CURRENT ADVANCES IN MEDICINE

EDITOR
Yasin GÜZEL

AUTHORS
Ayse CETIN
Deniz İPEK
Dilek ÖZTAŞ
Emel GÜÇLÜ CİHAN
Furkan ASAN
Hadijah MBONDE
Nazik Meziyet DİLEK
Nurten ÖZÇALKAP
Ozlem SEN
Salih MOLLAHALİLOĞLU
Serkan ALTUNTAS
Süleyman Sıtkı URALOĞLU

İKSAD
Publishing House

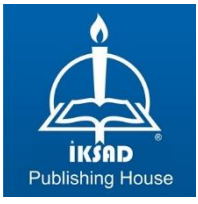
CURRENT ADVANCES IN MEDICINE

EDITOR

Yasin GÜZEL

AUTHORS

Ayse CETIN
Deniz İPEK
Dilek ÖZTAŞ
Emel GÜÇLÜ CİHAN
Furkan ASAN
Hadijah MBONDE
Nazik Meziyet DİLEK
Nurten ÖZÇALKAP
Ozlem SEN
Salih MOLLAHALİLOĞLU
Serkan ALTUNTAS
Süleyman Sıtkı URALOĞLU



(The Licence Number of Publicator: 2014/31220)
TURKEY TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

ISBN: 978-625-8323-47-4
Cover Design: İbrahim KAYA
July / 2022
Ankara / Turkey
Size = 16x24 cm

CONTENTS

PREFACE

Every day, medicine advances at a lightning speed. It is the responsibility of all healthcare workers, regardless of field, to keep up with current medical discoveries.

We wanted to convey recent developments from several fields in this book. We believe that this book will provide all of our colleagues with up-to-date information on the latest advances in diagnosis, treatment, and care protocols.

We would like to thank everyone at the Iksad Publishing House who supported us with this endeavor. We hope can always meet in the light of science and knowledge. We would also want to express our gratitude to all of the authors who contributed the most to the publication of this book.

Yasin GÜZEL, MD[1]

[1] Department of Cardiovascular Surgery, Çukurova University Medical School, Adana, Turkey

CHAPTER 1

PROTEIN METABOLISM DISORDERS AND NEW PRODUCT DEVELOPMENT STUDIES FOR PATIENTS WITH PHENYLKETONURIA

Dr. Nazik Meziyet DİLEK[1]

[1] Selçuk University, Akşehir Kadir Yallagöz School of Health, Nutrition and Dietetics, Konya, Türkiye, meziyettemel@hotmail.com,ORCID: 0000-0003-2506-2067

1. INTRODUCTION

It is reported that enzyme deficiencies playing role in protein metabolism lead to anomalies in the metabolism of amino acids and accumulation of toxic substances in body. As a result of this accumulation, it is expressed that, especially brain, kidneys and liver, the various organs of the body are affected; that beginning time and intensity of symptoms have individual differences and that the symptoms of interest may be much more intensive in catabolic periods like huger and infection (Atik Altınok, 2019).

In disorders of amino acid metabolism, as a healing way, precursor amino acid intake is generally restricted. While intake of one or more essential amino acids leading to illness is reduced in such a way that it will meet the lowest need for the body, it is necessary that intake of energy-vitamin-mineral and trace amounts of elements provide natural growth and development (Zeybek, 2004). While precursor mixes of amino acid not containing precursor amino acid are used in meeting daily protein need, daily needs of protein should be met with natural foods containing low protein and protein-removed or reduced products such as bread, macaroni and milk, which are specifically produced for the individuals suffering from this illness. In this study, it has been attempted to be given information about, particularly phenylketonuria (PKU), the various diseases of protein metabolism and studies of developing the new product, carried out to enrich the diets of PKU patients.

1.1. Maternal Phenylketonuria

Maternal phenylketonuria is a type of phenylketonuria seen in pregnant women with phenylketonuria. If such women are not treated during their pregnancies, microcephaly, mental deficiency, congenital heart disease, intrauterine growth retardation, dysmorphic face and many fetal damage form. For example, if mother has classical phenylketonuria, the incidence of mental deficiency in her infant is 92%, the incidence of distinct microcephaly is 74%. If mental deficiency coming with maternal deficiency cannot be prevented, the infants having mental deficiency coming with phenylketonuria, which

will be eliminated with scanning and early treatment, will replace with infants having mental deficiency coming with maternal phenylketonuria, which is a dangerous situation. As a conclusion, preventing mental deficiency via nutritional treatment in the female patient with phenylketonuria, it is necessary to give a possibility to them for gaining fertility. The results of multi-centered phenylketonuria study group point out that nutritional therapy with low phenylalanine started within the prenatal and postnatal ten weeks may prevent fetal exposure (Köksal & Gökmen, 2013).

1.2. Phenylketonuria

Phenylketonuria is a congenital metabolic disease, characterized by the damage the phenylalanine and its metabolites (phenyl pyruvic acid, phenyl lactic acid, phenyl acetic acid) accumulating in the blood especially generate in the brain, due to deficiency of phenylalanine hydroxylase enzyme secreted by liver as a result of that phenylalanine that is an essential amino acid cannot be metabolized in the body (Scriver, 2007).

Phenylketonuria is a genetic disease and was discovered as a result of the study carried out by Asbjörn Fölling on two mental deficient children in 1934. Phenylketonuria is the first neonatal metabolic dysfunction defined. Beginning from that phenyl pyruvic acid is defined as a phenylalanine metabolite, Fölling accepted that there was phenylalanine metabolism dysfunction in these children. In the further periods, it was demonstrated that there was no phenylalanine accumulation in the bloods and spinal liquids of the other mentally deficient individuals, who fling pyruvic acid with urine, and that there was no phenylalanine hydroxylase enzyme activity (which transform phenylalanine into tyrosine) in their livers. After 20 years from discovery of phenylalanine, in 1953, it was observed that a diet was useful, which can prevent mental deficiency in this disease. In the early, 1963, by beginning to continuously follow phenylketonuria, mental deficient, which can be seen in all individuals being born as the patient of phenylketonuria, was prevented (Köksal & Gökmen, 2013).

In the parents of the individuals with phenylketonuria, there are two genes that are responsible for producing phenylalanine hydroxylate, one of which is normal and the other is dysfunctional. An infant, who takes a dysfunctional gene from mother and father, is born with the disease of phenylketonuria. While a child, who takes a dysfunctional gene from only one of the parents, is carrier like his/her mother and father, he/she does not show disease symptoms. A child taking healthy genes from mother and father is completely healthy. When both mother and father are carriers, it is reported that the probability of child to be born with phenylketonuria disease reaches rather high values like 25%, therefore, that disease risk is more as a result of kin-marriage. It is reported that phenylketonuria can be treated in case that it is diagnosed in neonatal period, however, in case that it cannot be treated, that it leads to heavy mental deficiency (Köksal & Gökmen, 2013).

It is reported that the incidence of phenylketonuria is 1/15,000 in USA; 1/10,000 in United Kingdom; 1/60.000 in Japan; 1/18.000 in Japan (Dönmez, 2002; Özalp, 2000), In Turkey, it is reported that this disease is seen in one of 4500 neonatal (Demirkol, 2003; Özalp, 2000; Tokatlı, 1999). Also in Turkey, the reasons such as the fact that one per 20-25 people is carrier of disease of interest and that kin-marriages are very prevalent in East and South-East Anatolian regions largely increase the incidence of disease (Demirkol, 2003).

Due to the fact that Guthrie microbiological assay test is an important place in phenylketonuria scanning can be applied to hundreds of children in a short time, there is no need for a specific instrument and it is very cheap, this test has been commonly used since 1960s (Wright, Brown, & Davidson-Mundt, 1992). In our country, it is stated that phenylketonuria scanning program has been applied by Ministry of Health since 1963 (Baykal, Huner, & Cakmakcı, 1998; Demirkol, 2003).

In this disease, for preventing cerebral damage, in as short time as possible – within the first 7 days after birth, it is stated that it is necessary to begin dietary therapy and continue this diet lifelong. Nowadays, although there are therapy options preventing toxic effects

on central neural system, there is not any therapy providing healing. Therapy is based on phenylalanine restriction in diet and administrating sufficient amount of tyrosine, which cannot form due to enzyme blockage, via diet (Blau, 2006; Scriver, 2007).

It is reported that the patients with phenylketonuria should consume the meat, milk and dairy products and foods such as egg and chicken, which contain high protein, thus, high phenylalanine, in very limited amounts or not lifelong. However, that phenylalanine is an essential amino acid requires it to be taken in certain amounts. In the individuals having this disease phenylalanine need is met from natural resources such as vegetable and fruit that relatively contain less protein. In meeting daily protein need, amino acid mixes not phenylalanine are used. In order to be able to keep phenylalanine level in the blood, it is reported that it is necessary to be provided at least 80-90% of protein need from amino acid mixes in infancy period and at least 50% of it, in childhood and adolescent periods (Taylor, Moore, & Davidson, 1984). The level of phenylalanine level in the blood should be kept at 2-6 mg/dL in the children less than age and 10; 2-15 mg/dL in the individuals over age 12. It is also necessary to arrange daily amount of tyrosine as 100-120 mg/kg/day (Blau, 2006; Coşkun, 2003; Giovannini, Verduci, Salvatici, Fiori, & Riva, 2007). In phenylalanine–restricted diet, carbohydrates such as maize starch and sugar and edible oils form important energy resources. Since children with phenylketonuria are under risk in terms of deficiency of selenium, zinc, copper, iron, calcium, vitamin B_2, B_6, B_{12}, folic acid, their growths and developments should be closely followed and also reviewed in terms of vitamin and mineral deficiencies of interest (Arnold, Kirby, Presto, & Blakely, 2001; Darling, Mathias, O'regan, & Naughten, 1992).

1.3. Homocystinuria

It is stated that 3 malfunctions have seen in breakdown of methionine metabolism from essential amino acids, whose structure contains sulfur lead to homocystinuria. There are 3 types of homocystinuria as Type 1(cystathionine β-synthetase,), Type 2 (cobalamin metabolism disorder,) and Type 3 ($N^{5\text{-}10}$ methylene

tetrahydrofolate reductase enzyme deficiency). Incidence of homocystinuria that is an autosomal recessive transitive disease is known to be 1/200000.

In the healthily delivered babies, developing the cases such as growth retardation, mental deficiency, convulsion in some patients, light color, osteoporosis, cataract, blindness, trombonist anomaly, coagulopathy, cerebral hemorrhage in the next ages, a heavy disease forms and death may occur. As a result of decrease of cystine and cysteine, light color is seen on hairs and nails (Köksal & Gökmen, 2013). Although homocystinuria is a disease, which can be caught by scanning tests in neonatal periods, diagnosis is generally established as a result of that symptoms emerge in the late periods (Zeybek, 2004).

Dietary therapy should contain methionine –limited and cysteine –rich nutrients. It is stated that it is necessary to keep protein intake within about 3 g/kg/day and to continuously follow the level of albumin in the blood. Energy, as in all limited diets containing protein, is given at high level to prevent catabolism. Energy requirement should be met from special energy modules, oils and carbohydrates (Köksal & Gökmen, 2013).

1.4. Genetic Tyrosinemia

Type 1 tyrosinemia is defined as protein metabolism disorder, which forms due to deficiency of fumarylacetoacetate hydrolase enzyme functioning in the last stage of tyrosine metabolism (Zeybek, 2004). In this disease, which emerge with the symptoms such as heavy liver, kidney and central neural system disorders, mental deficiency, rickettsia, development retardation, hepatomegaly, hepatitis, vomit and diarrhea, it is necessary to keep tyrosine level in the blood below 10 mg/dl via diet (Köksal & Gökmen, 2013).

In the applications of therapy, it is necessary to restrict phenylalanine and tyrosine in diet, adjust protein needs of patient according to liver functions, not to increase solid load in kidney and provide normal growth in the child. The first phases of disease are important, and it is suitable to restrict methionine. Protein should be given at the level of 1 g/kg per day. In addition to dietary therapy,

riketsia developing as a result of genetic tyrosinemia should be also treated (Köksal & Gökmen, 2013).

