

# CURRENT ADVANCES IN MEDICINE - II

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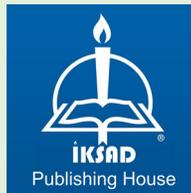
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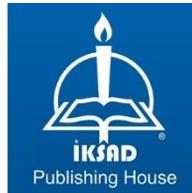
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## **CONTENTS**

### **PREFACE**

*Bayram KELLE, Assoc. Prof. Dr. ....1*

### **CHAPTER 1**

#### **FORMS OF RAISING CHILDREN**

##### **PRIVACY EDUCATION FOR CHILDREN**

*Dilek DEMİR , Assist. Prof. Dr*

*Çiğdem Müge HAYLI , Assist. Prof. Dr.....3*

### **CHAPTER 2**

#### **STAINING OF MICROORGANISMS IN PATIENT SPECIMENS OR PRODUCED CULTURES IN MEDICAL MICROBIOLOGY LABORATORY**

*Dilek VURAL KELEŞ, Assist. Prof. Dr.....17*

### **CHAPTER 3**

#### **SPICES, THEIR USAGE in MEDICAL APPLICATIONS, AND THEIR ANALYSIS with DIFFERENTIAL SCANNING CALORIMETRY**

*Bircan DİNÇ, Assist. Prof. Dr.*

*Tahsin ERTAŞ MSc.*

*Recep ÜSTÜNŞOY, MSc.....31*

## **CHAPTER 4**

### **S100B AS A BIOMARKER IN TRAUMATIC BRAIN INJURY**

*Ali Kemal ERENLER, Assoc. Prof., MD.*

*Mehmet YILMAZ, MD.*

*Türker YARDAN, Prof., MD*.....55

## **CHAPTER 5**

### **NECK PAIN**

*Özlem KALEOĞLU ASLAN, MD*.....67

## **CHAPTER 6**

### **SACRAL STRESS FRACTURES**

*Özlem KALEOĞLU ASLAN, MD*.....79

## **CHAPTER 7**

### **GREATER TROCHANTERIC PAIN SYNDROME**

*Gökhan BAŞAR, MD.* .....89

## **CHAPTER 8**

### **PROBIOTIC – PREBIOTIC and HEALTH**

*Nazan TOKATLI DEMIROK, Assist.Prof.Dr.*.....101

## **PREFACE**

This book contains 7 important topics. In addition to some common orthopedic problems, there are sections on biomarkers for traumatic brain injury, which is an important health problem, child education, microbiology, and alternative treatments.

We hope that this book will be useful to all our colleagues working in the field of health. We would like to thank the valuable authors who contributed to the creation of the book and devoted their valuable time to this purpose, and the family of İKSAD Publishing, who made it possible to publish the book.

Assoc. Prof. Dr. Bayram KELLE<sup>1</sup>

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**CHAPTER 1**

**FORMS OF RAISING CHILDREN**

**PRIVACY EDUCATION FOR CHILDREN**

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## **1. INTRODUCTION**

The position of the individual in the society has been changing rapidly in the last century, depending on the economic, social, scientific, technological and political developments. Throughout history, the human spirit and society have evolved and changed together. In this development, the mediator element that establishes the bond between the individual and the society is the child-rearing styles of the parents, who are an element of the society. Child-rearing moods (styles) differ from each other in every society, in every culture and in every historical period. These differences on the other hand, it is closely related to child abuse and neglect, in other words, to what extent children are traumatized by their families (Çelikkol, 2002; DeMause, 1998).

With the increase in the use of technology and social media in recent years, we see that the incidents of violence and sexual abuse against children are increasing day by day. As these problems worry every segment of society, they also raise the question of what we can do to raise awareness of our children. In this context, one of the most important points we need to know is that sexual education is not privacy education and that privacy education should be given in the family at the age of 0-6 (Livingstone ve Sarı, 2020).

The aim of this study is to explain the child rearing styles and the importance of privacy education in children.

## **Child Rearing Styles**

### **Who is the child?**

A child, a person between infancy and adolescence. Generally, childhood begins after the ability to speak and walk; It is accepted that the childhood period ends with the adolescence period, when sexual development begins. But these definitions are relative and do not have strict limits.

Biologically, a child (plural children) is a human being between the stages of birth and puberty or between the developmental period of infancy and puberty. The legal definition of child generally refers to a minor, otherwise known as a person younger than the age of majority. Children generally have fewer rights and responsibilities than adults. They are classed as unable to make serious decisions (O'Toole, 2013; TheFreeDictionary, 2022).

## **Child Rearing Patterns**

### ***Defining Childrearing Attitudes***

The child learns to express himself and to be a self-directed individual from his family. Especially parental attitudes play a fundamental role in the formation of personality. Research on the child-rearing characteristics of children's families necessitated the examination of the parent-child relationship (Erginbay, 2014).

### ***Why is Child Rearing Important?***

Since every child has the right to a good life, it is important for the future to grow up healthier and more confident in every aspect. This process differs according to the child's upbringing (Şükrü ve Şençiçek, 2015).

### ***Child Rearing Patterns***

Healthy families want to raise responsible children who look to the future with confidence. In this context, parenting attitudes of parents are important in shaping the future life of the child. Unrest in the family affects children negatively. There is no single, real way to raise a child. Studies have shown that every family has a unique child-rearing style.

In general, child-rearing attitudes can be classified as follows;

1. Democratic Attitude
2. Authoritarian Attitude
3. Free Attitude
4. Overprotective Attitude
5. Overly Tolerant and Loose Attitude
6. Unbalanced Attitude (Şanlı ve Öztürk, 2012).

***Democratic Attitude:*** It is an attitude in which control and attention are carried out in balance. As an attitude, everyone is given importance and the personality is respected. What is wanted to be done is not enforced, but applied in accordance with the rights and rules. Democratic parents evaluate the child's wishes on a rational level.

***Authoritarian Attitude:*** It is the attitude of the parents that exposes the child to a strict discipline and expects the child to strictly obey the rules. Children are expected to abide by the rules set by their parents. Parents who practice this attitude do not want their children to be independent. Children who grow up in this family are afraid and have trouble acting autonomously. The child has problems in

communicating with the environment and cannot develop a sense of self-confidence in the face of the harsh and rigid attitude of the family.

***Free Attitude:*** It is the attitude of parents who do not take sufficient responsibility for their children, are indifferent and insensitive to needs. Directing or managing children is out of the question in this type of family. Parents generally show a careless attitude.

***Overprotective Attitude:*** It is the attitude of the parents, who are attached to the child with love and are constantly protected. It is an attitude where all the needs of the child are met by the family. Parents undertake all the responsibilities that the child should gain in the process. In this attitude, the child's individualization process is ignored. The family does not allow the child to express himself in any field. The child sees himself/herself as a part of the family throughout his/her life as he/she is constantly controlling and intrusive.

***Overly Tolerant and Loose Attitude:*** Overindulgent parents have poor control and high acceptance of the child. During the child's personal and social life, the family does not interfere in anything. The child makes decisions based on his own will. The child, who cannot gain discipline in early childhood, will have problems both himself and his mother and father in the future. Overly pampered children do not adapt to social life easily because they are not accustomed to recognizing rules.

***Unbalanced Attitude:*** The parents behave quite inconsistently in the decision stages about the child. In any event, the father may react negatively to a situation that the mother welcomes. There is a clear understanding of discipline of the mother and father towards the child. Children who grow up in such families may experience role conflict in their future lives (Şanlı ve Öztürk, 2012; Yavuzer, 2005; Kulaksızoğlu, 2004).

## **2. PRIVACY EDUCATION FOR CHILDREN**

### **What is Privacy?**

It is the sum of all the feelings and thoughts that the person does not want to share with others in her life and private life (TDK, 2022). Privacy as a phenomenon; private, not open to everyone, not desired to be heard, seen and known. It is defined as an area closed to foreigners, which is not included except for the conditions and can be discussed in three different sections. These; spatial privacy, individual privacy and information privacy. Spatial privacy, close physical encompasses the protection of the environment. Individual privacy, such as physical observations or attacks on one's moral values. It covers protection against unfair interference. Information privacy is the collection, storage, processing and use of personal data. Control of distribution was owned by the individual (Budak, 2018; Güneş, 2017; Çetin, 2015).

### **Privacy Training**

Privacy education is a “respectability” that directly touches the child's personality and soul, and nourishes both the child and his/her environment (Güneş, 2015). “Privacy education is more about awareness of oneself and other people's private/private space besides

sexual information to arrive, to protect his private space in social life, to respect the privacy of other people, to be between himself and his environment.

### **How Do I Know If My Child Is Ready for Privacy Education?**

Although it is an education that is acquired within the family between the ages of 0-6, education is basically given at the age of 3 and after. However, education should continue until adolescence. The biggest reason for this is that the information needs of the child who changes and develops physically and cognitively will increase in every period. The scope of the questions will expand, the child's need to learn more details will increase. During this period, the family should be able to give the child age-appropriate answers (Şahin, 2003).

Families who have difficulty in talking about sexuality should not turn the child away, ask for some time and talk to the child when they are ready. The family should benefit from scientific and fact-based information while giving information to the child. Privacy education in children is often given when questions about sexuality begin to be asked. However, the main thing to pay attention to is how often the child stays away from the primary caregiver in the care of others (Çakır, 2015).

### **What Does Child Privacy Education Include?**

Includes information such as setting healthy limits” (Akcan, 2018). In order for the child to develop in a healthy way and to protect himself, families should teach privacy to their children.

In the pre-school period, privacy education should be given to the child correctly. How parents will give this education, what to pay attention to, how to answer children's questions so that They should have knowledge about what they will give (Eminarslan et.al, 2017). Privacy for children by the Ministry of Family and Social Policies. In the report, which was prepared with the aim of what can be done to raise awareness, it is stated that the awareness of privacy is it was emphasized that it should be brought to the child from the beginning give (Eminarslan et.al, 2017).

Families should be encouraged to provide privacy education from a young age to children who will become adults of the future.

Undoubtedly, its role is very important. When the literature is examined, it is seen that there are not enough studies on privacy education in children. is seen. However, examining the views of families on privacy education, training to be prepared for families. It is extremely important for direction (Çakır, 2015).

### **How Should Privacy Education be Given to Children?**

A child's awareness of privacy is born when he begins to notice, touch and wonder about his own body. During this period, the child may ask why their genitals are different from that of a friend or sibling of the opposite sex. The child “Where did I come from?”, “How was my brother?”, “Why do you have breasts, why does my father not?”, “Why does my father have a beard?” Ask questions about differences such as (Öcal, 2021).

The child's questions should be answered by the parent asking the question. While answering the questions, "Shame on you!, you are younger, you can ask them later." Reactions that will embarrass the child and cause him to withdraw should be avoided (Çalışkan, 2019).

The parent may not know how to answer the question. In this case, the child can clearly say, "I don't know how I can explain the answer to this question in a way that you can understand. I'll think about it/research and share it with you tomorrow." Such clear and real information should be given. Explanation must be given to the child within the promised time.

While raising the awareness of privacy, the child should be the right role model. Before giving privacy education, the right role model must be presented to the child. The child should not watch the family in the bathroom or toilet, and the parents should not dress or undress in the presence of the child. The child should be taught not to open closed doors without permission, and to knock on the door and wait for permission. Likewise, the child's own privacy should be respected when entering his room. Even if the door of the room is open, the family should ask if I can enter by knocking on the door before entering (Jago et.al., 2011).

Kissing and being kissed should also be with the child's request and permission. The child should not be pressured such as "let's kiss your uncle, kiss your aunt" or "let them kiss you". If the child wants to kiss someone else, he should kiss with his own consent and will. In the same way, permission should be asked from the child when kissing the child, including family members. The child should not be loved or kissed in ways that the child does not like. When the child is curious

about nudity, attention should be paid to domestic clothing as well. The child should not be allowed to walk around the house naked or in underwear. Like parents, their children should be ensured to comply with the dress code at home (Ünal, 2019).

Children should also be informed about who can see their private parts. “Don't show it to anyone but your parents!” When it is said, it will not allow the pediatrician or the teacher if he misses it. The child should be told where their private areas are. While doing this, pictures and dolls can be used. However, the family should not show it on themselves and on the child. Books and videos written in this context can also be used. Children should be told that these special areas should not be touched or seen by anyone other than their parents, doctor and teacher. If there is a relative other than the parents who are directly involved in the care of the child, that person should also join this list. The child should be taught to say "NO" when he/she dislikes a touch or kiss (Ünal, 2019; Altınsoy ve Erdoğan, 2022).

*As a result;* Children easily recognize negative emotions, irritating glances and touches, tone of voice, and messages expressing threat and danger. However, the child, who lives in the field of trust in the family, has difficulty in naming these negative feelings he notices when he opens up to the outside world without receiving education. The child does not like the one who harms him, he shows restlessness when he sees it. But he finds it difficult to explain the reasons for this feeling. The privacy education given by the family also teaches the child how to express these feelings. This information shared by the family makes the child feel that he can safely tell his family everything when faced with a threat.

