative Phase

When the ERAS protocols are examined in intraoperative phase, it is known that they are classified as anaesthesia protocol, selection of surgical incisions, prevention of intraoperative hypothermia, multimodal management of postoperative nausea and vomiting, perioperative fluid management and use of drains. Apart from these components, since the patient is infected with COVID-19, it is extremely important to ensure the personal protection of healthcare professionals during surgery (Ljungqvist, 2014; Neville et al., 2014).

Medical personnel should be careful in COVID-19 infected patients who need to undergo a surgical procedure. It is claimed that the safety of healthcare workers and patient safety during the operation should be ensured meticulously. It is necessary to prevent the risk and contamination by having minimum number of personnel enter the operating room and by limiting the entry and exit (Brindle & Gawande, 2020; Forrester et al., 2020).

The most important step for healthcare professionals during surgery is the provision of personal protection. The entire team in the operating room is required to ensure hand hygiene and wear PPE before the patient enters the operating room. After contact with the infected patient, different surfaces or objects should not be touched and gloves should be replaced. Since aerosolized particles are likely to be produced in tracheal intubation, non-invasive ventilation, tracheostomy, cardiopulmonary resuscitation, intubation and manual ventilation before bronchoscopy, they are associated with a high risk of COVID-19 contamination. For this reason, it is strongly recommended to use FFP3 mask during these procedures. Considering the susceptibility of the conjunctiva to viral contamination, it

is important to wear a face shield or goggles to protect the eyes from the potential exposure of viral particles (Tompkins & Kerchberger, 2010; Coccolini et al., 2020; Peng et al., 2020; Wang, 2020).

Therefore, every employee in the operating room need to use gown, gloves, face shield and mask. Medical personnel are recommended to use a new N95 mask and face shield, if possible, for each operation. In cases of limited protective equipment, N95 masks can be used in more than one case. A face shield or surgical mask can be used on the mask to extend the lifetime of the N95 mask. The use of chlorine bleach or alcohol solution to sterilize N95 masks is not recommended as it damages the integrity of the mask. It is reported that it is possible to use these masks by heating them up to 70 ° C (160 ° F) for 30 minutes (Cui, 2020).

Face shields, on the other hand, can be disinfected and reused. However, the proper donning and doffing of the equipment in question is very important. In order to provide personal protection, hands should be kept away from the face, the touched surfaces should be restricted, torn or heavily soiled gloves should be replaced and hand hygiene should be ensured (Suen et al., 2018; Andonian, 2019).

It is established that all materials and equipment that will not be used during the operation of COVID-19 infected patients must be removed from the operating room. The items should be disposable and should be discarded after the operation. Only necessary and disposable materials should be present in the operating room. If possible, disposable laundry should be preferred during the surgery. The laundry used (sheets, pillowcases, sleepers, etc.) should not come into contact with different areas during collection, they should be placed directly in special containers. Containers must be sealed and sent immediately for cleaning and sterilization (Brindle & Gawande, 2020; Coccolini et al., 2020).

The issue of how to dispose the intraoperative and postoperative wastes (such as aspirator content, organs, faeces, urine, used surgical materials) of COVID-19 infected patients is still unknown. How to send the specimens collected during surgery to pathology and how the preparations will be stored are still uncertain. However, standard practices are recommended for these wastes (Kamer & Çolak, 2020).

Intraoperative management of COVID-19 infected surgical patient;

- All suspected or infected patients should be treated with the utmost care.
- Electro-medical devices (ultrasound) and surfaces, protective sheaths should be used and sterilized at the end of the operation.
- All personnel in contact with the patient should wear personal protective equipment.

- Safe Surgery Checklist should be applied.
- The mobility of infected patients within the hospital should be restricted as much as possible.
 - Disposable material should be preferred.
 - Materials for each intervention must be minimized.
 - Nurses should contact the surgeon and provide all necessary materials and equipment before surgery begins.
 - In order to provide the materials needed during the operation, a person outside the operating room should be assigned and contacted by phone.
 - The doors of the operating room must be closed after the patient enters.
 - Nurses, surgeons, anaesthesiologists, technicians should enter the operating room in time to minimize exposure to infected patients.
 - Personnel who are in direct contact with the patient are required to wear double gloves.
 - If intubation will not be performed, the patient must wear a mask.
 - Personnel participating in the intervention should not leave the operating room during the procedure.
 - High air exchange cycles are recommended in the operating room (>25 exchanges / hour).
 - Clinical documents should be kept outside the operating room.
 - At the end of each intervention, all disposable materials must be discarded, and all surfaces and electro-medical devices must be properly cleaned and disinfected.
 - Personal protective equipment removal areas should be established to prevent the virus from being transmitted to the healthcare personnel.
 - Operating room and personal protective equipment donning and doffing areas should be sterilized as soon as possible after each procedure.
 - The materials used during the surgery should be discarded in double bags.
 - After each procedure, all relevant personnel should shower as much as possible.
 - After the operation, the clinic or intensive care where the patient will be isolated should be determined.

- The postoperative recovery phase is recommended to be performed in the operating rooms before being transferred to the clinical / intensive care unit.

- Patient transfer routes should be planned precisely and the shortest route should be preferred (Ademuyiwa et al., 2020; Coccolini et al., 2020; Wang et al., 2020).

III. Postoperative Phase

If a new case is to be taken after the finished surgery, it is reported that a minimum of one hour break between cases is required to ensure environmental cleaning and hygiene. All contaminated surfaces should be cleaned. All materials that are not used during surgery but are present in the room should be considered as contaminated (Ti et al., 2020; Wax et al., 2020).

It is recommended that patients who do not require intensive care should be followed up by a special staff member in the operating room. In clinical and intensive care, however, isolation measures should be applied. While the patient is being taken out of the operating room, the way of departure and arrival should be kept open by the security or healthcare personnel and the patient transfer should be done quickly (Brindle & Gawande, 2020).

It is reported that patients infected with COVID-19 should be placed in isolated rooms with separate medical devices and that operating rooms with negative pressure (below 5 Pa) should be present. It is stated that the surgical team should be isolated for 14 days (Luo & Zhong, 2020; Yu et al., 2020).

If contamination occurs for any reason during patient transfer (ex; vomiting), sanitation should be performed. It is recommended to use chlorine-derivative solutions for more than one minute while performing surface and electro-medical sanitization (Coccolini, 2020).

When the ERAS protocols are examined in postoperative phase, it is established that they are composed of the following components: the use of nasogastric tube, urinary catheter, blood sugar management, stimulation of gastrointestinal motility, postoperative analgesia, postoperative nutrition, early mobilization, discharge and follow-up, and the control of the results. Accordingly, approaches to COVID-19 are also required when planning the postoperative care of the patient (Ljungqvist, 2014; Neville et al., 2014).

In post-operative care of COVID-19 infected patient, medical personnel are advised to use PPE. There is no consensus on the fact that patients should be treated for COVID-19 in addition to the disease-specific treatment in post-operative care. However, since respiratory problems are

higher than normal in COVID-19 infected patients, it is reported that a multidisciplinary approach involving an anaesthesiologist and a pulmonologist is required. COVID-19 infected patients may also be treated for the corona virus after surgery. There is no consensus on the treatment due to the daily updates of treatments during this process. Since the treatments have side effects and their effectiveness is not proven, consent of the patient must be obtained before starting treatment (Kamer & Çolak, 2020).

Postoperative management of COVID-19 infected surgical patient;

- It is recommended that patients who do not require intensive care should be followed up in the operating room.
- If not possible, they should be monitored and treated in isolated intensive care units or in isolated rooms in accordance with hospital protocols.
- It should not be forgotten that contamination should be confined to a room, the patient should be transferred to the room he used during the preoperative period.
- The nurse who will transfer the patient must wear PPE.
- The complete file of the patient should be obtained.
- If contamination occurs during patient transfer, sanitation should be performed.
- During the transfer of patients from the operating room to the clinic or intensive care unit, a path with the least contact should be determined and the paths and elevators used should be disinfected appropriately after the transfer.
- Follow-up and treatment of the patient for COVID-19 should be carried out during the clinical or intensive care process, in addition to routine postoperative nursing care (Peng et al., 2003; Luo & Zhong, 2020; Yu et al., 2020).

In summary, the virus named COVID-19 has become a pandemic today. Management of preoperative, intraoperative and postoperative phases is very important in COVID-19 infected patients who need to undergo surgical intervention. In addition to the treatment and care of patients during these phases, extra precautions should be taken because of the high risk of transmission.

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CHAPTER XVIII
SLEEP AND HEADACHE

*Müjgan Arslan**

Introduction

The relationship between sleep and headache has been known for over a century. The headache disorders are strongly modulated by sleep, and the sleep disorders are frequently characterized by headache. In addition to their clinical overlap, headache and sleep disorders both involve disruption of highly conserved brainstem and diencephalic networks that have homeostatic functions.

1. Sleep

Sleep is a behavioral state that alternates with waking, relative to which it is characterized by a heightened threshold to sensory input, attenuation of motor output, characteristic changes in central and peripheral physiology, and diminished conscious awareness. Two major types of sleep, rapid eye movement (REM) and non-rapid eye movement (NREM), are characteristic of all mammals and alternate with regular periods, indicating that they are timed by a reliable oscillator (Sheldon, 2005).

Functions of sleep

The basic function of sleep largely remains a mystery. Most of what we understand about the function of sleep has evolved from studies that have examined the impact of experimentally induced sleep loss or pathologic sleep conditions on a host of physiologic and neurobehavioral systems in humans. What we do know is that adequate sleep is a biologic imperative that appears necessary for sustaining life, as well as for optimal functioning (Richardson, 2007).

Sleep architecture

The architecture of sleep is based on the recognition of three distinct states: wake, NREM sleep, and REM sleep. These stages are defined by distinct polysomnographic features of electroencephalographic (EEG) patterns, eye movements, and muscle tone. NREM sleep may be viewed as a period of relatively low brain activity during which the regulatory capacity of the brain continues to be active and in which body movements are preserved. Respiratory and cardiovascular parameters are regular in NREM sleep. After about the age of 6 months, NREM may be further

* (Asst Prof.); Süleyman Demirel University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, Isparta, Tuekey. E-mail: mujganarslan@yahoo.com

divided as follows: Stage 1 sleep occurs at the sleep-wake transition. Initial stage 1 typically lasts from 30 seconds to 5 minutes. Recall of fragmented visual imagery (hypnogogic hallucinations) as well as brief involuntary muscle contractions (hypnic jerks) may occur, both of which are considered to be normal phenomena in most cases. Stage 2 sleep is usually considered the initiation of “true” sleep. It is characterized by bursts of rhythmic rapid EEG activity called sleep spindles and high-amplitude slow-wave spikes called K complexes. The initial stage 2 period lasts from 5 to 25 minutes. Stage 3 is also known as “deep” sleep, slow-wave sleep (SWS), or delta sleep. This stage of sleep is dominated by delta waves, characterized by high-voltage, low-frequency activity. Respiration is slowest and most regular during SWS, and parasympathetic activity is high. The highest arousal threshold also occurs during SWS; the lowest arousal threshold is in stage 1. The initial SWS period is about 30 to 45 minutes and is followed by a brief arousal. REM sleep is characterized by desynchronized cortical activity and the highest brain metabolic rate, dreaming, absence of skeletal muscle tone, lack of normal thermoregulation, and episodic bursts of phasic eye movements that are the hallmark of REM sleep. In young infants, REM sleep is termed “active sleep” and is characterized by frequent muscle twitches and grimaces. Until age 3 months, infants also enter sleep through REM. REM-associated alterations in autonomic parameters and changes in control of breathing not only result in irregular respiration and heart rate, but also typically increase the severity of sleep-disordered breathing during this stage of sleep. The first REM sleep period occurs about 70 to 100 minutes after sleep onset and lasts for about 5 minutes ((Sheldon, 2005; Richardson, 2007; Delrosso, 2017).

Sleep cycles

NREM and REM sleep alternate throughout the night in cycles of about 90 to 110 minutes (50 minutes in infancy and gradually lengthening to adult levels at about school age). Brief arousals normally followed by a rapid return to sleep often occur at the end of each sleep cycle (4-6 times per night); this pattern of normal arousals plays an important role in the etiology of problematic nightwakings in infancy and childhood. The relative proportion of REM and NREM sleep per cycle changes across the night, such that SWS predominates in the first third of the night and REM sleep in the last third. That is, REM sleep percentage increases and SWS percentage declines over the course of the night. The potential clinical significance of this timing of sleep stages is important. The amount and timing of each sleep stage are also affected by a multitude of extrinsic factors. For example, the amount and depth of SWS is determined by prior sleep loss, as well as the time of sleep onset and the length of prior wakefulness. SWS is relatively “protected” by its appearance early on in

the nocturnal sleep period and is also preserved at the expense of other sleep stages when total sleep amounts are restricted. There is also typically a marked increase in SWS during nights of recovery sleep following sleep restriction. REM sleep also demonstrates this rebound phenomenon in recovery sleep, further highlighting the physiologic importance of these two sleep stages. Increased arousals, such as those occurring as in relation to obstructive sleep apnea or periodic limb movements, result in sleep fragmentation and reduced amounts of SWS and REM sleep. The relative percentage of sleep stages on a given night is also a reflection of other factors, such as circadian rhythm disruption associated with jet lag, stress, and medications, which have both direct effects and withdrawal effects, especially on SWS and REM sleep (Kirsch, 2014; Culebras, 2007).

Neuroanatomy and physiology of sleep

Although it shares many features with the awake state, sleep is not merely the absence of wakefulness and vice versa. There are many highly complex neural networks and related processes that actively control the three distinct states of wakefulness, NREM sleep, and REM sleep. Furthermore, sleep is an active process during which many physiologic, metabolic, and neurobehavioral functions continue to occur. Wakefulness is promoted by ascending projections that originate in neurons located in the brain stem (reticular formation) as well as hypothalamic pathways. These largely excitatory neurons relay sensory input to the thalamus, hypothalamus, and basal forebrain and activate vast areas of the cortex to increase wakefulness; their activity is suppressed during sleep. Cholinergic neurons of the dorsal midbrain and pons (pedunculopontine [PPT] nucleus and the laterodorsal tegmental [LDT] nucleus) also demonstrate increased activity during wakefulness and REM sleep and decreased activity during NREM sleep; they send excitatory projections to the thalamus, where they then regulate cortical activity and allow the flow of information through the thalamus to and from the cortex (thalamocortical activation). Cholinergic neurons located in the basal forebrain also send projections throughout the cortex, hippocampus, and amygdala; their activity is high during wakefulness and REM sleep and low during NREM sleep.

A number of neurotransmitters and neuropeptides actively modulate and influence wakefulness promotion. These include acetylcholine and a number of aminergic neurotransmitters; including histamine in the tuberomammillary nucleus (posterior hypothalamus); dopamine in the ventral tegmental area, substantia nigra, posterior hypothalamus, and brain stem; serotonin in the median and dorsal raphe; and norepinephrine (NE) in the locus coeruleus (midbrain). While coordinated activity across these arousal systems is necessary for complete and sustained wake states, each aminergic pathway may mediate different functions of wakefulness. For example, histamine appears to be the major arousal-promoting

neurotransmitter at wake onset; NE increases cortical activation, particularly under conditions of stress and in the presence of novel stimuli; and dopamine may be more likely to promote wakefulness under conditions of motivation or physical activity. Finally, neurons in the lateral or posterior hypothalamus that produce hypocretin (also called orexin) are also active during the wake state; a deficiency of hypocretin has been shown to be the primary etiology of both the excessive daytime sleepiness and cataplexy in narcolepsy. Hypocretin/orexin function also appears to be linked to control of feeding behaviors, locomotion, and autonomic functions. The ventrolateral preoptic area (VLPO) in the anterior hypothalamus is a major sleep-promoting area of the brain; most likely distinct subregions of the VLPO control NREM and REM sleep. During sleep, especially SWS, VLPO neurons are active and exhibit high firing rates. VLPO neurons send projections to all major wake-promoting regions, including the tuberomammillary nucleus, locus ceruleus, and LDT and PPT. These inhibitory neurons are believed to induce sleep by coordinating the inhibition of all the wake-promoting cholinergic and aminergic regions. Most VLPO neurons release the inhibitory neurotransmitter *g*-aminobutyric acid (GABA) at their sites of projection, while some utilize the inhibitory neurotransmitter galanin. The control of REM sleep involves the interaction of brain stem cholinergic and aminergic neurons in a complex feedback loop; neurons releasing acetylcholine are disinhibited by the suppression of aminergic neurons during REM. REM-associated muscle atonia is linked to inhibition or loss of excitation of motor neurons in the brain stem and spinal cord via the medulla; these pathways originate in the LDT and PPT and involve neurotransmitters, including acetylcholine, glutamate, and glycine (Sheldon, 2005; Richardson, 2007; Culebras, 2007).

Sleep regulation

On a basic level, sleep and wakefulness are usually described as being regulated by two highly coupled processes operating simultaneously (the “two process” sleep system):

Homeostatic process (“process S”): This process primarily regulates the length and depth of sleep. The homeostatic drive may be related to the accumulation of adenosine and other sleep-promoting chemicals (“somnogens”), such as cytokines, during prolonged periods of wakefulness. This sleep pressure appears to build up more quickly in infants and young children, thus limiting the duration of sustained wakefulness during the day and necessitating short periods of daytime sleep.

Endogenous circadian rhythm (“process C”): This process influences the internal organization of sleep and timing and the duration of daily

sleep-wake cycles. It also governs predictable patterns of alertness throughout the 24-hour day. The “master circadian clock” that controls sleep-wake patterns is located in the suprachiasmatic nucleus (SCN) in the ventral hypothalamus; however, we now know that “circadian clocks” are present in virtually every cell in the body and help to govern the timing of various other physiologic systems in the body. Circadian rhythms are generated by the expression of specific “clock genes.” The circadian timing system develops rapidly in the first 6 months of life as a result of the combined influence of neurodevelopmental maturation and social and environmental cues.

The relative level of sleepiness or alertness existing at any given time during a 24-hour period is partially determined by the duration and quality of previous sleep as well as time awake since the last sleep period. Interacting with this “sleep homeostat” is the 24-hour cyclic pattern or rhythm characterized by clock-dependent periods of maximum sleepiness (“circadian troughs”) and maximum alertness “circadian peaks”. In addition, relative sleepiness and wakefulness are influenced by many other variables, including individual factors, the nature of the task being performed, and environmental and physiologic factors that may “unmask” but do not cause sleepiness. Finally, a phenomenon known as “sleep inertia,” defined as a period of incomplete arousal characterized by confusion, disorientation, cognitive slowing, and irritability occurring immediately upon waking in the morning or after a nap, especially from SWS, may further compromise alertness levels. Because the human circadian clock is actually slightly longer than 24 hours, intrinsic circadian rhythms are synchronized or “entrained” to the 24-hour-day cycle by environmental cues called “zeitgebers.” In the absence of zeitgebers, circadian rhythms are desynchronized or “uncoupled” from one another. The most powerful of these zeitgebers is the light-dark cycle; light signals are transmitted to the suprachiasmatic nucleus via the circadian photoreceptor system within the retina, which switch the body's production of the hormone melatonin off or on by the pineal gland. The biomarker core body temperature, which falls just prior to sleep onset, and hormone levels are also linked to circadian timing. Circadian rhythms are also synchronized by other external time cues, such as timing of meals and alarm clocks. Thus, daytime schedules that are not consistently regulated may further exacerbate circadian disturbances such as delayed sleep-phase disorder. Finally, research also supports an important role for genetics in determining intrinsic circadian clock periodicities and, thus, influencing individual circadian preference for sleep-wake cycle timing or chronotype (Stickgold, 2009; Sheldon, 2005; Culebras, 2007; Delrosso, 2017).