1.5. Organic Acidemia

Organic acidemia is a disease characterized as a result of that organic acids, which accumulate organic acids in body liquids make toxic effect, depending on the various enzyme deficiencies, is autosomal recessive transitive genetic metabolism disorder, which shows itself with very different clinical symptoms, especially serious acidosis attacks that threatens life in the first days of the life. Incidence of disease is generally around 1/5000. Among them, methylmalonic acidemia and propionic acidemia are of disease groups the most seen in our country (Köksal & Gökmen, 2013).

1.6. Maple Syrup Urine Disease

In this disease forming due to dysfunction in valine, isoleucine and leucine metabolisms, there is a maple syrup odor in the urine. Maple syrup urine disease forms as a result of dysfunction in activity of the branched chain ketoacid dehydrogenase enzyme complex (Köksal & Gökmen, 2013). In the cases, where the early therapy does not start, acidose, convulsions, conscious changes, coma and as a consequence, death occurs (Zeybek, 2004). While world incidence of this disease, which is a recessive transitive genetic metabolic disease is reported as 1/185000, incidence in our country 1/200000 (Köksal & Gökmen, 2013).

2. THE STUDIES CARRIED OUT ON LOW PROTEIN PRODUCTS FOR PHENYLKETONURIA PATIENTS

Phenylketonuria is a genetic metabolic disease, whose symptoms can be prevented via appropriate dietary therapy, when it can be diagnosed in the first days following birth. The only therapy in phenylketonuria, which can be applied at the moment, is dietary therapy, and it must continue lifelong (Köksal & Gökmen, 2013). Although dietary therapy is successful in preventing mental deficiency when it is applied together with birth, it is suggested that lifelong sustaining low phenylalanine contained diet has obvious difficulties

and, in phenylketonuria patients treated with only diet, there is evidence associated with insufficient development and dietary results (Enns et al., 2010). Therefore, there is need for new approaches about dietary management of phenylketonuria.

In this study, in order to produce protein hydroxylates suitable for the consumption of phenylketonuria patients, it is studied whether or not to use agricultural wastes as pre-substance in the production of adsorbents is suitable for removing phenylalanine from sample solutions. Adsorbent was prepared by treatment of waste substances with H_3PO_4 and activation at 350 ºC. Group adsorption studies were carried out at 25, 35 and 45°C. On the adsorbents prepared, depending on pH of the solution, the different mechanisms for phenylalanine adsorption were observed. Hydrophobic interactions between carbon rings on adsorbent surface and phenyl rings of adsorbate molecules were accepted as dominant mechanism. Adsorption kinetics were found satisfying, and maximum intake capacity of phenylalanine was compared with synthetic adsorbent values in the literature. The results, in order to remove phenylalanine from aqueous solutions, showed using agricultural waste as material for absorbent production promised potential (Clark, Alves, Franca, & Oliveira, 2012).

In addition to normal milk proteins, in a study carried out to first demonstrate that modified milk production is suitable, in order to identify phenylalanine κ- casein in their milk, genetic-modified rabbits were created. One gene containing encoded section of rabbit κ- casein gene was modified by oligonucleotide direct mutagenesis. 4/5 of phenylalanine amino acids in mature protein underwent mutation, and gene making was used to form two genetically modified rabbit lines. Genetically modified rabbits produced high amount of recombinant κ-casein in their milk, which cause reduction in average sizes of casein micelles. It can be digested by κ- casein chymosin with low phenylalanine and can be separated in opposite phase column from the other milk proteins and natural peers by means of ingle step HPLC method. As a result of this study, it was concluded that casein with low phenylalanine produced from genetically modified animals could be

used dietary substitute product to meet specific needs of certain consumer groups (Baranyi et al., 2007).

In another study, successive hydrolyses of whey were examined by using carboxypeptidase -A fixed on trypsin, chymotrypsin and agarose –glioxil. Phenylalanine was separated by the action of carboxypeptidase -A from hydrolysates. So, sufficient amount of protein resource was provided for phenylketonuria patients. By means of carboxypeptidase-A, the role of the first two pre-hydrolyses on phenylalanine efficiency was studied. Whey proteins, using different enzyme-substrate rates, were hydrolyzed by trypsin and chymotrypsin to different hydrolyze degrees, respectively. Analysis showed that more suitable substrate was obtained for carboxypeptidase A, when whey was only hydrolyzed by chymotrypsin using an approach specific to aim, between immobilized chymotrypsin proteolysis, an empirical model was developed for a certain reaction rate enabling to predict concentration of hydrolyzed peptide bonds. While Michaelis-Menten type of equation failed in presenting time assessment of hydrolyzed peptide bonds in group test, this model was relatively suitable for empirical data (Galvão, Pinto, Jesus, Giordano, & Giordano, 2009).

In another study carried out, a new concept of enzymatic membrane reactor was presented whey containing low phenylketonuria was aimed to be used in the production of protein hydrolysates for diets of phenylketonuria. Whey proteins was hydrolyzed by first chymotrypsin and then, using a filter, by carboxypeptidase A, fixed on agarose gel particles kept in reactor. In addition, a mathematical model of enzymatic membrane reactor was presented, and its validity was confirmed (Cabrera-Padilla, Pinto, Giordano, & Giordano, 2009).

Even though restriction of dietary phenylalanine is the building block of phenylketonuria therapy, in Europe, there are no unanimously prepared fundamentals. In a study carried out associated with this, using a questionnaire prepared, detailed information was obtained about routine dietary therapy of phenylketonuria from 10 European center, each of which is represented by dietician or physician. All centers were scanned within the first ten days for phenylketonuria. The responsibilities, roles and educations of dieticians and nutritionists

show diversity. In some centers, while dieticians are responsible for adjusting diet, in the other centers, diets are prepared by physicians. In defining target phenylalanine concentration, doses of protein substitutes, permitted daily phenylalanine intake systems and foods that can be consumed without restricting, certain differences were seen. 80% of centers support those infants with phenylketonuria are nourished by breast milk as well as protein substitutes. As a result, in adjusting phenylketonuria diet and support systems designed to help patients for making this, it was identified that there were important differences between European centers (Ahring et al., 2009).

Phenylketonuria patients form risk group for micronutrient unbalances. Optimum nutrient, in the cases, where especially intake of nutrient from unnatural resources form an important part of diet, is rather compelling. In the follow of dietary therapy of the patients with phenylketonuria, micro nutritional supplement is a requirement, and vitamins and minerals must be added to amino acid mixes without phenylalanine together or individually. In a study, in which published articles since 1990 were reviewed, deficiency of vitamins and minerals was defined. Insufficiencies of biological micronutrient, first of all, were reported for zinc, selenium, iron, B_{12} vitamin and folate (Robert et al., 2013).

In a study, in 16 foods, specially prepared for phenylketonuria patients, amino acid content and amount of protein were identified, and the most suitable results were compared with the data of various international food composition. As expected, all foods contain low proteins (0.67-3.15 g/100 g). The highest protein content was found in boiled rice and potatoes. It was seen that these foods contained phenylalanine in the highest amounts (respectively, 158.51 and 62.65 mg/100 g). When the results are compared to the different data of composition, small deviations were observed (Pimentel, Alves, Costa, Torres, et al., 2014).

In another study, carried out on 16 foods, which were specially prepared for phenylketonuria by considering the nutritional composition, phenylalanine and tyrosine contents, fatty acid profile and amounts of E and B_{12} vitamins, the composition of sample cooked was

identified as 15.5-92 g/100 g water, 0.7-3.2 g/100 g protein, 0.1-25 g/100 g total fat and 5-62 g/100 g carbohydrate. Fatty acid profile and amounts of vitamin E reflected the type of edible oil used. All samples were found weak in terms of vitamin E (0.3-0.8 μg/100g). It was seen that boiled rice had the highest phenylalanine content with 50.3 mg/ g protein (Pimentel, Alves, Costa, Fernandes, et al., 2014).

In a study carried out, for the production of bread with low phenylalanine, 5 different gums were added to maize starch in the rates of 1, 2 and 3%. The best results were obtained by adding xanthan gum and carrageenan mixes (Özboy, 2002).

In another study of bread with low phenylalanine, maize and rice starches, treated in microwave for 2, 4 and 6 minutes, were used. Analysis results showed that viscosity fell, while acidity, water bonding ability, swelling power, solubility and amylose content increase as a result of treatment with both sorts of starch. The best microwave application was identified as 2 and 4 minutes and breads were produced. As a result of sensorial analyses, in terms of taste and general appearance, the best results were obtained from modified maize starch, treated for 2 and 4 minutes, and modified rice starch, treated for 2 minutes. Results showed that as a result of treatment of both modified starches for 2 and 4 minutes, staling in bread delayed and freshness increased (Ayman, Zahran, & Omaima, 2009).

In a study, carried out, for the production of test bread with low phenylalanine, gliadin–removed wheat flour and hydrocolloids were used. For the production of wheat flour with low phenylalanine, gliadin aqueous alcohol solution, which as wheat protein fraction, was used and extracted. When compared with control group, phenylalanine content of the samples of gliadin–free bread decreased by 43.2%. While removal of gliadin negatively affects the rheological features and swelling quality of dough, addition of hydrocolloids positively affected them. Results showed that the use of pectin and hydroxyl methyl cellulose (respectively, 2% and 3%) together with gliadin-free wheat flour was suitable for production of acceptable bread (Mohsen, Yaseen, Ammar, & Mohammad, 2010).

In a study carried out Shamy, which is highly prevalent sort of bread in Egypt and Middle-East, was produced as low-phenylalanine for phenylketonuria patients. Formulations were prepared based on replacement of wheat flour (low protein) with maize starch in different rates. In order to delay stalling in bread and develop texture, pectin and carboxymethylcellulose were used. Even though sensorial analyses show that all sample is acceptable, the best results were obtained from the sample producing with addition of 30 g of wheat flour, 2 g of pectin and 2 g of carboxymethylcellulose. When this sample is compared to control group, it was seen that phenylalanine content decreased by 62% (Yaseen, Abd-El-Hafeez, & Shouk, 2011).

In another study of bread, in order to produce tin with low phenylalanine, replacing wheat flour with maize starch in the different rates, 4 formulations were formed. In the production of bread for adjusting texture and developing sizing rate, pectin and carboxymethylcellulose were used. As a result of analyses carried out, as the best formulation, the samples containing 66 g of wheat starch, 30 g of wheat flour, 2 g of pectin and 2 g of carboxymethylcellulose were accepted. When these samples are compared with control group, it was seen that phenylalanine and protein contents decreased in the rates of 67% and 65% (Yaseen, Shouk, Enssaf, & Ashour, 2012).

In a study, where it was aimed to produce cookie with low phenylalanine, formulations were prepared by replacing with wheat flour with the different starches and, as texture regulatory, pectin and carboxymethylcellulose were preferred. As a result of the analyses, although all formulations were found acceptable, it was concluded that the most suitable formulation for cookie production is the one consisting of 84 g of maize starch, 10 g of wheat flour, 3 g of pectin and 3 g of carboxymethylcellulose In these samples, it was seen that the contents of phenylalanine and protein decreased by 88% and 79%, respectively (Yaseen, Shouk, & Bareh, 2014).

In another study, macaroni containing low phenylalanine for phenylketonuria. For this aim, based on replacement of wheat flour with maize starch in different amounts, formulations were formed and, in order to texture, pectin and carboxymethylcellulose were added to

the formulations. As a result of the analyses carried out, it was concluded that the most suitable formulation for phenylketonuria was the formula, which contains 66 g of maize starch, 30 g of wheat flour, 2 g of pectin and 2 g of carboxymethylcellulose and, compared to the control group, that the contents of phenylalanine and protein were 68% and 70%, respectively. As a result of cooking tests, cooking loss of this sample was found largely higher than that of control group but it was seen that addition of hydrocolloids reduced cooking loss (Yaseen & Elhafeez, 2011).