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## CHAPTER 2

# STAINING OF MICROORGANISMS IN PATIENT SPECIMENS OR PRODUCED CULTURES IN MEDICAL MICROBIOLOGY LABORATORY

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## INTRODUCTION

Staining is a reaction of colouring agent on the chemical and structural components of microorganisms (Beveridge et al., 2001). Stains are composed of positive and negative ions and are divided into acidic, basic and neutral stains according to their pH levels. Many of the bacterial stains are basic stains, positively charged and also called cationic stain. They bind to negatively charged components (phosphate groups of nucleic acids (DNA and RNA) of the bacterial cells and the organism becomes directly stained (Bartholomew et al., 1952; Altındış et al., 2013). Acidic stains are negatively charged and can not stain bacteria due to their negatively cytoplasmic components. Acidic stains such as Indian ink and nigrosin are mainly used for background staining (Singh et al., 2022). Neutral dyes stain both acidic and basic components and are usually used in microbiology in staining tissue and blood smears, in order to better visualize some parasites and shaped elements of blood (Bartholomew et al., 1952).

The first step before the staining process is the preparation of a smear on a slide. A clinical sample or a culture is used to prepare the smear (Madison et al., 2001). To prepare the smear from the culture, first a small drop of water is placed on a clean slide and the bacteria taken by touching a single colony with a fire-sterilized and cooled inoculation loop is placed on the dry edge of the water drop on the slide, then spread homogeneously with circular movements with water and left to air-dry completely. If the smear is to be prepared from a clinical sample rather than a culture, the specimen taken with the help of a swab stick is spread on a clean slide and left to dry at room temperature (Harrigan et al., 1998; Günalp et al., 2003).

The second important step before the staining process is the fixation of the prepared smear on the slide. The purpose of this process is also to ensure that the microorganisms spread on the slide do not leave their places during washing and decolorization in the staining process in addition to preserve its morphology and structure. There are two methods is used to adhere bacteria to the slide, heat fixation or methanol fixation (Günalp et al., 2003; Haley et al., 1978; Jones et al., 1984).The most widely used fixation in microbiology is heat fixation. Before the staining process is started, heat fixation is made by the flame of a Bunsen burner 3 or 4 times at a distance that will not kill the bacteria, with smear side facing up, and left to cool (Altındış et al., 2013; Günalp et al., 2003).

## **1. GRAM STAINING**

Gram staining, which is the most widely used staining method in microbiology, was introduced to the medical world by the Danish bacteriologist Hans Christian Joachim Gram in 1884 (Madani et al., 2003). With this method, bacteria are stained in different colors according to the differences in their cell wall structures and examined in two groups as gram positive and gram negative according to the color they appear (Harvey et al., 2007). The smear, which is prepared as described above, is taken to the staining stand, firstly is stained with the crystal violet called primer stain for two minutes and washed (Beveridge et al., 2001; Altındış et al., 2013). At this stage, both gram positive and gram negative bacteria in the smear take up the stain and appear purple (Harvey et al., 2007). At second step, Lugol's solution called mordant is added on the smear for one minute, in order to ensure

that the crystal violets can better penetrate bacteria and do not flow during decolorization, and then washed (Smith et al., 2005). The third step is the decolorization stage. At this step, alcohol or acetone is added onto the slide for 15 seconds and washed. With the decolorization, the stains on the cell wall of gram negative bacteria are removed with the help of alcohol and the cell wall becomes colorless, while gram positive bacteria retain purple color (Madani et al., 2003; Harvey et al., 2007; Bilgehan et al., 1994). The last fourth step is the staining of colorless gram negative bacteria. For this process, carbol fuchsin (or safranin), which gives pink or red color to gram negative bacteria, is added to the smear for about 30 seconds and washed, and the slide is dried with bibulous paper and examined. When immersion oil is dripped onto the stained smear and viewed under a microscope, gram positive bacteria appear purple and gram negative bacteria appear pink or red (Altındış et al., 2013; Günalp et al., 2003; Bilgehan et al., 1994; Smith et al., 2005).

The procedure performed in the Gram staining method is given below, respectively.

On the fixed smear by heat fixation:

1. Apply crystal violet -wait for 2 minutes -wash with water
2. Apply Lugol's solution -wait for 1 minute -wash with water
3. Add alcohol (or acetone)-wait for 15 seconds -wash with water
4. Apply carbol fuchsin (or safranin) - wait for 30 seconds - wash with water (Altındış et al., 2013; Günalp et al.,

2003; Harvey et al., 2007; Bilgehan et al., 1994; Smith et al., 2005).

The slide, which is dried by pressing lightly with bibulous paper, is examined under a microscope by dropping immersion oil with 100x objective.

## **2. METHYLENE BLUE STAINING**

The methylene blue stain is widely used to show leukocytes and microorganisms in stool samples. Methylene Blue is also used for *Neisseria meningitidis* and *Haemophilus influenzae* in spinal fluid and to observe metachromatic granules of *Corynebacterium diphtheriae* (Günalp et al., 2003; Bilgehan et al., 1994; Mahon et al., 2018). The sample is spread on a slide and dried in air, it is fixed through the flame of a Bunsen burner. Methylene blue, which will give the blue color to leukocytes and microorganisms, is applied onto the fixed sample on the slide for 1-3 minute and then washed with water. After the slide is dried, immersion oil is dropped and examined under a microscope under 100x objective (Altındış et al., 2013; Günalp et al., 2003; Bilgehan et al., 1994).

## **3. ZIEHL-NEELSEN STAINING**

This method, also known as AFS (Acid resistance or acid fast staining), stains Mycobacteria (known as acid fast bacteria) and some bacterial species (*Actinomyces*, *Nocardia*, *Isospora*, *Cryptosporidium*) with similar characteristics, which are not stained by gram staining due to their differences in cell wall structures (Altındış et al., 2013; Harvey et al., 2007, Kalyan et al., 2018). For the staining of

these bacteria, basic stains must be applied intensively and with the effect of heat on the prepared smear. Therefore, acid-fast bacteria do not leave the stains they have acquired on their cell walls during the decolorization stage (Bilgehan et al., 1994, Kalyan et al., 2018). Carbol fuchsin (primary stain) is heavily applied on the prepared smear in the first step of the AFS. In order for Carbol Fuchsin to penetrate through Mycobacterial lipoidal wall and enters into cytoplasm, heat is applied to the slide from the bottom for heat the stain until about 60 °C. While applying heat, care is taken to ensure that the stain does not boil, and the stain that decreases with the effect of heat is added on the slide and care is taken not to dry out. After staining, it is washed with clean water to remove excess stain (Altındış et al., 2013; Günalp et al., 2003; Bilgehan et al., 1994; Kalyan et al., 2018). In the second step, the smear is decolorized an acid-alcohol mixture (3% HCL in 95% alcohol) and is washed with clear water. At this stage, acid-fast bacteria resist decolorization and retain the pink color with carbol fuchsin, but other bacteria on the slide are decolorized and remain colorless (Harvey et al., 2007, Kalyan et al., 2018). In the third step, methylene blue (counterstain) is applied on the smear for decolorized bacteria. At the end of the period, the slide is washed with water, dried between bibulous paper, immersion oil is applied and examined in a 100X objective. Under the microscope, acid-resistant bacteria are observed in red or pink color and other bacteria are observed in blue color (Altındış et al., 2013; Günalp et al., 2003; Kalyan et al., 2018).

The steps performed in the AFS method are listed below.

On the fixed smear by heat fixation:

1. Apply Carbol fuchsin at 60 °C -wait for 5 minutes -wash with water
2. Apply Acid alcohol mixture (decolorizing agent) -wait for 2 minutes -wash with water
3. Apply Methylene blue (counter stain) - wait for 1 minute - wash with water (Altındış et al., 2013; Günalp et al., 2003; Bilgehan et al., 1994; Kalyan et al., 2018).

#### **4. NEISSER STAINING**

Neisser staining is a method used to assess the presence of metachromatic bodies (Babes-Ernst polar bodies) of *Corynebacterium diphtheria* (Bilgehan et al., 1994; Morton et al., 1942; Köhler et al., 2004). Before starting the staining, the sample is spread on a slide and slide is fixed through the flame of a Bunsen burner. The Neisser stain, which is a prepared solution of two volume of Neisser A solution (aqueous iron-methylene blue solution) and one volume of Neisser B solution (aqueous-alcoholic crystal violet solution) is added onto the slide such a way that it completely covers it. At the end of the period, the preparation is dried with bibulous paper without washing it. The metachromatic bodies become visible as dark brown with the stain. Then, the chrysoidine (counter stain) is added onto the slide and the preparation is dried immediately between bibulous paper without washing it. The body of the bacterium is stained by chrysoidine are observed in light yellow color (Altındış et al., 2013; Günalp et al., 2003; Harvey et al., 2007; Bilgehan et al., 1994; Morton et al., 1994).

The steps performed in the this method are listed below.

1. Apply Neissers stain solution-wait for 10-15 seconds - dry with bibulous paper
2. Apply Chrysoidine (counter stain) - wait for 3 seconds - dry with bibulous paper.
3. After the slide is dried, immersion oil is dropped and examined under a microscope under 100x objective (Altındış et al., 2013; Günalp et al., 2003; Bilgehan et al., 1994).

## **5. INDIA INK STAINING**

It is a staining method used to see the capsule of *Cryptococcus neoformans* in CSF. The CSF is centrifuged for 5 to 10 minutes and sediment is taken. A few drops of Indian ink is placed onto slide. Then sediment is added onto the dripped ink and mixed with in. After the preparation is covered with a coverslip, immersion oil is dropped and examined under a microscope under 100x objective. Cryptococci are identified by their large, transparent capsules that displace the India ink particles. *Cryptococcus neoformans* is observed with a clear or halo capsule against a black staining background (Harvey et al., 2007, Emmons et al., 1970).

## **6. LACTOPHENOL COTTON BLUE METHOD**

Lactophenol cotton blue stain is a mounting medium and staining agent used to examine the fungal species. Lactophenol cotton blue stain contains phenol, lactic acid, cotton blue (Aniline blue) that stain chitin on the walls of fungi and glycerol. With this method, *Rhizopus*, *Aspergillus*, *Penicillium*, *Fusarium*, *Candida*, *Mucor* can be seen better. A drop of lactophenol blue is added on a clean slide and the

sample taken from cultures of fungi with an inoculating wire is spread within stain. It is examined under a microscope with 10X and 40X objectives (Walsh et al., 2018; McGinnis et al., 2012).

The steps performed in the this method are listed below.

1. Place a drop of Lactophenol Cotton Blue on the slide
2. Place fungus sample in the drop stain on the slide.
3. Spread fungus sample within stain.
4. Place a coverslip on preparation (Walsh et al., 2018; McGinnis et al., 2012).

## **7. POTASSIUM HYDROXIDE (KOH) METHOD**

In this examination method, potassium hydroxide (KOH) digests the bacteria and tissues (skin, hair and nails ) surrounding the fungi. A drop of 10% potassium hydroxide solution is placed on a slide. Then, the clinical specimen is added in solution on slide. The hyphae and conidia of fungi can be examined under a microscope with 10X and 40X objectives.( Harvey et al., 2007; Tille et al., 2015).

## **8. GIEMSA STAINING**

Giemsa staining method, which carries the German chemist and bacteriologist Gustav Giemsa's name, is a staining technique used in the diagnosis of parasites in malaria and other parasites in the blood or bone marrow and the examination of the blood cells (Barcia et al., 2007). Also, Giemsa's stain is used to show the presence of *Dientamoeba fragilis* and *Blastocystis hominis* in feces (Intra et al., 2019).