Autonomic and peripheral physiological changes in sleep

In NREM sleep relative to waking, heart rate, blood pressure, and respiration rate decline, sympathetic outflow of the autonomic nervous system decreases, and parasympathetic activity increases. In REM, heart rate, blood pressure, and respiration rate again rise but, as noted previously, muscle tone remains actively suppressed. In REM, homeostatic responses are attenuated. In the transition from NREM to REM sleep, sympathetic activity increases and parasympathetic activity decreases (Overeem, 2018).

Sleep as an intrinsic biologic process and as a learned behavior

While our scientific understanding of the structure, organization, regulation, and development of sleep has advanced significantly in the past decades and has elucidated much regarding the genetics and neurobiology of sleep, it is clear that sleep is also impacted by multiple psychosocial factors, ranging from exposure to environmental toxins, to cultural variables, and to community standards. While much of the chronobiologic “hard-wiring” of sleep is immutable, the plasticity of the developing brain makes it likely that sleep and its relationship to other neural systems in the brain are also highly susceptible to environmental influences. Furthermore, the construct that much of sleep behavior is learned behavior not only underscores the importance of developing health-promoting sleep behaviors early in childhood but also emphasizes the role that parents and healthcare providers may play in shaping and modifying sleep behaviors in children (Miglis, 2017).

2. Headache

Nociception involves a complex network of interacting brain structures with distinct physiologic and pharmacologic properties.

Neuroanatomy and physiology of headache

The principal innervation of the cranium and its contents comes from the trigeminal nerve. After synapsing on the relevant sensory, all of these afferents converge on the trigeminal nucleus caudalis (TNC), the principal brainstem nucleus subserving pain and temperature sensation in the head and neck. The brain parenchyma proper does not sense pain.

From the TNC, pain and temperature information passes up the trigeminal lemniscus to the ventral posteromedial thalamus (VPM) and then to the somatosensory cortex. However, trigeminal nucleus caudalis efferents also supply the insular and limbic cortices, which are thought to process visceral and affective aspects of pain, respectively. These efferents pass through a highly interconnected brainstem and diencephalic circuit, which interacts at multiple levels with autonomic control centers. The TNC also has direct relays to the hypothalamus via the trigeminohypothalamic tract.

It has yet to be determined which of the peripheral and central components of craniofacial pain are primarily involved in the generation of different types of headache disorders; however, it is likely at higher brainstem and diencephalic circuit levels that the pain and sleep systems interact. Collaterals from neurons in the TNC synapse on the nucleus of the solitary tract (NTS) and the parabrachial nucleus (PBN). The NTS is the principal nucleus for viscerosensation, and is thus involved in cardiovascular, respiratory, and gastrointestinal reflex loops. The PBN has similar viscerosensitive functions, but is a higher level regulator, and communicates to a greater extent than the nucleus of the solitary tract visceral information to the hypothalamus, thalamus, and cortex.

Via collaterals to the nucleus of the solitary tract and PBN, TNC neurons are thus in contact with all components of central autonomic control. These include networks for modulation of pain, arousal and sleep, sympathetic and parasympathetic outflow, and neuro-endocrine function. In turn, by virtue of entry into these networks, nociceptive information becomes amenable to feedback from other modalities. It is at these network levels that sleep and headaches are likely connected.

Interestingly, both the posterior hypothalamic area and the immediately adjacent lateral hypothalamic area and dorsomedial hypothalamus contain orexinergic neurons. Orexinergic neurons have been implicated in both narcolepsy and in modulation of nociceptive activity in the trigeminal nucleus caudalis. As will be seen below, narcolepsy may be associated with an increased prevalence of migraine and tension-type headache. And though so far there is no direct evidence, the circadian nature of several headache disorders strongly suggests involvement of the SCN. Thus, though there is likely not a specific “headache generator” in the hypothalamus, it is possible that dysfunctional hypothalamic activity contributes to both altered sleep/wake function as well as altered pain processing via its orexinergic neurons (Culebras, 2007; Overeem, 2018).

3. The clinical relationship between sleep and headache disorders

Sleep and headache share a well-recognized, bidirectional relationship, with complex and incompletely understood interactions. The physiology of sleep shares many features with the pathophysiology of headache disorders, both in terms of the neuroanatomical pathways and the neurotransmitters that are involved.

Migraine is a recurring headache disorder characterized by attacks of unilateral, pulsating head pain, that is, moderate to severe in intensity, with associated photophobia and phonophobia, and/or nausea and vomiting. The changes in sleep patterns can precipitate migraine attacks. In addition, sleep disorders contribute to the evolution of episodic migraine into its chronic form. The use of behavioral sleep modification techniques may

result in an improvement in migraine frequency, and may be an effective adjunctive therapy in reverting chronic migraine back to its episodic form. Sleep complaints are prevalent among migraineurs, with difficulty initiating or maintaining sleep. This is particularly striking in chronic migraine, where patients almost invariably report modifiable poor sleep habits, nonrestorative sleep, and shorter sleep durations. Migraine attacks exhibit clear circadian timing, with attacks more likely to occur between the hours of 4 a.m. and 9 a.m. A disturbance of circadian rhythms in migraine is also supported by “circa-septan” and circannual patterns that vary according to localization in the northern or southern hemisphere. Parasomnias such as sleep terrors, somnambulism, sleep bruxism, restless legs syndrome, and nocturnal enuresis are also seen more commonly in migraineurs. The mechanisms underlying the association of these disorders with migraine are poorly understood. Migraine attacks may be related to specific sleep stages, although the exact mechanisms remain unclear. Nocturnal arousal from sleep with migraine may be more likely during REM sleep. In addition, migraine appears more likely to occur following a night’s sleep with excessive durations of slow wave and REM sleep. Some patients report that daytime napping can trigger migraine attacks, and this has been reported to occur following naps in which slow wave or REM sleep is obtained.

Cluster headache (CH) is a distinct primary headache disorder characterized by attacks of excruciating pain, typically in a unilateral retro-orbital distribution, with associated ipsilateral autonomic features. It occurs more commonly in males. In its typical episodic form, patients experience “clusters” of headaches occurring up to 8 times per day, with periods of remission in between, each lasting between 20 and 180 minutes. CH is a disorder intricately associated with sleep, often with a predictable chronobiological pattern. CH often awakens the patient at the same time each night (circadian rhythmicity), and tends to occur in clusters in the same season each year (circannual periodicity). The circadian nature of cluster headache strongly suggests alterations in hypothalamic and brainstem circuits. Cluster headache shows an association with REM sleep, as well as abnormalities in melatonin and cortisol secretion.

Hypnic headache is the only primary headache disorder, that is, defined specifically in relation to sleep. The underlying pathophysiology remains poorly understood. It has been proposed that hypnic headache may in fact represent an age-related phenotypic change of another sleep-related primary headache disorder such as migraine. Hypnic headache may also coexist with other primary headache conditions such as migraine or tension-type headache. Hypnic headaches typically begin in the 5th decade of life, more often in females, and are generally moderate to severe in intensity and bilateral in distribution. Migrainous features have been

reported. Hypnic headaches tend to occur at a specific time each night, earning the nickname “alarm clock headache.” This points strongly toward an underlying disorder of chronobiology. Hypnic headache may be associated with age-related alterations in sleep physiology. Hypnic headache does not appear to be strictly associated with a specific sleep phase, and attacks can occur in both REM and non-REM sleep, even within the same individual. Structural neuroanatomical changes reported in hypnic headache patients further support a circadian disorder, with decreased gray matter volume in the posterior hypothalamus, a key structure in sleep physiology. Similar structural changes have been reported in narcolepsy patients. Age-related melatonin deficiency has also been proposed as a pathophysiological mechanism in this condition.

Narcolepsy is thought to be due to a selective loss of orexinergic neurons in the lateral hypothalamus. These neurons are active in arousal as well as in the inhibition of REM sleep. Their loss is thought to contribute to the disruption of sleep-wake transitions and dysregulation of REM activity that characterize narcolepsy (Miglis, 2017; Kirsch, 2014; Culebras, 2007).

The shared neurobiological substrate of sleep and headache

The transition between wake and sleep is controlled by the interaction between arousal and sleep-promoting pathways in the brain. Many of the structures, pathways, and neurotransmitters involved in this process are also implicated in headache pathophysiology. The convergence between sleep and headache pathways primarily localizes to diencephalic and brainstem structures. Sensory afferents mediating headache pain converge on the TNC of the brainstem, which then project to the ventral posteromedial nucleus of the thalamus. These TNC projections interact with numerous brainstem and diencephalic nuclei as they ascend through the midbrain, including the periaqueductal grey matter (PAG), locus coeruleus, hypothalamus, limbic system, and autonomic brainstem centers such as the solitary nucleus and the parabrachial nucleus. Of equal importance in headache pathophysiology are the descending pathways that modulate nociceptive pathways in the TNC. Brainstem structures including the rostroventromedial medulla, nucleus raphe magnus, locus coeruleus, and PAG modulate the trigeminovascular pathway and are influenced by descending inputs from the thalamus and hypothalamus (Kirsch, 2014).

The hypothalamus is a key structure linking headache and sleep

Hypothalamic structures are vital for maintaining wakefulness. In particular, the posterior hypothalamus is a critical wake-promoting area. Histaminergic neurons of the tuberomammillary nuclei project widely to cortical and diencephalic structures to promote wakefulness. In the lateral hypothalamus, a specific population of orexin-containing neurons

reciprocally excite many activating monoaminergic systems, and also project to the basal forebrain and cortex. These orexinergic projections are thought to have a specific role in coordinating the activity of other activating systems, maintaining the stability of the waking state, and preventing inappropriate sudden onset of sleep. Orexin-containing neurons are deficient in patients with narcolepsy, a condition characterized by inappropriate sleep onset as well as intrusion of a REM-like-state into waking hours. In migraine, hypothalamic involvement has long been hypothesized, as premonitory symptoms suggest localization to this region. The distinctive rhythmicity of CH and hypnic headache is also highly suggestive of hypothalamic involvement. Neuroendocrine abnormalities provided the first objective evidence supporting hypothalamic dysfunction in CH (Overeem, 2018).

The brainstem contains key structures linking headache and sleep

Key structures in the overlap between headache and sleep localize to the brainstem, including the locus coeruleus, ventral PAG, and dorsal raphe nucleus. These cell bodies send monoaminergic projections to thalamic nuclei, the lateral hypothalamus, basal forebrain, and cortex. They are important in maintaining arousal, and are highly active during wakeful hours (Overeem, 2018).

Abnormal serotonin signaling in the brainstem may link headache and sleep

The main CNS serotonergic nucleus is the dorsal raphe nucleus. This is a key antinociceptive structure that is also involved in promoting wakefulness. A significant role for serotonin in migraine pathophysiology has long been suspected. Serotonin metabolism in migraineurs is abnormal, both during and between attacks. Plasma serotonin is abnormally low in the interictal phase in migraineurs, and increases during attacks, while the inverse is observed in plasma measures of plasma 5-HIAA, the principle metabolite of serotonin.

Serotonergic activity of the dorsal raphe nucleus is characterized by circadian rhythmicity. Waking hours are associated with tonic activity of the serotonergic neurons, which decreases during slow wave sleep, becoming almost totally quiescent during REM sleep. These fluctuations in serotonergic activity may also contribute to the relationship between migraine and sleep (Overeem, 2018).

The role of adenosine in headache and sleep pathophysiology

Adenosine plays a role in both pain and sleep, acting primarily on A1 and A2A receptors in the CNS. The pain-related effects of adenosine vary in a receptor-dependent manner, with A1 stimulation having antinociceptive effects, and A2A stimulation resulting in pain. The sleep-

promoting effects of adenosine are primarily mediated via A1 receptor activation, with some contribution from A2A receptor activation. Adenosine also facilitates the effects of vasoactive intestinal peptide (VIP) and calcitonin-related gene peptide (CGRP), molecules known to be important in migraine pathophysiology. Adenosine exerts its somnogenic effect via inactivation of the CaV2.1 channel. In addition, a specific haplotype of the A2A receptor has been identified, that is, associated with the development of migraine with aura.

Adenosine is most likely an endogenous somnogen, which accumulates with energy consumption during the day. An area that appears particularly sensitive to adenosine concentrations is the basal fore-brain, whose cholinergic nuclei are involved in arousal. In opposite fashion, the sleep-promoting ventrolateral preoptic nucleus of the hypothalamus is excited by adenosine. Moreover, adenosine concentrations vary over the sleep–wake cycle, increasing with prolonged wakefulness, and decreasing with sleep, consistent with the kinetics of an endogenous somnogen (Overeem, 2018).

The role of melatonin in headache and sleep pathophysiology

Melatonin is synthesized from serotonin by pinealocytes in the pineal gland. It is secreted nightly, its timing closely entrained by activity from the SCN, which itself integrates incoming light information from the retinohypothalamic tract. In conditions of natural lighting, melatonin secretion begins when daylight dims, peaks at midnight, and decreases in the later night hours. Thus, melatonin helps maintain the daily sleep–wake cycle.

Melatonin may also have analgesic effects, and disordered melatonin secretion may result in headache. Mechanisms underlying the interaction between melatonin and headache may include potentiation of GABAergic inhibition of pain pathways, modulation of 5-HT signaling, reduced production of proinflammatory cytokines, inhibition of nitric oxide synthase, antioxidant effects, and the induction of cytokines acting at opioid receptors (Overeem, 2018).

The role of orexin in headache and sleep

The orexin system of the hypothalamus is activated in response to stimuli such as emotion and stress via inputs from the insular cortex and amygdala, the biological clock via inputs from the dorsomedial nucleus of the hypothalamus, and sleep–wake transitions via inputs from the VLPO and dorsal raphe nucleus. Activation of the orexinergic system results in behavioral changes including increased wakefulness, feeding, and sympathetic tone. Orexinergic neurons from the posterolateral hypothalamus interact in a complex manner with the TNC, and may

facilitate or inhibit TNC nociception by receptor-specific pathways (Culebras, 2007).

Cortical spreading depression may link headache and sleep

Cortical spreading depression (CSD) is a spreading wave of neuronal and glial depolarization that is thought to correlate with the aura preceding migraine headache in a subset of patients. It results in neurogenic inflammation, as well as activation of meningeal nociceptors and the TNC. CSD also affects the physiology of subsequent sleep, with pronounced changes persisting for hours. As the percentage of non-REM sleep increases, REM sleep decreases, and slow-wave activity is increased ipsilateral to the side of CSD stimulus (Overeem, 2018).

Headache itself can result in sleep disturbances

Sleep disturbance is increasingly prevalent with increasing headache frequency, and can be predicted by other headache-related factors including headache severity and headache-related disability. Pain makes restful sleep very difficult because it is associated with a state of stress and heightened alertness. However, painful stimuli alone do not explain the sleep disturbance reported by patients with pain disorders, as painful stimuli during sleep in healthy subjects result in only brief cortical arousals and postural adjustments without significant sleep disruption or recollection of disrupted sleep the following day. Chronic pain, however, has been associated with increased activity of systems modulating ascending activation as well as nociception, such as the raphe magnus (Culebras, 2007).

Sleep disorders can cause or exacerbate headache

Typically healthy people who do not experience regular headaches will develop sleep deprivation headaches when provoked; these tend to be dull, frontal, and aching in nature.

The mechanism underlying this relationship is poorly understood. The two processes may be associated with a shared neurobiological substrate in the hypothalamus. Alternatively, a causal relationship may exist, possibly secondary to hypoxia (Culebras, 2007).

Conclusions

Much remains to be discovered about both sleep and headache disorders and their interactions. They are intricately related, both in clinical presentation and physiology. Shared anatomical pathways may help to explain this relationship, studies suggest that the hypothalamus may be

involved in both processes. The roles of shared neurotransmitters such as serotonin, adenosine, melatonin, and orexin lend further support to this relationship. These physiological intersections not only provide insights into the underlying headache pathology but also suggest novel therapeutic approaches. Sleep and headache disorders cause or exacerbate each other in a complex, bidirectional manner, perhaps as a reflection of their shared neurobiological substrate. The common comorbidities of depression and anxiety further modify and complicate this relationship, and are associated with lower quality of life indices. The conclusions can be more certain: treatment of headache cannot be optimized without attention to sleep hygiene and sleep disorders.

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CHAPTER XIX
NEUROPLASTICITY IN THE DEVELOPING BRAIN

*Müjgan Arslan**

Plasticity is one of the most prominent features of the central nervous system (CNS). It is a complex process that is heightened during time-sensitive periods of pre and postnatal brain development and continues to a lesser degree throughout adolescence. Neuroplasticity refers to the inherently dynamic biological capacity of CNS to undergo maturation, change structurally and functionally in response to experience and to adapt following injury. This malleability is achieved by modulating subsets of genetic, molecular and cellular mechanisms that influence the dynamics of synaptic connections and neural circuitry formation culminating in gain or loss of behavior or function (Dennis et al, 2013:37).

Neuroplasticity may involve changes in the efficacy of already existing synaptic contacts, formation of new synaptic contacts or elimination of existing ones, large-scale changes in dendritic or axonal arborization, and production or signaling of neuromodulators or neurohormones (Citri and Malenka, 2008: 33).

Stages of brain development

Brain development is a complex and protracted process, beginning in the third gestational week and continuing well into early adulthood. Normal developmental processes are subject to a variety of exogenous and endogenous influences, from the molecular events of gene expression to environmental inputs. At varying time points during gestation, neurons are generated and migrate to predetermined areas. Once they reach their destinations they begin connecting with specific neuron groups, thus forming neural networks that will subsume future functions. Individual brain structures and cortical layers demonstrate different maturation timetables, based on a series of precise and genetically predetermined stages, the outcome of which is partially determined by the outcome of previous stages of development, but is also vulnerable to both intrinsic and extrinsic influences (Anderson et al, 2011:134).

The prenatal period

This period is characterized by dynamic activity and is primarily concerned with gross structural formation. A series of processes (neurulation, proliferation, migration, dendritic development,

* Asst Prof, Süleyman Demirel University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, mujganarslan@yahoo.com

synaptogenesis, differentiation and apoptosis) enable the transformation of the primitive neural tube into a series of complex neural networks comprising the CNS. Interruptions to development during this period are likely to have a significant impact on cerebral development. The early CNS development is precisely regulated so that appropriate numbers of cells are formed at predetermined times and in well-defined regions. Prenatal and perinatal development are characterized by expanding cortical connectivity, linked to increases in the number and size of cortical regions.