Glycomacropeptide is an important peptide, which forms as a result of hydrolyze of proteins in milk and which is also in whey, and forms as a result of activity of chymosin enzyme in κ-casein (Harper, 2004). Glycomacropeptide is the only protein that naturally forms and does not contain phenylalanine, is the most contained 3rd protein in whey, following β-lacto globulin ve α-lacto albumin and forms 15-25% of a total of whey proteins (Lim, van Calcar, Nelson, Gleason, & Ney, 2007). In addition to many biological functions of glycomacropeptide or peptides derived from it (stimulation of cholecytokinin (the hormone that regulates energy and food intake) released from intestinal cells, prevention of platelet increase and support of beneficial intestinal bacteria (for example, *Bifidobacteria*), glycomacropeptide is important with its low phenylalanine-containing structure. Therefore, it is suggested that glycomacropeptide can be used as a supplement in the foods of phenylketonuria patients' nutrients not containing phenylalanine (Harper, 2004).

In a study carried out, as protein resource for phenylketonuria patients, the powder pudding mix containing glycomacropeptide, which is isolated from whey, and pudding, which contains dried blueberry, was produced. As a result of the analyzes made in the six different pudding mixes containing glycomacropeptide isolate and dried blueberry, it was concluded that the most suitable formulation in terms of sensorial features and viscosity was the pudding containing 15% glycomacropeptide isolate and 15% dried blueberry (Ergül & Karakaya, 2013).

In a study, in order to make delicious foods and drinks with low phenylalanine such as sports drink, drinks with chocolates, pudding and cracker, the use of glycomacropeptide was studied. The analyses made showed that functional features of glycomacropeptide were suitable for using in semi-liquid foods and drinks like especially pudding. It was concluded that glycomacropeptide supplemented with the limited essential amino acids would provide an alternative resource of protein for the individuals with phenylketonuria (Lim et al., 2007).

In a study carried out, for increasing purity of glycomacropeptide and supplementing the foods with essential amino acids, it was aimed to develop calculation of mass balance. For this aim, glycomacropeptide obtained from whey was supplemented with amino acids (histidine, leucine, methionine, tyrosine and tryptophan) and mixing it with non-milk sourced cream, pudding with strawberry. The puddings produced were replaced with amino acids formulas of 15 phenylketonuria treated in clinic and then tested. The results showed that the foods produced with high rate profiled glycomacropeptide and completed nutritionally presented more alternatives to provide quality of life and adaptation for phenylketonuria patients (LaClair, Ney, MacLeod, & Etzel, 2009).

In another study carried out related to this, due to the fact that it naturally contains low phenylalanine, glycomacropeptide was found highly suitable for phenylketonuria diet. In order to increase diversity of protein resources for phenylketonuria diet, it was concluded that nutritionally full acceptable medical foods and drinks could be made by glycomacropeptide (Van Calcar & Ney, 2012).

In this study, making in vitro protein digestion, flour, whose phenylalanine content was reduced, was obtained. For this aim, flour was treated by maize sprouts containing FAL enzyme, and then again drying flour, phenylalanine content was obtained reduced phenylalanine contented flour was obtained for PKU patients (Büyükkurt, 2017).

3. CONCLUSION

In this study, protein metabolism disorders were discussed, and the studies if new product development were tried to be compiled for phenylketonuria. Due to fact that the only therapy method is diet, there is a need for new products for especially phenylketonuria patients, the other protein metabolism disorders, which will not impede the growth and development of disease, which will not cause malnutrition and which will contain energy and whole nutritional elements in balanced way. For this aim, even though product development studies are carried out for these patients, they are not still at sufficient level.

Cereals and its products, which form an important part of daily diet, come in the various forms on market shelves. Although cereals and its products are so various, the individual who suffer from protein metabolism disorders, especially phenylketonuria, face to a serious restriction in consumption of these products. Even though there are specific products for phenylketonuria, their prices are rather high and they are not prevalent are the other problems these patients experience. Lifelong sustaining restricted diet has its nutritional effects as well as psychological effects, especially on children. In addition, that product diversity is less makes difficult the patient to adapt dietary therapy. For reducing the effect of restricted diet on the patients, it is necessary to increase product diversity. When all of these are considered, in order to present more alternatives to patients, for increasing product diversity and facilitating reach of patients to these products, there is a need to carry out more studies.

REFERENCES

Ahring, K., Bélanger-Quintana, A., Dokoupil, K., Ozel, H. G., Lammardo, A. M., MacDonald, A., van Rijn, M. (2009). Dietary management practices in phenylketonuria across European centres. *Clinical nutrition, 28*(3), 231-236.

Arnold, G. L., Kirby, R., Presto, C., & Blakely, E. (2001). Iron and protein sufficiency and red cell indices in phenylketonuria. *Journal of the American College of Nutrition, 20*(1), 65-70.

Atik Altınok, Y. (2019). Doğuştan Aminoasit Metabolizması Bozukluğu Hastalıklarında Tıbbi Beslenme Tedavisi.

Ayman, E., Zahran, G., & Omaima, S. (2009). Evaluation of bread free of phenylalanine for phenylketonuria patients. *Annals of Agricultural Science (Cairo), 54*(2), 349-358.

Baranyi, M., Hiripi, L., Szabó, L., Catunda, A. P., Harsányi, I., Komáromy, P., & Bősze, Z. (2007). Isolation and some effects of functional, low-phenylalanine κ-casein expressed in the milk of transgenic rabbits. *Journal of biotechnology, 128*(2), 383-392.

Baykal, T., Huner, G., & Cakmakcı, T. (1998). İstanbul ilinde fenilketonuri yenidoğan taraması sonuçları. *Biyokimya Dergisi, 23*, 43-46.

Blau, N. (2006). Disorders of phenylalanine and tetrahydrobiopterin metabolism. Hoffmann Jl CJ. *Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases, Berlin Heidelberg*, 26-34.

Büyükkurt, Ö. K. (2017). Fenilketonüri hastalarına yönelik fenilalalnin içeriği azaltılmış bir un geliştirilmesi.

Cabrera-Padilla, R. Y., Pinto, G. A., Giordano, R. L., & Giordano, R. C. (2009). A new conception of enzymatic membrane reactor for the production of whey hydrolysates with low contents of phenylalanine. *Process Biochemistry, 44*(3), 269-276.

Clark, H. M., Alves, C. C., Franca, A. S., & Oliveira, L. S. (2012). Evaluation of the performance of an agricultural residue-based activated carbon aiming at removal of phenylalanine from aqueous solutions. *LWT, 49*(1), 155-161.

Coşkun, T. (2003). *Amino asit metabolizması ve bozuklukları*: Alp Ofset Matbaacılık.

Darling, G., Mathias, P., O'regan, M., & Naughten, E. (1992). Serum selenium levels in individuals on PKU diets. *Journal of inherited metabolic disease, 15*(5), 769-773.

Demirkol, M. (2003). Fenilketonüri hastalığı. Retrieved from http://www.ntvmsnbc.com/news/156191.

Dönmez, S. (2002). *Okul öncesi yaş grubu klasik fenilketonürili hastaların beslenme durumunun değerlendirilmesi.* (Doktora Tezi). Hacettepe Üniversitesi, Ankara., Sağlık Bilimleri Enstitüsü.

Enns, G., Koch, R., Brumm, V., Blakely, E., Suter, R., & Jurecki, E. (2010). Suboptimal outcomes in patients with PKU treated early with diet alone: revisiting the evidence. *Molecular genetics and metabolism, 101*(2-3), 99-109.

Ergül, N., & Karakaya, S. (2013). Fenilketonüri hastaları için maviyemiş Vaccinium corymbosum L. içeren toz puding karışımı üretimi. *Akademik Gıda, 11*(2), 28-36.

Galvão, C. M., Pinto, G. A., Jesus, C. D., Giordano, R. C., & Giordano, R. L. (2009). Producing a phenylalanine-free pool of peptides after tailored enzymatic hydrolyses of cheese whey. *Journal of Food Engineering, 91*(1), 109-117.

Giovannini, M., Verduci, E., Salvatici, E., Fiori, L., & Riva, E. (2007). Phenylketonuria: dietary and therapeutic challenges. *Journal of inherited metabolic disease, 30*(2), 145-152.

Harper, W. J. (2004). Biological properties of whey components.

Köksal, G., & Gökmen, H. (2013). *Çocuk Hastalıklarında Beslenme Tedavisi (3. bs.). Ankara: Hatiboğlu Yayınları*. Retrieved from

LaClair, C. E., Ney, D. M., MacLeod, E. L., & Etzel, M. R. (2009). Purification and use of glycomacropeptide for nutritional management of phenylketonuria. *Journal of Food Science, 74*(4), E199-E206.

Lim, K., van Calcar, S. C., Nelson, K. L., Gleason, S. T., & Ney, D. M. (2007). Acceptable low-phenylalanine foods and beverages can be made with glycomacropeptide from cheese whey for individuals with PKU. *Molecular genetics and metabolism, 92*(1-2), 176-178.

Mohsen, S. M., Yaseen, A. A., Ammar, A. M., & Mohammad, A. A. (2010). Quality characteristics improvement of low-phenylalanine toast bread. *International journal of food science & technology, 45*(10), 2042-2051.

Özalp, İ. (2000). Yenidoğanda fenilketonüri ve Hiperfenilalaninemilerin taranması. *Katkı pediatri dergisi, 21*, 175-184.

Özboy, Ö. (2002). Development of corn starch-gum bread for phenylketonuria patients. *Food/Nahrung, 46*(2), 87-91.

Pimentel, F. B., Alves, R. C., Costa, A. S., Fernandes, T. J., Torres, D., Almeida, M. F., & Oliveira, M. B. P. (2014). Nutritional composition of low protein and phenylalanine-restricted dishes prepared for phenylketonuric patients. *LWT-Food Science and Technology, 57*(1), 283-289.

Pimentel, F. B., Alves, R. C., Costa, A. S., Torres, D., Almeida, M. F., & Oliveira, M. B. P. (2014). Phenylketonuria: protein content and amino acids profile of dishes for phenylketonuric patients. The relevance of phenylalanine. *Food chemistry, 149*, 144-150.

Robert, M., Rocha, J. C., Van Rijn, M., Ahring, K., Bélanger-Quintana, A., MacDonald, A., . . . Goyens, P. (2013). Micronutrient status in phenylketonuria. *Molecular genetics and metabolism, 110*, S6-S17.

Scriver, C. R. (2007). The PAH gene, phenylketonuria, and a paradigm shift. *Human mutation, 28*(9), 831-845.

Taylor, C., Moore, G., & Davidson, D. (1984). The effect of treatment on zinc, copper and calcium status in children with phenylketonuria. *Journal of inherited metabolic disease, 7*(4), 160-164.

Tokatlı, A. (1999). *Tarama testleri, Pediatride Gelişmeler*. Sinem Ofset, 370, Ankara.

Van Calcar, S. C., & Ney, D. M. (2012). Food products made with glycomacropeptide, a low-phenylalanine whey protein, provide a new alternative to amino acid–based medical foods for nutrition management of phenylketonuria. *Journal of the Academy of Nutrition and Dietetics, 112*(8), 1201-1210.

Wright, L., Brown, A., & Davidson-Mundt, A. (1992). Newborn screening: the miracle and the challenge. *Journal of Pediatric Nursing, 7*(1), 26-42.

Yaseen, A., A., , Shouk, A., A., , Enssaf, M., A., E., , & Ashour, M., M., S. (2012). Production and evaluation of low phenylalanine pan bread. *Journal of Applied Sciences Research, 8(12)*, 5799-5805.

Yaseen, A., & Elhafeez, S. A. (2011). Low phenylalanine pasta. *International Journal of Nutrition and Metabolism, 3*(10), 128-135.

Yaseen, A., Shouk, A., & Bareh, G. (2014). Production of functional biscuits for phenylketonuria patients. *World Applied Sciences Journal, 31*(5), 692-697.

Yaseen, A. A., Abd-El-Hafeez, A., & Shouk, A. (2011). Low phenylalanine Egyptian shamy bread. *Polish journal of food and nutrition sciences, 61*(4).

Zeybek, A., Ç. (2004). *Sağlıkta ve Hastalıkta Beslenme Sempozyum Dizisi* Paper presented at the Cerrahpaşa Tıp Fakültesi Sürekli Tıp Eğitimi Etkinlikleri, İstanbul Üniversitesi.