Giemsa stain contains giemsa powder, glycerol and methanol. Thin and thick blood smears is used to Giemsa's stain for identifying Plasmodium parasites. To prepare a thin smear, the patient's blood is placed on clear slide and is spread over the surface of the slide with the edge of another clean slide. The thin smear is dried in air and fixed with methanol for 2-3 minutes. After it is rinsed in distilled water, giemsa solution (diluted 1:10 with distilled water) is applied on slide for 20 min. Then, it is rinsed in distilled water and dried in air, immersion oil is applied and examined by 100x-objective (Cheesbrough et al., 1999; Isenberg et al., 1992). *Plasmodium* species and blood cells is examined in thin blood smear under the microscope. *Plasmodium* species in erythrocytes are seen as blue-violet with red nucleus (Güenalp et al., 2003; Cheesbrough et al., 1999; Isenberg et al., 1992). To prepare a thick smear preparation, the patient's blood is placed on clear slide and is spread over the surface of the slide to form thick film. While preparing the thick smear, unlike the thin smear, it is air-dried without any fixation in order to break up the erythrocytes and to better observe the parasites. Then Giemsa solution (diluted 1:50 with distilled water) is applied on slide for 1hr. Then, it is rinsed in distilled water and dried in air. It is examined in a 100x objective to detect the presence of *Plasmodium* (Güenalp et al., 2003; Cheesbrough et al., 1999; Isenberg et al., 1992)

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**CHAPTER 3**

**SPICES, THEIR USAGE in MEDICAL  
APPLICATIONS, AND THEIR ANALYSIS with  
DIFFERENTIAL SCANNING CALORIMETRY**

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## 1. INTRODUCTION

Spices are important elements in world cuisines. Spices have not only remained in kitchens for centuries but have also created important research and usage areas in medicine and health. Medical and health-related research is becoming increasingly popular. In this study, the contents of spices and their use in medicine and health are briefly mentioned considering the data in the literature. In addition, thermal analyses were performed using the Differential Scanning Calorimetry (DSC) method. Glass transition temperature ( $T_g$ ) values of 19 spices were measured by DSC analysis. The  $T_g$  value, on the other hand, gives us information about the physical properties while showing the temperature at which molecular variations begin in solids. It is also known that keeping the storage conditions below the  $T_g$  value will create healthier storage conditions.

The herbal products obtained by grinding the roots, leaves, fruits, seeds, buds, flowers, and bark of the plants that we use for their scent, taste, and appetizing properties are called spices. General information about some important spices in the literature is given under separate headings. Glass transition temperature ( $T_g$ ) is one of these characteristics and it houses much information about the thermodynamical, physical, mechanical, and electrical properties of a matter (Wu, 1999). It is considered as the change in either direction between the rubbery and glass states. It gives us information about storage temperatures. It should be under  $T_g$  values (Roos, 2010).

### 1.1. Black pepper (*Piper nigrum*)



**Figure 1:**Black pepper

Black pepper is a dark brown spice (Figure 1) obtained by drying the fruits of plants belonging to the *Piperaceae* family. Considering the ratios of vitamins and minerals, six grams of ground black pepper powder provides trace levels of other vital minerals, protein, and dietary fiber, as well as moderate amounts of vitamin K (13 percent DV), manganese (18 percent DV), and iron (10 percent DV) (Goswami & Malviya, 2020). Due to its antioxidant, antibacterial, and gastro-protective properties, it is an important nutritious food (Butt et al., 2013).

### 1.2. Red hot pepper (*C. frutescens*)

Chilli Pepper is a red that is toned down with a bit of brown (Figure 2). Red spicy chili peppers are mostly water, with 9% carbs, 2% protein, and 0.4 percent fat. Vitamin C and vitamin B6 are abundant in chili peppers, and



**Figure 2:**Red hot pepper

each 100-gram serving contains 40 calories (USDA, 2022). Topical ointments, dermal patches, and nasal sprays containing capsaicin, a fiery chemical found in chili peppers, are used to relieve pain (Fattori et al., 2016). According to a preliminary study published in 2022, frequent eating of chili peppers was linked to a decreased risk of mortality from cardiovascular illnesses and cancer (Kaur et al., 2021).

### 1.3. Thyme (*Thymus Vulgaris*)



**Figure 3:**Thyme

Fresh thyme is more open and flavorful while dried thyme is long-keeping and the color of its dried form has shades between dark green and brown (Figure 3). Thymol (8.55 mg/g) is the most prevalent volatile compound in thyme

leaves, according to gas chromatography data. Carvacrol, 1,8-cineole, -terpineol, and linalool are among the other components (Purcell et al., 2016). In the same published study, antibacterial properties are shown. Basil contains several them as well and antioxidant properties are found in some (Lee et al., 2005). Thymol, an antiseptic, is a component in Listerine and other commercially available mouthwashes (Peirce, 1999).

### 1.4. Rosemary (*Rosmarinus officinalis*)

Rosemary has deep sage green (Figure 4) and negligible nutritional benefits. Certain amounts are frequently used to flavor foods, such as one teaspoon (1 gram). Rosemary extract has been found to extend the shelf life and maintain the heat stability of rancid omega-3-rich oils (Talbot, 2016). Rosemary is an antibacterial herb as well (Nieto et al., 2018).



**Figure 4:**Rosemary

### 1.5. Basil (*Ocimum basilicum*)



**Figure 5:**Basil

Dried basil leaves range in color from green to brown (Figure 5). Basil is claimed to have medicinal effects in folk medical techniques such as Ayurveda and traditional Chinese

medicine (Ríos-Rodríguez et al., 2021). The essential oil has been proven to have insecticidal and insect repellent qualities, as well as possible toxicity to mosquitos (Maurya et al., 2009). *Lactiplantibacillus Plantarum* and *Pseudomonas spp.* These are two bacteria that the essential oil of the leaf and/or terminal shoot is effective against (Davidson et al., 2013).

### 1.6. Peppermint (*Menthabalsamea*)

Dried powder peppermint has a green color with a silver wash (Figure 6). Menthone (40.7%) and menthol (23.4%) are the main constituents, but peppermint also contains (+/-)-menthyl acetate, 1,8-cineole, limonene, beta-pinene, and beta-caryophyllene (Schmidt et al.,



**Figure 6:**Peppermint

2009). Peppermint oil is being examined in the lab for its potential to heal irritable bowel syndrome in the short term, and it is recognized to offer medicinal benefits in traditional medicine for mild ailments (Ruepert et al., 2011).

### 1.7. Fennel (*Foeniculum vulgare*)



**Figure 7:**Fennel

Dried fennel seed has an aromatic, anise-like flavor, it is brown or green when fresh, then as the seed ages, it slowly turns a dull grey color (Figure 7). Fennel was highly esteemed by the ancient Greeks and Romans, who used it for medicine, nutrition, and insect repellent (Drost Dan, 2020). Rich in minerals and vitamins. Used in the production of Absinthe (an alcoholic drink) (Garden, 2022).

### 1.8. Coriander (*Coriandrum sativum*)

Cilantro leaves look similar to parsley and the dried form of the spice changes in color from white to yellowish-brown (Figure 8). Vitamin A, C, and K are all abundant in coriander. In the Coca-Cola secret recipe, coriander is mentioned as one of the original components (Pendergrast, 1994). In a preliminary study, coriander essential oil inhibited gram-positive and gram-negative bacteria such as *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. Coli* (Silva et al., 2011).



**Figure 8:**Coriander

### 1.9. Cinnamon (*Cinnamomum Verum*)

Dried powder cinnamon is a dark, muted, autumnal orange with a pumpkin spice undertone (Figure 9). Around 11% of ground cinnamon is water, 81 percent carbohydrates



**Figure 9:**Cinnamon

(consisting of 53% dietary fiber), 4% protein, and 1% fat. Ground cinnamon has a lot of calcium (100 percent DV), iron (64 percent DV), and vitamin K (30 percent DV) in a 100-gram serving (Gul & Safdar, 2009). Cinnamon supplementation experiments with lipid measures revealed that it may cut total cholesterol and triglycerides, but no significant changes in LDL or HDL cholesterol were seen (Maieran et al., 2017).

### 1.10. Anise (*Pimpinella anisum*)



**Figure 10:** Anise

In traditional European herbal medicine, anise was primarily used for its carminative properties (reducing flatulence) (Beynes, 1878). The color of star anise seeds is dark reddish-brown color while the smaller anise seeds look more like

fennel seeds (Figure 10). Its seeds have been employed as an appetite stimulant, tranquilizer, and diuretic in Turkish folk medicine (Baytop, 1999).

### 1.11. Clove (*Syzygium aromaticum*)

Dried cloves are dark brown in hue with a reddish tinge (Figure 11). Clove oil containing eugenol has been used in traditional medicine for a long time, and there is evidence that it is useful for dental pain and



**Figure 11:** Clove

other forms of pain (Milind & Deepa, 2011). Its also used for aromatherapy (Tabatabaeichehr & Mortazavi, 2020).

### 1.12. Linseed or Flax (*Linum usitatissimum*)



**Figure 12:**Linseed

Linseed has smooth seeds and the color of the seeds is either brown or golden/yellow (Figure 12). Moreover, they have a nutty taste and are full of nutrients, including carbs, fat, and fiber. Linseed species are nutritionally comparable and have

the same amount of short-chain omega-3 fatty acids (Adorian et al., 2022). Flax seeds are 7 % water, 18 % protein, 29 % carbs, and 42 % fat. Flax seeds have substantial quantities (20 % DV) of protein, dietary fiber, numerous B vitamins, and dietary minerals in 100 grams (3.5 oz) as a reference quantity (Data, 2022). Flax seed or its derivatives may lower total and LDL cholesterol in the blood, according to a study published between 1990 and 2008, with significant effects in women and persons with high cholesterol (Pan et al., 2009).

### 1.13. Cumin (*Cuminum cyminum*)

There are varieties of cumin and the most used type has a brownish-yellow color (Figure 13) while some types have black, green, and white colors. There is no scientific proof that it might be used as a medication or medicine (Drugs.com, 2022). Cumin oil's main



**Figure 13:**Cumin

volatile components are cumin aldehyde, cymene, and terpenoids, which are employed in a range of flavors, fragrances, and essential oils (Bettaieb et al., 2011). Cumin seeds offer a significant percentage of the Daily Value for fat (particularly monounsaturated fat), protein, and

dietary fiber in a 100-g reference quantity. B vitamins, vitamin E, and a variety of dietary minerals, including iron, magnesium, and manganese, are all present in significant proportions.

#### 1.14. Nutmeg (*Myristica fragrans*)



**Figure 14:**Nutmeg

Nutmeg is a dark-leaved evergreen tree (Figure 14) and it is cultivated for its seed, mace, and seed covering. Nutmeg seeds show strong antioxidant and antimicrobial potential against gram-positive and gram-negative bacteria, as well as against various pathogenic fungi (Gupta et al., 2013). An overdose might cause delirium, anxiety, disorientation, headaches, nausea, dizziness, dry mouth, eye irritation, and forgetfulness (Ehrenpreis et al., 2014). It is used in the perfumery and pharmaceutical industry such as toothpaste and cough syrups (Crask, 2012).

#### 1.15. Sumac (*Rhus*)

Sumac has a deep red hue color (Figure 15) and is one of the most instantly recognizable spices of the Middle East. Sumac has a low amount of Vitamin C. *Rhus coriaria* cultivars have the highest value of vitamin C (39 mg/kg) (Kossah et al., 2009).



**Figure 15:**Sumac

Dietary sumac lowers blood pressure in individuals with hypertension, according to clinical research, and can be used as adjuvant therapy (Ardalani et al., 2016).

### 1.16. Sesame(*Sesamum Indicum*)



**Figure 16:**Sesame

The colors of sesame seeds can be white, black, red, brown (Figure 16), and tan. Dried whole sesame seeds have 573 calories per 100 g (3.5 oz) and are made up of 50% fat, 5% water,

23% carbs (including 12% dietary fiber), and 18% protein (Tunde-Akintunde & Akintunde, 2004). Both systolic and diastolic blood pressure have been reported to be lower in the literature (Khosravi-Boroujeni et al., 2017). In research, sesame has been proven to lower fasting blood glucose and hemoglobin A1c levels (Sohouli et al., 2022). It also helps to lower oxidative stress indicators and lipid peroxidation levels (Vittori Gouveia et al., 2016).

### 1.17. Ginger (*Zingiber officinale*)

Dried ginger powder can have colors from dark yellow through light brown (Figure 17) to pale buff. Raw ginger is made up of 79 percent water, 18 percent carbs, 2% protein, and 1% fat (Kiyama, 2020). Raw ginger has 333 kilojoules (80 kcal) of dietary energy per



**Figure 17:**Ginger

100 grams, as well as minor levels of vitamin B6 (12 percent DV), magnesium (12 percent DV), and manganese (11 percent DV), but is generally micronutrient deficient. It may aid digestion and has anti-inflammatory qualities, according to some studies (Terry et al., 2011). Ginger can help obese people lose weight and boost HDL cholesterol levels, according to a 2018 study (Maharlouei et al., 2019).

### 1.18. Turmeric (*Curcuma longa*)



**Figure 18:**Turmeric

Turmeric powder has colors from bright yellow-orange to reddish-orange (Figure 18) and it comprises around 60–70 percent carbohydrates, 6–13 percent water, 6–8% protein, 5–10 percent fat, 3–7% dietary minerals, 3–7% essential oils, 2–7% dietary fiber, and 1–6% curcuminoids (Nelson et al., 2017).

Based on their long-standing traditional use, the European Medicines Agency concluded in 2019 that turmeric herbal teas or other forms taken by mouth could be used to ease minor digestive disorders such as fullness and gas (Rahmat et al., 2021).