The post-natal period

Post-natal development is primarily associated with elaboration of the CNS, with differentiation and maturation progressing into adolescence and early adulthood. It appears to follow a set sequence, with early development characterized by growth of short cortico-cortical connections, rapid synaptogenesis and dendritic development, myelination and development of local circuitry. All progress in a largely hierarchical manner (Anderson et al, 2011:134, Ismail et al, 2017:21).

Types of brain plasticity in developing brain

1. Developmental plasticity

Normal developmental plasticity

It is a complex genetically encoded, time-dependent and sequenced maturational process that is closely regulated by intrinsic homeostatic mechanisms and is influenced by extrinsic environmental experiences. Developmental neuroplasticity is an inclusive term that involves fundamental changes in neurogenesis, neuronal cell migration, synapse formation and structural and functional neural networks specialization leading to behavioral acquisition of motor and non-motor developmental milestones and adaptation to a constantly changing environment through learning and memory (Johntson, 2004: 26).

Four special features of developmental plasticity are especially important. The first feature is found in the cells lining the subventricular zone of the lateral ventricles and cells in the hilus of the dentate gyrus. Both regions contain stem cells that remain active throughout life. The cells in the subventricular zone produce both glial and neural progenitor cells that can migrate into cerebral grey or white matter. Stem cells in the dentate gyrus generate new neurons at a slow but steady pace throughout life, although there is a decline with ageing. The functional role of these cells is not totally understood, but they do integrate with the existing neurons and likely play a role in the formation of new memories. A second special feature is that three types of plasticity can be distinguished in the

developing brain: experience independent, experience-expectant, and experience-dependent. Experience-independent plasticity results from the fact that the genome generates a rough approximation of connectivity that is modified by both internal and external events. Neurons that are active together increase their connections, whereas those that are not coincidentally active weaken their connections. Experience-expectant plasticity occurs mostly during early postnatal development. It means normal, generalized development of neuron connections that occur as a result of common experiences that all humans are exposed to in a normal environment. Experience-dependent plasticity, a process whereby the connections of ensembles of neurons are modified by experience, begins in early postnatal life and continues for a lifetime. A third special feature is the speed at which dendrites, and especially dendritic spines, can modify their structure to form or delete synapses in response to experience, possibly in a matter of minutes. A fourth special feature is the presence of critical periods, especially for experience-expectant plasticity (Kolb, 2013).

Impaired developmental plasticity

Pathological states can disturb normal developmental homeostasis and induce aberrant developmental neuroplasticity contributing to abnormal neurophysiological and behavioral phenotype.

2. Adaptive plasticity

Neural plasticity is neither good nor bad, it follows its rules toward structural and functional changes in connectivity, allowing experience to change our brain. If the outcome of the changes favors adaptive responses of the individual, we talk of adaptive plasticity. Experience-dependant structural synaptic plasticity is abundant in the developing brain and is thought to represent the neurobiological substrate for learning and memory formation.

3. Reactive plasticity

Changes in the strength of synapses and reorganization of neuronal circuits also play important roles in brain plasticity. Synaptic plasticity refers to changes in the strength of neurotransmission induced by activity experienced by the synapse in the past (Ismail et al, 2017:21).

4. Excessive/ destabilizing plasticity

Loss of homeostatic synaptic regulation leads to an abnormally intense response to excitatory or inhibitory plasticity induction stimuli which increases risk of developing clinical neurologic pathologies (Drubach et al, 2004:79).

Critical periods in development

Brain maturation is not linear, but is punctuated by a series of developmental spurts. Linked with these stepwise processes is the concept of ‘critical’ or ‘sensitive’ periods. Sensitive periods are hallmarks of early development, which result in either particularly good, or conversely, particularly poor outcomes. They mark phases of increased plasticity, when specific brain circuits are maximally sensitive to acquiring certain kinds of information to establish interconnections with other systems. Within the context of healthy development, critical periods are times when neural networks are most sensitive to environmental influences. Brain disruption or insult during a critical period is thought to be particularly detrimental, causing a cessation of development or altering its course. If this progression does not occur appropriately it may never occur, there may be delay in ongoing development of damaged brain regions, or asynchrony with respect to the sequential establishment of neural connections.

The factors that govern the opening and closing of critical windows are partially understood. Functioning inhibitory circuits are needed to open some critical periods, The closing of critical periods may involve the physical stabilization of synapses and network structure by myelin or extracellular structures (Knudsen, 2004).

Mechanism of recovery

Brain insult, results in a ‘cascade’ of events, some detrimental and some beneficial, with the balance depending on the type of insult incurred and the nature of the subsequent pathology. Insult results in destruction or disruption of neural networks, via death of neurons and glia, damage to axonal tracts, alterations to neurotransmitter systems and disruption to vasculature. Destroyed neurons are not replaced and abnormal axons struggle to spontaneously regenerate, impacting neural circuitry and altering the cellular environment. Secondary processes, such as inhibitory influences of glial cells and associated scarring, hinder recovery processes and compete with neuroprotective responses, to determine the degree of recovery.

Recovery mechanisms can be grouped into two general classes; restitution and substitution. Restitution suggests that, as the damaged brain heals, neural pathways are reactivated and functions are restored. Substitution theories refer to recovery via transfer/reorganization of functions from damaged brain tissue to healthy sites (Jankowska and Edgley, 2006:12).

1. Restitution of function

Diaschisis

The period of rapid recovery of function immediately following brain insult, which reflects the generalized nature of impairment in the early stages post-insult and involves biochemical mechanisms and genomic alterations in protein synthesis, not just restricted to site of insult and necrotic tissue, but also involving distant brain regions.

Regeneration

Regeneration, the process by which damaged neurons, axons and terminals regrow and establish previous neuronal connections.

Sprouting

Remaining nerve fibres develop branches that occupy sites left empty by damaged neurons, thus re-innervating unoccupied areas in the vicinity of the lesion, and facilitating synaptic contacts. Although the efficiency of this process is inhibited by the action of glial cells at the site of injury axons may regrow, but only a subset will reach their appropriate destination, resulting in incomplete or maladaptive recovery.

Denervation supersensitivity

This process suggests that post-synaptic cells, deprived of their characteristic synaptic inputs, will develop increased sensitivity to any neurotransmitter substance leaking from pre-lesion neurons, via the emergence of new receptors and a larger surface area. Thus supersensitivity facilitates activation of post-lesion pathways and restitution of normal functioning.

Molecular genetic processes

Underpinning neural plasticity at all levels are two mechanisms that modulate the effects of neurotransmitters, protein phosphorylation and regulation of gene expression. These have been identified as potentially protective in the context of insult. Protein phosphorylation is the molecular mechanism by which neural activity is modulated via regulation of ion channels and neurotransmitter receptors, signal transduction pathways, and neurotransmitter synthesis and release to the expression of genes in the nucleus that underlie synaptic changes. Regulation of gene expression is also a key mechanism that produces quantitative and qualitative changes in the protein components of neurons. Neurotransmitters continually regulate neuronal gene expression. Typically, changes to the CNS mediated by protein phosphorylation have a rapid onset, are more readily reversible, and have a shorter duration compared with neural plasticity

mediated by gene expression. However, both processes mediate the long-term effects of experience on the brain. The changes induced through protein phosphorylation and gene expression result in alterations in the function and efficacy of synapses, in the transmission of information by individual neurons, and ultimately in communication within neural networks (Anderson, 2011:134, Warraich and Kleim; 2010: 125, Cicchetti and Blender, 2006: 1094).

2.Substitution of function

Anatomical reorganization

There are possible scenarios for functional reorganization: 1. Interhemispheric reorganization: functions transfer to the analogous site in the non-damaged hemisphere; 2. Intrahemispheric reorganization: reorganization of functions within the damaged hemisphere; and 3. Intrahemispheric maintenance: skills subsumed by damaged tissue are maintained within that tissue, resulting in maximum dysfunction. The precise factors that govern which of these options occurs are not well understood, but appear to be dependent on factors including the nature; size and laterality of brain damage; distribution of the neural network underpinning the impaired skills; as well as timing of insult with respect to developmental stage of the child.

Interhemispheric transfer is based on the premise that the contralateral hemisphere has some capacity to subsume skills lost due to brain insult, and so is most likely to occur in the context of unilateral brain damage, and during infancy.

Intrahemispheric transfer is more commonly described in the context of unilateral focal lesions, where there is adjacent healthy tissue to take up skills normally subsumed by damaged tissue.

Finally, intrahemispheric maintenance is generally associated with poorest outcome, and can be caused by brain insult at any time through childhood, including the prenatal period. It is most likely following bilateral or diffuse insults, where little healthy brain tissue is available to support reorganization (Anderson et al, 2005: 116).

Neural and functional plasticity

Neural plasticity, the brain's response to the environment, refers to physiological processes, and can be observed at molecular, cellular, neurochemical and neuroanatomical levels, and at the level of brain systems or, in the context of insult, through neural recovery processes, such as regeneration and axonal sprouting. In contrast, functional plasticity refers to behavioural change or recovery occurring in response to environmental or injury-related events. Contrary to the parallel processes

seen for normal neural and cognitive development, once the genetically predetermined sequence of brain maturation has been interrupted, neural recovery may not necessarily translate to functional recovery (Kozlowski and Schallert: 1998).

Timing issues

Age at insult and time since insult

It is generally agreed that the age at which a child sustains brain insult will influence their development and mastery of neurobehavioural skills, although the relationship is not a simple one. Time since testing is also important, due to: 1. The rapid recovery that occurs postinsult; and 2. The potential for children with early brain insult to struggle to keep pace with their peers developmentally, due to neurobehavioural impairments resulting from their insults. As a consequence, studies that report performance in the acute stages post-injury may reflect transient impairments that will recover with time, or fail to identify impairments in skills that are yet to develop (Anderson et al, 2009).

Emerging deficits

While children may function normally immediately post-insult, over time, and with increasing environmental demands, they may fail to make age-appropriate developmental gains or ‘grow into’ seemingly new deficits (Westmacott et al, 2009).

Delayed development

Others have described delayed skill acquisition, but gradual catch up following early brain insult, with some evidence of delayed onset of neuropathology (Stiles et al, 2009).

Clinical implications

Throughout the early phases of brain damage and rehabilitation, neural networks are gradually restored to some degree around the lesion itself, while secondary brain regions in a distributed network are often recruited to progressively compensate and, depending on the extent of damage to a given region, may perhaps adopt some of the functions of the damaged area. Plasticity is crucial to recovery, but the rates and extent of recovery vary considerably between individuals. Whereas individual factors such as lesion size and location, mechanism of damage and demographics all affect the extent and rate of recovery.

Genetic variation in any of these components could influence each individual’s capacity for brain plasticity and could explain the variability encountered in motor rehabilitation efficacy. Those individuals with a

greater capacity for adapting and favorably altering cortical connections have a theoretical advantage with regard to brain recovery. Further, genetic differences may also influence the amount or type of rehabilitation therapy required to induce cortical plasticity and concomitant functional recovery (Anderson et al, 2011:134).

The endpoint of plasticity is not always beneficial and can lead to significant maladaptive outcomes depending on the nature and extent of the neuropathogenic process, the stage of neurodevelopment during which it occurs as well as the integrity of homeostatic regulatory mechanisms (Flor, 2008: 8).

Implications for intervention

We do know that the processes that occur following brain injury are rather dynamic and can be progressively modified via both internal and external factors. Improved understanding of the mechanisms associated with brain insult and recovery provides the opportunity for designing effective interventions. Neurorehabilitation seeks to overcome the disabilities of neurologically impaired patients by looking for strategies to bring about functional improvement and amelioration of neurological deficits.

The apparatus of neuromodulation is diverse and the cortical, subcortical and spinal targets vary depending on the primary pathological process and downstream effects on the neural networks, pathways and synapses. Neuromodulation that alters mechanisms of neuroplasticity is an inclusive term that should include behavior, pharmacological, electrical and biological approaches. Each one of these approaches interacts preferentially with multiple components of plasticity in the young brain, probably influencing different regulatory mechanisms.

Spontaneous post-injury neuroplasticity or therapeutic stimulation may play an adaptive role in modifying the functional organization of the remaining cortical tissue, leading to clinical improvements. The critical period closes as molecular brakes dampen plasticity, and alter the balance between excitation and inhibition. A key point is that it is possible to reopen the critical period by manipulating this balance chemically. Plasticity mechanisms are enhanced in the developing brain so that children can recover more fully from brain injuries than adults. Activity-dependent plasticity at synapses combined with reorganization of motor and sensory maps in the brain is one of the major mechanisms for adaptive plasticity after injury. Exercise as well as direct electrical stimulation of the brain can enhance recovery if these interventions are designed to take advantage of the brain's intrinsic plasticity mechanisms.

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CHAPTER XX
VASCULAR COMPLICATIONS OF BEHCET'S DISEASE

*Didem Melis Oztas**

Introduction

Behçet's disease is a chronic, systemic vasculitis (1). The diagnosis of the disease is based on the clinical criteria of International Study Group for Behçet's (2). The pathology was first described in 1937 by a Turkish dermatologist, Hulusi Behçet (3). It is more common in the Eastern Mediterranean and the Far East (4). Turkey has the highest prevalence with 40–370 per 100000 people (5). While Japan, Korea, Iran, Iraq, and Saudi Arabia has high incidence of disease (4), it is seen less common in North America, Northern Europe, USA, UK, and German, (5).

Behçet's disease is an inflammatory disease and affects both males and females but it is more severe especially in 30 to 40 years old young men (6). The severity of the disease decreases with older age. Male to female ratio is approximately 3:1 (4). The pathology includes mouth and/or genital ulcers, mucocutaneous lesions, affects central nervous system, and causes ocular, vascular, articular, digestive tract, and inflammatory eye lesions (1). While aphthous stomatitis, genital ulcers, eye and skin lesions are major criterias; vascular involvement is considered as a minor sign (7).

Cardiac involvement is quite rare and contains intracardiac thrombosis, aneurysm of coronary arteries (Figure 1, Figure 2), endomyocardial fibrosis, aneurysm of septum, ventricle, aorta or sinus of Valsalva (8). Also, because of fibrous intimal thickening, acute myocardial infarction may be seen in patients with angiographically normal coronary arteries (3).

Although Behçet's disease is considered as a mixture of both autoinflammatory disorders and autoimmune diseases (9); the activity of immune system increases against environmental and auto-antigens in pathology (4). Idiopathic recurrence of systemic inflammation occurs in autoinflammatory pathologies (5). Also, autoimmune diseases contain hypergammaglobulinemia and female dominance and Behçet's disease does not include these characteristics. Hence, the pathology is considered as an autoinflammatory disease rather than autoimmune disease (4). Anti-lymphocyte and anti-cardiolipin antibodies are used for diagnosis (4). The

*(MD) Bagcilar Training and Research Hospital, Department of Cardiovascular Surgery.
e-mail: didemmelisoztas@gmail.com

prognosis of the disease is unpredictable due to absence of significant laboratory markers that show disease activity. Based on the association of Behcet's disease and endothelial dysfunction, endothelial progenitor cells and circulating endothelial cells are thought to be new markers which play a role in the pathogenesis of vasculitis. (9).

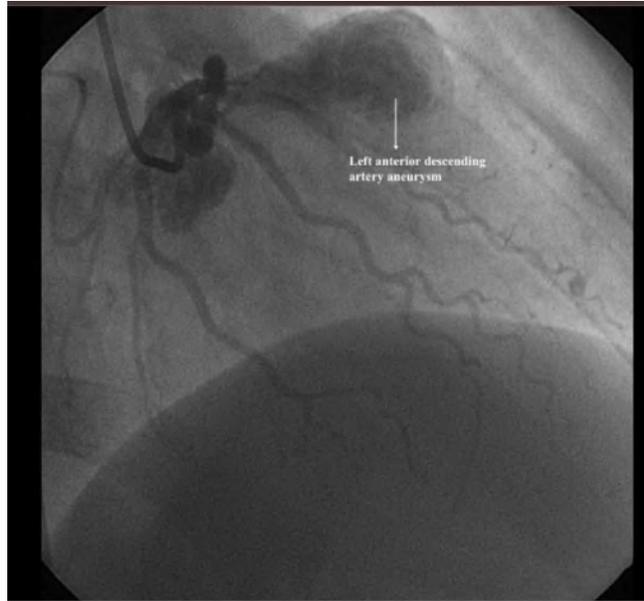


Figure 1. Angiographic image of left anterior descending artery aneurysm

In the management of the pathology, the protocol is generally determined according to affected systems. The pathology is individual based on age, sex, system involvement and severity of disease. The main aim of therapy is to provide and maintain remission and amend quality of life. The treatment of the pathology includes corticosteroids, immunosuppressives such as TNF alpha inhibitors, biologic agents, nonsteroid antiinflammatory drugs, colchicine and many other drugs according to disease manifestations (10).

Vascular Complications of Behcet's Disease

Behcet's disease is a neutrophilic vasculitis (4). Symptoms are generally related on vasculitis and various sizes of arteries and veins may be affected (11). Endothelial damage based on vasculitis causes hypercoagulability and this may lead to thrombosis (9) Thrombosis may cause many vascular pathologies associated with complications of disease and also stroke may occur due to cerebral arterial thrombosis (11).

Vascular involvement in Behcet's disease is called vasculo-Behcet's disease (12). The pathology affects both arteries and veins. The incidence of vasculo-Behcet is 7.7 to 38% of Behcet's disease patients and mortality occurs approximately in %20 of severe cases (5). The lesions include arterial occlusions, aneurysmal formations and vein thrombosis, thrombophlebitis etc. The major cause of mortality in vasculo-Behcet's disease is rupture of aneurysms (3).

Arterial Complications

Arterial complications of Behcet's disease is especially significant because of mortality risk. This complication is responsible for over 25% of all Behcet's disease related deaths (12). Arterial involvement may be in aneurysm formation or occlusive lesion. Mostly, venous involvement is thought to be more common than arterial lesions in Behcet's disease (4, 5). Abdominal and thoracic aorta, pulmonary arteries, iliac and femoral arteries are mostly affected arteries. Involvement of coronary arteries is very rare (4).

Aneurysms are most severe pathology of vasculo-Behcet's disease because of high risk of rupture (13). The aneurysm in Behcet's disease is different from atherosclerotic aneurysm. Vasculitis destroy media, leads to arterial dilatation and fibrosis. The aneurysm in Behcet's disease is characterized by recurrence, the recurrence may be seen in different locations (5). Pulmonary artery aneurysms are more common in patients with Behcet's disease than general population (12). Aortic aneurysms cause high risk due to risk of rupture and it is major cause of death in patients (2, 12). The clinical situation is associated with severity of disease and anatomic location of lesion. Aortic aneurysms are mostly progress rapidly, leads to rupture and causes retroperitoneal hematoma or hemoperitoneum.

While aneurysm occurs mostly in abdominal aorta, aneurysms of ulnar, celiac, subclavian, tibioperoneal, iliac, superior mesenteric arteries are reported very rare (4). Aneurysm formation is approximately %60 of arterial lesions in Behcet's disease (5). The most common aneurysm type is saccular aneurysm, the symptoms are related to compression to other structures (4). Invasive diagnostic methods are beneficial but risky because new aneurysm may occur. Noninvasive methods such as contrast-enhanced tomography and magnetic resonance angiography are other options (5).