CHAPTER 2

BETA-AMYLOID DEPOSITS AT THE VESSEL WALL: CEREBRAL AMYLOID ANGIOPATHY

M.D. Furkan ASAN[1]

[1] Lokman Hekim Van Hospital, Neurology Department, Van/Turkey, furkan61asan@gmail.com, ORCID ID: 0000-0003-3062-5872

INTRODUCTION

Cerebral amyloid angiopathy (CAA) is the major cause of spontaneous intracerebral hemorrhages in advanced age. CAA, which occurs as a result of beta-amyloid deposits that develop on the vessel walls of small arteries and capillaries, also contributes to cognitive decline in the elderly (Smith & Greenberg, 2009). Although intracranial lobar hemorrhage and microhemorrhages are the main presentations of CAA, transient focal neurological episodes and cerebral microinfarcts may also be observed during the course of the disease. (Boulouis et al., 2016).

With the increase in magnetic resonance imaging (MRI) techniques, the diagnosis of CAA has become easier over time. As a matter of fact, the Boston criteria used in the diagnosis are mainly based on pathological and radiological findings. Lobar, cortical or cortico-subcortical microhemorrhages together with superficial siderosis constitute the imaging findings (Smith & Greenberg, 2003).

Alzheimer's disease (AD) which has neuronal amyloid beta deposition pathophysiologically was detected only in less than 50% of CAA patients (Vinters, 1987). This shows that although the two diseases have similar pathophysiology at the molecular level, they are essentially two different clinical entities.

Due to the predisposition to intracerebral hemorrhage, the necessity of using anti-coagulant and/or antiaggregant in CAA patients leads to many difficulties in the management of these patients.

In this section, we will summarize the clinical and radiological features as well as the pathophysiology of CAA.

1. EPIDEMIOLOGY OF CAA

CAA is a disease with a markedly increased incidence with age. In the autopsy series, the prevalence was found to be 10-15% over the age of 60, but this rate has reached 40% over the age of 80 (Masuda et al., 1988). In the post-mortem examination performed on individuals over the age of 85, the prevalence of CAA was found to be around 70% (Tanskanen et al., 2012). In the subjects examined in the autopsy series, amyloid pathology of vessels was found to be more prominent in the frontal and parietal lobes (Masuda et al., 1988; Tanskanen et al., 2012). The autopsy series have shown different

results in terms of gender predisposition. Hence, a clear predilection to any gender cannot be specified.

The association of CAA with AD has been investigated for many years. CAA pathology was also detected in 80% of the patients with AD-related neuronal plaque. On the other hand, CAA pathology was found in 30% of the patients without AD pathology (Brenowitz et al., 2015). Despite similar pathophysiological mechanisms, the observation of CAA pathology without neuronal amyloid deposits is still a matter waiting to be explained.

Since the definitive diagnosis of CAA is based on pathological findings, it is very difficult to accurately determine its frequency in living individuals. However, in a study using the clinical and radiological data in the subjects over 60 years of age, its prevalence was found to be 13.3%. (Akoudad et al., 2015).

2. PATHOPHYSIOLOGY OF CAA

2.1 Physiology of Beta-amyloid Peptides

Beta-amyloid peptides, which are involved in the pathophysiology of CAA and AD, are formed by proteolytic degradation of amyloid precursor protein (APP) by alpha and beta secretases. As a result of the degradation of APP in this way, peptides called amyloid beta 1-40 (Aβ-40) and amyloid beta 1-42 (Aβ-42) are formed. There are three main pathways for the elimination of Aβ proteins from the brain parenchyma (Figure 1):

- Direct enzymatic degradation of Aβ peptides in the brain parenchyma by the several proteases (Farris et al., 2007).
- Passage of Aβ peptides into plasma by crossing the blood-brain barrier with the help of LDL receptor-related protein-1 (LRP-1). In this way, the elimination rate is higher for Aβ-40 compared to Aβ-42 (Bell et al., 2007; Shibata et al., 2000).
- Passage of Aβ peptides into plasma from the interstitial fluid via perivascular drainage. This pathway runs at a much slower rate than the LRP-1 pathway under normal conditions (Preston et al., 2003).

- Depending on the disruptions in the elimination pathways of the beta-amyloid proteins, different amyloid-related clinical conditions, especially CAA and AD, occur.

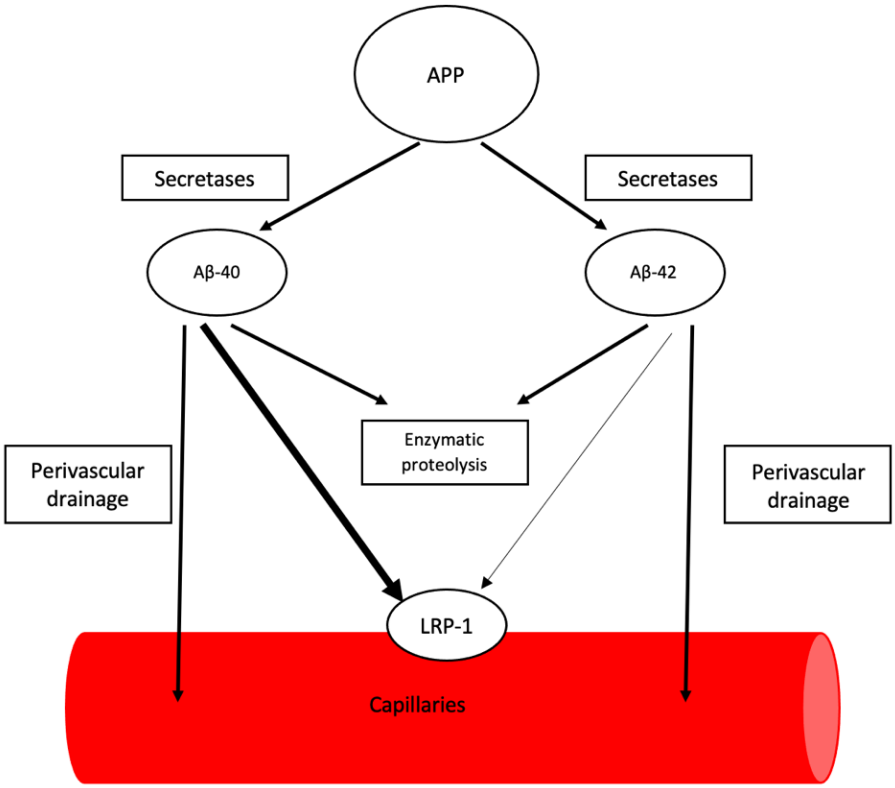


Figure 1: Physiologic degradation and elimination of the beta-amyloid peptides (APP: amyloid precursor protein, Aβ-40: Amyloid beta 1-40, Aβ-42: Amyloid beta 1-42; LRP-1: LDL receptor-related protein-1)

2.2 Pathophysiology of CAA

CAA is characterized by the accumulation of the beta-amyloid peptides in the media and adventitia layer of the leptomeningeal and cortical arteries and capillaries. While the amyloid peptide Aβ-42 accumulates in AD as neuritic plaques, Aβ-40 accumulation is predominantly seen in CAA. (Smith & Greenberg, 2009).

The source of beta-amyloid accumulated in CAA is thought to be neuronal cells. It is thought that beta-amyloid produced in neuronal cells and passed to capillaries via perivascular drainage pathway accumulate in the vessel wall (Weller et al., 1998). This hypothesis is supported by the fact that beta-amyloid deposits initially have been observed in the periphery of arteries where interstitial fluid drainage is intense. On the other hand, it is known that smooth muscle cells of the vessel wall can also produce APP and beta-amyloid, and even increase their own production after exogenously given beta-amyloid (Davis-Salinas et al., 1995). Therefore, smooth muscle cells may also contribute to beta-amyloid production and thus CAA development.

- Many different studies have been conducted to explain the reason for the significantly increased prevalence of CAA with aging. The findings of these studies can be summarized as follows:
- Decreased expression of the beta-amyloid-degrading enzymes such as neprilysin may force beta-amyloids to be excreted via the perivascular drainage pathway (Miners et al., 2006).
- The slower functioning of the LRP-1 pathway with aging may refer to more redirected beta-amyloid peptides to the perivascular drainage pathway for the elimination (Shibata et al., 2000).
- According to the models created, it is thought that perivascular drainage of brain interstitial fluid and beta-amyloid peptides is triggered by vessel pulsation. As a result of arteriosclerosis in the arterial walls, the vessel wall becomes stiff with aging (Nagasawa et al., 1979; Schley et al., 2006). This stiffness may interrupt the perivascular drainage from the vessel wall.
- It is known that structural changes, like increased collagen deposition and thickening, occur in the vascular basement membrane with aging (Farkas et al., 2000). The vascular basement membrane, which has become thicker and less permeable, may also be one of the factors that impair perivascular drainage of beta-amyloid.

Beta-amyloid deposition in the media and interstitial layers leads to loss of smooth muscle cells, focal aneurysm formation, and separation of the outer part of the vessel wall from the inner layers (McCarron et al., 1999;

Revesz et al., 2003). These important pathological changes in the vessel walls are demonstrated as the main reason for the bleeding tendency in patients.

3. NEUROLOGIC MANIFESTATIONS AND IMAGING FEATURES OF CAA

As a result of small vessel-related pathologies, CAA is seen together with some different clinical conditions, both hemorrhagic and non-hemorrhagic. Although lobar hemorrhages are the most common presentation, cognitive decline, microinfarctions, and transient focal neurological episodes may be other presentations.

The definite diagnosis of CAA relied on pathological findings. However, a probable diagnosis of CAA may be done by using the modified Boston criteria (Table 1). These criteria are based on clinical and radiological imaging findings. Cortical or cortico-subcortical microbleeds, single or multiple lobar hematoma(s), and focal or disseminated superficial siderosis form the basis of imaging findings (Smith & Greenberg, 2003).

Table 1: Modified Boston criteria for the diagnosis of CAA

Definite CAA	Full post-mortem examination reveals lobar, cortical, or cortico-subcortical hemorrhage and pathological evidence of severe CAA
Probable CAA with supporting pathology	Clinical data and pathological tissue (evacuated hematoma or cortical biopsy specimen) demonstrate a hemorrhage as mentioned above and some degree of vascular amyloid deposition
Probable CAA	● Appropriate clinical history ● Patient ≥55 years-old ● MRI findings: ○ Multiple hemorrhages restricted to lobar, cortical, or cortico-subcortical regions (cerebellar hemorrhages allowed) ○ A single lobar, cortical, or cortico-subcortical hemorrhage and focal (≤3 sulci) or disseminated (≥3 sulci) cortical superficial siderosis
Possible CAA	● Appropriate clinical history ● Patient ≥55 years-old

	● MRI findings: ○ A single lobar, cortical or cortico-subcortical hemorrhage ○ Focal or disseminated cortical superficial siderosis

3.1 Hemorrhagic Manifestations of CCA

3.1.1 Lobar Intracerebral Hemorrhage

Lobar intracerebral hemorrhage (L-ICH) is the most common presentation of the CAA. It may occur in any lobes of the brain; however, the posterior region of the brain (temporal and occipital lobes) is most likely (Yamada, 2000). Cortical or subcortical location of lobar hemorrhages is the clinical correlation of pathophysiological changes being more pronounced in cortical and leptomeningeal arteries. Cerebellum is the rare location of CAA-related hematomas (Cuny et al., 1996). However, hemorrhages of the deep brain structures are atypical for CAA.

The clinical presentation of L-ICH is dependent on localization. Focal neurologic deficits, seizures, headache, and drowsiness may be observed in the course of the L-ICH.

3.1.2 Cerebral microbleeds

Cerebral microbleeds (CMB) constitute one of the most common imaging findings of CAA, which is also included in the modified Boston criteria. Hemosiderin remains in the surrounding parenchyma, within the macrophage, after microhemorrhages that develop as a result of injuries to the vessel. Due to the paramagnetic properties of hemosiderin, it creates a "susceptibility effect" in MRI. Therefore, it causes hypointensity in the “gradient echo” (GRE) or in the “susceptibility weighted images” (SWI) sequences, which are sensitive to the susceptibility effect. It has been reported that the SWI sequence is more sensitive than the GRE in detecting CMBs (Cheng et al., 2013). In any case, MRI is the appropriate imaging modality for the detection and follow-up of CMBs. However, it should be noted that hypointense lesions detected on MRI due to the blooming effect are bigger than the actual amount of hemosiderin and may cause overestimation in the severity of the disease (Greenberg et al., 2009).