### 1.19. Black cumin (*Nigella sativa*)

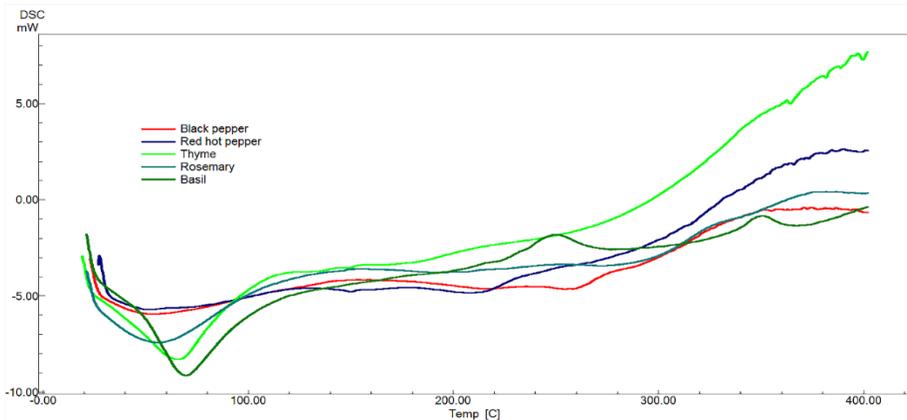
A meta-analysis of clinical studies indicated very modest evidence that *N. sativa* can lower blood pressure in the systolic and diastolic, there's also some evidence that different black seed extracts (Figure 19) can lower triglycerides, LDL, and total cholesterol while raising HDL (Gharby et al., 2015). Oils account for 32–40% of the overall makeup of *N. Sativa* seeds (Sahebkar, 2014).



**Figure 19:**Black cumin

## 2. RESEARCH AND FINDINGS

Differential scanning calorimetry (DSC) is a method for evaluating the heat capacity of small material samples. DSC can measure glass transition temperatures ( $T_g$ ), melting temperatures ( $T_m$ ) and enthalpy changes ( $\Delta H$ ) can be calculated via DSC. To find DSC thermograms all spices are weighted as 10 mg. Then, spices were analyzed thermally between 30-400 °C under a Nitrogen atmosphere (flow rate:30 ml/min) with a 10 °C/m in heating rate. The calculation of  $T_g$  has been made via T60 software.



**Figure 20:** DSC thermograms of B.Pepper, R.Pepper, Thyme, Rosemary, and Basil.

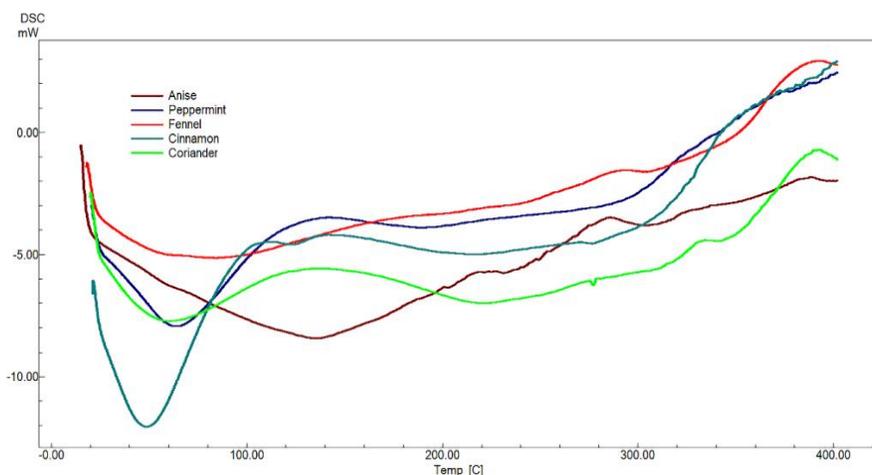
According to the DSC analysis, the glass transition temperatures for black pepper, paprika, thyme, rosemary, and basil were calculated as approximately 26, 30, 39, 28, and 47 °C, respectively, and the DSC thermograms are shown in

Figure. In the DSC curve of black pepper, although the peak of vitamin K between 65-70 °C is not very evident, specific heat changes observed between 250-300 °C and 350 °C indicate the presence of iron and manganese (Ali et al., 2021; Ortiz & Aranda, 1999; Osipov et al., 2019). Small peaks

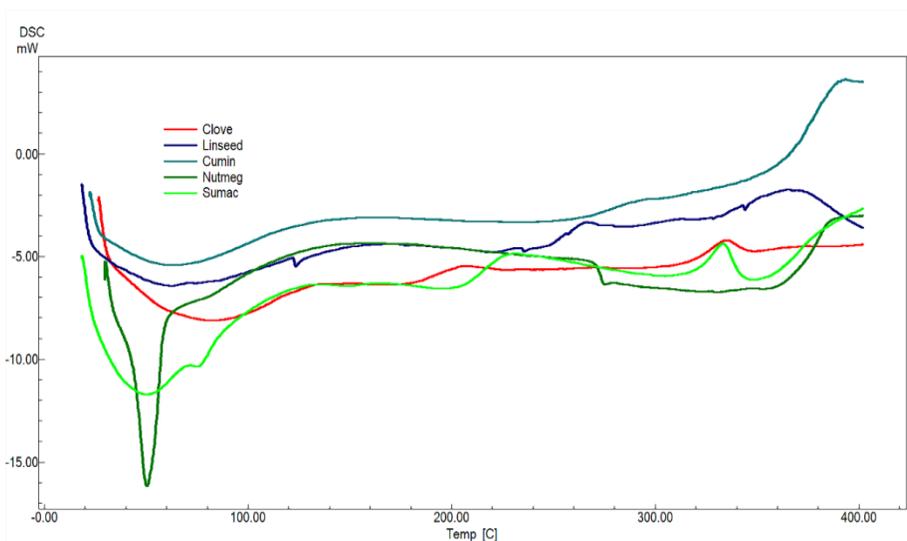
between 150-200 °C on the DSC thermogram of red pepper indicate the presence of Vitamin C and Vitamin B6 (Sugibayashi et al., 2020). The peak seen at 65 °C in the DSC curve of thyme is the peak that appeared in the studies performed after the thyme extraction (Gonccedil et al., 2013). The characteristic curves seen after 70°C and 200 °C in the DSC curves obtained from the previous rosemary extract are also present (Micić et al., 2021). The peak seen between 200-250 °C is compatible with the DSC curve for basil that appeared in previous measurements in the range (Martins et al., 2011).

For mint, fennel, coriander, cinnamon, and anise, the T<sub>g</sub> values were calculated as 32, 24, 30, 30, and 64 °C, respectively, and DSC thermograms are given in

Figure 11. The changes seen in the DSC curve for coriander between 300-400 °C are in line with the curve obtained from spice previously (Wieczorek et al., 2011).



**Figure 11:** DSC thermograms of Anise, Peppermint, Fennel, Coriander, and Cinnamon



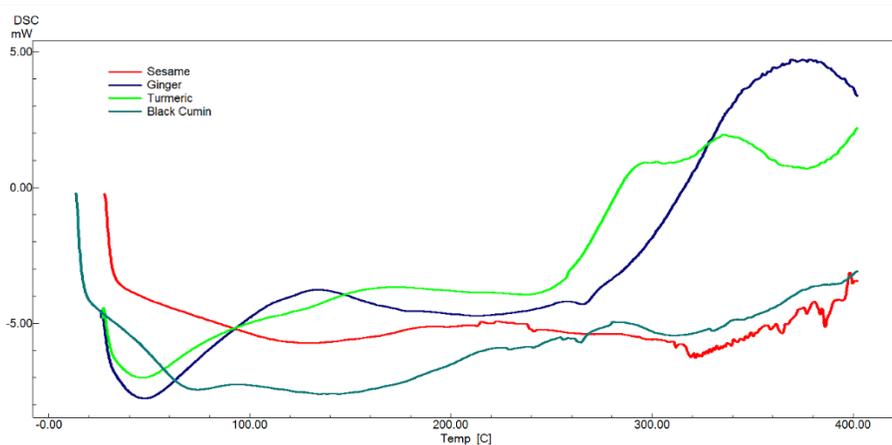
**Figure 22:** DSC thermograms of Clove, Linseed, Cumin, Nutmeg, and Sumac.

In the following analyses, the  $T_g$  values for clove, flax seed, cumin, nutmeg, and sumac were determined as 32, 23, 27, 30, and 26 °C, respectively, and DSC thermograms are given in Figure 22.

Finally, sesame, ginger, turmeric, and black cumin were subjected to DSC analysis, and  $T_g$  values were measured at 32, 29, 30, and 55 °C, respectively, and DSC thermograms are given in Figure 23. Peaks after 50°C and 300 °C are in line with the DSC curves previously made for ginger (Oliveira et al., 2019). Specific heat changes after 150°C and 300 °C in DSC measurements for black cumin are consistent with the changes in its DSC curve (Carrión-Prieto et al., 2017).

While some of the spices examined here have DSC data in the literature, the data of some of them are similar at certain temperature ranges. The difference in the mass of the spice used and the change in the heating rate changes the characteristic of the DSC curve (Araújo et

al., 2010). Therefore, these differences arise. Another reason for these differences may be that the proportions of the components in the spices vary according to the place where the plant is grown. The results obtained include the calorimetric evaluation of spices sold in Turkey.



**Figure 23:** DSC thermograms of Sesame, Ginger, Turmeric, and Black Cumin.

In addition to Tg values, onset, end set, and transition energy per mg data, which are the detailed data of the analysis, are given in

**Table1.**

**Table1:** Onset, Endset, Transition, and Tg values of the spices.

Spice	Onset (°C)	Endset(°C)	Transition(mW/mg)	Tg(°C)
1. Black pepper	23.34	28.64	-0.28 ± 0.02	26.34
2. Red hot pepper	28.06	32.00	-0.27 ± 0.03	30.06
3. Thyme	20.69	29.82	-0.49 ± 0.04	39.52
4. Rosemary	22.22	29.45	-0.33 ± 0.03	28.60
5. Basil	21.60	34.80	-0.73 ± 0.07	47.26
6. Peppermint	21.01	30.06	-0.49 ± 0.04	32.00
7. Fennel	20.12	25.35	-0.32 ± 0.03	24.12
8. Coriander	-67.61	28.87	-0.32 ± 0.03	30.61
9. Cinnamon	22.99	30.74	-0.55 ± 0.06	30.78

10. Anise	-67.60	51.40	-0.43 ± 0.04	64.43
11. Clove	27.80	33.99	-0.59 ± 0.05	32.44
12. Linseed(Flax)	19.94	24.56	-0.46 ± 0.05	23.02
13. Cumin	23.24	29.10	-0.31 ± 0.03	27.61
14. Nutmeg	50.04	49.32	-1.06 ± 0.09	30.56
15. Sumac	17.73	28.21	-0.63 ± 0.05	26.19
16. Sesame	29.12	33.95	-0.51 ± 0.04	32.85
17. Ginger	26.82	32.12	-0.32 ± 0.01	29.53
18. Turmeric	28.04	32.12	-0.25 ± 0.01	30.34
19. Black Cumin	43.84	66.11	-0.25 ± 0.01	55.06

### 3. CONCLUSION

In the study, 19 different spices were subjected to thermal analysis with DSC. As a result of the analysis, glass transition temperatures were calculated for 19 different spice varieties and the lowest Tg value was found to be between 25-30 °C. In addition, in light of this data obtained, there was no problem in storing the aforementioned spices under room conditions. Since it is known that molecular variations will start when the Tg temperature is exceeded, it is recommended not to increase the storage conditions above these temperatures.

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## CHAPTER 4

# S100B AS A BIOMARKER IN TRAUMATIC BRAIN INJURY

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## **INTRODUCTION**

Traumatic brain injury (TBI) is defined as an injury resulting in impaired brain functions (Lippi and Cervellin, 2016). Traumatic brain injury results in many deaths, disabilities, and the consumption of healthcare resources each year. All these facts have motivated researchers to investigate prognostic biomarkers in patients with TBI (Erenler, Yalçın and Baydin, 2015); (Lorente, 2017); (Yalçın and et al., 2017).

The S100B is known as a calcium binding peptide and is widely investigated and used as an indicator of glial activation and/or death in many disorders of the central nervous system (CNS). S100B is a useful neurobiochemical marker of brain damage such as in circulatory arrest, stroke, neurodegenerative diseases (such as Alzheimer's Disease) and TBI (Yardan, Erenler, Baydin, Aydin and Cokluk, 2011). In this narrative review, our aim was to clarify the diagnostic and prognostic role of S100B protein in TBI in the light of current literature data.

## **1. MATERIAL AND METHODS**

This review was conducted by entering keywords “S100B” and “traumatic brain injury” into scientific database Pubmed/MEDLINE. Recently published articles –particularly within the last three years– were included into the study. Articles without full-texts or without explanatory abstracts were excluded. Also, articles written in languages other than English were excluded.