Aorta is the most affected artery and pulmonary artery follows it (5t). Pulmonary artery aneurysm is the most common pulmonary lesion and generally occurs in main pulmonary arteries. Its' incidence is approximately 1%–10% in patients with Behcet's disease (5). Immune mediated response is thought to be cause of pulmonary artery aneurysm, the neovascularization and degeneration of vascular wall plays a role in the

formation of aneurysm (14). The most common symptom is hemoptysis, the rupture of aneurysm and fatal hemoptysis results poor prognosis (5). The mortality rate of rupture of pulmonary artery aneurysm is approximately %50. The rupture may be a result of damage based on inflammation in arterial wall and injury aggravation by damaged nutrient vessels (3). Mortality occurs approximately %30 of the patients with pulmonary artery aneurysm within 2 years (14).

The intracranial aneurysms are very rare in vasculo-Behcet's disease. The most affected artery is middle cerebral artery and results mostly subarachnoid hemorrhage due to aneurysm rupture (5).

Thrombosis is another complication of disease but aneurysm or pseudoaneurysm are more common (2). Arterial embolism may be also due to intracardiac thrombosis formation. Pseudoaneurysm formation in aorta is often and usually arise from posterior or lateral walls of the aorta (5). Also following surgical therapies in the anastomosis lines, after invasive interventions in the access sites and in the stented regions of the coronary arteries, pseudoaneurysm may occur (4). Acute dissection is rare in vasculo-Behcet's disease (14, 15).

Venous Complications

Venous system complications are mostly thought to be more common in Behcet's disease rather than arterial involvement (4). Thrombosis is more common in male patients and prevalence ranges between 10%–30% (5). Thrombosis is seen in earlier ages of male patients (16). Thrombosis of lower extremity veins is the most frequent manifestations of venous complications (17). Bilateral involvement is more common in patients with Behcet's disease. Recanalization chance is low and more collaterals occur (16). Lower extremity vein thrombosis may be the first symptom of the disease (18) and post-thrombotic syndrome occurs approximately in 50% of the patients with deep vein thrombosis (17).

Although deep vein thrombosis is the most common form of venous involvement, inferior vena cava, superior vena cava, pulmonary arteries, suprahepatic vessels, and cardiac cavities may also be affected (17). Atypical thrombosis may also affect portal vein, cerebral sinuses, suprahepatic veins resulting with Budd-Chiari syndrome (19). Vena cava thrombosis and Budd Chiari syndrome has high risk of mortality (5).

Superficial thrombophlebitis is common in patients following vein puncture. Patients with iliac and/or femoral vein thrombosis presents with venous claudication. In central nervous system thrombosis may cause cerebral venous thrombosis. Central venous thrombosis is approximately 7–30% of all central nervous system pathologies in Behcet's disease in different series. Also, central venous thrombosis may be the presenting

symptom in up to 20% of patients. Superior sagittal sinus and transverse sinus are affected mostly. The symptoms of the pathology are headache, papilledema, vomiting, and focal disorders and may cause severe complication such as visual loss (5).

Pathogenesis of Vascular Complications

Although the pathogenesis of vascular involvement of vasculo- Behcet's disease is unclear, there are some mechanisms which are thought to be reasons of disease. Arterial stiffness is found to be increased in patients this is blamed for increased pressure wave reflections and aortic blood pressure (20, 21). Neutrophils have an important role on the pathway leading to thrombosis by promoting inflammation (19). Neutrophils release superoxide anion radicals and lysosomal enzymes and these factors increase lymphocytic infiltration, cause occlusion of vasa vasorum and transmural necrosis. In the end this cascade results with true aneurysm by damaging the wall of large arteries and impairing local blood flow. However if perforation occurs, this causes pseudoaneurysm (4). Additionally, matrix metalloproteinase proteins are thought to be an important part of the pathway of aneurysm formation. In some series matrix metalloproteinase proteins are considered as a significant marker of Behcet's disease (5).

Also, fibrinogen, thrombin, factor Xa and factor VIIa are thought to foster inflammation. As well as anticoagulant drugs, immunosuppressive drugs are useful for the management of thrombosis and this supports the role of inflammation in thrombosis (19). There are some studies that show the effect of endothelial cell injury in vascular inflammation. Injury causes releasing of immune complexes and leads to hypercoagulability and thrombosis (14). In studies, while the increased proinflammatory and T-helper type 1 cytokines, adhesion molecules such as VCAM-1 and ICAM-1, tissue factor, soluble CD40L, matrix metalloproteinase-9, free oxygen radicals have provided unusual coagulation. Decreased nitric oxide disrupts platelet activity and lipid peroxidation (22). Inflammation results thrombosis by causing increased platelet aggregation and deterioration of fibrinolysis (14). Also, the von Willebrand factor, hyperhomocysteinemia, and antiphospholipid antibodies were found to be contributed in thrombus manifestation. However, thrombus in cardiac chambers is very rare. The reasons are thought to be the dynamic blood flow and decreased risk of endocardial damage due to circulating immune complexes (14).

Management of Vascular Complications

The management of vascular complications in Behcet's disease contains medical and surgical therapy (3) (Figure 2). The treatment should be

organized by multidisciplinary teams such as rheumatology, cardiovascular surgery, internal medicine, radiology, and thrombologists (5).

In general management, the therapy of Behcet's disease includes corticosteroid therapy and immunosuppressives. Cyclosporine, azathioprine, anti-tumor necrosis factor (TNF) agents, and interferon- α (IFN- α) are the other drugs which can be used in therapy (3). The standard therapy of deep vein thrombosis is not different from traditional therapy in the patients with Behcet's disease. The thrombolytic therapy can be considered in some selected cases. In the case of intracardiac thrombosis, thrombolytic therapy for recurrent right ventricular thrombosis and use of colchicine, anticoagulant therapy, and corticosteroids are presented in the literature (5).

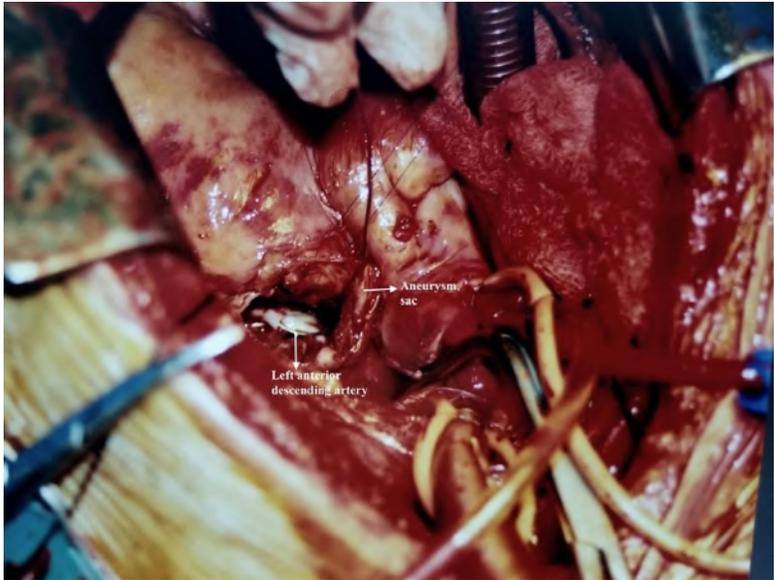


Figure 2: Perioperative image of left anterior descending artery aneurysm.

Previously, in vascular pathologies of Behcet's disease such as aneurysm, open surgical repair was preferred. But, then, some researches revealed that recurrence may be seen in patients and endovascular approaches may be alternative in the treatment (3). Endovascular aortic repair is considered as a safe alternative especially for ruptured aneurysm and may prevent pseudoaneurysm formation which may occur after surgical therapy (12). Although the combination of endovascular and immunosuppressive therapy are used for treatment, the recurrence of aneurysm and pseudoaneurysm in the access site is still an important issue

(3). Also, supportive medical therapy together with surgical treatment is preferred treatment modality for peripheral arterial aneurysms, nowadays. Anastomotic pseudoaneurysm is major complication of surgery with %12.9 5-year cumulative incidence following surgery (12).

Surgical treatment is an important therapy when organ or life threatening situations occur such as aneurysm, occlusion or stenosis of significant arteries (5). There are some key points that improves surgical outcome. The medical therapy which is given before and after the surgery reduce inflammation, complication and provides good outcome (4). The medical treatment which provides decreased systemic or local inflammation before elective surgeries should be considered to prevent complications (5). The autologous arteries or veins which is thought to be used for operation should be considered if they are not affected with vasculitis. The extreme manipulation of aorta should be hesitated. The patients with Behcet's disease suffer more bleeding depending on anticoagulant, thrombolytic therapies and decreased plasminogen activation and fibrin in active disease (4).

In conclusion, Behcet's disease is a multisystemic autoimmune vasculitis with certain, sometimes life threatening vascular complications. Moreover, cardiovascular complications during the course of the disease are the major determinants of mortality and morbidity in affected patients. It is important to provide remission with various types of immunosuppressive agents otherwise complications may occur. In case of vascular complications, multidisciplinary team approach should be provided for the care of the patients. Surgery should be hesitated otherwise mandatory such as in case of life threatening complications. When an intervention is definitely required, less invasive precautions such as endovascular measures should be taken, as new aneurysm or pseudoaneurysm formation at the anastomosis lines are not seldom. When conventional surgery is mandatory, it should be preferred when the disease is in remission state. Otherwise, prior to emergent procedures, pulse immunosuppressive therapy should be administered during the perioperative period.

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CHAPTER XXI
GENERAL APPROACH TO PULMONARY EMBOLISM

Hakan Kartal & Gökhan Erol***

Definition of Pulmonary Embolism

Pulmonary thromboembolism (PTE) occurs when clotted blood enters the pulmonary artery circulation. Most PTE is caused by deep vein thrombosis (DVT) in the legs, arms or pelvis, and sometimes from the jugular vein and inferior vena cava. Much more rarely, oil droplets, neoplastic cells, air, amniotic fluid or foreign substances (Iv drug addicts, catheter parts in various iv interventions, etc.) can form embolism ^(1,2).

Epidemiology

It is difficult to determine the epidemiology of pulmonary embolism; because it may remain asymptomatic or diagnosed accidentally, some cases of PTE may present with sudden death⁽³⁾.

According to the data of the United States, the average annual incidence of VTE is about 1/1000, it increases with age and increases approximately 10 times after the age of 80 compared to 45-50 years old⁽⁴⁾.

While the mortality of PE is about 25-30% in untreated cases, the mortality decreases to 2-8% in treated ones^(5,6).

Recurrence is observed in 5-23% of VTE cases despite treatment^(7,8). The highest risk of recurrence is 6-12 months after the end of treatment. Recurrence rates are higher in patients with cancer and hereditary thrombophilia. After the anticoagulant treatment was completed, the patients with high D-dimer levels were found to have a high recurrence rate. The average annual incidence of venous thromboembolism (VTE) is between 23-269 / 100000. Considering the cases that are clinically silent and lost without diagnosis, the annual incidence is estimated to be higher than this. The risk of venous thromboembolism increases with age. The incidence of VTE doubles for every 10 years over the age of 40 ^(9,10).

* (MD); Department of Cardiovascular Surgery, Gulhane Training and Research Hospital, Ankara, Turkey E-mail: hakhankartal@gmail.com

** (MD). Department of Cardiovascular Surgery, Gulhane Training and Research Hospital, Ankara, Turkey E-mail: drgekhanerol@gmail.com

Risk Factors

Factors leading to intravascular coagulation: vascular endothelial damage, hypercoagulability and stasis were described by Virchow in 1856. In 75% of cases of venous thromboembolism, acquired and / or hereditary factors that lead to one of these three factors are detected⁽¹¹⁾.

Virchow Triad	
Stasis Immobilizations:	post-operative period, pregnancy and postpartum period, congestive heart failure.
Endotelial Damage:	Burn, lower extremity orthopedic surgeries, sepsis, varices and posttromboflebitic syndromes
Hypercoagulability:	Congenital Antithrombin III deficiency, Protein C deficiency, Protein S deficiency, Factor V Leiden mutation, Hyperhomocysteinemia, Factor VIII increase (> 150 IU/dl), Prothrombin 20210 gene variant, acquired Major abdominal and pelvic surgery, having previously DVT for any reason, malignancies, estrogen-containing oral contraceptives, long trips.

In idiopathic cases where no risk factor is detected, the probability of occult cancer and thrombophilia is high^(12,13). Major trauma, surgery, lower extremity fractures and dislocations, spinal cord injury are powerful provocative factors for VTE^(10,14). Surgical intervention in the last 45-90 days increases the risk of VTE 6-22 times⁽⁴⁾. Oral contraceptive use in fertile women is the most common risk factor for VTE. ⁽¹⁵⁾ The highest risk during pregnancy is in the third trimester of pregnancy and in the first 6 weeks of postpartum, and the risk has increased 60 times compared to non-pregnant women ⁽¹⁶⁾. The risk of VTE in postmenopausal women receiving hormone replacement therapy varies according to the drugs they use ⁽¹⁷⁾.

Infection is a common trigger factor for hospitalization in VTE cases. In addition, the use of blood transfusion and erythropoiesis stimulating agents also increases the risk of VTE⁽¹⁴⁾. If we consider VTE as part of the cardiovascular system diseases, smoking, obesity, high cholesterol, hypertension (HT) and diabetes can be considered direct or indirect common risk factors ^(18,19).

Myocardial infarction (MI) and heart failure increase the risk of PE⁽²⁰⁾. In contrast, patients with VTE have an increased risk for MI and stroke⁽²¹⁾.

VTE risk factors are ⁽²²⁾	
Genetic Risk Factors,	Acquired Risk Factors
Antithrombin III deficiency	Nephrotic syndrome
Protein C deficiency	Stroke
Protein S deficiency	Obesity
Activated Protein C resistance: (Factor V Leiden)	Long-period travel
Prothrombin G20210A mutation	Major surgery (Pelvic, abdominal)
Hyperhomocysteinemia	Immobilization
Factor VIII increase	Cancer
Congenital dysfibrinogenemia	Congestive heart failure
Anticardiolipin antibodies	Myocardial infarction
Plasminogen deficiency	Oral contraceptive use
Factor VII deficiency	Hormone replacement therapy
Factor IX increase,	Central venous catheter
	Chemotherapy Spinal Cord Injury (SCI)
	Polycythemia vera
	Pregnancy / Postpartum
	Trauma

Pathophysiology

With the formation of pulmonary embolism, first of all, due to the release of dead space ventilation, then the release of mediators such as histamine, serotonin, bronchoconstriction in bronchioles, ventilation / perfusion (V / Q) imbalance, diffusion reduction and shunt resultant hypoxemia develop. Linear atelectasis occurs within 24-48 hours due to tachypnea induced hypocapnia and decreased surfactant in the segment whose perfusion is impaired ⁽⁴⁾.

In patients without additional comorbidity, in PE where the pulmonary arterial bed is blocked by 20% thrombus, pulmonary artery pressure is tried to be kept close to normal by supporting mechanisms such as the opening of new flow areas.

The right ventricular blood flow volume and heart rate are increased, and the cardiac blood pulse volume is maintained close to normal. However, in people with 30-40% of pulmonary arterial bed occlusion, these supportive mechanisms are not sufficient and anatomic obstruction and vasoconstriction cause an increase in PVD while a proportional decrease in arterial compliance, and a moderate pressure increase in the right heart in response to an increase in pulmonary vascular resistance ^(23,24).

Sudden increase in pulmonary vascular resistance as a result of massive embolism, in which more than 50% of the vascular bed suddenly clogs, causes right ventricular dilation, worsening the contractile property of the right ventricular myocardium. Right ventricular pressure increase and volume loading increase wall tension and myositis flexion. This leads to prolonged right ventricular contraction time. Elongation at the time of right ventricular contraction causes the interventricular septum to bend into the left ventricle in early diastole ⁽²⁵⁾.

Desynchronization of the ventricles worsens with the development of the right bundle branch block. As a result, filling of the left ventricle in early diastole is prevented, cardiac output decreases, so systemic hypotension and hemodynamic instability develop ^(3,26).

With the activation of the endogenous thrombolytic system of the body within a few days, recanalization of the occluded vessels begins and is completed to a large extent within 10-14 days. It takes 4-8 weeks to be fully recanalized. However, only half of the cases develop complete resolution, the other half organizes thrombus and remains residual thrombus ⁽⁴⁾.

One of the uncommon local consequences of pulmonary embolism is infarction. It is not often seen because the pulmonary paranchime has three potential sources of oxygen: pulmonary arteries, bronchial arteries, and airways (29). Two of these three sources should be compromised in order for the infarction to occur. Therefore, infarction is rare in patients without cardiopulmonary disease association. Infarction occurs in approximately 20% of patients with significant cardiac and pulmonary disease with defects in both bronchial arterial flow and airways. In patients with left ventricular disorder, high pulmonary venous pressure can reduce bronchial flow and result in infarction (27).

Clinical signs and symptoms

The path to diagnosis in pulmonary thromboembolism (PTE) begins with clinical suspicion. For this, the presence of risk factors should be taken into account in addition to the initial symptoms and signs. PTE should be suspected especially in patients who present with dyspnea and tachycardia, whose chest X-ray is normal and whose condition cannot be explained by another disease. According to the results of the autopsy studies, it was determined that most of the cases had no suspicion of PTE before death (28).

It should be noted that common symptoms and physical examination findings such as dyspnea, tachypnea, tachycardia and chest pain in pulmonary embolism cases are not specific to this disease. Clinical findings can range from a symptom-free table to sudden death as a result of a massive attack. Clinical diagnosis in trauma or intensive care patients is much more difficult and unreliable. In patients with no previous cardiopulmonary problem in pulmonary embolism, suddenly developing dyspnea and follow-up are the most common symptoms and signs. Pleuritic pain with dyspnea and follow-up is found in more than half of the cases. Hemoptysis occurs in less than 10% of cases. Clinical symptoms and findings may vary depending on the size, number (single / multiple), localization of the embolus, whether or not infarction develops, rate of resolution, recurrence, age of the patient and reserve of cardiopulmonary functions. The main symptoms and findings related to PTE can be seen in Table-1. Application with syncope in the elderly is more common than in young people. On the other hand, side pain and hemoptysis are less common in the elderly (29).

When a large thrombus reaches the lungs, it settles in the bifurcations of the main pulmonary artery or lobar branches and causes hemodynamic impairment. In this table, which is seen in approximately 25-30% of cases, attacks with sudden onset of dyspnea, tachypnea and tachycardia are in the

foreground. There is anxiety and a feeling of substernal pressure. In 60-70% of cases, smaller thrombus can reach the peripheral and small pulmonary arteries, initiating the inflammatory response affecting the parietal pleura, causing pleuritic chest pain and pleural fluid. In peripheral obstructions, infarction is more likely to develop and is around 10%. Vascular problems of the underlying cardiopulmonary disease facilitate the development of infarction. Hemoptysis is more common in these cases. Dyspnea and hypoxemia findings are prominent in occlusion of central vessels. When 50% or more of the pulmonary vascular bed is blocked, a sudden onset dyspnea develops with hypotension and / or shock. The blockage is mostly bilateral. Cyanosis, apathy, oliguria, mental confusion, severe tachypnea, tachycardia and hypotension (Systolic blood pressure <90 mmHg) are detected. Pulmonary 2nd sound can be hard, right ventricular S3, venous fullness, and pansystolic murmur due to tricuspid regurgitation along the left edge of the sternum can be detected. Pulmonary embolism clinical pictures; It is classified as massive, submassive and non-massive. Massive pulmonary embolism has acute right ventricular failure accompanied by hypotension-shock or cardiopulmonary arrest. In submassive pulmonary embolism, there are signs of right ventricular dysfunction (dilation and hypokinesis) detected in echocardiography in response to normal systemic blood pressure. In non-massive pulmonary embolism, systemic blood pressure and right ventricular functions are normal ⁽³⁰⁾.