In a study conducted with the amyloid PET, it was shown that CMBs detected on MRI occur in places where beta-amyloid accumulation is more intense (Gurol et al., 2012). Lobar-located CMBs are supportive for the diagnosis of CAA, as included in the modified Boston criteria. However, CMBs detected in the deep brain structures (basal ganglia, thalamus, pons, and bulbus) are not diagnostic for CAA.

3.1.3 Cortical superficial siderosis

In animal studies, it has been observed that cortical superficial siderosis (cSS) occurs as a result of hemosiderin accumulation in the subpial region after hemorrhages into the subarachnoid space (Koeppen et al., 1993). cSS was detected in approximately 60% of CAA patients (Linn et al., 2010). Ribbon-like hypointense areas following the sulci in cortical regions on SWI or GRE sequences of MRI are characteristic of cSS. Another reflection of the involvement of cortical and meningeal arteries pathophysiologically is the distribution of cSS. It is thought that the micro-bleedings to the subarachnoid space, which develop due to damage in the cortical/meningeal arteries, play a major role in the formation of cSS. Opening of cortical lobar hemorrhages to the subarachnoid region may cause similar findings. However, when MRIs of CAA patients were examined, no adjacent L-ICH was detected in any of the patients with cSS (Linn et al., 2010). On the other hand, cSS was not found in any types of intracerebral hemorrhages except CAA. Thus, cSS is more specific for CAA than CMBs.

Transient focal neurologic episodes (TFNE) are the unique clinical entities related to the cSS. TFNE are attacks of aura-like, short-term, transient sensory or motor symptoms that develop and end within seconds/minutes (Charidimou et al., 2015). Clinically, it is important to distinguish them from transient ischemic attacks. Because the use of erroneous antiaggregant or anticoagulant medications will come up with an increased risk of intracerebral hemorrhage.

3.2 Non-Hemorrhagic Manifestations of CCA

3.2.1 White Matter Hyperintensities and Ischemic Infarctions

White matter hyperintensities (WMH) are the lesions detected in T2-FLAIR sequences of MRI. Although they are not specific to CAA, they can occur during the course of the disease as a result of microvascular damage and increase in number gradually. The presence of posteriorly located WMH was found to be an independent predictor for CAA, correlated with the distribution of the vascular pathology of CAA. Even in cases of pathologically confirmed CAA without L-ICH and CMB, WMH location was found to be predictive (Thanprasertsuk et al., 2014). Similarly, a PET study showed that the intensity of amyloid deposition was correlated with white matter signal hyperintensities in CAA patients (Gurol et al., 2013).

Silent ischemic infarcts can be detected in 15% of the patients with CAA (Kimberly et al., 2009). These infarcts are predominantly located in the cerebral cortex and subcortical white matter. Further, the presence of these infarcts is found to be associated with the number of hemorrhages in the GRE sequences.

3.2.2 Perivascular Spaces

Dilated perivascular spaces (DPVS), also known as Virchow-Robin spaces, are spaces that appear isointense with cerebrospinal fluid in all sequences on MRI. They may be located in the basal ganglia or the white matter (WM-DPVS). Some evidence has been reported that WM-DPVS may be associated with CAA (Martinez-Ramirez et al., 2013). Further, a potential association between the degree of DPVS and CAA severity also had been reported (van Veluw et al., 2016).

The pathological link between dilated perivascular space and CAA severity is not yet clear. However, disruption of perivascular drainage of amyloid beta peptides around the dilated perivascular spaces may be a possible explanation. Further studies are needed on this issue.

3.2.3 Cognitive Impairment

It is very difficult to determine the frequency of CAA-related cognitive impairment precisely because it overlaps with other conditions that

may cause cognitive impairment that occurs with advanced age. However, the frequency of CAA in dementia patients was found to be higher than those in subjects with intact cognitive function (Keage et al., 2009). On the other hand, as we mentioned before, CAA pathology was found in 80% of AD patients. Therefore, the coexistence of two intertwined diseases with similar pathophysiology creates difficulties in determining the true frequency of CAA-related cognitive impairment.

In a study conducted to investigate which of the cognitive components had affected severely in patients with CAA, a significant decrease was found in episodic memory and perceptual speed in patients with moderate-to-severe CAA but not mild-to-moderate CAA (Arvanitakis et al., 2011). It has been stated that recurrent microinfarcts may contribute to cognitive decline in patients with CAA. (Soontornniyomkij et al., 2010). In a diffusion tensor imaging study conducted in patients with CAA, a decrease was found in the global efficiency of structural brain networks. This decrease was found to be independent of intracerebral hemorrhage. (Reijmer et al., 2015). Based on these data, it can be thought that small vessel disease caused by CAA, independent of the development of bleeding, causes a decline in different functions of the brain over time as a result of microvascular damage in different parts of the brain. In support of this, the cognitive impairment that develops in patients with CAA, unlike AD-type cognitive decline, primarily affects functions such as episodic memory and perceptual speed (Arvanitakis et al., 2011).

3.2.4 Cerebral Amyloid Angiopathy-Related Inflammation

CAA-related inflammation (CAA-Ri) is a clinically and pathologically distinct form of CAA. CAA has subtle immune reactions with monocyte/macrophage-lineage cells (Yamada et al., 1996). However, in rare cases, severe inflammation around the vessels may occur with beta-amyloid deposition. In these clinical conditions, which are the most catastrophic cases of beta-amyloid deposition, subacute regressions in cognitive functions, seizures, headaches, and focal neurologic deficits can be seen depending on the shedding area of the affected artery. The detection of the APOE ε4/ε4 genotype at a rate of 77% in CAA-Ri and 5% in isolated CAA supports that some genetic factors are predictive for CAA-Ri (Kinnecom et al., 2007).

Radiologically, unifocal or multifocal, cortico-subcortical or deep white matter hyperintensities, typically asymmetric, and extending to the immediately adjacent subcortical white matter are detected. Mild parenchymal or meningeal contrast enhancement may be observed (Auriel et al., 2016). Along with these findings, there are also findings such as microbleeds and superficial siderosis in SWI or GRE sequences, as in the diagnostic criteria of CAA.

The clinical distinction of CAA-Ri from other diseases of beta-amyloid deposition is important because of its good response to immunotherapies. White matter lesions and clinical findings detected in CAA-Ri may improve after intravenous steroids or different immunosuppressant treatments (Eng et al., 2004).

CONCLUSION

Beta-amyloid pathologies will be encountered more frequently in the current century due to the increase in the average life expectancy. With a better understanding of the pathophysiological processes that cause beta-amyloid deposition, potential targeted therapies are also on the agenda. CAA, which occurs as a result of beta-amyloid deposition in the vessel wall, to reach potential treatments in the future also depends on the pathophysiological processes that will be better understood.

In recent years, CAA has started to be better recognized and detected more easily with the developments in radiological imaging methods, especially MRI. The CAA diagnosis is important for managing patients since it has not only hemorrhagic but also non-hemorrhagic neurological consequences.

REFERENCES

Akoudad, S., Portegies, M. L. P., Koudstaal, P. J., Hofman, A., van der Lugt, A., Ikram, M. A., & Vernooij, M. W. (2015). Cerebral Microbleeds Are Associated With an Increased Risk of Stroke: The Rotterdam Study. *Circulation*, *132*(6), 509–516. https://doi.org/10.1161/CIRCULATIONAHA.115.016261

Arvanitakis, Z., Leurgans, S. E., Wang, Z., Wilson, R. S., Bennett, D. A., & Schneider, J. A. (2011). Cerebral amyloid angiopathy pathology and cognitive domains in older persons. *Annals of Neurology*, *69*(2), 320–327. https://doi.org/10.1002/ana.22112

Auriel, E., Charidimou, A., Gurol, M. E., Ni, J., Van Etten, E. S., Martinez-Ramirez, S., Boulouis, G., Piazza, F., DiFrancesco, J. C., Frosch, M. P., Pontes-Neto, O. V. M., Shoamanesh, A., Reijmer, Y., Vashkevich, A., Ayres, A. M., Schwab, K. M., Viswanathan, A., & Greenberg, S. M. (2016). Validation of Clinicoradiological Criteria for the Diagnosis of Cerebral Amyloid Angiopathy-Related Inflammation. *JAMA Neurology*, *73*(2), 197–202. https://doi.org/10.1001/jamaneurol.2015.4078

Bell, R. D., Sagare, A., Friedman, A. E., Bedi, G., Holtzman, D. M., Deane, R., & Zlokovic, B. V. (2007). Transport pathways for clearance of human Alzheimer's amyloid β-peptide and apolipoproteins E and J in the mouse central nervous system. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *27*(5), 909–918. https://doi.org/10.1038/sj.jcbfm.9600419

Boulouis, G., Charidimou, A., & Greenberg, S. M. (2016). Sporadic Cerebral Amyloid Angiopathy: Pathophysiology, Neuroimaging Features, and Clinical Implications. *Seminars in Neurology*, *36*(3), 233–243. https://doi.org/10.1055/s-0036-1581993

Brenowitz, W. D., Nelson, P. T., Besser, L. M., Heller, K. B., & Kukull, W. A. (2015). Cerebral amyloid angiopathy and its co-occurrence with Alzheimer's disease and other cerebrovascular neuropathologic changes. *Neurobiology of Aging*, *36*(10), 2702–2708. https://doi.org/10.1016/j.neurobiolaging.2015.06.028

Charidimou, A., Linn, J., Vernooij, M. W., Opherk, C., Akoudad, S., Baron, J.-C., Greenberg, S. M., Jäger, H. R., & Werring, D. J. (2015). Cortical superficial siderosis: Detection and clinical significance in

cerebral amyloid angiopathy and related conditions. *Brain: A Journal of Neurology*, *138*(Pt 8), 2126–2139. https://doi.org/10.1093/brain/awv162

Cheng, A.-L., Batool, S., McCreary, C. R., Lauzon, M. l., Frayne, R., Goyal, M., & Smith, E. E. (2013). Susceptibility-Weighted Imaging is More Reliable Than T2*-Weighted Gradient-Recalled Echo MRI for Detecting Microbleeds. *Stroke*, *44*(10), 2782–2786. https://doi.org/10.1161/STROKEAHA.113.002267

Cuny, E., Loiseau, H., Rivel, J., Vital, C., & Castel, J. P. (1996). Amyloid angiopathy-related cerebellar hemorrhage. *Surgical Neurology*, *46*(3), 235–239. https://doi.org/10.1016/0090-3019(96)00158-9

Davis-Salinas, J., Saporito-Irwin, S. M., Cotman, C. W., & Van Nostrand, W. E. (1995). Amyloid beta-protein induces its own production in cultured degenerating cerebrovascular smooth muscle cells. *Journal of Neurochemistry*, *65*(2), 931–934. https://doi.org/10.1046/j.1471-4159.1995.65020931.x

Eng, J. A., Frosch, M. P., Choi, K., Rebeck, G. W., & Greenberg, S. M. (2004). Clinical manifestations of cerebral amyloid angiopathy-related inflammation. *Annals of Neurology*, *55*(2), 250–256. https://doi.org/10.1002/ana.10810

Farkas, E., De Jong, G. I., de Vos, R. A., Jansen Steur, E. N., & Luiten, P. G. (2000). Pathological features of cerebral cortical capillaries are doubled in Alzheimer's disease and Parkinson's disease. *Acta Neuropathologica*, *100*(4), 395–402. https://doi.org/10.1007/s004010000195

Farris, W., Schütz, S. G., Cirrito, J. R., Shankar, G. M., Sun, X., George, A., Leissring, M. A., Walsh, D. M., Qiu, W. Q., Holtzman, D. M., & Selkoe, D. J. (2007). Loss of Neprilysin Function Promotes Amyloid Plaque Formation and Causes Cerebral Amyloid Angiopathy. *The American Journal of Pathology*, *171*(1), 241–251. https://doi.org/10.2353/ajpath.2007.070105

Greenberg, S. M., Vernooij, M. W., Cordonnier, C., Viswanathan, A., Al-Shahi Salman, R., Warach, S., Launer, L. J., Van Buchem, M. A., Breteler, M. M., & Microbleed Study Group. (2009). Cerebral microbleeds: A guide to detection and interpretation. *The Lancet. Neurology*, *8*(2), 165–174. https://doi.org/10.1016/S1474-4422(09)70013-4