The articles were evaluated by three reviewers independently for accuracy. If there were studies from the same source, the more recent or more informative study was selected. Disagreements between

reviewers were settled by discussion or consulting the fourth reviewer. A total of 29 articles were included into the study.

## **2. THE STRUCTURE OF S100B PROTEIN**

S100 is a relatively small protein of 9–14 kDa (Thelin, Nelson and Bellander, 2017). This 92 amino-acid protein is encoded by the S100B gene located on chromosome 21q22.3 (Lippi and Cervellin, 2016). It has a biological half-life of approximately 30 minutes (Rezaei and et al., 2017). Its main functions are known to be calcium hemostasis, cell differentiation and cell cycle progression, inhibition of apoptosis and neurogenesis. In the CNS, the protein exists mainly as the homodimer S100BB or heterodimer S100AB. Together, these proteins constitute the S100B family. When an injury occurs in brain tissue, astrocytes release the stored S100B. In summary, S100B is released from the cerebrospinal fluid (CSF) to serum and its drainage could be affected by the glymphatic clearance as well as the blood-brain barrier permeability (Thelin, Nelson and Bellander, 2017).

High levels of S100B, may show the expression of astrocyte damage in patients with TBI (Lorente, 2017).

## **3. DISCUSSION**

Biochemical markers may help assess disorders of the CNS. Especially in recent years, there has been an increased interest in the clinical use of brain markers such as S100 proteins. Many researchers have performed S100B measurements in patients suffering from TBI (Yardan, Erenler, Baydin, Aydin and Cokluk, 2011).

Brain injury is a common reason for Emergency department (ED) admission and typical management of mild TBI involves

computed tomography (CT) of the brain to identify complications such as intracranial haemorrhage and cerebral contusions (Norlund, Marke, Geijerstam, Oredsson and Britton, 2006).

In a study with 1449 patients, it was shown that the clinical use of S100B in TBI management reduces the use of CTs by approximately one-third (Allouchery and et al., 2018). Use of S100B in clinical practice may reduce not only radiation exposure but also healthcare costs.

It was also shown that S100B measurements could predict the likelihood of head CT abnormalities in paediatric patients with GCS scores of 14 to 15. However, we do not have enough evidence that S100B levels can replace clinical examinations or CT to identify paediatric patients with mild head injuries. Kelmendi et al. suggested that S100B might be used to identify low-risk patients to prevent their unnecessary exposure to radiation (Kelmendi and et al., 2018).

In a meta-analysis, it was revealed in 8 studies that, S100B serum analysis as a part of the clinical routine could significantly reduce the number of CT scans performed on children with mild TBI. Sampling should take place within 3 hours of trauma (Oris and et al., 2018).

In a study by Calcagnile et al., a reduction in costs was obtained by implementation of S100B in patients with TBI in the ED. Adding S100B to existing guidelines for mild TBI seems to reduce CT usage and costs, especially if guideline compliance could be increased (Calcagnile, Anell and Undén, 2016).

It was also shown that when measurement of S100B was added to the clinical decision rules for a cranial CT scan in mild TBI patients,

a 30% reduction in radiation exposure was obtained (Yardan, Erenler, Baydin, Aydin and Cokluk, 2011); (Biberthaler, Linsenmeier, Pfeifer, Kroetz, Mussack and Kanz, 2016).

The main challenge seems to be the determination of cut-off value for S100B in TBI. It was shown that S100B concentrations vary rapidly in the first 24–48 h. Hence, there is a need for identification of a standardized window for sampling (Ercole, Thelin, Holst, Bellander and Nelson, 2016). The cut-off value used for ruling out a positive CT scan in patients with mild TBI varied between 0.10 and 0.60  $\mu\text{g/L}$  across different studies (Lippi and Cervellin, 2016). In a study, the accuracy of surviving prognosis was found to be 76% in case of S100B protein level  $\leq 0.25 \mu\text{g/l}$  and Neuron-Specific Enolase (NSE) level  $<19 \mu\text{g/l}$ . A personalised prognostic model was proposed as a predictor of the outcome of severe TBI in children on the first day after trauma (Meshcheryakov, Semenova, Lukianov, Sorokina, and Karaseva, 2018). In severe TBI patients, high initial serum S100B levels of  $>0.7 \mu\text{g/dl}$  were found to be associated with 100% mortality (Kellermann, Kleindienst, Hore, Buchfelder and Brandner, 2016). Further studies are needed for determination of an accurate cut-off value in order to decrease the total healthcare cost for patient management.

In post mortem studies, NSE and S100B levels are significantly elevated subsequent to a fatal TBI in CSF. However, a similar increase in both markers in blood could not be obtained (Sieber, Dreßler, Franke, Pohlers and Ondruschka, 2018).

S100B levels were also found to be useful in predicting unfavorable outcome in patients with TBI. A correlation between transcranial protein S100B gradient and development of brain death

was determined (Ballesteros, Rubio-Lopez, San Martín, Padilla, López-Hoyos and Llorca, 2018).

In another study, patients with mild TBI had higher S100B serum levels than the healthy subjects. It was proposed that measurement of S100B within 6 hours of mild TBI accurately predicts risk of intracranial lesion in patients with a GCS score of 15 and at least one neurological symptom (Egea-Guerrero and et al., 2018).

When S100B and NSE were compared, prognostic capacity of S100B was higher than NSE in predicting short and long-term mortality in severe TBI patients (Rodríguez-Rodríguez and et al., 2016). In pediatric patients, serum levels of S100B and NSE are known to be related to not only initial GCS scores, but also related to 6-month GCSs one week after TBI. Thus, serial measurements of serum S100B and NSE are recommended to assess brain damage and clinical outcome of pediatric patients with TBI (Park and Hwang, 2018).

In another study, NSE was to a greater extent correlated to multitrauma the first 48 hours following injury, whereas the effect of extracerebral trauma on S100B levels appears limited to the first 12 hours. S100B was found to be superior in outcome prediction and a more clinically useful biomarker than NSE for TBI patients (Thelin and et al., 2016).

S100B was also compared with proenkephalin (P-ENK). In a study, it was shown that in patients with TBI, S100B and P-ENK levels have elevated. Additionally, serum levels of both markers are found to be elevated in patients with multiple lesions when compared to patients with a single lesion. Serum S100B and P-ENK levels were proposed as predictors of mortality in patients with TBI (Yalçın and et al., 2017).

The original articles investigated in this review are summarized in the table.

## **CONCLUSION**

Serum S100B levels may predict the progress or the prognosis of many kinds of diseases, such as cerebrovascular diseases, neurodegenerative diseases, motor neuron diseases, traumatic brain injury, schizophrenia, depression, diabetes mellitus, myocardial infarction, cancer, and infectious diseases. Given that the activity of S100B has been implicated in the pathological process of these diseases, S100B should not be simply regarded as a biomarker, it may also function in therapeutic processes of these diseases (Chong, Changyaleket, Xu, Dull and Schwartz, 2016). However, according to our results, we can not recommend use of S100B solely as a diagnostic and prognostic marker in TBI. Although it seems superior than other trauma markers and useful in reducing CT use particularly in mild TBI, there is a lack of evidence that support clinical decision can be based on S100B measurements. Clinical symptoms, physical examination and CT findings are still the gold standard for evaluation of patients with TBI.

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## **CHAPTER 5**

### **NECK PAIN**

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## **INTRODUCTION**

Neck pain is one of the most common problems among musculoskeletal pain, and 40-70% of people have experienced neck pain at least once in their lives. The cervical spine is a vital structure that supports the entire weight of the head, while protecting the spinal cord, nerve roots and vertebral arteries. At the same time, being the most mobile part of the spine, it is exposed to high mechanical stress and is the part of the spine that is most subject to degenerative and traumatic changes (Frontera & Joel A DeLisa, 2010).

### **1. EPIDEMIOLOGY**

Neck pain ranks as 4th leading causes of disability in the world (Popescu & Lee, 2020a). Although it is more common in women, it occurs with a frequency ranging from 5.9% to 38.7% in the adult population, and the incidence of neck pain within a year has been found to be 40% (Vos et al., 2008).

### **2. RISK FACTORS**

Among the risk factors for neck pain, the most frequently encountered in systemic reviews is the inability of the work environment to provide ergonomic working conditions, and the maintenance of an unphysiological neck posture at the desk for a long time (May et al., n.d.; Popescu & Lee, 2020a).

In addition, in reviews of many studies, female gender, age, low social-work support, smoking cessation, history of low back or neck pain, working in fixed positions for a long time and heavy lifting were found to be associated with neck pain (Devereaux, 2003).

### 3. ETIOLOGY

Neck pain can be caused by different anatomical structures and many different diseases. In addition to primary cervical spine pathologies, pathologies of other regions such as shoulder, cranio - cervical junction and temporo-mandibular joint (TMJ) may also cause neck pain. The etiology of the majority of patients who applied to the outpatient clinic with neck pain is muscle tension (strain), Myofascial Pain Syndrome, and trigger points (TP), which is the cause of referred pain. (Cohen, 2015). We can group the etiology of neck pain; as inflammatory, degenerative, traumatic, neoplastic, infectious, metabolic and other causes (Table 1). Causes other than the musculoskeletal system may be: Thyroiditis, pharyngitis, tracheitis, lymphadenitis; pericarditis , diaphragmatic infection, aortic dissection , aortic aneurysm.

**Table 1:** Etiology of Neck Pain

<b>Inflammatory</b>	<b>Infectious</b>
Rheumatoid Arthritis	Septic Arthritis
Ankylosing Spondylitis	Discitis
Juvenile idiopathic Arthritis	Osteomyelitis
Psoriatic Arthritis	Meningitis
Reiter's Syndrome	Epidural Abscess
Polymyalgia romatica	
Giant Cell Arthritis	<b>Metabolic</b>
Polymyositis - Dermatomyositis	Paget
	Osteoporosis
<b>Degenerative</b>	Osteomalacia

Osteoarthritis	
Spondylosis	<b>Neoplastic</b>
Radiculopathy	Primary: Neurofibroma , Spinal Cord Tumor , Meningeoma
Myelopathy	Metastases
	<b>Other</b>
<b>Trauma</b>	Diffuse Idiopathic Skeletal Hyperostosis
Whiplash	Thoracic Outlet Syndrome
Sprain/Strain	Myofascial Pain syndrome
Tramvatic Disc Herniation	Fibromyalgia syndrome

#### **4. CLASSIFICATION**

Neck pain can be classified according to the duration and cause of the pain.

##### **4.1. According to the duration of the pain: acute, subacute, and chronic**

Neck pain is divided into 3 groups according to how long it has been present: acute, subacute, and chronic. Pain lasting less than 6 weeks is classified as acute, those lasting 6-12 weeks are subacute, and pain lasting longer than 3 months is classified as chronic. It is known that the prognosis of short-term pain is better (Popescu & Lee, 2020a). While most patients with acute neck pain recover within 1 week, approximately 10-30% of them become chronic (Bogduk & Mercer, 2000).

#### **4.2. According to the mechanism of pain: Mechanical and Neurological Secondary to other pathologies:**

Non-traumatic neck pain is divided into two in terms of having neurological and mechanical components. Pathologies related to cervical intervertebral disc, cervical zygapophyseal joint, facet joint, ligaments and atlanto-axial joint are mechanical causes. Radiculopathy due to nerve compression or irritation as a result of disc herniation, foraminal stenosis, spinal stenosis or pathologies with one or more of these are classified among the neurological causes of neck pain (Cohen, 2015; Popescu & Lee, 2020b).

In the etiology of neck pain that can lead to neurological deficit: disc herniation causing nerve cord compression, severe foraminal stenosis or disc-osteophyte complex, or myelopathy with spinal stenosis may be considered. Myelopathy is the name given to the clinical picture of muscle weakness accompanied by fine motor skill and balance disorder resulting from spinal cord compression. Ossification of the posterior longitudinal ligament is a rare cause of spinal cord compression and myelopathy. Separating mechanical pain from neurological is very important in order to reach the correct diagnosis and give appropriate treatment (McLean et al., 2010; “The epidemiology of neck pain: what we have learned from our population-based studies,” 2003).

There is a wide spectrum among the differential causes of neck pain. Conditions that we should not forget among the differential causes when making the right diagnosis: coronary artery disease, infections (discitis, osteomyelitis, retropharyngeal abscess, meningitis, dense fracture), tumor (multiple myeloma, metastasis), rheumatological

diseases (polymyalgia rheumatica, calcium pyrophosphate storage disease in the atlanto-axial joint, fibromyalgia), vascular pathologies (vertebral-carotid dissection), thoracic outlet syndrome should be kept in mind (McLean et al., 2010; Popescu & Lee, 2020a; “The epidemiology of neck pain: what we have learned from our population-based studies,” 2003).