Symptoms and Clinical Signs in Pulmonary Thromboembolism	
Symptoms	Findings
• Dyspnea	• Tachypnea (> 20 / min)>
• Pricking chest pain	• Tachycardia (> 100 / min)>
• Hemoptysis	• Rals
• Palpitations	• DVT findings
• Retrosternal chest pain	• Fever (> 38 o C)>
• Syncope/presyncope	• Gallop rhythm

It is beneficial in the empirical diagnosis and treatment approach to classify patients with suspected PTE by scoring according to symptoms, findings and risk factors they carry, as clinically low, medium and high

probability. For this purpose, two scoring is widely used. The first of these scoring; *Wells scoring*, also known as "*Canadian*" scoring.

Wells (<i>Canadian</i>) Clinical Evaluation of Pulmonary Thromboembolism (22)	
Finding	Points
Presence of DVT symptoms and signs	3.0
Alternative diagnosis unlikely	3.0
Tachycardia (> 100 / min)	1.5
History of immobilization or surgery in the past 4 weeks	1.5
Previous history of DVT or pulmonary embolism	1.5
Hemoptysis	1.0
Cancer presence	1.0
<i>* Total score:</i>	
<i>* <2.0 points: Low clinical probability</i>	
<i>* 2.0- 6.0 points: Moderate clinical probability</i>	
<i>* > 6.0 points: High clinical probability</i>	
<i>* or ≤ 4 point: PE clinical probability poor</i>	
<i>* > 4 points: PE clinical probability is strong</i>	

The second scoring is the modified Geneva scoring.

Modified Geneva Scoring ⁽²²⁾	
Finding	Points
> 65 years old	1 point
Previous history of DVT or pulmonary thromboembolism;	3 points
A history of surgery or limb fracture within a week;	2 points
The presence of active cancer:	2 points
Pain in the unilateral lower limb:	3 points
Hemoptysis;	2 points
Heart rate: 75-94 / minute;	3 points
Heart rate:> 95 / minute;	5 points
Pain with palpation of the leg or edema-swelling in one-sided leg;	4 points
<i>* 0-3 points: Low probability</i>	
<i>* 4-10 points: Medium probability</i>	
<i>* ≥11 points: High probability</i>	

In an analysis involving 4 studies with pulmonary angiography controlled studies performed in patients admitted to the emergency clinic with suspicion of PTE, in cases where Wells and Geneva scoring were applied; The prevalence of PTE was 10% in those with a low probability, 30-40% in the medium probability and 67-81% in the high probability ⁽³¹⁾.

Diagnosis in Pulmonary Thromboembolism

The most important step in the diagnosis of pulmonary thromboembolism; to suspect the disease. The absence of clinical and physical examination findings specific to pulmonary thromboembolism makes diagnosis difficult. Lung x-ray, electrocardiography (ECG), biochemical examinations and arterial blood gases contribute to clinical

evaluation, but these tests are useful in excluding other diagnoses, they cannot be sufficient for definitive diagnosis ⁽⁹⁾.

The anticoagulant treatment initiated with suspicion of pulmonary embolism carries a high risk of bleeding, requiring confirmation or exclusion of the diagnosis as soon as possible. Only 25% of patients with suspected PTE can be diagnosed directly with objective tests (spiral CT, Doppler USG) ^(32,33,34).

Chest Radiography

In 20-40% of patients with PTE without cardiopulmonary disease, chest radiography is normal. In most patients with suspected PTE, a chest X-ray is performed to look for an alternative cause to the patient's symptoms. Hampton hump and the Westermark sign are rare, but increases the suspicion of pulmonary embolism ⁽³⁵⁾.

Electrocardiography

ECG abnormalities are common in patients with suspected pulmonary embolism, but are not specific ⁽³⁶⁾. The most common findings are tachycardia and nonspecific ST segment and T wave changes (70%) ⁽³⁷⁾. Abnormalities that suggest PTE, such as the S1Q3T3 pattern, signs of right ventricular loading, new incomplete right bundle branch block are rare (less than 10%) ⁽³⁶⁾.

Arterial Blood gas

Arterial blood gas (ABG) measurements and fingertip saturation have a limited role in the diagnosis of PE. In ABG, hypoxemia, hypocapnia and respiratory alkalosis are usually detected ⁽³⁸⁾.

D-dimer

D-dimer levels are high in plasma due to the activation of coagulation and fibrinolysis in acute thrombosis cases. The negative predictive value of the D-dimer test is high and acute PTE and DVT are unlikely when the D-dimer level is normal ⁽³⁾.

D-dimer level can also be found in cases such as surgical intervention, trauma, kidney diseases, malignancies, severe infections, SLE, pregnancy ⁽⁹⁾. The positive predictive value of increased D-dimer is low and is not used to confirm PTE diagnosis ⁽³⁾.

Lung Scintigraphy

Ventilation / Perfusion scintigraphy is a diagnostic test with a low and allergic reaction used for suspected PE. Perfusion scintigraphy is a sensitive but nonspecific test to detect perfusion defects in the pulmonary artery⁽³⁹⁾. Radiation exposure is 2-6 times lower than CT angiography (2-6 mSv vs. 1.1 mSv)^(40,41).

Due to the low radiation exposure and no contrast agent, V / Q scintigraphy is a preferable method with low clinical probability and it is a preferable method in patients who had normal lung radiography, outpatients with a history of contrast-related anaphylaxis and patients with severe renal failure⁽⁴²⁾.

Spiral CT Angiography

High-resolution multi-detector CT (HRCT) has become the preferred method for imaging pulmonaryvascular structures in patients with suspected PE after the introduction of angiography. Contrast spiral CT angiography can directly show the thrombus in the pulmonary artery bed to the segmental level.

According to the PIOPED II study, the negative predictive value of negative CT was 96% in those with a low-risk group, and 89% in the medium-risk group, while only 60% in the high-risk group. In contrast, the positive predictive value of positive CT was 92-96% in the medium or high-risk group, while 58% in the low-risk group. Therefore, clinicians should be careful in the mismatch between HRCT results and clinical probability risk groups (43).

Attention should be paid to creatinine values for contrast imaging. This method should not be preferred in patients with renal insufficiency and creatinine clearance below 50 ml / min⁽⁹⁾.

Lower Extremity Venous Ultrasonography

In the majority of cases, PTE originates from the lower extremity DVT. Venous compression ultrasonography (USG) has 90% sensitivity and 95% specificity in symptomatic DVT.^(44,45)

Magnetic Resonance Imaging (MRI)

It can be used as an alternative to CT angiography in pregnant women in patients with renal impairment or allergy to contrast media⁽⁹⁾. Its effectiveness in showing subsegmental pulmonary arteries is low⁽⁴⁶⁾.

Echocardiography

Acute PTE can cause increased right ventricular pressures and dysfunction, which can be detected by echocardiography. Negative echocardiography cannot exclude the diagnosis of PE since its negative predictive value is between 40-50% ^(47,48,49).

On the other hand, right ventricular loading or dysfunction may also occur in the absence of acute PTE, and in the presence of concomitant cardiac or respiratory disease ⁽⁵⁰⁾.

Right ventricular dilation is found in at least 25% of patients with PTE and is an important finding used in risk assessment of the disease ⁽³⁾.

Disruption of the right ventricular ejection pattern or reduction of the contractility of the right ventricular free wall relative to the apex has a high positive predictive value for the diagnosis of PE even if there is concomitant cardiorespiratory disease ⁽⁵¹⁾.

Routine echocardiography is not recommended among the diagnostic examinations of normotensive PTE cases that are hemodynamically stable ⁽⁴⁷⁾.

In contrast, the absence of right ventricular loading or dysfunction findings in patients who are hemodynamically unstable, theoretically excludes the diagnosis of high-risk PTE. In addition, pericardial tamponade, acute valve dysfunction, severe global or regional Left Ventricular Dysfunction, aortic dissection, such as the differential diagnosis of diseases that may be the cause of shock is useful ⁽³⁾.

Pulmonary Angiography

Conventional pulmonary angiography is considered the gold standard test that provides definitive diagnosis in PTE. However, because CT angiography with similar diagnostic accuracy is a less invasive technique, pulmonary angiography is not preferred ⁽⁵²⁾.

Cardiac Troponins

Right ventricular dilatation due to acute right heart failure due to pulmonary thromboembolism increases the oxygen requirement of the right ventricle. Right coronary artery circulation decreases, and as a result, micro-infarction occurs in the right ventricular muscles and troponin release increases. Increased troponin level indicates right ventricular

dysfunction. Increased serum troponin levels have been associated with an increased risk of death. ^(53,54).

Treatment Approach

Bed rest is not required for deep vein thrombosis (DVT) unless there is pain and swelling in the leg. On the other hand, although there is no scientific data on the necessity of bed rest in pulmonary thromboembolism (PTE), the general trend is that patients are not standing up for 24-48 hours ⁽²²⁾.

Hypoxemia and respiratory failure is the most important complication caused by ventilation / perfusion incompatibility in massive and some submassive PTE patients. In this case, oxygen therapy is required and the nasal cannula or facial mask should be arranged so that saturation is above 92%. Oxygen supplement treatment prevents worsening of increased pulmonary vascular resistance and right ventricular ischemia. When respiratory failure deepens despite oxygen supplementation therapy, O₂ support should be provided by mechanical ventilation. However, mechanical ventilation prevents venous return to the heart by increasing intrathoracic pressure. On the other hand, its hyperinflation may increase pulmonary vascular resistance and lead to right ventricular dysfunction. In addition, sedative hypnotics used for intubation prevent catecholamine discharge and vasodilation, thereby increasing the risk of cardiovascular collapse by lowering cardiac output. This risk should be taken into account during application ⁽⁵⁵⁾.

In cases where mechanical ventilation is unavoidable, PEEP should be kept to a minimum to keep the plateau pressure level below 30 cmH₂O and a low tidal volume (approximately 6 mL / kg) should be used ⁽⁵⁶⁾.

In patients with VTE with right ventricular dysfunction, fluid therapy should be carefully administered to correct possible hypovolemia ⁽⁵⁷⁾.

In hemodynamically unstable massive PTE patients, if fluid replacement does not improve the patient's condition, it is necessary to urgently start vasopressor medications. Because increasing fluid treatment will lead to further impairment of the patient's ventricular function. For this purpose, norepinephrine, dopamine and epinephrine can be used ^(57, 58).

Acute PE is a clinical picture that requires rapid risk classification and treatment selection, as it displays a wide spectrum in terms of clinical and prognosis. The main factors in risk determination in patients diagnosed with acute PE are clinical evaluation, the presence of RV dysfunction, and

the rise of biomarkers in the blood that indicate RV infarction. While low-risk patients can exhibit an excellent prognosis only under anticoagulant therapy and even with outpatient treatment, close hemodynamic monitoring, respiratory and inotropic support should be provided to high-risk patients, and mechanical ventilation and ECMO support should be available for immediate use if necessary ⁽⁵⁹⁾.

Anticoagulation alone is not sufficient in the treatment of high-risk patients, in addition, advanced treatment options such as systemic thrombolytic, pharmacomechanical catheter-based excipients, vena cava filter or surgical embolectomy are required ^(60, 61).

In patients with high clinical suspicion, if the risk of bleeding is not high, rivaroxaban, apixaban or edoxaban, which are direct factor Xa inhibitors, low molecular weight heparin (LMWH), standard heparin (SH) or new oral anticoagulant (NOAC) drugs can be used until the diagnosis is excluded (1)

In the presence of moderate clinical suspicion, if diagnostic tests will result in more than 4 hours, and in low-clinical suspect patients, if diagnostic tests will result later than 24 hours, it is recommended to start anticoagulant therapy. In case of massive PTE suspicion, high dose unfractionated heparin (to keep aPTT > 80sec) should be started ⁽⁶²⁾.

When the diagnosis is clear, heparin treatment should be continued for at least five days. Initial treatment is 3 weeks for rivaroxaban and 7 days for apixaban. In cases where anticoagulant therapy is contraindicated, vena cava inferior filters should be placed ⁽³⁰⁾.

In proven nonmassive and submassive cases, traditional oral anticoagulants (coumadin) are added to the treatment within the first 24 hours. In patients with low risk of bleeding, giving 10 mg for the first 2 days enables INR to reach the desired value (2-3) more quickly, thereby shortening the length of hospital stay. It is safer to start warfarin treatment at a dose of 5 mg / day, especially in patients at risk of bleeding and above 75 years of age ⁽⁶²⁾.

If the initial treatment of the patient was performed with an oral direct Factor Xa inhibitor (rivaroxaban or apixaban), maintenance therapy is continued with the maintenance dose of the same drug. Dabigatran, a direct thrombin inhibitor, or a factor Xa inhibitor, edoxaban, which is one of the new oral anticoagulants, can be used instead of warfarin during the maintenance period after heparin treatment.

When the diagnosis of massive PTE accompanied by a hypotension and / or shock is confirmed, thrombolytic therapy is applied if there are no major contraindications. Patients who are treated with thrombolytic therapy are then switched to standard heparin infusion without bolus dosing with aPTT control. Cases with massive PTE with contraindications or no response to thrombolytic therapy are evaluated for catheter or surgical embolectomy ⁽³⁰⁾.

Primary thrombolytic therapy is controversial in submassive PTE cases with right ventricular dysfunction on echocardiography. In cases of submassive PTE selected by risk assessment, if the risk of bleeding is not high and the clinic is deteriorating, it is recommended to start salvage thrombolytic therapy ^(62, 56)

Having a hypotension or shock clinic indicates that the patient is at high risk. The primary treatment of these patients is to ensure rapid reperfusion; catheter embolectomy with thrombolytic therapy or alternatively surgical or percutaneous intervention ⁽³⁰⁾.

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CHAPTER XXII

UPDATE FOR METABOLIC STONE DISEASES IN CHILDREN

*Osman Kose**

Introduction

Urinary stone diseases are considered to be occurring rarely in the pediatric age group; however, studies in recent years have shown increases especially in the prevalence and incidence of ureter stones (1). Although they have a prevalence of about 2-3%, pediatric stone diseases have a recurrence risk that may vary between 6.5 - 54% (2). Besides, it is an important health problem due to high morbidity and risk of end-stage renal failure (3). It has a multi factorial etiology such as race, gender, genetics, climate, dietary habits (4). In developing countries, endemic pediatric Stone diseases are often limited to the bladder. This is associated with decreased phosphate intake and may often manifest as ammonium acid, urate or uric acid stones. Again, studies in recent years have shown that incidence of stone disease has increased with increased animal protein consumption in parallel with the rising standards of living in developed countries (5).

Although, urinary stone diseases are common in all pediatric age groups, the average age at diagnosis is between 4.2 - 9.4% (1).

Hereditary factors should be considered in case of children who are diagnosed earlier than these age groups (6). In pediatric group, the objective in the treatment of urinary Stone disease covers removing the stone, preventing recurrences, maintaining renal functions, preventing urinary system infections, and correcting the anatomical and underlying metabolic problems. The majority of children with urinary tract stone disease exhibited ≥ 1 metabolic risk factors. Metabolic risk factors should be evaluated in all children with urinary stone disease to provide appropriate treatment (7). Mohammadjafari et al. concluded the similar and noted that Patients should be carefully evaluated considering this point of view (8).

Unfortunately, incidence of urolithiasis in pediatric age groups have been increasing in developed countries mainly due to changes in daily routine diet. Additionally, urolithiasis must be suspected in the face of abdominal pain even central or diffuse pain in younger children when there

* (Asst Prof, FEBU); Katip Celebi University, Faculty of Medicine, Department of Urology, IZMIR - TURKEY, e-mail: oskose@gmail.com

is a positive family history even though specific urinary symptoms such haematuria and dysuria may be lacking (9).

The medical treatment are used in the urinary system stone diseases and these are specific to the type of stone and is effective only in a small group of stones. Alkalization in uric acid stones may also be effective for cystine Stones when used in combination with thiols; besides urine acidification is another method used in infection stones. Objectives of medical treatment cover preventing the formation of new Stones, preventing of the growth of existing stones and thus reducing the surgical need and hence, morbidity. In light of all of these, medical treatment may be considered as a preventive treatment. In order to start medical treatment, exact diagnosis should be made. Therefore, metabolic investigations covering stone analysis, urine and serum analysis gain importance.

In this compilation, general recommendations in pediatric urinary Stone diseases and treatment options specific to the type of Stone or metabolic disorder are reviewed.

General recommendations

The first general recommendation in all urinary stone diseases is abundant fluid intake. Urine production increases with abundant fluid intake and insoluble concentration in urine and super saturation may be reduced. In studies conducted, children with Stone diseases have been detected as taking less fluid than the ones in the control group (10). Lande et al. have reported that calcium oxalate, calcium phosphate and uric acid super saturation do not occur when urine amount is more than 1ml/kg (11). Fluid intake is a critical component of prevention of stones. This is done by effectively reducing the lithogenic factors including calcium, oxalate, uric acid and cystine; besides, the only treatment for patients with primary xanthinuria is large fluid intake. Curhan et al. reported that drinks such as coffee and tea reduced stone formation while grapefruit juice increased stone formation in a study conducted in adult females with stone (12). The reason for this may be that it increases the tendency to oxalate stones due to its high oxalate content. To the best of our knowledge, there are no such studies conducted in pediatric stone patients in the literature. Milk, other fruit juices and water are fluid sources that may be recommended to the pediatric stone patients to be consumed in excess. Surely, alkaline drinks such as lemonade are more advantageous than acid ones in terms of the risk of stone formation (13).

In studies conducted, increased calcium and sodium in urine have been shown to be associated with the increased dietary sodium intake (14). Frassetto et al. emphasized that the chloride in the excess dietary sodium chloride intake may lead to low degree metabolic acidosis (15). By this means, bone mineralization may deteriorate and may also contribute to stone formation. The fact that excess dietary salt intake increases the risk for stone formation has been associated with excess salt intake in developed countries. The reverse mechanism is seen in the case of excess dietary potassium intake (16). Potassium salts generally come from alkali salts such as potassium citrate. Potassium citrate reduces urinary calcium excretion (17). Potassium salts are taken from fruits and vegetables in diet. Sodium and potassium have opposite effects on blood pressure similar to their opposite effects on urinary calcium. Excess dietary sodium increases blood pressure whereas excess dietary potassium decreases blood pressure.