Gurol, M. E., Dierksen, G., Betensky, R., Gidicsin, C., Halpin, A., Becker, A., Carmasin, J., Ayres, A., Schwab, K., Viswanathan, A., Salat, D., Rosand, J., Johnson, K. A., & Greenberg, S. M. (2012). Predicting sites of new hemorrhage with amyloid imaging in cerebral amyloid angiopathy. *Neurology*, *79*(4), 320–326. https://doi.org/10.1212/WNL.0b013e31826043a9

Gurol, M. E., Viswanathan, A., Gidicsin, C., Hedden, T., Martinez-Ramirez, S., Dumas, A., Vashkevich, A., Ayres, A. M., Auriel, E., van Etten, E., Becker, A., Carmasin, J., Schwab, K., Rosand, J., Johnson, K. A., & Greenberg, S. M. (2013). Cerebral amyloid angiopathy burden associated with leukoaraiosis: A positron emission tomography/magnetic resonance imaging study. *Annals of Neurology*, *73*(4), 529–536. https://doi.org/10.1002/ana.23830

Keage, H. A. D., Carare, R. O., Friedland, R. P., Ince, P. G., Love, S., Nicoll, J. A., Wharton, S. B., Weller, R. O., & Brayne, C. (2009). Population studies of sporadic cerebral amyloid angiopathy and dementia: A systematic review. *BMC Neurology*, *9*, 3. https://doi.org/10.1186/1471-2377-9-3

Kimberly, W. T., Gilson, A., Rost, N. S., Rosand, J., Viswanathan, A., Smith, E. E., & Greenberg, S. M. (2009). Silent ischemic infarcts are associated with hemorrhage burden in cerebral amyloid angiopathy. *Neurology*, *72*(14), 1230–1235. https://doi.org/10.1212/01.wnl.0000345666.83318.03

Kinnecom, C., Lev, M. H., Wendell, L., Smith, E. E., Rosand, J., Frosch, M. P., & Greenberg, S. M. (2007). Course of cerebral amyloid angiopathy–related inflammation. *Neurology*, *68*(17), 1411–1416. https://doi.org/10.1212/01.wnl.0000260066.98681.2e

Koeppen, A. H., Dickson, A. C., Chu, R. C., & Thach, R. E. (1993). The pathogenesis of superficial siderosis of the central nervous system. *Annals of Neurology*, *34*(5), 646–653. https://doi.org/10.1002/ana.410340505

Linn, J., Halpin, A., Demaerel, P., Ruhland, J., Giese, A. D., Dichgans, M., van Buchem, M. A., Bruckmann, H., & Greenberg, S. M. (2010). Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy(CME). *Neurology*, *74*(17), 1346–1350. https://doi.org/10.1212/WNL.0b013e3181dad605

Martinez-Ramirez, S., Pontes-Neto, O. M., Dumas, A. P., Auriel, E., Halpin, A., Quimby, M., Gurol, M. E., Greenberg, S. M., & Viswanathan, A.

(2013). Topography of dilated perivascular spaces in subjects from a memory clinic cohort. *Neurology*, *80*(17), 1551–1556. https://doi.org/10.1212/WNL.0b013e31828f1876

Masuda, J., Tanaka, K., Ueda, K., & Omae, T. (1988). Autopsy study of incidence and distribution of cerebral amyloid angiopathy in Hisayama, Japan. *Stroke*, *19*(2), 205–210. https://doi.org/10.1161/01.STR.19.2.205

McCarron, M. O., Nicoll, J. A., Stewart, J., Ironside, J. W., Mann, D. M., Love, S., Graham, D. I., & Dewar, D. (1999). The apolipoprotein E epsilon2 allele and the pathological features in cerebral amyloid angiopathy-related hemorrhage. *Journal of Neuropathology and Experimental Neurology*, *58*(7), 711–718. https://doi.org/10.1097/00005072-199907000-00005

Miners, J. S., Van Helmond, Z., Chalmers, K., Wilcock, G., Love, S., & Kehoe, P. G. (2006). Decreased expression and activity of neprilysin in Alzheimer disease are associated with cerebral amyloid angiopathy. *Journal of Neuropathology and Experimental Neurology*, *65*(10), 1012–1021. https://doi.org/10.1097/01.jnen.0000240463.87886.9a

Nagasawa, S., Handa, H., Okumura, A., Naruo, Y., Moritake, K., & Hayashi, K. (1979). Mechanical properties of human cerebral arteries. Part 1: Effects of age and vascular smooth muscle activation. *Surgical Neurology*, *12*(4), 297–304.

Preston, S. D., Steart, P. V., Wilkinson, A., Nicoll, J. a. R., & Weller, R. O. (2003). Capillary and arterial cerebral amyloid angiopathy in Alzheimer's disease: Defining the perivascular route for the elimination of amyloid beta from the human brain. *Neuropathology and Applied Neurobiology*, *29*(2), 106–117. https://doi.org/10.1046/j.1365-2990.2003.00424.x

Reijmer, Y. D., Fotiadis, P., Martinez-Ramirez, S., Salat, D. H., Schultz, A., Shoamanesh, A., Ayres, A. M., Vashkevich, A., Rosas, D., Schwab, K., Leemans, A., Biessels, G.-J., Rosand, J., Johnson, K. A., Viswanathan, A., Gurol, M. E., & Greenberg, S. M. (2015). Structural network alterations and neurological dysfunction in cerebral amyloid angiopathy. *Brain*, *138*(1), 179–188. https://doi.org/10.1093/brain/awu316

Revesz, T., Ghiso, J., Lashley, T., Plant, G., Rostagno, A., Frangione, B., & Holton, J. L. (2003). Cerebral amyloid angiopathies: A pathologic, biochemical, and genetic view. *Journal of Neuropathology and*

Experimental Neurology, *62*(9), 885–898. https://doi.org/10.1093/jnen/62.9.885

Schley, D., Carare-Nnadi, R., Please, C. P., Perry, V. H., & Weller, R. O. (2006). Mechanisms to explain the reverse perivascular transport of solutes out of the brain. *Journal of Theoretical Biology*, *238*(4), 962–974. https://doi.org/10.1016/j.jtbi.2005.07.005

Shibata, M., Yamada, S., Kumar, S. R., Calero, M., Bading, J., Frangione, B., Holtzman, D. M., Miller, C. A., Strickland, D. K., Ghiso, J., & Zlokovic, B. V. (2000). Clearance of Alzheimer's amyloid-β1-40 peptide from brain by LDL receptor–related protein-1 at the blood-brain barrier. *Journal of Clinical Investigation*, *106*(12), 1489–1499.

Smith, E. E., & Greenberg, S. M. (2003). Clinical diagnosis of cerebral amyloid angiopathy: Validation of the Boston criteria. *Current Atherosclerosis Reports*, *5*(4), 260–266. https://doi.org/10.1007/s11883-003-0048-4

Smith, E. E., & Greenberg, S. M. (2009). Beta-amyloid, blood vessels and brain function. *Stroke; a Journal of Cerebral Circulation*, *40*(7), 2601–2606. https://doi.org/10.1161/STROKEAHA.108.536839

Soontornniyomkij, V., Lynch, M. D., Mermash, S., Pomakian, J., Badkoobehi, H., Clare, R., & Vinters, H. V. (2010). Cerebral microinfarcts associated with severe cerebral beta-amyloid angiopathy. *Brain Pathology (Zurich, Switzerland)*, *20*(2), 459–467. https://doi.org/10.1111/j.1750-3639.2009.00322.x

Tanskanen, M., Mäkelä, M., Myllykangas, L., Notkola, I.-L., Polvikoski, T., Sulkava, R., Kalimo, H., & Paetau, A. (2012). Prevalence and severity of cerebral amyloid angiopathy: A population-based study on very elderly Finns (Vantaa 85+). *Neuropathology and Applied Neurobiology*, *38*(4), 329–336. https://doi.org/10.1111/j.1365-2990.2011.01219.x

Thanprasertsuk, S., Martinez-Ramirez, S., Pontes-Neto, O. M., Ni, J., Ayres, A., Reed, A., Swords, K., Gurol, M. E., Greenberg, S. M., & Viswanathan, A. (2014). Posterior white matter disease distribution as a predictor of amyloid angiopathy. *Neurology*, *83*(9), 794–800. https://doi.org/10.1212/WNL.0000000000000732

van Veluw, S. J., Biessels, G. J., Bouvy, W. H., Spliet, W. G., Zwanenburg, J. J., Luijten, P. R., Macklin, E. A., Rozemuller, A. J., Gurol, M. E., Greenberg, S. M., Viswanathan, A., & Martinez-Ramirez, S. (2016). Cerebral amyloid angiopathy severity is linked to dilation of

juxtacortical perivascular spaces. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *36*(3), 576–580. https://doi.org/10.1177/0271678X15620434

Vinters, H. V. (1987). Cerebral amyloid angiopathy. A critical review. *Stroke*, *18*(2), 311–324. https://doi.org/10.1161/01.str.18.2.311

Weller, R. O., Massey, A., Newman, T. A., Hutchings, M., Kuo, Y.-M., & Roher, A. E. (1998). Cerebral Amyloid Angiopathy. *The American Journal of Pathology*, *153*(3), 725–733.

Yamada, M. (2000). Cerebral amyloid angiopathy: An overview. *Neuropathology: Official Journal of the Japanese Society of Neuropathology*, *20*(1), 8–22. https://doi.org/10.1046/j.1440-1789.2000.00268.x

Yamada, M., Itoh, Y., Shintaku, M., Kawamura, J., Jensson, O., Thorsteinsson, L., Suematsu, N., Matsushita, M., & Otomo, E. (1996). Immune reactions associated with cerebral amyloid angiopathy. *Stroke*, *27*(7), 1155–1162. https://doi.org/10.1161/01.str.27.7.1155

CHAPTER 3

DIAGNOSIS & TREATMENT OF INGUINAL BLADDER HERNIA

Deniz İPEK MD[1]

Süleyman Sıtkı URALOĞLU MD[2]

[1]Department of Urology Yalova State Hospital, Yalova, Turkey. ORCHID ID: 0000-0002-8124-3885

[2] Department of Urology Yalova State Hospital, Yalova, Turkey. ORCHID ID: 0000-0003-4076-5385

INTRODUCTION

Bladder hernia was first defined by Levine in 1951 and has been known as a rare condition since then (Madani, Nikouei, Aval, Enshaei, Asadollahzade ve Esmaeili, 2013). The prevalence of bladder hernia ranges between 1 – 4%, but in obese men over the age of 50, this rate may increase up to 10% (Habip, 2017).

The majority of bladder hernias are detected during surgery and only 7% of them can be diagnosed before surgery. In fact, 16% of these hernias are observed due to complications such as bladder injury and leakage in the postoperative period. The terminology of hernia is generally indicated by its localization as the inguinoscrotal hernia pouch is called scrotal cystocele. In addition, the sac may include diverticulum, bladder or ureter tissue parts (Khan, Chaudhry and Feinman, 2016).

1- ETIOLOGY OF THE INGUINAL BLADDER HERNIA

The main risk factors for the hernia development can be stated as gender (more frequent in males), advanced age, chronic urinary obstruction, weak pelvic muscle system and obesity (Kraft, Sweeney, Fink, Ritenour and Issa, 2008).

In most cases, the prominent pathology underlying the bladder hernia can be attributed to the obstruction of the bladder output. Others can be expressed as weakness of the pelvic floor muscles, chronically shifted bladder, decreased bladder tone and obesity (Calıskan, Türkmen ve Sungur, 2018; Wagner, Arcand and Bamberger, 2004) .

2- CLINICAL SYMPTOMS OF THE INGUINAL BLADDER HERNİA

Since there is no significant specific symptom in most cases, the diagnosis is usually delayed. In symptomatic patients, there are non-specific symptoms such as groin swelling, dysuria, hematuria and urine trapping (Wagner et al., 2004; Fisher, Hollenbeck, Montgomery and Underwood, 2004). In some severe cases, patients manually compress the scrotum after voiding in order to discharge the bladder and rise to 2 – stage urination. In much more advanced cases, the ureter is also curled with hernia sac that curves cause obstruction and hydronephrosis. This obstruction can cause side pain and sometimes even renal failure (Caliskan et al., 2018).