In cases of myelopathy, osteomyelitis, discitis, urinary stool incontinence and possible malignancy, which are considered as red flags, specific laboratory tests (sedimentation, CRP, whole blood analysis, protein electrophoresis, etc.) should be requested and advanced imaging methods such as Magnetic Resonance (MR) and Computer Tomography (CT) imaging should be used to reach the diagnosis as soon as possible. If there is no pathology found in imaging methods and chronic pain is mentioned, myofascial pain syndrome should be kept in mind (Popescu & Lee, 2020a).

## **5. RADIOLOGICAL EVALUATION OF THE CERVICAL REGION**

### **5.1. Radiography**

Although CT and MR have come to the core in defining many diseases by taking thin sections, conventional X-ray maintains its importance and place in the evaluation of bone structure as it is economical, practical and easily accessible. Indications for X-ray examination of the spine are degenerative changes, inflammatory changes and trauma. Changes in intervertebral disc height may indicate degenerative changes in bone structure. At the same time, hereditary or developmental anomalies can be recognized by X-ray imaging. In X-

ray examination of the cervical spine, flexion-extension radiographs are very important in demonstrating possible articular defects, lysis, and also in monitoring post-op changes (especially after fusion surgeries).

### **5.2. Computed Tomography**

With multi-slice CT, we can see the areas we want to examine in cross-sections in different plans, including axial, coronal and sagittal. With conventional CT devices, the bone structure of the spinal canal, nerve roots, intervertebral discs, neural foramina can be evaluated. However, MR imaging is often needed to evaluate the spinal cord and the soft tissue (Freund & Sartor, 2006).

### **5.3. Magnetic Resonance Imaging**

Today, the gold standard for imaging the spinal cord is MRI. MRI is indicated for neck pain that does not go away despite 4 weeks of regular conservative treatment. CT and MRI should be preferred in the diagnosis of persistent, acute progressive or chronic neurologic deficits in the diagnosis of neck and back pain. In studies of Ashkan et al. (Cohen, 2015), MRI is shown to be more sensitive than neurophysiological studies (93% vs. 42%) and has a higher negative predictive value (25% vs. 7%). However, neurophysiological studies can only show us the damage after neural changes.

Technically, both can be helpful in diagnosis, but MR should be preferred if access to it is possible. At this point,

- Its superiority in evaluating soft tissue,
- spinal structures in a multi-planar way,
- The absence of radiation are its advantages.

Line with the guidelines and technical parameters, in MR imaging: intervertebral discs, ligaments, vertebral joints, spinal cord, extraforaminal nerve roots and intervertebral foramen can be distinguished quite nicely. Although bone structures are more difficult to select in MRI than in CT; discs, capsular ligaments, spinal cord and the rest of the spinal canal can be visualized much more optimally with MRI (Freund & Sartor, 2006).

#### **5.4. Myelography and CT myelography**

Myelography is distinguished from others as it is an invasive imaging method. As a basic principle, non-invasive imaging methods should be chosen primarily. If myelography is to be planned, post myelography CT imaging should also be planned. Lumbar puncture is usually made between L2-L3 or L3-L4 and a water-soluble contrast agent of 15-18 ml is given at this level. MR and CT Myelography is indicated when it fails to explain acute symptoms such as paraplegia (Oğuz, 2015).

#### **5.5. Discography**

Discography is an invasive imaging method used to evaluate the intervertebral disc, which is rarely used today. A contrast agent containing 0.4-1.5 ml of iodine is given to the patient. In the meantime, lateral X-Ray images of the patient are taken. Any leakage into the epidural area is considered a positive finding. Unlike lumbar punctures, cervical punctures are performed anteriorly. The most common complication is discitis, mostly secondary to esophageal injury. Complications resulting in vascular, or nerve tissue injury are very rare (Oğuz, 2015).

### **5.6. Scintigraphy**

Technetium scintigraphy (  $^{99}$  Tc-MDP) with methylene diphosphate is a very important method used in the evaluation of primary or metastatic bone lesions, fractures, sacroiliitis , osteomyelitis and discitis . The most important feature that distinguishes this method from others is that it can show early osteoblastic activity. In this way, pathological disorders can be distinguished at an early stage (Oğuz, 2015).

### **5.7. Ultrasonography**

With ultrasonographic (USG) imaging, muscles and ligaments can be distinguished quite nicely and clearly. Besides being very useful in determining a possible inflammation, tear or edema, it can also be used to evaluate vertebrae (Oğuz, 2015).

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**CHAPTER 6**  
**SACRAL STRESS FRACTURES**  
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## **INTRODUCTION**

Sacral stress fractures (SSFs) were first described by Laurie in 1982 (Deschamps Perdomo ABEF et al., 2015). Pentecost et al. classified sacral stress fractures into two groups depending on their mechanism as of insufficiency and fatigue fractures (Pentecost et al., 1964) . The mechanism behind the insufficiency fractures is the deficiency in bone strength and structure such as osteoporosis or metabolic bone diseases; often called as fragility fractures. Fatigue fractures are the result of excessive and repeated force exerted on the normal bone. As a result of an uneven biomechanical stress directed to the osteoporotic sacral bone thus creating the fractures (Ayhan et al., n.d.). Sacral Insufficiency fractures (SIFs) generally present in osteoporotic elderly patient with highly painful low back and buttock pain without any history of fall or trauma. They are generally misdiagnosed due to their nonspecific vague symptoms and it's not being visible on plain radiograph. With the increased life expectancy, the incidence of SIF, morbidity and mortality rate is rising.

### **1. EPIDEMIOLOGY**

Non-osteoporotic sacral fractures incidence was estimated as 2.1 cases per 100,000 people, whereas osteoporotic fractures were to have an incidence of 1–5% in elderly patients (Emmanuele Santolini et al., 2020). A recent study included 250 patients who have admitted to the emergency department with pelvic pain complaint. They have practiced pelvic Computed Tomography (CT) to all of them in suspicion of pelvic fracture. As a result, 46 patients were diagnosed with pelvic fracture and among them 11 (4.4%) of them had SIFs (Tamaki et al., 2017). Its mortality and morbidity rate are high thus diagnose and treatment of these cases are highly crucial. The difference in the incidence of the SIF may be due to its vague symptoms and often being misdiagnosed.

### **2. PATHOPHYSIOLOGY**

During a normal cycle of gait, with the flexion and extension of the opposite legs there is a biomechanical torsional stress loaded on the

sacrum. So, the sacroiliac joints (SIJ) absorb this stress and helps the normal gait pattern. When there is a pathology in SIJ the torsional stress is not being eliminated and transferred directly to sacrum. In cases where the sacrum is not strong such as osteoporosis the sacrum cannot withstand the stress and we face SIFs. We can conclude that in patients with SIF there is both SIJ pathologies and weak sacrum (Vleeming et al., 2012) .

### **3. RISK FACTORS**

Most of the underlying etiology behind SIF is osteoporosis in elderly patient. The second most prominent group to SSF is the athletes. Because of overuse, stress is loaded on sacrum. Weightlifters, runners, bicyclists, fitness instructors, aerobics, basketball players are more prone to SSFs. In a study where there are 312 asymptomatic runners under the age of 18 who went through MRI screening, they found that 1.6% of them had silent SIFs (Bencardino et al., 2017). In addition to this, metabolic diseases such as rheumatoid arthritis, Paget, osteomalacia, hyper parathyroidism, osteodystrophy, steroid induced osteopenia, previous thoracolumbar or lumbosacral fusion surgery, joint arthroplasty, obesity, pregnancy, pelvic radiation, sacral metastasis, sacral chordoma may change the sacral bone structure and lead to SIF (Ayhan et al., n.d.; Bencardino et al., 2017; Bostel et al., n.d.; Hmida et al., 2018; Kawamoto et al., 2018; Speziali et al., 2015).

### **4. CLINICAL PRESENTATION**

Patients usually present with vague symptoms and often misdiagnosed. Most commonly they have nonspecific low back, buttock, hip pain. The clinic of the patient generally gets mixed with facet arthropathy, lumbar strain and lumbar disc hernia. Very rarely they have radicular pain as a result of nerve compression due to the oedema around the sacrum. Sitting, walking, or standing generally propagates the pain. Although there is not a specific examination test and mostly the entire examination is completely normal some tests may help us to diagnose. In patients with low back pain (especially in elderly) painful palpation of the sacrum (Squish Test), positive

Gaenslen and FABER test may suggest us to consider sacral insufficiency fracture for our differential diagnosis (Ayhan et al., n.d.; Öztürk et al., n.d.).

In elderly patients who have resistant low-back pain and have risk factors for osteoporosis further diagnostic measures should be taken. Plain radiographs generally don't show any diagnostic sign. However, it is recommended in the first line in order to rule out any other pathologies such as pelvic fractures. Computed tomography (CT), magnetic resonance imaging (MRI), and three-phase bone scans help diagnose SIF. MRI is the gold standard recommended imaging method in diagnosing SIF (Lotan et al., n.d.).

## **5. MANAGEMENT**

The first line protocol in treating SIF is conservative management: Controlling the pain and immobilization. Analgesics such as opioids, acetaminophen, and nonsteroidal anti-inflammatory drugs (NSAIDs) or injection therapies and physical therapy modalities such as TENS, magnetic area therapy are recommended to control the pain. There is a reluctance in using NSAIDs since they may impair enchondral ossification in the bone healing process. Bed rest for 3-6 months after the fracture is highly recommended.

In addition to this coping with the osteoporosis underlying the fracture is crucial. Serum 25-OH vitamin D level needs to be kept over 30 ng/dL for adequate bone mineralization. Daily consumption of 1200 mg calcium and 800 IU vitamin D is recommended by authorities (Tang et al., 2007). The first line anti osteoporosis drug are bisphosphonates such as alendronates, ibandronates or zoledronic acids. The mechanism behind bisphosphonates is their inhibiting bone resorption. Another medication alternative is denosumab which is a RANKL inhibitor thus inhibiting osteoclast activity. There is also teriparatide which is a recombinant form of parathyroid hormone. This hormone therapy is acting on osteoblasts. It helps generate bone formation. Many studies have shown that Teriparatide helps new bone formation for SIF cases (Aspenberg et al., n.d.).

## **6. SURGERY**

### **6.1. Sacroplasty**

First performed on patients with pelvic metastatic lesions, it is a minimally invasive procedure where the cement is injected into the bone to support the structure and reduce the symptoms. In the study of Kortman et al. (Kortman et al., n.d.) they show that VAS score of the SIF patients have reduced drastically after sacroplasty. So, sacroplasty is an effective low risk procedure to help reduce the pain and disability.

### **6.2. Stabilization**

Trans iliac or trans sacral screw stabilization is another technique for those patients who are not responding to the conservative therapy. In recent articles it is written that this procedure also helps reduce pain and disability of the patient for at least 1 year (Emmanuele Santolini et al., 2020).

## **7. CONCLUSION**

Sacral stress fractures are often seen in osteoporotic elderly patients with nonspecific low back pain which cause high morbidity and mortality rates. They are often misdiagnosed due to nonspecific clinical presentation and requiring advanced imaging techniques such as MRI for the diagnose. Thus, clinicians should ask for MRI in cases of high SIF suspicion.

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## CHAPTER 7

# GREATER TROCHANTERIC PAIN SYNDROME

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## **INTRODUCTION**

Greater trochanteric pain syndrome (GTPS) also called by the name of trochanteric bursitis (TB) or greater trochanteric bursitis (GTB) is a group of pathologies related to the lateral hip, buttock and thigh pain which encompasses trochanteric bursitis, gluteus medius and minimus tendinopathy or tears and iliotibial band syndrome presenting with lateral hip pain(Karpinski & Piggott, 1985).

Karpinski et al. developed the term GTPS in 1985 observing the patients with lateral hip pain. Since the patients did not exhibit any inflammatory signs such as swelling, heat, crepitus or fluctuation this was not a bursitis case (Karpinski & Piggott, 1985). Other studies have supported this thesis including Bird et al. 2001 (Silva, Adams, Feinstein, & Arroyo, 2008). In their study 24 patients of lateral hip pain were evaluated using magnetic resonance imaging and they figured out that most patients had gluteus medius tendon pathology. Among them only 2 patients had isolated trochanteric bursal inflammation (Bird, Oakley, Shnier, & Kirkham, 2001).

Greater trochanteric bursitis generally presents with lateral hip pain. The pain increases by walking, sitting up from a sofa or laying down on the affected side. The patient complains about the localized pain around the greater trochanter(4).

On physical examination we find tenderness around the greater trochanter with palpation. Active abduction or passive adduction may increase the discomfort.

We generally encounter with GTPS in the middle aged, overweight women. According to the previous studies greater pelvic width is related to the greater tension over iliotibial band. The tension

over the iliotibial band is directed on the greater trochanter thus agitating the syndrome (Silva et al., 2008).