In stone diseases accompanied by hypercalciuria, reduction of urinary calcium excretion is recommended. This may be ensured by reducing the dietary animal protein load, that is by reducing the acid load (18). Acids that form when these dietary animal proteins are metabolized lead to bicarbonate secretion from the bones. Therefore, bone resorption that cause osteopenia and hypercalciuria occur (19). Nouvenne et al. reported that limiting animal protein and salt intake increased recurrences compared to calcium intake at a normal level in patients with recurrent calcium oxalate stone and hypercalciuria (20). Apart from these, low dietary calcium intake leads to decreased oxalate that binds with calcium in the intestines and increased urinary oxalate excretion (21). Moreover, high amount of dietary vitamin C, sucrose and fructose intake may lead to Stone formation. On the other hand, high levels of magnesium intake decrease the risk of stone (22).

Furthermore, Shavit et al reported importance of body mass index on urinary stone disease (23). They revealed that weight loss may reduce stone recurrence but these needs more studies for exact results (23).

Dietary recommendations should be explained to families in the treatment of pediatric urinary stone diseases and most importantly, it should be noted that dietary habits do not change overnight.

Medical treatment

Hypercalciuria

Calcium oxalate and calcium phosphate stones constitute the majority of urinary system stones in children. About 30%- 50% of children who

develop stones have hypercalciuria (24). Metabolic calculi accounted for 48% of the patients with idiopathic hypercalciuria as the main cause (25).

Alemzadeh-Ansari et al. noted similar findings and reported that high rate of metabolic abnormalities in infants with urolithiasis (26).

Hypercalciuria is the most common cause of pediatric urolithiasis. It means that urinary calcium excretion is above 4mg/kg/day or that urinary calcium/creatinine ratio is above 0.21 in older children. In many children 24 hour urine collection is not practical and calcium-creatinine ratio in the urine is used to estimate daily calcium excretion. It should be noted that urinary calcium excretion increases with age. Urinary calcium oxalate and phosphate should also be measured for an optimal evaluation (27).

Hypercalciuria usually occurs as a result of disturbances in one or more of the 3 systems such as increased gastrointestinal calcium absorption, disturbances in bone formation and resorption and renal loss (27). Hypercalciuric calcium stones are categorized as normo-calcemic and hypercalcemic.

Hypercalciuria is not the only factor, but it is associated with many factors. The most common cause in children and adults is idiopathic hypercalciuria. Idiopathic hypercalciuria is defined as hypercalciuria despite the absence of hypercalcemia and occurs in patients without any apparent cause. The gene(s) responsible for familial idiopathic hypercalciuria have not been defined yet, but it is observed as having autosomal dominant character. 4% of asymptomatic healthy children have evidence of idiopathic hypercalciuria (28), and 40%-50% of these children have positive family history for urolithiasis (29).

When hypercalciuria is observed, some issues must be ruled out for the diagnosis of idiopathic hypercalciuria. In definition, blood calcium level of the patient should be normal. In patients with hypercalcemic hypercalciuria, hyperparathyroidism and D hypervitaminosis should be investigated and when clinically detected, prolonged immobilization, sarcoidosis, malignancy, juvenile idiopathic arthritis, excess corticosteroids, adrenal failure or Williams syndrome should be considered. Children with hypercalcemic hypercalciuria should be evaluated in terms of hypoparathyroidism and autosomal dominant hypocalcemic hypercalciuria (mutation in calcium receptor function). Although patients with normocalcemic hypercalciuria are often diagnosed with idiopathic hypercalciuria; prematurity, history of diuretic use (furosemide and acetazolamide), anticonvulsant use (topiramate and zonisamide),

ketogenic diet, Dent disease, Bartter syndrome, familial hypomagnesemia and nephrocalcinosis (AHHNK) with hypercalciuria, distal renal tubular acidosis (dRTA), hereditary hypophosphatemic rickets with hypercalciuria (HHRH) and potential medullar sponge kidney must be ruled out and kept in mind in the first assessment.

Genetic conditions associated with normo-calcemic hypercalciuria

Dent disease is a condition based on X and occurs as a result of the mutation in CLCN5 gene. This condition is characterized by molecule weighted proteinuria, nephrocalcinosis, hypercalciuria, nephrolithiasis and chronic kidney disease. Clinical picture is usually insidious and asymptomatic throughout the childhood period; signs and symptoms of nephrocalcinosis and hypercalciuria are not common in children. The damage occurs in proximal tubular function and rarely it may manifest as a part of glycosuria, aminoaciduria, metabolic acidosis and Fanconi syndrome associated with hypophosphatemia. In a limited number of patients, Dent phenotype occurs with the mutation in the OCRL gene (Dent 2). This condition is also associated with the oculocerebrorenal syndrome of Lowe.

Bartter syndrome is an autosomal recessive condition characterized by loss of salt in kidneys, hypokalemia, metabolic alkalosis, hypercalciuria and normal serum magnesium levels. The disease typically manifests itself as salt deficiency, polyuria, dehydration, emesis, and constipation and growth retardation in children below six years of age. Severe polyhydramnios, prematurity and rarely sensorineural hearing loss are distinctive characteristics of the disease. Mutations in SLC12A, KCNJ1 and BSND genes (type 1, type 2 and type 4 Bartter syndrome, respectively) typically result in serious dysfunction in the thick ascending limb of the loop of Henle in the neonatal period (neonatal Bartter syndrome). Mutations in the C1CKB gene (type 3 Bartter syndrome) usually lead to medium level dysfunction in the thick ascending limb of the loop of Henle. It is usually seen out of the neonatal period (classic Bartter syndrome).

AHHNK is usually seen in childhood together with seizures or tetany accompanying hypomagnesemia. Other clinical findings are recurrent urinary tract infections (UTI), polyuria, polydipsia, growth retardation, nephrolithiasis and progressive kidney failure (30). AHHNK is an autosomal recessive condition. It occurs with the mutations of both CLDN-16 and CLDN-19 genes. Homozygous CLDN-16 or CLDN-19 mutations are associated with disturbances in the integrity of the tight junctions in the ascending limb of Henle, magnesium, calcium loss in the urine and the

resulting hypomagnesemia. Patients usually develop a classical triad composed of hypomagnesemia, hypercalciuria and nephrocalcinosis. In case of the combination of CLDN-19 mutations, deep visual disturbances characterized by macular coloboma, considerable myopia and horizontal nystagmus may be seen (31).

Primary dRTA is a hereditary disease and is characterized by systemic acidosis that occurs as a result of the loss of the ability of the distal tubule to properly acidize the urine. Growth retardation, polyuria, polydipsia, hypercalciuria, hypocitraturia, nephrocalcinosis, kidney stones and hypokalemia are common findings in infancy. Primary dRTA may be autosomal dominant (SLC4A1 gene) or recessive (ATP6V1B1 or ATP6V0A4 genes).

Failure to release H⁺ ions from α -intercalated cells is caused by vacuols H⁺-ATPase (ATP6V1B1 or ATP6V0A4 genes) or a damaged Cl⁻/HCO₃⁻ anion exchanger-1 (SLC4A1 gene). Patients with ATP6V1B1 mutations may have hearing loss or neural type hearing loss.

HHRH is rare autosomal recessive disease that is caused by a mutation in the SLC34A3 gene. This condition results in loss of function in the type IIc sodium phosphate carriers of the proximal tubule. Decreased renal phosphate reabsorption results in deep hypophosphatemia, normokalemia, rickets and bone ache. In addition, hypercalciuria and nephrolithiasis are detected. It may occur as a result of the stimulation of 1,25-dihydroxyvitamin D synthesis triggered by hypophosphatemia. Increased synthesis causes excess urine calcium losses in the face of increased gastrointestinal reabsorption of calcium and normal calcium levels (32).

When there is hypercalciuria, environmental factors that increase calcium excretion such as high sodium consumption and ketogenic diet should be considered. In treatment, sodium restriction, large fluid intake, and a diet rich in proteins and poor in oxalate should be recommended according to weight and age. Tiazid diuretics prevent calcium excretion through proximal and distal renal tubules. Another diuretic is amiloride. These diuretics may improve calciuria; however, they may also cause abnormalities such as weakness, nausea, orthostatic hypotension, hypercholesterolemia and electrolyte abnormalities. Potassium citrate may be used in hypercalciuria associated with dRTA since it improves metabolic acidemia and hypokalemia and it brings urinary calcium and citrate excretion to normal levels (33). In addition to all of these, a combination of tiazid diuretics and potassium citrate may also be used (34).

Patients with idiopathic hypercalciuria may also be treated with potassium citrate (35). This treatment reduces urinary calcium excretion while increasing urinary citrate excretion. Moreover, mineral density of the bone also increases. Urinary pH of the patients should be monitored. At very high pH levels, formation of calcium phosphate stones may become easier (36). Penido et al. reported decreased bone mineral density in one third of hypercalcemic children (37). Parallel to this study, Freundlich et al. reported that alendronate may have beneficial effects on bone tissue and calcium excretion in children with osteopenia and urinary stone disease (38).

Hyperoxaluria

Oxalate is the last product of the metabolic path of glyoxylate and ascorbic acid. It is secreted primarily by kidneys. A majority of the daily oxalate excretion (80-85%) is derived from normal metabolic homeostasis and diet constitutes the rest (10-15%). Daily urinary oxalate excretion is usually less than 50 mg/ d/1.73m². Due to the challenge of 24-hour urine collection in little children, urinary oxalate/creatinine ratio is used to estimate the oxalate excretion. Increased urinary oxalate excretion may occur due to a hereditary metabolic condition, (primary hyperoxaluria (PH)), more often due to oxalate reabsorption or increased oxalate precursors.

Primary hyperoxaluria

Type 1 and type 2 PH are rare; they are autosomal recessive diseases in which endogenous oxalate production is increased. Excess oxalate production by the liver leads to nephrocalcinosis and nephrolithiasis which result in increased oxalate excretion. Calcium oxalate deposits cause progressive kidney damage. Its clinical Picture may vary from end stage renal failure in newborns to possible random stone disease in adulthood. The diagnosis is often eludes observation and there may even be cases where it is realized after the loss of the transplanted kidney (39).

Type 1 PH occurs due to the mutations in the AGXT gene which causes functional damage in hepatic peroxisomal enzyme alanine-glyoxylate aminotransferase (AGT). Deficiency leads to the accumulation of glyoxylate, glycolate and oxalate in urine. Pyridoxin is the basic cofactor for proper AGT activity and rarely, prolonged vitamin B6 deficiency may mimic type 1 PH. Type 2 PH occurs due to the mutation in the GRHPR gene which leads to dysfunction in glyoxylate reductase-hydroxypyruvate reductase enzyme activity. Increased oxalate and L-glyceric acid are

excreted through the kidney (40). Type 2 PH is a lighter form than type 1 PH, but it is not benign. Recently, a third form type III PH has been defined in 8 families who have hyperoxaluria and mutations in the DHDPSL gene (41). The exact mechanism of the hyperoxaluria that occurs in Type 3 PH has not been cleared yet.

Secondary hyperoxaluria

In secondary hyperoxaluria, there are both high amounts of dietary oxalate (or oxalate precursors) intake and the dysfunction that causes increased absorption of the dietary oxalic acid into the intestinal system. Gastrointestinal absorption varies in opposite directions with dietary calcium intake and calcium-dependent diet increases oxalate absorption and hypercalciuria (42). Oxalate is a side product of ascorbic acid metabolism and high dose of vitamin C is also associated with hyperoxaluria. Increased diet absorption is usually characterized by disturbance in fat absorption or chronic diarrhea. Among secondary causes of hypercalciuria is inflammatory bowel disease associated with gastrointestinal disease, celiac disease, exocrine pancreatic insufficiency (cystic fibrosis), biliary tract disease and small intestine resection or short bowel syndrome. Pathogenesis in these cases is caused by the presence of free fatty acids that bind to the calcium in the intestinal lumen. This results in free, absorbable, unbound oxalate formation.

Oxalobacter formigenes not only reduces oxalate, it also changes the oxalate released endogenously in the intestines. Thus it reduces the oxalate in blood and in urine and it may be applied orally in PH treatment (43). Moreover pyridoxine is used to decrease oxalate excretion in PH. Pyridoxine is an important cofactor of AGT. About 10%-30% of children with PH type 1 are sensitive to pyridoxine (>30% decrease in urinary oxalate excretion). Particularly, they may ensure protection of renal functions in patients who are homozygous for Gly170Arg and Phe152Ile mutations within the appropriate treatment period. Treatment should be started in patients with suspicious PH type 1 (2-5 mg/kg/g) and it should be titrated until a diagnosis is made and a response is received (8-10 mg/kg/g). High doses of pyridoxine are known to trigger sensory nephropathy. There is no apparent evidence showing that pyridoxine additions are beneficial unless there is a real pyridoxine deficiency.

In PH, liver and kidney transplant are the best treatment methods in patients who developed chronic renal failure. It is essential to prevent excess dietary intake, increase oral calcium intake and to improve gastrointestinal disorders in the treatment of hypercalciuria.

Hypocitraturia

Citrate is an important stone inhibitor that prevents the growth of calcium oxalate and calcium phosphate crystals by binding to calcium in urine. Additionally, it can prevent new stone formation. It is adjusted in the proximal tubule both by absorption and by metabolism. Hypocitraturia is defined as a citrate/creatinine ratio of below 180 mg/g in men, and below 300 mg/g in women. Intra-cell acidosis of the proximal tubule occurs due to the hypocitraturia associated both with metabolic acidosis and increased citrate absorption in the proximal tubule. Consequently, ketogenic diet, medications (topiramate, zonisamide and acetazolamide), dRTA and chronic diarrhea are often associated with hypocitraturia. Incomplete dRTA may occur in the absence of apparent systemic acidosis or in hypokalemia. This may usually be neglected in the face of hypocitraturia in case no provocative acid loading test is conducted. Nevertheless, hypocitraturia is idiopathic although many cases are rich animal protein and little amounts of fibre vegetables, potassium that lead to low dietary citrate excretion (44). Elmaci et al. reported the most frequent metabolic abnormalities in preschool-age children with urolithiasis were hyperuricosuria and hypocitraturia (45). A comprehensive investigation of stone disease in children presenting with hematuria and urinary tract infection is important to prevent the development of renal parenchyma scarring (45).

Potassium citrate is an ideal medical option in treatment. The treatment is safe except for its minor gastrointestinal effects.

Hypomagnesuria

It may occur in dietary magnesium deficiency. Magnesium may form a complex with oxalate and it reduces calcium oxalate super-saturation in urine and it may also prevent oxalate reuptake. It is essential to increase dietary intake in treatment. It is also very important to evaluate magnesium level in urine that hypomagnesiuria is also an important risk factor in adults and children.

Hyperuricosuria

Uric acid excretion is greater in children than in adults. The highest fractional excretion (Fe) is in newborns (Fe 30%-50%). It reaches adult values (Fe 8%-12%) in adolescence (46). Hyperuricosuria is defined as uric acid excretion greater than 815 mg/d/1.73m². When the glomerular filtration rate (GFR) is adjusted, uric acid excretion is relatively stable after 2 years of age. When GFR is greater than 0.56 mg/dL in children older than

two years who do not have toilet training, hyperuricosuria may be detected in spot urine. This value may be calculated by using the formula, “Urinary uric acid (mg/dL) x Plasma creatinine (mg/dL) / Urinary creatinine (mg/dL)”.

Hyperuricosuria is the greatest risk for stone formation especially at low urine pH. Hereditary purine metabolism disorders are lymphoproliferative diseases and diseases associated with polystemia and apparent hyperuricemia hyperuricosuria. Rarely a condition known as hereditary hyperuricemia characterized by hyperuricosuria, nephrolithiasis and activity induced renal failure is also detected. Mutations in both SLC22A12 gene and SLC2A9 gene that code the urate transport in the proximal tubule are known causes for its formation (47). Other causes of hyperuricosuria include excess purine intake (animal protein), hemolysis, uricosuric medications (probenesid, salicylates and losartan), cyanotic congenital heart disease, melamine toxicity and idiopathic (familial) causes. Moreover, a phenomenon primarily detected in adults is referred to as hyperuricosuric calcium oxalate stones. In this case, hyperuricosuria forms the fundamental basis for the formation of oxalate stones without or minimal uric acid content (epitaxy).

Hereditary diseases of the Purine metabolism

Phosphoribosyl pyrophosphate synthetase superactivity (PRPSS) is hereditary based on X and is formed by the mutation in the PRPS1 gene. Increased PRPSS activity is associated with excess purine production. The following purine degeneration results in hyperuricemia, gout, hyperuricosuria and uric acid nephrolithiasis. Nerve development abnormalities, neural type hearing loss are seen in some affected individuals (48). Hypoxanthine-guanine phosphoribosyl transferase (HPRT) deficiency is a purine metabolism disorder in the neonatal period which occurs due to the mutations in the HPRT1 gene associated with excess uric acid production. Complete deficiency of HPRT activity is associated with Lesh-Nyhan syndrome characterized by mental retardation, spastic cerebral palsy, choreoathetosis, uric acid Stones, and self-destructive behaviors. Children with partial HPRT deficiency may be phenotypically similar to the patients who have complete phenotypical deficiency or moderate neurological symptoms. Kidney stones, uric acid nephropathy, kidney obstruction or gout may be the first signs of the disease.

First line treatment is urine alkalization and usually potassium citrate is used. Restricting excess dietary animal protein intake in patients may result

in increased purine intake and increased uric acid production, and it may contribute both to uricosuria and acidic urine. Allopurinol (4–10 mg/kg/g, adults maximum 300 mg/g) is necessary in both hyperuricemia and hyperuricosuria such as PRPSS or HPRT deficiency. Xanthine dehydrogenase inhibition with allopurinol may lead to xanthine accumulation and its excess excretion in the urine. Rarely, secondary xanthuria with xanthine Stones may be detected in children in long term treatments. If there are co-morbid findings of hypercalciuria, hyperoxaluria or hypocitraturia, allopurinol may also be a treatment option for the treatment of hyperuricemic calcium oxalate stones (49).

Cystinuria

Cystinuria is an autosomal recessive disease caused by mutations in both SLC3A1 and SLC7A9 genes which results in irregular amino acid transport in the proximal tubule. Cystinuria is characterized by urinary hypersecretion of cystine and lysin, ornitin and arginin, which are among dibasic amino acids. Normal individuals release cystine at a rate of 50-60 mg/g/1.73 m² (less than 30 mg daily). Patients who are homozygous for cystinuria have a cystinuria excretion of more than 400 mg/ g/1.73m² (varies between 400 mg and 3000 mg) (50). Patients typically have renal colic and urolithiasis in the second and third decades of their lives. However, they may also have staghorn stones in the neonatal period. Weak solubility of cystine in urine causes precipitation in collecting duct systems. If this is not treated, it usually results in frequently recurring kidney stones and renal failure in the long run. Related UTI is frequent; combined cystine and struvite stones may be detected (51).