3- DIAGNOSIS OF INGUINAL BLADDER HERNIA

Many patients have difficulties in urination. However, the most significant complaint is swelling in the groin and scrotum (Figure 1). It is a very important finding that the necessity of draining the scrotum by pressure on the binary urine or hernia can be counted a diagnostic finding. At the same time, the hernia sac cannot be reduced by pushing from the outside. For these reasons, the majority of patients apply to general surgery outpatient clinics with suspicion of inguinal intestinal hernia rather than urology clinics.

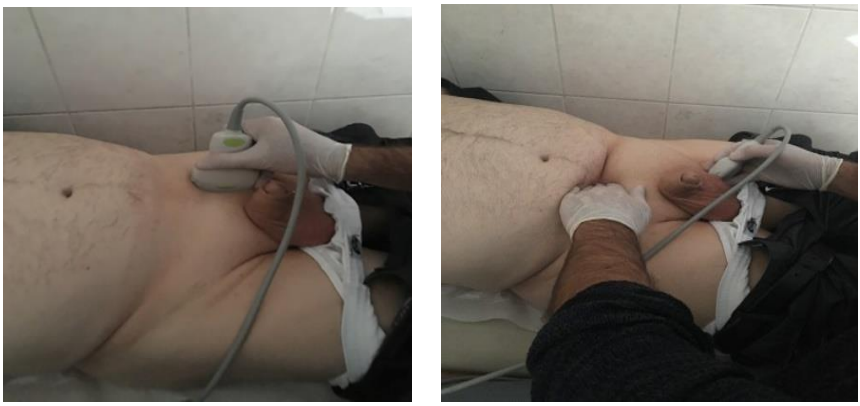
Picture 1: Right inguinal bladder

There are many different methods in the diagnosis of inguinal bladder hernia. Abdominal USG, cystography, cystoscope, uroflowmetry, abdominal CT and abdominal MRI can be counted as the most common methods (Ugur, Atci, Oruc, Akkucuk and Aydogan, 2016).

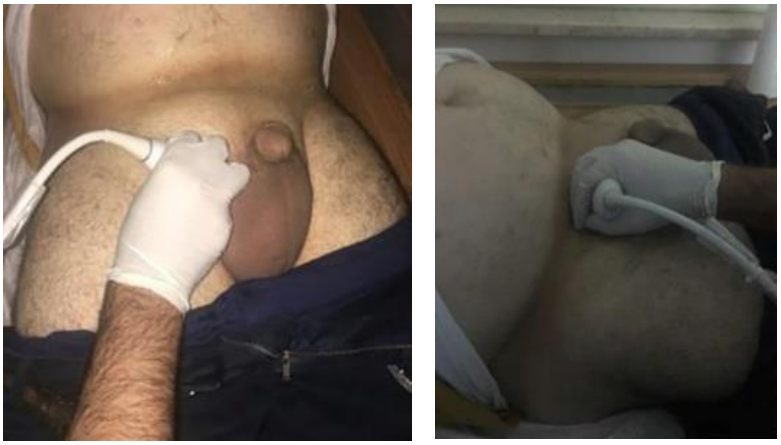
3.1 Ultrasonography (USG)

Ultrasonography is relatively cheap and prevents the patient from being exposed to radiation, but depends on the experience of the physician. Ultrasonography should be performed with a full bladder in the supine position. The abdominal USG examines the upper urinary system, bladder, prostate, groin channel and scrotum via convex probe. Beginning from the scrotum, hernia pouch is investigated and the probe can be fixed by identifying the unification location in the groin area of the bladder and hernia sac.

The Valsalva Maneuver is performed by increasing the abdominal pressure by coughing or straining the patient (Ugur et al., 2016; Taskovska and Janez, 2017).



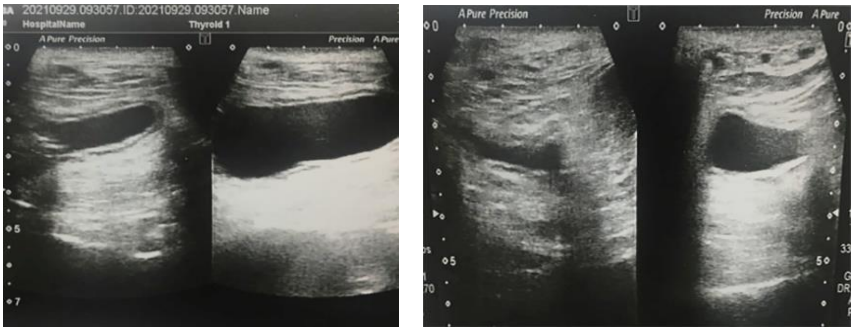
Pictures 2: Left inguinal region examination



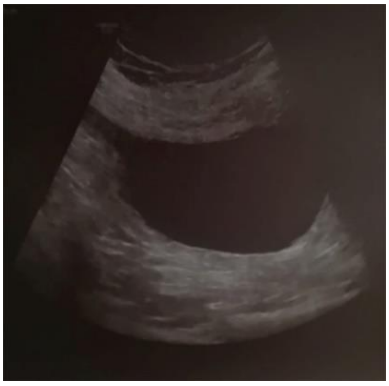
Picture 3: Right inguinal region examination

In patients who cannot conduct valsalva maneuver adequately, pressure can be applied to the bladder from the suprapubic region with the other hand. An increase in the size of the hernia sac can be detected due to the increased amount of urine passing through the herniated canal with the help of increasing pressure (Moufid K., Touiti D., Mohamed L., 2013). With this pressure increase, hernia neck enlargement caused by the bladder wall can also be detected in the USG imaging (Mahadevappa, Suresh, Natarajan and Thomas, 2009). During the USG the bladder wall forming the hernia sac and

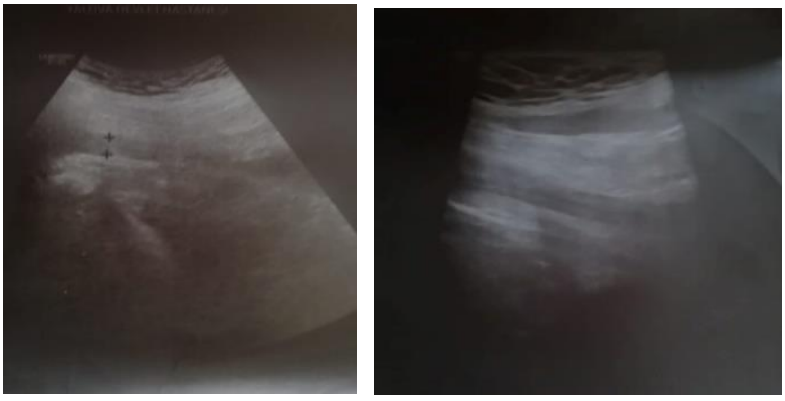
the canal wall are hyperechoic, and the inside of the canal is anechoic due to urine on USG.



Pictures 4: Before Valsalva & After Valsalva



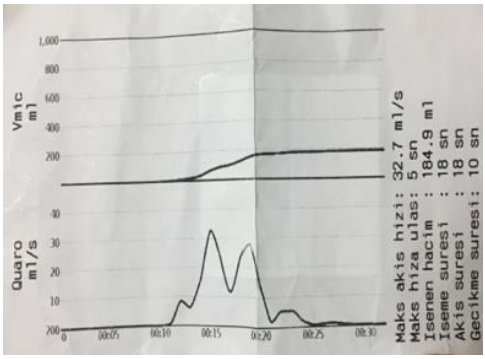
Picture 5: Inguinal Canal Transvers Section



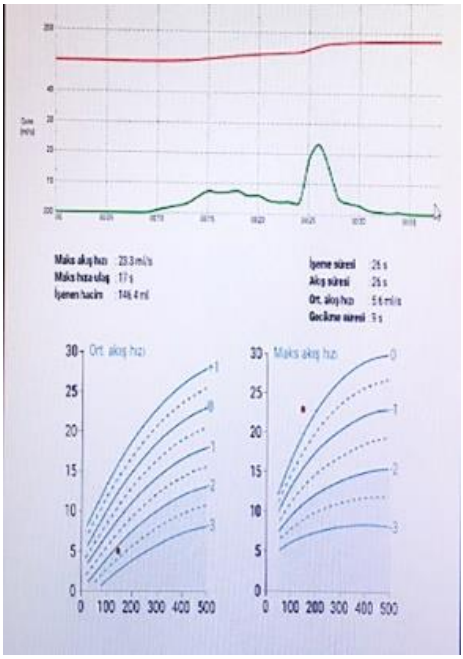
Pictures 6: Before Valsalva After Valsalva

3.2 Uroflowmetry

Uroflowmetry is a non-invasive test used to evaluate infravesical obstructions. It is widely utilized in urology clinics to evaluate many patients. In male patients with a complaint of swelling in the scrotum, especially with a high body mass index, bilateral voiding or voiding by pressing on the scrotum can be easily detected in the uroflowmetry test which is an important finding in inguinal bladder hernia (Oruç, Akbulut, Ozozan and Coşkun, 2004).



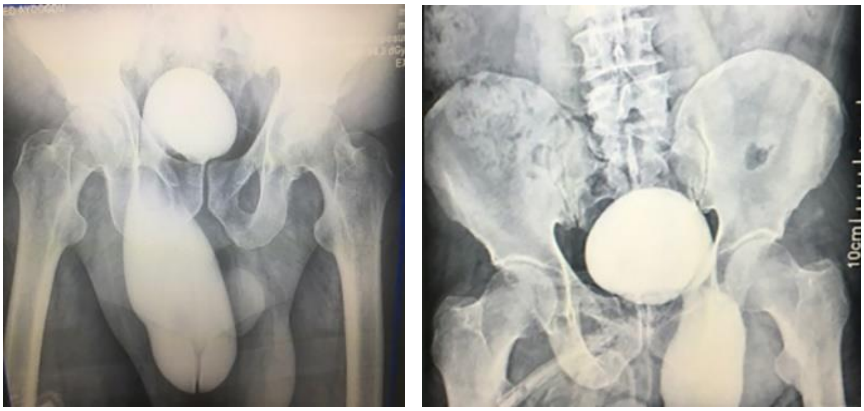
Picture 7: Dual urinary excretion pattern



Picture 8: Micturition by pressing on the scrotum

3.3 Cystography

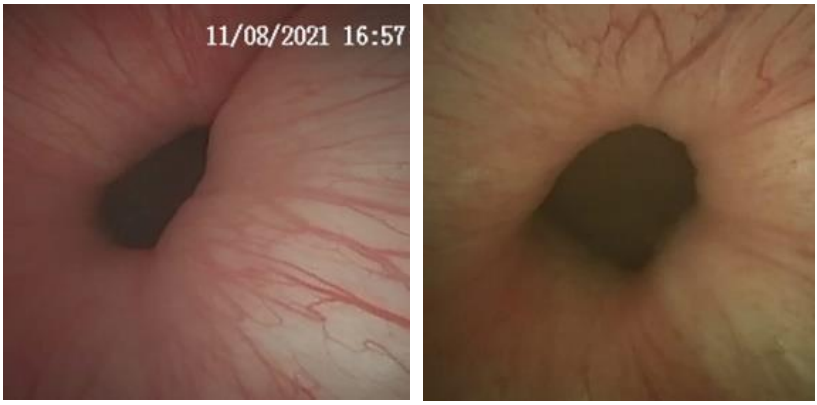
The golden standard of inguinal bladder hernia diagnosis is cystography, but it has several disadvantages such as inability to show additional pathologies in narrow neck hernias and has high rate of false negativity. Cystogaphy is cheaper than other imaging methods *(CT and MRI)*, thus it is one of the most accurate tests but the patient is exposed to radiation. Cystography can be performed in cases where a diagnosis with abdominal ultrasound is suspected but uncertain. Cystourethrography, which reveals the "dumbbell" or "dog ear" shape of the bladder, is the most sensitive test for diagnosing an inguinal bladder hernia. The disadvantage of cystography is its cost and requirement of technical personnel and materials. On the other hand the patient is exposed to radiation (Yong, Siaw, Yeoh and Lee, 2013).