Imaging is partially helpful. We may see gluteal tendinopathy, inflammation around the bursa or nothing at all. Since there are no specific diagnostic criteria clinical examination is crucial.

## **1. ETIOLOGY**

Repeated microtrauma or any kind of trauma to the hip may be the cause beneath the pathology. It can also be idiopathic. Tendinopathies of gluteus medius and/or minimus due to excessive use are very common. Cycling or stair climbing causing repeated hip abduction results in inflammation of the trochanteric bursa. Immobile patients who lay on their side thus giving pressure to the greater trochanter also initiate the cascade of bursitis. The main etiology beneath is tendon tear or tendinosis of gluteus medius or minimus or both causing the pain around the greater trochanter (Jacobson et al., 2016).

## **2. EPIDEMIOLOGY**

Generally seen in middle aged female and younger active male and female patients. The ration of GTB of female to man is around 2-3 (Jacobson et al., 2016). Nearly 15% of women and 8% of men develop GTB once in their lives (Jacobson et al., 2016). Repetitive jumping, running, climbing stairs, cycling, squatting may cause this syndrome in active younger patients.

### **3. SYMPTOMS AND EXAMINATION**

The pain is elicited from the lateral side of the hip. It generally is unilateral. Patient generally localize the lateral hip pain on the femoral greater trochanter. This pain may interrupt sleep and mobilization increases the pain. Bed rest, anti-inflammatory drug usage and cold pack compression helps reduce the inflammation thus the pain (Labrosse et al., 2010).

Gold standard examination is palpation which elevate the pain. Single leg rise and FABERE, FADIR test examination should not be causing any pain. Localized pain over greater trochanter of femur with deep palpation is a positive test sign (Fearon et al., 2014). There are no color or temperature change over the inflamed area since it is not an infectious disease. While we force the hip for abduction the pain is relieved due to decreased tension over iliotibial band (ITB) (Labrosse et al., 2010).

### **4. EVALUATION**

Imaging is mainly necessary to rule out any pelvic fractures. After the physical examination if we suspect GTPS we need to eliminate any kind of fracture or osseous pathologies. In order to do that we should be getting pelvic AP and femoral two-way x-ray plain radiography. In the case of TB no specific finding is seen on the plain graph. If you have further suspicion, MRI will show inflammation of the bursitis with increased signal on T2 weighted images. Furthermore, Ultrasonography imaging can show the excessive fluid around the bursa.

## **5. MEDICAL TREATMENT**

Conservative treatment is always the preferable one. Since it is not an infectious pathology antibiotics are not necessary. Oral Non-Steroidal Anti-Inflammatory drugs (NSAIDs) are the first line medical treatment if there are no counterindication such as gastrointestinal irritation or kidney deficiency. Oral NSAIDs can help relieve the pain and rule out the inflammation around the bursa.

### **5.1. Physical Therapy**

It is crucial to diminish the inflammation by abduction of the hip thus relieving the stress on ITB. Rest is recommended with cold pack or hot pack application to the inflamed area. Modifying the running and jumping exercises which may increase the inflammation is necessary. Stretching and strengthening the hip and the surrounding muscles are significant (Ganderton, Semciw, Cook, Moreira, & Pizzari, 2018).

### **5.2. Corticosteroid Injection**

Corticosteroid injection is preferred on patients who are resistant to the conservative treatment or oral medication. Being an anti-inflammatory drug, it helps reduce the pain with dissolving the inflammation on the bursa. Usually applied with no imaging device by orthopedics or physical therapy and rehabilitation medicine specialist. It is preferable to do the procedure under ultrasound guidance. Generally, lidocaine or marcaine is added to the injection material to reduce the pain and target the local inflammation. Studies have shown that exercise has similar effects with corticosteroid injections (Mellor et al., 2018).

### **5.3. Platelet Rich Plasma**

Platelet Rich Plasma (PRP) is a hot topic related to musculoskeletal pathologies. It is generated by autologous blood withdrawn from the patient. After centrifuge of the blood, the plasma which is rich with the thrombocytes gets injected on to the inflamed area. It is believed that PRP propagates first inflammation then growth factors. These include, platelet-derived endothelial growthfactor (PD-EGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), insulin-like growthfactor (IGF), vascular endothelial growth factor (VEGF)and basic fibroblast growth factor (bFGF) (Oderuth et al., n.d.). These cytokines and growth factors help rejuvenate the damaged tissue. The studies have shown that PRP injection help reduce the pain and increase mobilization up to 3 to 6 months. Due to the lack of standardized procedures and the heterogenicity of the clinical studies no high evidence suggestion is given for PRP (Ali, Oderuth, Atchia, & Malviya, n.d.; Oderuth et al., n.d.).

### **5.4. Extracorporeal Shock Wave**

Extracorporeal Shock Wave Therapy (ESWT) is an alternative treatment method for GTPS. There are studies showing significant improvement of the symptoms similar to corticosteroid injection.

### **5.5. Surgical Treatment**

For those who are resistant to the conservative therapy surgery might be an alternative. It is generally performed on patients with rheumatologic diseases where the bursa is inflamed persistently. So, the bursa is removed surgically.

## **6. DIFFERENTIAL DIAGNOSIS**

Femoral fractures, snapping hip, hamstring avulsion injury, strain and sprain of the hip circle should be kept in mind for differential diagnosis (Philip D Nowicki, Carl Wierks, & Lisa Maskill, 2013). We should also be cautious about femoro acetabular impingement syndrome for the athletes who have ongoing persistent hip pain.

## **7. PROGNOSIS**

Prognosis of GTPS is quite great. Generally conservative treatment dissolves the pain. Within days of NSAID usage or corticosteroid injection the pain disappears and the inflammation of the bursa dies out. For those who are not responding to the conservative treatment we should keep in mind that there might be repetitive ongoing trauma, patient not complying with rest or having rheumatologic disease background.

## **8. COMPLICATIONS**

The complications of GTPS is mainly related to the NSAIDs usage and corticosteroid injection. Gastrointestinal and renal side effect should always be kept in mind since it may trigger gastrointestinal bleeding and kidney insufficiency(Maldonado, Youssefzadeh, Wydra, Sherman, & Gerhardt, 2021). For those who are under anticoagulant treatment, having gastrointestinal bleeding history or kidney functional deficiency we should be thinking over medicine subscription. Other than that, corticosteroid might elevate blood sugar and blood tension. It may also create irritation or bleeding of the injection site (Brutzkus JC, Shahrokhi M, & Varacallo M, 2022).

## **9. PATIENT EDUCATION**

Stretching and strengthening exercises is helpful in preventing any cases of GTPS. However traumatic and idiopathic cases are non-preventable. It is crucial to comfort the patient and counsel that conservative treatment is almost always enough for the situation to dissolve. We should also warn the patient about the side effects of NSAID use and corticosteroid injection. Patient should be aware of the fact that recovery may take up to a couple of weeks (Fearon et al., 2014).

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**CHAPTER 8**  
**PROBIOTIC – PREBIOTIC and HEALTH**  
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## **1.INTRODUCTION**

### **1.1. Probiotic**

The basis of food fermentation reaches back many years and even in those times, this process was chosen due to obtaining better shelf-life and the desired quality. Symbiotic relationship between humans and bacteria has led to investigate potential beneficial properties of microorganisms. Awareness of the relationship between intestinal health and human health extends back to the period of Hippocrates who stated ‘all diseases begin in the gut’. Research in the modern era began with a study called ‘Enterobacteria of infants and their relation to digestion physiology’ by Theodor Escherich in 1886 and a study called ‘The use of microorganisms (*Lactobacilli*) to treat vaginal infections’ by Albert Doderlein in 1892 (Zimmer, 2001; Mizock, 2015).

Russian scientist Eli Metchnikoff is known as the father of the probiotic concept, and was the first person to recommend the use of probiotics in today’s sense. Eli Metchnikoff completed the first scientific explanations related to the beneficial effects of lactic acid bacteria, stating that regular use of fermented milk products containing *Lactobacilli* ensured a long and healthy life in his book ‘*The Prolongation of Life*’ (Ranadheera et al., 2010). Later studies were performed showing that yogurt contained beneficial microorganisms protecting the gut from harmful microorganisms and that these microorganisms were effective in prevention and treatment of diseases (Tripathi and Giri, 2014).

In 2002, the Food and Agriculture Organization/World Health Organization Working Group (FAO/WHO) stated the most up-to-date

definition of probiotics was ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (FAO, WHO, 2002).

For a microorganism to be probiotic, it must have a certain portion of the features; be safe, acid and bile resistant, tolerant of oxygen and heat, resistant to antibiotics, able to show antimicrobial properties, show anticancerogenic and antiallergic effect, develop the intestinal system. Foods containing  $10^7$  colony forming units and g rates of live microorganisms with probiotic characteristics, led by lactic acid bacteria and bifidobacteria, are defined as ‘probiotic food’ (Erkmen, 2010).

## **1.2. Prebiotics**

Gibson and Roberfroid (1995) was defined prebiotics as a ‘nondigestible food ingredient that selectively stimulating the activity and/or growth of bacteria in the colon, and in this way improves host health. This definition overlaps to a large extent with the definition of dietary fiber, apart from the selectivity for certain species. This selectivity was determined in research performed about bifidobacteria encouraged by intake of materials like fructooligosaccharide, inulin, transgalactosylated oligosaccharide, soya oligosaccharide etc. Prebiotics; must not be hydrolyzed or absorbed in the upper section of the gastrointestinal system. Must complete selective fermentation of beneficial bacteria in the colon, change colonic microbiota to become healthier, and selectively benefit the health of the host, preferably (Vrese and Schrezenmeir, 2008).

### 1.3. Symbiotic

An important method in managing microflora is to use combinations of probiotics and prebiotics. To ensure this, live microorganisms are added to a specific substrate supporting their development. For example, when fructooligosaccharide supplementation is made in addition to *Bifidobacterium*, the viability of the microorganism is increased by preparing the substrate for fermentation (Ray and Bhunia, 2016).

The health benefits of probiotics and prebiotics were investigated in this book chapter.

## 2. BENEFICIAL EFFECTS OF PROBIOTICS and PREBIOTICS

### 2.1. Support of intestinal flora

Probiotic microorganisms may fight against pathogens due to their competitive abilities and their ability to bind to mucosa. Adherence of *Lactobacillus spp.* to the intestinal surfaces occurs by the lipoteichoic acid adhesion property. At the same time, exopolysaccharides produced by some probiotic microorganisms are stated to have significant effects on adhesion (Sun et al., 2007).

Symbiotic combinations of probiotics (*L. plantarum*, *L. paracasei* or *B. bifidum*) in addition to inulin, oligofructose or oligosaccharide ensure increases in *bifidobacteria* and *lactobacilli* and inhibit a variety of pathogenic bacterial strains (*Clostridium difficile*, *C. perfringens*, *C. histolyticum*, *E. coli*, *Campylobacter jejuni*, *E. faecalis*, *Enterobacterium spp.*, *Listeria monocytogenes*, *Staphylococcus aureus*,

*Bacteroides vulgatus*, *Salmonella enteritidis* or *S. typhimurium*) (Collado et al., 2008).

*Saccharomyces boulardii* and *B. infantis* reduce inflammation levels in the intestinal system and fungal colonization in the gastrointestinal system (Goldin and Gorbach, 2008).

## **2.2. Viral and bacterial infections diarrhea**

Diarrhea in gastrointestinal infections is due to bacteria (*E. coli*, *Listeria*, *Shigella*, *Salmonella*), protozoa (*Entamoeba*, *Giardia*, *Microsporidium*), viruses (rotavirus) or metazoans. For treatment of diarrhea due to infection, *Saccharomyces boulardii* used alone or with *bifidobacterium* was determined to display beneficial effects (Dubey et al., 2008).

The use of *L. plantarum*, *L. rhamnosus* GG, *L. reuteri* and *L. acidophilus* among *Lactobacillus*, and *Enterococcus* SF 68 species was identified to be effective for diarrhea (Watson and Preedy, 2010).

### **2.2.1. Antibiotic-sourced diarrhea**

Diarrhea (cause antibiotic) linked to imbalances in intestinal microbiota in 20% of patients. Treatment with antibiotics generally causes diarrhea, while most diarrhea associated with antibiotics was determined to be due to the toxin-producer *Clostridium difficile* (Dendukuri et al., 2005).

A randomized study (double-blind, placebo controlled) identified that the administration of *L. casei*, *L. bulgaricus* and *S. thermophilus* to healthy people and patients receiving antibiotic treatment significantly reduced the incidence of diarrhea (Hickson et al., 2007).

A study of mice identified that use of inulin, oligofructose or oligosaccharide alone or with prebiotics with symbiotic effects was effective on rotavirus and other diarrhea diseases induced by *C. difficile* (Lewis et al., 2005).