Irregular cystine transport in cystinuria primarily results from the dysfunction in heavy (rBAT) and light (b0, 1AT) subunits (rBAT/ b0, 1AT) in the heteromeric amino acid transporter. Cystinuria is originally categorized into two classes: type 1 and non-type 1 (type 2 and type 3). The distinction is based on urine cystine concentration pattern compulsory heterozygote and estimated mode of inheritance. There is classical autosomal recessive inheritance in type 1 and normal cystine excretion is seen. On the contrary, non-type 1 heterozygotes (type 2 and type 3) show moderate and high urinary cystine excretion. Types 2 and 3 vary among themselves. There is a quasi-normal increase in plasma levels of cystine after oral cystine administration in type 3 homozygotes (52).

Homozygous mutations in the SLC3A1 gene which code rBAT are associated with type 1 cystinuria and homozygous mutations in the SLC7A9 gene which code b0,1AT are associated with type 2 and 3

cystinuria. A new classification system has been developed. In this system, patients who are homozygous for SLC3A1 mutations are determined as cystinuria type A, patients who are homozygous for SLC7A9 mutations are determined as cystinuria type B, and mutations in both SLC3A1 and SLC7A9 genes are considered as type AB (53).

There is little evidence concerning restriction of proteins which have high cystine content; however, animal protein intake may help increase urinary pH in patients with cystinuria. Children who have stones are recommended not to take increased amounts of protein; but proper protein intake for growth and nutrition according to age should be recommended. The objective in treatment is to ensure the concentration and amount of urine in which cystine may dissolve. This is ensured by large fluid intake and medical treatment. The 2 most frequently used agents are D-penicillamine and α -mercaptopyrionylglycine (tiopronin). Cystine is formed as a dimer of cysteine and these agents work by reducing the disulphide bonds that bind the 2 molecules of cystine. Thiol group combines with cysteine and forms a more soluble product which is a combination of excreted cysteine and the product. D-penicillamine has a wide range of adverse events including febrile reactions, gastrointestinal complaints, liver dysfunctions, taste disturbances, bone marrow suppression, metal deficiencies, membranous glomerulopathy, myasthenia gravis and skin eruptions (elastosis perforans serpiginosa) (6). α -mercaptopyrionylglycine has a similar incidence of adverse events, but it may be slightly lower. Evaluation of liver enzymes, complete blood count, urine analysis, copper and zinc levels should be regularly studied. Special studies (solid-phase trial and high performance liquid chromatography) may assist in distinguishing between urinary cystine and cysteine-drug complexes and in long-term treatment.

Although captopril containing disulfidryl is a drug that may be used in treatment, its hypotensive effects should be taken into account.

Infection stones

Infection stones are seen in 2-24% of children who have been diagnosed with kidney stones (6). They constitute 75% of the stones diagnosed in European children. They are usually seen in children below 6 years of age. 80% of the patients are male. Infection stones in the urinary system are seen more commonly in patients with anatomic and functional disorders that cause stasis. Infection stones occur due to infections induced by organisms that ensure hydrolysis of urea with the urease enzyme and which form ammonium and bicarbonate as a result (27). *Proteus*, *Providencia*,

Klebsiella pneumoniae, *Pseudomonas aeruginosa*, *Serratia*, *Enterobacter*, *Staphylococcus* are bacteria that produce urease. The compound at high pH which does not produce urease but is rich in ammonium is magnesium and the bacterium which help Stone formation by facilitating precipitation of phosphate is *Escherichia coli* (33).

Infection stones contain ammonium phosphate, carbonate apatite and monoammonium urate. Ammonium phosphate is the main constituent of many infection stones. Ammoniac damages the urothelial glycosaminoglycan layer which is the defense mechanism against bacteria. These stones may require intervention following long-term antibiotherapy as of their structure. The incidence of this stone is gradually decreasing thanks to the developments in infection diagnosis and treatment (53).

The treatment is composed of extracting the stones and correcting the underlying anatomical and/or functional obstruction. In the long-term treatment and follow-up, antibiotherapy according to urine culture antibiogram is important. Urinary acidification and a balanced fiber diet that is poor in phosphate helps in the treatment.

Orotic acid stones

Hereditary orotic aciduria is a rare genetic disease. It occurs due to orotate-phosphoribosyl-transferase and orotidine5-phosphate-decarboxylase enzyme deficiency responsible for transformation of orotic acid into uridine-5-phosphate. Consequently, orotic acid excreted in urine increases and it crystallizes forming orotic acid stones. Uridine is used in the treatment (33).

2,8-dihydroxyadenineuria

It is an autosomal recessive disease and there is adenine – phosphoribosyl transferase defect. As a result, there is excess accumulation of 2,8 dihydroxyadenine. It is very similar to uric acid stones; however it may be distinguished by metabolic and stone analysis. It may be treated by allopurinol therapy and regulation of diet (54).

Xanthuria

Xanthuria is a rare disease and its inheritance is autosomal recessive. Xanthin, which takes part in the formation of uric acid, the final product of purine metabolism, is seen as a result of oxidase enzyme deficiency. Urinary excretion of hypoxanthin and xanthin increases. Urinary solubility of xanthin is minor. Allopurinol may be used in the treatment of this

disease. Dietary purine intake should be reduced and a lot of fluids should be taken.

Finally, hypomagnesiuria, hypocitraturia, and hypercalciuria are the most important risk factors for stone formation in adults and pediatric patients (55). Dietary changes, more fluid intake, decreased salt usage, increased potassium intake are useful for preventing stone diseases in children. According to these, balanced consumption of fruit and vegetables and a low consumption of chocolate and cola according to general nutritional guidelines, although no studies have assessed in pediatric stone formers the effect of fruit and vegetables supplementation on urinary citrate and the effects of chocolate and cola restriction on urinary oxalate in pediatric stone formers. Despite the low level of scientific evidence, a low-protein (< 20 g/day) low-salt (3 liters/day) is strongly advised in children with cystinuria (56). Nevertheless, infantile patients should be separated from all pediatric groups and coexistence of systemic disorders, anatomic anomalies at high frequencies may indicate a role of distinct pathologic mechanisms (57). Besides urinary infections should be detected more details in infants.

In the daily pediatric urology practice, detailed examination is necessary in cases where urolithiasis is seen. Detailed medical history should be recorded first and physical examination should be conducted. Familial history of stones, additional diseases and medications used should be recorded. Metabolic and nonmetabolic problems which may be the reason for stone diseases should be kept in mind. In urolithiasis seen in the pediatric age group, the most common non-metabolic disorders may include vesicoureteral reflux disease, uroteropelvic junction obstructions, neurogenic bladder and other micturition disorders (58). Then simple clinical assessment may be made. In the simple assessment, first simple urine analysis and ultrasound are among the tests that may be requested. It may be useful to consider the following algorithm to reach an exact diagnosis after reaching pre-diagnosis with examinations to be made in the urine and other clinical information.

Following simple clinical examinations; pH, calcium, phosphorus, magnesium, oxalate, sodium, potassium, uric acid, citrate, cystine, creatine should be investigated in urine collected over 24 hours and the urine amount should be recorded. Moreover, urine culture is recommended (58). On the other hand, sodium, potassium chloride, calcium, phosphorus, magnesium, creatine, blood urea nitrogen, alkaline phosphatase, uric acid, intact parathyroid hormone should be investigated in the serum and

complete blood count should be made. The probability of stone formation is high if the calcium/citrate ratio in the 24-hour urine is above 0.326 (59). In pediatric urolithiasis that is commonly seen in daily clinical practices, when it is time to decide on the treatment after diagnostic stages; first, second and third line treatment options are considered according to the cause. It is recommended that the following algorithms are followed for the most commonly seen pathologies and their practical treatments. As mentioned above, it is crucial to apply general therapy recommendations in pediatric urolithiasis including large fluid intake and dietary modifications in each stage of these lines of treatment.

If the patient had spontaneously passes a stone or a stone was acquired as a result of ESWL and/or surgical procedures, a stone analysis should definitely be made. After an exact diagnosis is made according to the results of the stone analysis and metabolic analysis, a metaphylaxis should be given as mentioned above (60).

In conclusion; urolithiasis, which is common also in our community in the pediatric and adult age groups, requires detailed examinations. Delays in diagnosis and treatment may lead to serious consequences that may develop into renal failure. Today, dietary modifications should be offered to families who have children with urolithiasis. Stone analyses are mandatory and metabolic analyses with urine evaluations are required. Metabolic abnormalities are common in pediatric urolithiasis and they are also responsible for stone recurrences. Recurrence of stones and renal damages may be prevented with the help of early diagnosis, detailed metabolic examination as well as appropriate follow-up and treatment protocols. In view of all these, pediatric urolithiasis needs more interest relevance.

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CHAPTER XXIII

ROLE OF EMBOLIZATION IN HEMOPTYSIS TREATMENT

*Yilmaz Onal**

Introduction

Hemoptysis is the blood that comes from the lower respiratory tract by coughing. Mild to moderate hemoptysis is a common problem that usually responds to treatment of the underlying pathology. However, various diseases can cause massive hemoptysis (MT) which needs more aggressive therapy. The term 'MT' describes hemorrhages of 300-600 ml and more over a 24 hour period, and the mortality risk is reported to be 50-85% in these cases only when conservative treatment is performed (O'Dell, 2017: 263-265). Therefore, it is important to reveal the underlying cause and the source of bleeding, especially in cases with MT. Most common causes include bronchiectasis, chronic obstructive pulmonary disease, tuberculosis and malignancy (**Table 1**).

Bronchial artery embolization (BAE) is considered the treatment of choice in massive and recurrent hemoptysis. It is essential to know the anatomy of the bronchial arteries and collateral vessel pathways to figure out the pathophysiology of bleeding. For embolization, various embolic materials can be used, such as coils, microspheres, polyvinyl alcohol (PVA) particles or liquid agents (Mejía, 2016: 215-224).

The purpose of this chapter is to provide information about the pathophysiology and causes of hemoptysis, as well as embolization techniques to be used, embolizing agents and complications in treatment.

Pathophysiology

The lungs have a double arterial supply consist of pulmonary and bronchial arteries. The pulmonary arteries are responsible 99% of the arterial blood supply whereas bronchial arteries are for remaining 1% (Bruzzi, 2006: 3-22). Bronchial arteries are the most common source of bleeding in hemoptysis. In many acute and chronic lung diseases, the pulmonary circulation is reduced or occluded at the level of the pulmonary arterioles because of hypoxic vasoconstriction, intravascular thrombosis, or vasculitis. The reduced pulmonary arterial perfusion can lead to increase in bronchial arterial circulation. As a result bronchial arteries proliferate, enlarge, and become prone to rupture into the bronchial airways, causing

* (MD); Fatih Sultan Mehmet Training and Research Hospital, Radiology Department e-mail: dronalylmz@gmail.com

hemoptysis.

Causes of Hemoptysis	
Infection	Tuberculosis
	Fungus
	Chronic pneumonia
	Abscess
Cystic Fibrosis	
Bronchiectasis	
Neoplasm	
Trauma	
Anticoagulation	
Pulmonary arteriovenous malformation	
Vasculitis	
Pulmonary embolus	
Pulmonary hypertension	
Mitral stenosis	
Congestive heart failure	
Congenital	

Table 1: Underlying pathologies of hemoptysis.

Imaging

Diagnostic modalities for hemoptysis include radiography, bronchoscopy and Computed tomography (CT) of the chest (Hsiao, 2001: 861-867; McGuinness, 1994:1155-1162). Conventional radiography is a basic study but it may be useful in diagnosing and localizing pneumonia, tuberculosis or lung cancer. But radiographic findings are normal or nonlocalizing in 17%– 81% of patients with hemoptysis (Abal, 2001: 548-552). Hirshberg et al. showed that radiography was helpful in diagnosis in only 50% of patients in a study in which 208 hemoptysis patients retrospectively evaluated (Hirshberg, 1997: 440-444).

Bronchoscopy has long been considered by clinicians to be the primary method for diagnosis and localization of hemoptysis (Dweik, 1999: 89-105). It is especially important in the diagnosis of central bronchial lesions. It has been reported that, it can help to localize bleeding in 49%–92.9% of patients with hemoptysis (Abal, 2001: 548-552). Bronchoscopy has some disadvantages in the diagnosis and localization of massive, active hemoptysis. It is difficult to localize the bleeding site with bronchoscopy in patients with MT because of excessive blood in the bronchi.

The role of CT in the assessment of patients with hemoptysis has been approved (Naidich, 1995: 1-12). In multislice CT, alveolar bleeding is observed as an area in ground-glass density and its location can be evaluated. It is important to localize the bleeding area to guide both surgery and endovascular treatments. Bronchiectasis, chronic fibrotic changes, acute or chronic infections (tuberculosis, aspergilloma), tumoral lesions, edema and infarct signs can be detected in the lung parenchyma. CT may demonstrate lesions that may not be visible on conventional radiographs and contrast enhanced CT may help detect vascular lesions that can cause MT. CT findings can suggest a specific diagnosis in 50% of patients in whom bronchoscopy findings are nondiagnostic and in 39%-88% of patients in whom chest radiographs are nondiagnostic. CT can also help to localize the site of bleeding in 63%–100% of patients with hemoptysis (Millar, 1992: 39-44). It has been stated that CT and bronchoscopy are not competitive but complementary tools for evaluating patients with hemoptysis.

Bronchial artery anatomy

The bronchial arteries supply the trachea, extra and intrapulmonary airways, bronchovascular bundles, nerves, regional lymph nodes, visceral pleura, esophagus as well as the vasa vasorum of the aorta, pulmonary artery and pulmonary vein.

Interventionalists who perform BAE should have proper knowledge of bronchial artery anatomy. The bronchial arteries have variable anatomy in terms of origin, branching pattern, and course. They originate from the descending thoracic aorta, most commonly between the levels of the T5 and T6 vertebrae. 20%, the bronchial arteries have an aberrant origin and may arise from the subclavian, brachiocephalic, internal mammary, thyrocervical trunk, inferior phrenic, and pericardio-phrenic arteries or from the abdominal aorta. In 10%, the bronchial arteries arise from other segments of the descending thoracic aorta, and aortic arch (Stoll, 1988: 263-269).

Digital subtraction angiography

In angiographic imaging of bronchial arteries, after accessing the femoral artery, diagnostic catheter is advanced to descending aorta with the help of 0.35inc guidewire. A variety of different catheters should be available for optimal selective bronchial arterial catheterizations and may include Cobra, Simmons or Headhunter catheters. At the T5-T6 level, the aortic wall is searched with catheter tips and the bronchial arteries are attempted to be selectively catheterized. In cases where bronchial arteries cannot be catheterized, origin points can be seen with flush aortography by pigtail catheter. Opacification of bronchial arteries during selective angiography is achieved by hand injection of a non-ionic contrast medium. The rate and volume of injection are dependent on the size of the bronchial artery and the concurrent acquisition of images. In the images taken after selective catheterization, these features can be considered pathological; enlarged bronchial artery (>3 mm at orifice), tortuous bronchial arteries with parenchymal hypervascularity, bronchial artery aneurysms, bronchial artery to pulmonary vein shunting, active extravasation. Coaxial microcatheters allow superselective catheterization in cases where a secure catheter position cannot be achieved with a diagnostic catheter. Thus, inadvertent embolization can be avoided.

Endovascular therapy

For the first time in 1968, Newton and Adams reported that they performed selective transcatheter embolization to treat a spinal angioma (Newton, 1968: 873-976). Later, this form of treatment has found use in a wide range of diseases in various localizations, which are manifested by bleeding, to obstruct the feeding vessels. In 1975, Wagner et al. reported that in a MT case, they clearly demonstrated the source of bleeding that they could not detect by bronchoscopy but by pulmonary angiography (Wagner, 1975: 121-123). On the other hand Fellows et al. (Fellows, 1975: 551-556) performed selective bronchial arteriography in a case of cystic fibrosis with MT. They compared their findings with bronchoscopy results and suggested that bronchial arteriography is an effective diagnostic method in cases where bronchoscopy cannot be performed or is not sufficient to determine the source of bleeding.

Among the agents that can be used in embolization, PVA, microspheres, coil, cyanoacrylate or etilen vinyl alcohol copolymer can be used. However, PVA is important for filling the microvascular bed that causes bleeding and preventing collaterals that will develop. In addition, in case of re-bleeding after PVA embolization, it is important to be able to repeat the embolization from the same artery due to the absence of proximal vessel occlusion.

It is important to avoid embolic material that can pass through abnormal

bronchopulmonary anastomoses, as there is a risk of pulmonary infarction via bronchial artery-pulmonary artery shunts or systemic artery embolization via bronchial artery-pulmonary vein shunts. Bronchopulmonary anastomoses of up to 325 mm have been demonstrated in an anatomical study; embolic materials less than this diameter should not be used in BAE (Chun, 2010: 240-250). Embolic material should always be mixed with contrast medium so that it can be carefully observed with fluoroscopy (Marshall, 1997: 1221-1227).

With BAE, the aim is to reduce the systemic arterial pressure at fragile vessels in the inflammatory tissue. However, the fact that the agent used for the purpose of embolization should not pass bronchopulmonary anastomoses is the main point to be considered in preventing the development of pulmonary infarction. In addition, the embolic agent should not reach normal peripheral branches that supply bronchi, esophagus, pulmonary artery or aortic wall which can end with necrosis in these areas. On the other hand, permanent proximal embolization results with the development of distal collaterals in a short time, while also eliminating the possibility of reaching the source of bleeding in the next session. Considering these reasons, PVA particles with the opportunity to use in different sizes are the most suitable agents recommended for selective embolization of bronchial artery and systemic collaterals (**Figure 1**).

Pulmonary artery can be the source of bleeding in 5% of hemoptysis cases. Rasmussen aneurysms, which are often described together with chronic tuberculosis, may also develop as a complication of lung abscess, bronchogenic carcinoma or invasive aspergillosis and cause erosion in the adjacent pulmonary artery wall along with chronic inflammation. In the literature, pulmonary angiography is recommended in ongoing hemoptysis, especially in the presence of fibrocavitary tuberculosis despite the embolization of the bronchial artery and systemic collaterals, and it is reported that effective embolization can be performed using a “coil” following the detection of the bleeding source.

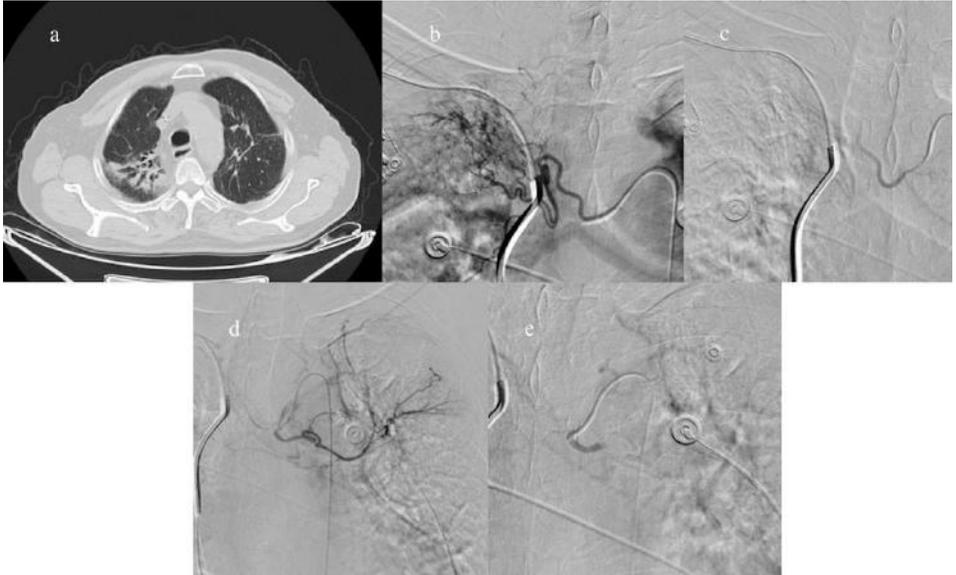


Figure 1: In a non-contrast CT examination performed due to MT of a 55-year-old male patient, bronchiectasis areas and fibrotic changes of both lungs in the upper lobe, more apparent on the right, are noteworthy (a). In the right bronchial artery selective imaging, hypertrophy in the bronchial artery, and marked hypervascularization in the lung parenchyma was detected, embolization was performed with 500-700 μ m particle. The control image showed that the bronchial artery was completely occluded. Left bronchial artery angiogram shows, contrast staining in the parenchyma, which indicates pathologic hypervascularization again. We observe that the bronchial artery is completely closed after particle embolization. Note the pacemaker of the patient.

Complications

The most common complication is chest pain with an incidence of 1.4-34.5% (Woo, 2013: 594-602; Fruchter, 2015: 55-60). The second most common dysphagia is 0.7-30%. Both complications are mostly temporary, they are considered to be ischemic and are treated palliatively. Spinal cord ischemia and related transverse myelitis, which are among the most feared complications, have been reported in 1.4-6.5%. Dorsal and ventral radicular spinal arteries can be visualized on angiography before embolization. Involuntary embolization of these branches does not lead to spinal cord ischemia and their visualization is not considered a contraindication for BAE (Uflacker R, 1985: 637-644). However, monitoring of the anterior spinal artery (Adamkiewicz artery) on angiographic images is significant for the risk of spinal cord ischemia.

Anterior spinal artery angiography can be distinguished by its typical 'hairpin' appearance. In this case, if possible, the distal of the anterior spinal artery should be superselectively catheterized with microcatheter and efforts should be made to avoid reflux by careful, slow injection. The anterior spinal artery cannot be seen in the CT sections evaluated before the procedure, so it is important to carefully examine the angiographic images taken in the same session as the BAE.

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CHAPTER XXIV
**PROSTATIC CALCULI: ETIOLOGICAL FACTORS,
DIAGNOSIS AND TREATMENT**

Mehmet Balasar & Gökhan Ecer** & Arif Aydın***
Mehmet Giray Sönmez *****

Introduction

Prevalence of prostatic calculi has increased due to increasing radiological imaging for detecting urological problems. Although prostatic calculi are common in daily urology practice, data on their prevalence in general population is inconclusive. (*Kim WB, 2011*) No systematic research study using suitable methods is available yet and standard prostatic calculi have not been defined. It has been accepted that the prevalence of prostatic calculi increases in proportion to age without generally causing clinically specific symptoms. It is considered that the present symptoms are mostly lower urinary system related. (*Kim WB 2011, Park SW 2010*) Prostatic calculi are mostly noticed during X-ray graphics, pelvic/transrectal ultrasonography (TRUS), Computed tomography (CT) scanning and frequently during prostate transurethral resections (TUR-P). (*Köseoğlu H, 2010*) Recently, as TRUS application increases, more researches have been made on prostatic calculi and some reports are available on the shape and composition of calculi. On the other hand, prevalence, formation mechanism, relation with benign or malign prostatic diseases and the clinical significance of the calculi are still unknown. In this book section, we aimed to present the details of the etiology, anatomy, clinic, classification and treatment of the prostatic calculi with the current literature.

1. Anatomy of the prostate and formation of prostatic calculi

BPH is the most common benign neoplasm in males. While prostate gland was considered and classified to be formed by different lobes before, zonal anatomy was started to be widely used after the definition by Mc Neal et al. According to this, the glandular tissue forming the prostate gland can be examined in three different sub-zones (Figure 1). While peripheral

* (Assoc. Prof.Dr.); Necmettin Erbakan University, Meram Medical Faculty, Department of Urology, Konya, TURKEY. drbalasar@gmail.com

** (M.D.); Necmettin Erbakan University, Meram Medical Faculty, Department of Urology, Konya, TURKEY. ecergokhan@gmail.com

*** (Asst Prof. Dr.); Necmettin Erbakan University, Meram Medical Faculty, Department of Urology, Konya, TURKEY. aydinarif@gmail.com

**** Assoc. Prof. Necmettin Erbakan University, Meram Medical Faculty, Department of Urology, Konya, TURKEY. drgiraysonmez@gmail.com

zone forms nearly 70% of prostate in a young adult, central zone forms 25% and transitional zone forms 5%. These zones have separate discharge canals.

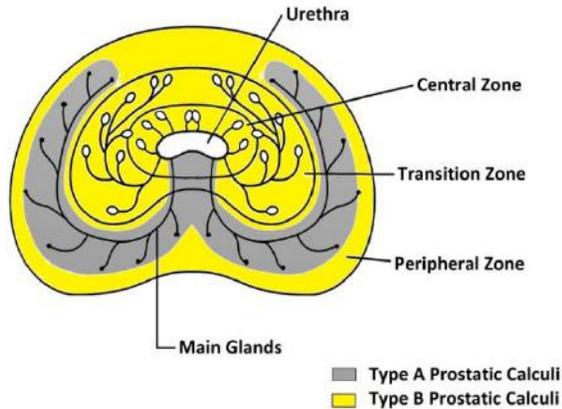


Figure 1: Classification of Prostatic Calculi Based on Localization

Although different theories are asserted with prostatic calculi analyses in etiology, a definite phase has not been identified. In the first study on this subject, Thomas claimed that prostatic calculi formation was a result of corpora amilacea calcification. (*Thomas BA, 1927*) Kirby et al. showed that intraprostatic urinary reflux and reflux urine components may contribute to prostatic calculi formation, In the study by Kirby et al, carbon particles given inside the bladder right before prostate TUR were shown in resected prostate tissue. This shows that reflux to intraprostatic and ejaculator canals during urinary discharge is not a rear phenomenon. The possibility of this condition is higher in obstructions caused by a mild urethral stricture or external sphincter spasm as in prostatitis. (*Kirby RS, 1982*) In their studies, Balasar et al. showed that most calculi formations in non-contrasted CT classification of prostatic calculi start from the urethral opening in peripheral zone main intraprostatic canal and then grew towards the intraprostatic canal proximal. This supports intraprostatic urinary reflux theory. (*Balasar M 2015, Balasar M 2019*) (Figure 1)

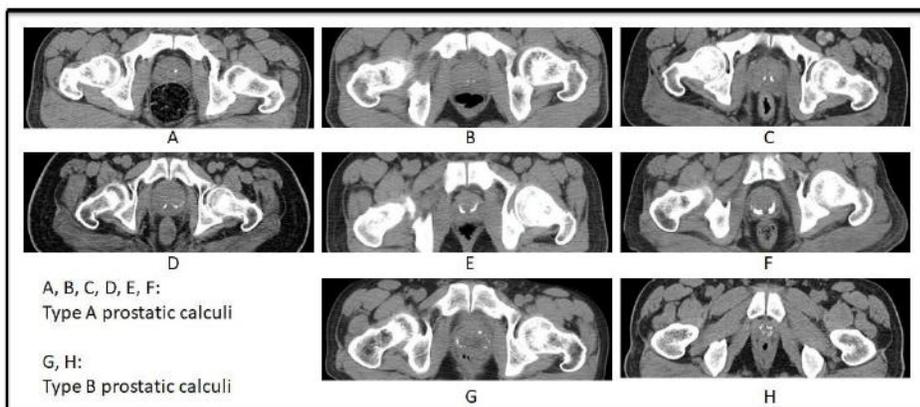


Figure 2: Classification of Prostatic Calculi Based on CT images

Inflammatory procedures in prostate may cause prostatic calculi formation as the result of the accumulation of hydroxyapatite crystals in corpora amilacea in the intraprostatic canals due to factors such as intraprostatic reflux and their mineralization with calcium. As also proven in previous studies, the main constituents of prostatic calculi are calcium phosphate or carbonate-apatite and calcium oxalate and hydroxyapatite. (*Park SW 2010, Sutor DJ 1974, Torres Ramirez C 1980*)

Prostate calculi are frequently detected in acute or chronic prostatitis patients, this may be the cause of chronic prostatitis. (*Lee SE, 2003*) Some authors claimed that prostatic calculi form as a result of calculi formed due to chronic infection and inflammation of prostate secretory canal and acini. (*Torres Ramirez C 1980, Klimas R 1985, Meares EM 1974*) Based on histopathological studies, while calculi in prostatic acini are related to chronic inflammatory infiltrate (lymphocytes and histiocytes), bigger calculi form in intraprostatic canals enlarged due to intraprostatic epithelium loss and the resultant fibrosis. (*Moore RA, 1936*) However, blockage of intraprostatic canals, bacterial biofilm production and dominant chronic inflammation may cause calcification at this stage. Also, prostatic calculi studies show that there is a relationship between prostatic bacterial infections such as *Escherichia coli* and *Pseudomonas* infections and prostatic calculi. (*Meares EM 1974, Eykyn S 1974*) But there is still confusion on whether prostatic calculi are the result or cause of infection.

In addition to increasing calculi formation in urinary system, hypercalciuria may be one of the underlying causes of calculi formation in intraprostatic canals. (*Sutor DJ, 1974*) In a pediatric study, prostatic calculi cases were found to be related to hypercalciuria and increased urine calcium/creatinine ratios. (*Al-Taheini K, 2007*)

Urine acidity is a fact known to increase calculi formation in the whole urinary system. It was shown that acidic urine is related to uric acid and calcium calculi. In patients with renal calculi in the studies performed in our clinic and in metabolic evaluation with more frequent prostatic calculi, it was observed to have a strong connection with high serum uric acid. While the percentage of prostatic calculi was 44.4% in 133 urinary system calculi patients included in the study, it was detected as 21% in the control group with 100 participants. (*Balasar M 2015, Balasar M 2019*)

2. Prostatic calculi prevalence and symptoms

In literature, differences are observed in prostatic calculi incidence in different societies due to different methods used for defining and detecting prostatic calculi. In a postmortem study performed with microradiographic examination, intraprostatic calcification was reported in up to 71% of the subjects. (*Thomas BA, 1927*) Studies were published especially on intraprostatic calcification detected with TRUS. Prostatic calculi was reported in 198 of 486 cases (40.7%) with a mean age of 61.9 (29-89) years examined during TRUS. (*Lee SE, 2003*) A similar study detected that 47.2% of males under 50 years of age and 86% of males over 50 years of age had prostatic calculi in 612 TRUS scannings following different urological complaints. (*Bock E, 1989*) In another TRUS-aided study, prostatic calculi was detected in 799 of 1.563 cases (51.1%) with a mean age of 49.5 (40-59 years). (*Kim WB, 2011*) Apart from TRUS, studies performed with transabdominal ultrasonography and CT were also published. In the study performed with transabdominal ultrasonography, prostatic calculi presence was reported as 7.35% in an adult group with a mean age of 40.9 (21-50 years). (*Geramoutsos I, 2004*) (TRUS was performed only in 16.83% of the cases)

In the study performed in our clinic, 233 patients who had non-contrasted CT imaging which is safer than US imaging were retrospectively examined and prostatic calculi was detected in 80 patients (34.3%) with a mean age of 44.8 (30-60 years). (*Balasar M, 2015*) Despite all these difference, it is generally accepted that prostatic calculi prevalence is rare in children and less in individuals under 40 years of age and it is accepted to be common especially in individuals over 50 years of age. (*Klimas R, 1985*)

BPH related prostatic calculi are generally asymptomatic. Chronic prostatitis related prostatic calculi can be closely related to lower urinary system symptoms. Leader et al stated that small prostatic calculi were clinically insignificant and formed physiologically with aging. (*Leader, AJ 1958*) They claimed that they can be considered as the symptom of an underlying disease like BPH although they may have clinical symptoms. On the other hand, in chronic prostatitis other than BPH, it may be a

permanent inflammation source in patients with pelvic pain. Prostatic calculi doesn't only serve as a colonizing area for infectious bacteria but also induces the closure of prostate secretory canals and may be resist antibiotics and other drugs. These results are supported by the findings of Shoskes et al on inflammation, bacteria colonization and symptom duration of prostatic calculi in patients with chronic pelvic pain syndrome. (*Shoskes DA, 2007*)

Kim et al didn't observe any difference in the evaluation of prostatic calculi patients with International Prostate Symptom Score. (*Kim SH, 2009*) On the other hand, Cha et al stated that when periurethral area contains prostatic calculus/calculi, it may cause less urinary flow and urinary retention may occur due to a big prostate. (*Cha WH, 2008*) In 2016, Park et al. used calculi load as a demonstrator to evaluate the relationship between prostatic calculi and lower urinary tract symptoms (LUTS) for the first time. While claiming that prostatic calculi presence wasn't an independent predisposing factor for mild or severe LUTS, they also claimed that increased prostatic calculi load is significantly related to storage symptoms in males. (*Park B, 2017*)

A significant relationship was reported between chronic prostatitis and sexual function disorders such as erectile dysfunction (ED), decreased sexual drive and premature ejaculation. On the other hand, the number of studies investigating the relationships between prostatic calculi and sexual function is limited. Again some researchers found that chronic prostatitis patients commonly had pain during ejaculation. Presence of prostatic calculus quite likely has a negative effect on ejaculatory functions of males or prostatic calculus may be increasing ejaculation problem in chronic prostatitis patients. However, no study investigating the relationship between prostatic calculus and male ejaculation function is available. (*Cao JJ, 2018*)

3. Prostatic calculi and prostate cancer

There is a limited number of studies on the relationship between prostatic calculi and Prostate Cancer (PCa) and the roles in PCa formation have not been clearly understood. Griffiths et al. US images of 221 patients diagnosed with PCa were examined and a concurrence ratio of 63% was observed between PCa and prostatic calculi. (*Griffiths GJ, 1987*) Hwang et al. examined the medical records of 417 patients who had TRUS guided prostatic biopsy and reported that patients diagnosed with PCa had prostatic calculi more commonly and when proven PCa was correlated with high Gleason score. (*Hwang EC, 2010*) In another study, Smolski et al. found that 78.1% of peripheral zone calculi were PCa related. (*Smolski M, 2015*) Yücel et al. showed a relationship between PCa and patients with prostatic calculi over 3 mm. (*Yucel C, 2018*) But contrary to the studies

mentioned above, Woods et al. examined the histopathological materials of 266 radical prostatectomy and 10 cystoprostatectomy cases and claimed that prostatic microstones were less related to PCa. (*Woods JE, 1998*) But, more studies are needed to examine the relationship between prostatic calculi and PCa in more detail.

4. Prostatic calculi and infertility

It was shown that chronic infection and inflammation observed in prostatic calculi have the potential of degrading the secretion ability of prostatic epithelium and this may cause the decreasing of semen quality and even after the removal of pathogens with antibiotics, semen parameters didn't return to normal in half of the patients. (*Domes T 2012, Ludwig M 2002*) Due to the formed biochemical damage of sperm cell membrane, it may cause spermiocyte fertilization failure even though the sperms have a solid structure. Another possible cause is the fact that symptomatic or asymptomatic chronic inflammation may damage fertility by causing channel obstructions and germ cell loss by affecting both testicles. (*Lu Y, 2013*)

5. Diagnosis in prostatic calculi

Prostatic calculi are generally detected through pelvic US and TRUS. Harada et al. separated prostatic calculi into two groups as Type A (small and dispersed ecos) and Type B (large and multiple ecos) using TRUS. (*Harada K, 1979*) (Figure 3)



Figure 3: USG image of prostatic calculi

Prostatic calculi can also be easily detected by CT scanning. Balasar et al classified prostatic calculi as Type A when they were located inside the

main prostate canals and as Type B when dispersedly located inside the prostate based on their locations in prostate on non-contrasted CT (Figure 2). On non-contrasted CT images, a small A type prostatic calculus is noticed first on prostate canal holes opening to urethra and it was detected that prostatic calculi grow bigger towards the periphery on main prostate canals with a shape resembling crescent. It was observed that prostatic calculi classified as Type B had an irregular distribution in prostate (Figure 1). Among all Prostatic calculi, while Type A located ones were detected with a ratio of 75%, Type B located ones were detected with a ratio of 25%. This supports the theory of calculi formation as the result of intraprostatic reflux which may cause inflammation and infection from related mechanism with prostatic calculi formation. (*Balasar M, 2015*)

Effects on prostatic calculi on serum prostate specific antigen (PSA) have not been completely proven. Some researchers reported a positive relationship between histological prostatitis and increased serum PSA and stated that PSA increases are related to the degradation degree of normal prostate histological structure by inflammatory cells. Lee et al were the first individuals researching the relationship between prostatic calculi and serum PSA in males with Prostate cancer or prostatitis and no relationship was found between prostatic calculi and high PSA levels. (*Lee SE, 2003*)

6. Treatment of prostatic calculi

Treatment is rarely recommended and follow-up can be considered since most prostatic calculi are asymptomatic and cause no complications. In the presence of acute or chronic prostatitis or severe symptoms, antibiotics constitute a valid treatment option. While alpha blockers can be used for the treatment of prostatic calculi causing LUTS, surgical resection is an alternative when large prostatic calculi fall in urethra or bladder and cause significant LUTS. (*Harada K 1979, Bedir S 2005, Dessombz A 2012, Goyal NK 2013*) (Figure 4) Based on the chemical constituents in etiology, treatments like potassium citrate or sodium thiosulphate can be given to overcome prostatic calculi but no study has been made to validate their efficiency.



Figure 4: Image of resected prostatic calculi

Conclusion

The relationship between prostatic calculi and prostatitis is controversial but the prevalence of prostatic calculi is reported to be high. Additionally, prostatic calculi doesn't only lengthen the duration of the LUTS but may also decrease the success of antibacterial treatment in the patients and this makes us consider that long-term antibiotic treatment is required in chronic prostatitis patients. Although LUTS is more severe in prostatic calculi patients, it is not an independent predictive factor for LUTS. But some studies paradoxically reported that average or large prostate calculi constitute an independent risk factor for average or severe LUTS. It was shown that PSA serum level isn't affected by prostate calculi and calculi presence is not related to increased PCa risk. On the other hand, the correlation between prostatic calculi in peripheral zone and PCa has been found to be statistically significant. The relationship between prostatic calculi and Gleason scores is also controversial. Some new studies found that it can play a significant role in sexual intercourse disorder in chronic prostatitis patients with prostatic calculi. It was recently reported that severe LUTS following TRUS-Biopsy in prostatic calculi patients may increase urinary retention and hematospermia incidence. Treatment is often unnecessary for prostatic calculi, but if a person has chronic pain or difficulty in urinating, it can be easily removed using transurethral electroresection or holmium laser. Despite all these results, higher number of detailed studies is required to ensure that the clinicians completely understand prostatic calculi which can easily go unnoticed.

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