### **2.2.2. Acute infectious (rotaviral) diarrhea**

Rotaviral diarrhea is characterized by vomiting followed by sudden watery diarrhea in a short time in infants from 6 months to 2 years of age. Treatment requires replacement of lost fluids and regulation of feeding. A study determined that *L. rhamnosus* GG and *L. reuteri* SD 2222 shortened the duration of watery diarrhea significantly (Szyman'ski et al., 2006).

A study by Dubey et al. (2006) stated that a probiotic mixture (a total of 90 billion bacteria including *L. bulgaricus*, *L. paracasei*, *L. acidophilus*, *L. plantarum*, *B. longum*, *B. infantis*, *B. breve*, and *S. thermophilus*) was effective in earlier recovery.

Waligora-Dupriet et al. (2007) identified that after supplementing children with 2 g/day FOS for 3 weeks significantly less fever, diarrhea, vomiting, and bloating occurred in children within 7-19 months.

### **2.2.3. Tourist (traveler) diarrhea**

Tourist diarrhea occurs in individuals from developed countries after going to tropical or subtropical regions (McFarland, 2007). The probability of developing diarrhea varies from 5-50% according to the country of travel (Watson and Preedy, 2010). Most tourist diarrhea is due to *Vibrio parahemolyticus*, *Vibrio cholerae*, *Salmonella*, enteroaggregative *E. coli*, enteropathogenic *E. coli*, *Yersinia*

*enterocolitica*, *Shigella*, *Aeromonas hydrophila*, *Campylobacter jejuni*, and *Plesiomonas shigelloides*, parasites, and viruses (McFarland, 2007).

Studies investigating the efficacy of *S. boulardii*, *L. acidophilus* and *B. bifidum* against tourist diarrhea concluded that probiotics can significantly prevent tourist diarrhea (McFarland, 2007).

Daily 12 g oligofructose intake was stated to be effective in preventing tourist diarrhea and reducing symptoms if present (Cummings et al., 2001).

#### **2.2.4. Diarrhea in people with suppressed immune systems**

Patients receiving radiotherapy and chemotherapy have increasing fungal infections in organs, led by the gastrointestinal system (*Candida albicans*), causing severe disorder of the intestinal system along with diarrhea. A study of 490 patients with radiation-linked diarrhea in Italy identified that the use of probiotic VSL#3 may prevent development of diarrhea and fungal infection (Delia et al., 2007).

#### **2.3. Urinary tract infections**

Probiotics may be used against urinary tract infections in individuals including preterm infants, especially (Watson and Preedy, 2010).

*L. reuteri* RC-14, *L. rhamnosus* GR-1, *L. fermentum* RC-14 and *L. acidophilus* were identified to significantly reduce urinary tract infections (Seung et al., 2007).

## **2.4. Lactose intolerance**

Lactose intolerance is characterized by gastrointestinal discomfort like nausea, cramps, bloating, abdominal pain and diarrhea after milk consumption linked to lack of, or inadequate, galactosidase (lactase) enzyme (De Vrese et al., 1997).

Consumption of fermented milk products like yogurt increases tolerance to lactose. At the same time, probiotics were identified to be effective in resolving side effects of intolerance (De Vrese et al., 2001).

A research by Oozeer et al. (2002) in mice identified that *Streptococcus thermophilus* and *Lactobacillus casei defensis* could hydrolyze lactose.

## **2.5. Inflammatory bowel disease**

It is reported that genetic, immunologic and psychological factors play roles in the pathogenesis of this disease (Mow et al., 2004).

Studies reported that *L. fermentum*, *L. casei*, *L. salivarius*, *L. reuteri*, *L. rhamnosus*, and prebiotics were effective in reducing inflammation in these diseases (Lara-Villoslada et al., 2006; Peran et al., 2006).

## **2.6. Crohn disease**

This causes intestinal discomfort, abdominal pain, vomiting, weight loss and diarrhea. Studies observed the use of some probiotics led by *S. boulardii* reduced the effect of diarrhea in Crohn patients (Schultz et al., 2004).

## **2.7. Irritable bowel syndrome**

Patients comprise a group with high heterogeneity with variable treatments implemented resulting mostly in disappointment. Sometimes

irritable bowel syndrome may develop acutely due to diarrhea, antibiotic treatment and mostly from viral causes (Cremonini and Talley, 2005).

Aerssens et al., (2008), determined a fall in the numbers of *lactobacillus* spp. and *bifidobacterium* spp. in some mucosal inflammatory situations, while elevated numbers of coliform group bacteria especially *E.coli* and *bacteriodes* increased intestinal gas production and this situation triggered irritable bowel syndrome.

The use of *Bifidobacterium animalis*, *Lactobacillus rhamnosus* GG, *E.coli* DSM 17252 reduced intestinal permeability by forming a protective layer on intestinal mucosa, reduced irritable bowel syndrome symptoms and ensured a reduction in abdominal bloating (Kajander et al., 2008; Enck et al., 2009).

Use of prebiotics in irritable bowel syndrome may cause bloating linked to the increase in fermentation, while the mild laxative properties may be effective in preventing constipation (Cummings and Macfarlane, 2002).

## **2.8. Constipation**

Constipation is a discomfort seen in all individuals characterized by fewer/denser stools, slower bowel movements and bloating (Fernandez-Banares, 2006).

A study with children have determined that different concentrations of the probiotics *L. reuteri* ATCC 55730 and *L. rhamnosus* and prebiotics fructooligosaccharides, galactooligosaccharides and inulin alone or in combination reduced

density of stools and increased frequency of defecation (Indrio et al., 2008).

### **2.9. Necrotizing enterocolitis**

Necrotizing enterocolitis is observed in the first weeks of life of infants with low birth weight predominantly causing neonatal intensive care admission. Most necrotizing enterocolitis cases are associated with prematurity, comprising only 5-25% of all neonates (Deshpande et al., 2007).

The results of meta-analysis of clinical study data found administration of probiotics to premature infants significantly reduced serious risks related to necrotizing enterocolitis and mortality (Alfaleh and Bassler, 2008).

Studies in neonatal patients admitted to intensive care units determined that patients administered *Lactobacillus acidophilus* and *Bifidobacterium* had reductions in both necrotizing enterocolitis incidence and death rates (Sodhi et al., 2008).

A study by Kanamori et al. (2004) investigating the effects of prebiotics in mice identified that inulin, oligofructose or oligosaccharides were effective on colitis alone or with symbiotic effects.

### **2.10. Colitis ulcerosa (ulcerative colitis)**

Colitis ulcerosa generally spreads through the rectum and lower section of the colon to the whole colon. Probiotics initially may be used to induce remission and to prevent recurrence of disease. Researchers reported that *E. coli* Nissle was effective to colitis ulcerosa (Henker et al., 2008).

Administration of *Bifidobacterium longum* with 6 g fructooligosaccharide and inulin for 4 weeks provided a reduction in ulcerative colitis symptoms, as shown in a study by Furrie et al. (2005).

A study by Bibiloni et al. (2005) identified that *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium infantis*, and *Bifidobacterium longum* reduced indications of colitis ulcerosa.

### **2.11. Colic and regurgitation**

It was determined that *L. reuteri* DSM 17938 prevented initiation of gastrointestinal disorders (Athalye et al., 2016).

A study of 200 healthy infants determined that administering 0.8 g/dL prebiotic oligosaccharides FOS/GOS (1:9) significantly reduced colic frequency and the number of regurgitation episodes after 2 weeks (Savino et al., 2003).

### **2.12. *Helicobacter pylori* colonization**

*H. pylori* is accepted as the etiologic vector involved in the risk of gastroduodenal ulcers and stomach cancer; hence, the early colonization of *H. pylori* is an important in determining of stomach cancer that may occur later. Development of chronic gastritis in all colonized individuals, most remain asymptomatic. These individuals cannot receive effective treatment with antibiotics due to high cost, high inefficacy and antibiotic resistance, in addition to the possible induction of an inverse effect. Probiotics may be used in diet as a tool to manage this risk in the population (Gotteland et al., 2006; Langat et al., 2006).

*Lactobacillus* spp. and *Bifidobacterium* spp. subspecies have the ability to prevent bacteriocin and/or organic acid production and adhesion to epithelium cells; for this reason, they were determined to have bactericidal effect under in vitro conditions against *H. pylori* (Gotteland et al., 2006).

Sýkora et al., (2005) reported the fermented product (containing *L. casei* dn-114001) was identified to significantly increase the eradication rate *H.pylori*.

### **2.13. Lymphocytic colitis and collagenous colitis**

Lymphocytic colitis and collagenous colitis are microscopic colitis where inflammation is not visible and biopsy is required for diagnosis. It is a rare intestinal disease with inflammatory changes characterized by chronic watery diarrhea with normal endoscopic and histopathologic findings. Probiotics like *Bifidobacterium animalis* subsp. *lactis* BB12 and *Lactobacillus acidophilus* LA-5 have not yet provided sufficiently positive results (Wildt et al., 2006; El-Matary et al., 2010). A study stated that the probiotic *E. coli* Nissle significantly reduced the rate of defecation (Tromm et al., 2004).

### **2.14. Management of allergies**

Allergies, appears that intestinal infections, industrial processing of food materials, changes in food consumption models and intestinal microbiota have caused allergic reactions to reach higher frequency (Grüber et al., 2007).

Probiotics regulate homeostasis of intestinal microbiota, and are stated to reduce allergy risks and symptoms (Von Berg et al., 2007).

*L. rhamnosus* was shown to increase immunologic tolerance by regulating atopic dermatitis and reducing levels of inflammatory markers (Wickens et al., 2008).

A study of 259 children investigating the effect of prebiotics on eczema identified that children administered galactooligosaccharide and fructooligosaccharide had reduced prevalence of atopic eczema (Moro et al., 2006).

### **2.15. Pre-probiotics and obesity, metabolic irregularity and trauma**

Obesity comprises a risk factor for related disorders including cardiovascular diseases, non-alcoholic fatty liver disease, and type 2 diabetes mellitus (Konturek et al., 2004).

Administration of *L. rhamnosus* GG and *B. lactis* Bb12 pregnant individuals from the first trimester of pregnancy was observed to cause a reduction in sugar amounts in blood and increased glucose tolerance during pregnancy and for 12 months after birth (Laitinen et al., 2008).

Rats with induced hypercholesterolemia were administered  $2 \times 10^6$  cfu/day *L. acidophilus* ATCC 43121 for 21 days and positive effects were observed on serum cholesterol and lipoprotein levels (Park et al., 2007).

Daubioul et al. (2002) added 10 g oligofructose in diet to obese rats for 7 weeks and reductions were observed in hepatic triacylglycerol, energy intake and body weight.

Mice given 5 g inulin per day for 8 weeks with a high fat and high sucrose diet were observed to have reductions in plasma triacylglycerol, fat acid, and glucose (Sugatani et al., 2006).

A study of healthy male individuals found 25 g lactulose and rhamnose consumption daily for 4 weeks provided a significant reduction in plasma triacylglycerol (Vogt et al., 2006).

The effects of probiotic cultures in clinical studies were researched in many studies. Kotzampassi et al. (2006) in a study of 65 patients with randomized severity of multi-cause trauma administered maltodextrin as placebo one time per day for 15 days to the first group and a symbiotic to the other group. The symbiotic comprised probiotics of *L. paracasei* ssp. *paracasei*, *L. plantarum*, *Leuconostoc mesenteroides*, and *Pediococcus pentosaceus* (each  $10^{11}$  cfu/g) and prebiotics of pectin, inulin, oat bran, and resistant starch. The duration of stay in intensive care significantly reduced in the group given symbiotics.

A study of 59 people researching the effects of prebiotics on calcium absorption reported the calcium absorption of the group administered a mix of inulin and oligosaccharide (70%-30%) significantly increased compared to the groups with only oligosaccharide and placebo (Griffin et al., 2002).

### 3. CONCLUSION

The *lactobacillus* spp. and *bifidobacterim* spp. used in food production are accepted as generally recognized as safe by the Food and Drug Administration (De Vrese and Schrezenmeir, 2008).

The increasing use of probiotics following their introduction to the world has illustrated that their safety is an important topic requiring focus. To monitor the safety of probiotics, isolation of probiotic bacteria from infectious patients and thus population-based surveillance is important. It is necessary to correctly identify the strain to know the susceptibility profile of any strain used in clinical studies. It should be possible to compare probiotic strains with clinically isolated strains using molecular methods. In recent times, many patent applications have been made related to materials proposed to be prebiotic. Prebiotics may be included in many food item groups to regulate and improve a balanced intestinal microflora and develop health.

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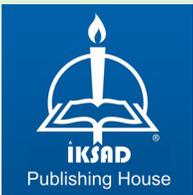
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