Picture 9: Right and left inguinal bladder cystography

3.4 Cystoscopy

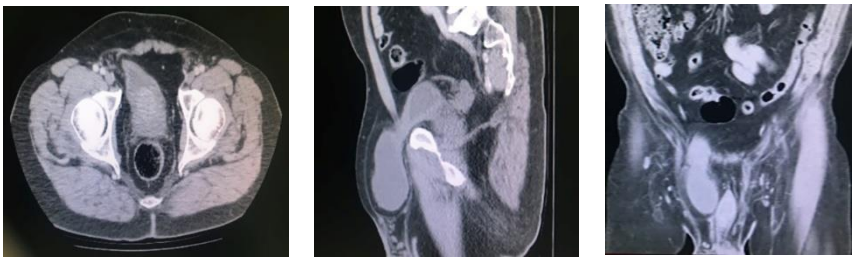
Today, in parallel with the advances in technology and the progress in endoscopic systems, the evaluation of the urethra and bladder with both rigid and flexible cystoscopes under local anesthesia is frequently performed in urology outpatient clinics. Cystoscopy is also recommended in patients with hematuria and suspected malignancy (Elkbuli, Narvel, McKenney and Boneva, 2019; Papatheofani, Beaumont and Nuessler, 2020). However, it is a costly method and requires experience.



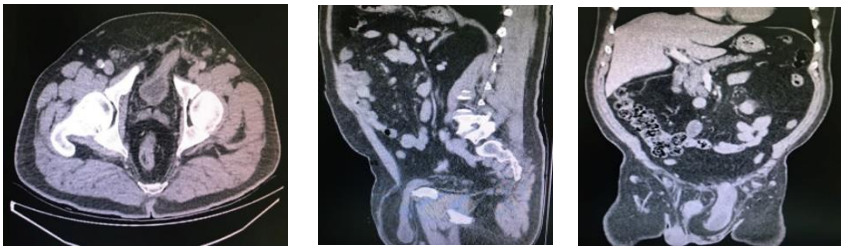
Pictures 10: Inguinal bladder hernia cystoscopic images

3.5 Computerized Tomography

Abdominal CT is not the first choice in the diagnosis of inguinal bladder hernia (Wang P, Huang, Ye, Gao, Zhang and Wu, 2018). It is preferred in cases where additional pathology or malignancy is suspected, evaluation of the relationship of bladder hernia with surrounding tissues are requested. CT is expensive and has accessibility disadvantages in some urban areas (Sarr, Ondo, Sow, Fall, Thiam, Sine B., et al., 2015; Hellerstein L.H., Sacks and Hellerstein D.K., 2018). Additional pathologies accompanying herniation can be shown on CT, but this technique has disadvantages such as higher cost and exposure of the patient to radiation compared to other diagnostic methods.



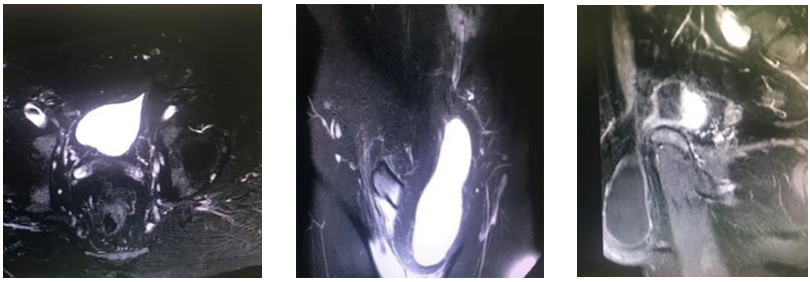
Pictures 11: CT images of the Right Inguinal Region



Picture 12: CT images of the Left Inguinal Region

3.6 Magnetic Resonance

MRI provides the best anatomical details and has the highest sensitivity and specificity. Abdominal MRI is not the first choice in the diagnosis of inguinal bladder hernia (Wang et al, 2018). In cases where additional pathology or malignancy is suspected, evaluation of the relationship of bladder hernia with surrounding tissues should be requested. The advantages of magnetic resonance imaging include the ability to show the relationship of the hernia with the peritoneal structures, accompanying additional pathologies, inflammatory changes and complications, and it does not involve radiation exposure. MRI is expensive and has accessibility disadvantages in some geographic locations. MRI reliability decreases in obese individuals (Sarr et al., 2015; Hellerstein et al., 2018).



Picture13: MR images of the Left Inguinal Region

4- DIFFERENTIAL DIAGNOSIS OF INGUINAL BLADDER HERNIA:

In the differential diagnosis of inguinal bladder hernia, inguinal intestinal hernia, hydrocele, spermatocele and scrotal abscess are the most prominent diseases that should be investigated. The physician can identify the intestinal loops in the inguinal canal in patients with inguinal hernia. They are easily separated from the bladder due to the peristaltic movements of the intestinal loops and the ability to detect the air and liquid inside. Incarcerated bowel can be easily distinguished by the predominance of pain in intestinal

hernias and the absence of painless bladder hernia (Mahadevappa et al., 2009).

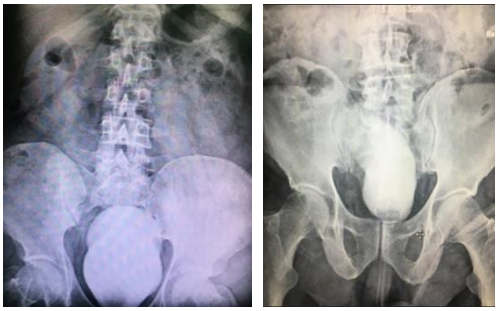
The differential diagnosis can be performed easily by the presence of hydrocele sac, spermatocele, epididymal cyst with thin-walled structures in the scrotum, unrelated to the bladder, not changing size with valsalva maneuver, and detecting all of the sac walls. In bladder hernia, it is observed that the hernia sac is thicker in relation to the bladder wall (Oruç et al., 2004). In scrotal abscess, the abscess wall may mimic the bladder wall in thickness, but hypoechoic content may be observed rather than anechoic content, and additional changes due to edema or inflammation may be observed in the surrounding tissues (Oruç et al., 2004).

5- TREATMENT OF INGUİNAL BLADDER HERNİA:

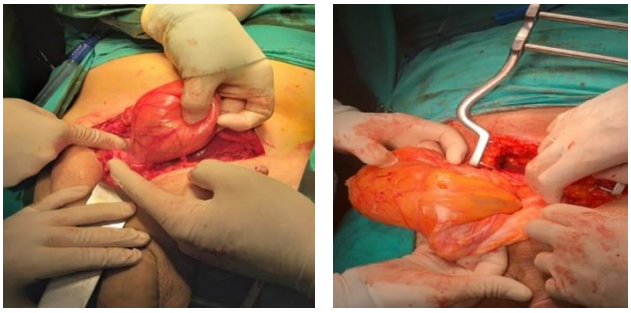
Surgical repair of hernia after bladder reduction is currently the standard of care. This technique consists of intraoperative reduction or, less commonly, bladder resection followed by herniorrhaphy. If the diagnosis is already known, catheterization is recommended before surgery. Prompt recognition of inguinal bladder herniation and appropriate preoperative imaging can help plan a modified surgical approach and reduce postoperative complications.

The only treatment for inguinal bladder hernia is surgery. Bladder scrotum, inguinal canal and hernia sac should be removed and hernia area should be repaired. The surgery can be performed as open surgery or laparoscopically. Although there are many different surgical techniques, the skin, subcutaneous and anterior leaf of the rectus sheath is usually opened with a pfannenstiel incision and blunt and sharp dissections are made by opening the rectus muscle to reach the pre-peritoneal space, until the Hasselbach triangle is visible. The bladder is filled and palpated, and the bladder is herniated to its anatomical location.

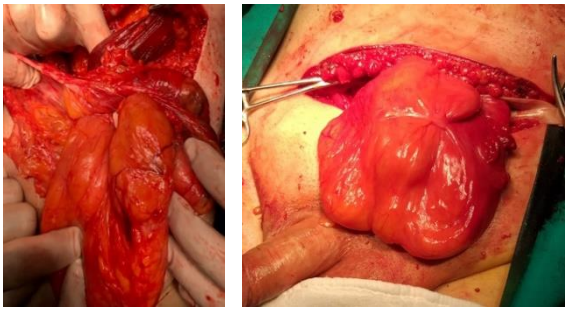
After bladder reduction, wall integrity is checked and Lichtenstein hernia repair is performed. Prolene mesh is fixed to the poupart ligament and the associated tendon to cover the large direct hernia area. After 3-7 days postoperatively, the control cystogram is taken and the foley probes are removed. An additional urological intervention is usually not performed.



Post-operative cystograms



Picture 14: Open surgery from the right inguinal region



Picture 15: Open surgery from the left inguinal region

6- COMPLİCATİONS İN THE TREATMENT OF INGUİNAL BLADDER HERNİA

Serious urological complications include urinary tract infections, obstructive uropathy, and even bladder infarctions that require sub-total cystectomy (Caliskan et al., 2018; Ugur et al., 2016). Associated pathologies include benign prostatic hyperplasia, hydronephrosis, vesicoureteric reflux, and scrotal abscesses. sicoureteric reflux, and scrotal abscesses.

DISCUSSION

Although many patients with bladder hernia do not have hernia-related **complaints, they usually have lower urinary tract symptoms, dysuria, hematuria, and prostate complaints. While these patients are frequently operated on in the general surgery departments, mistaking them for inguinal hernia, peri-operative diagnosis is made at a rate of 16% and complications develop in 12%. Another important issue is that the treatments performed due to lower urinary system symptoms, dysuria, hematuria and prostate complaints related to hernia usually fail and patients lose time with different applications instea**d of hernia treatment in urology outpatient clinics. (Wagner et al., 2004; Fisher et al., 2004). In patients with suspected bladder hernia in the inguinal canal, physical examination can be easily performed in outpatient clinic conditions. The diagnosis can be made easily in patients with symptoms and in suspicious patients (Yong et al., 2013).

Early diagnosis is important to minimize iatrogenic injuries to the bladder and prevent complications during inguinal surgery. Radiological imaging plays an important role in the prevention of possible complications such as perforation, renal failure and strangulation (Habip, 2017; Oruç et al., 2004). Abdominal ultrasound is the first choice because it does not harm the patient, as well as evaluates the upper urinary system. Additionally, ,it is easily accessible, has a low cost and has a high accuracy rate in expert hands. With physical examination, the diagnosis can be made easily in the outpatient clinic and complications that may occur during surgery can be prevented (Elkbuli et al., 2019).

Cystography can be performed in cases where abdominal ultrasound is suspected but the diagnosis is uncertain. Cystourethrography, which reveals the "dumbbell" or "dog ear" shape of the bladder, is the most sensitive test for diagnosing an inguinal bladder hernia. The disadvantage of cystography is that the patient is exposed to radiation and it is costly because it requires technical personnel and materials. Cystoscopy is also recommended in patients with hematuria and suspected malignancy (Papatheofani et al., 2020). However, transportation is difficult, it is costly and requires experience. It is not the first choice for the diagnosis of inguinal hernia in abdominal CT and MRI (Wang et al., 2018). In cases where additional pathology or malignancy is suspected, evaluation of the relationship of bladder hernia with surrounding tissues should be requested. CT and MRI are expensive and have accessibility disadvantages in some places. While exposure to radiation is observed in CT,

the reliability of MRI decreases in obese individuals (Sarr et al., 2015; Hellerstein et al., 2018).

Radiological imaging techniques have various advantages and shortcomings compared to each other. The gold standard in diagnosis is cystography, but this has disadvantages such as inability to show additional pathologies and false negative results in narrow neck hernias. Impaired bladder morphology, failure to visualize the base of the bladder on intravenous pyelography, or abnormal position of the distal ureter should suggest herniation. Additional pathologies accompanying herniation can be shown on CT, but this technique has disadvantages such as higher cost and exposure of the patient to radiation compared to other methods. Among the advantages of magnetic resonance imaging are that it can show the relationship of hernia with peritoneal structures, accompanying additional pathologies, inflammatory changes and complications, and it does not involve radiation exposure (Sarr et al., 2015; Hellerstein et al., 2018).

Treatment is surgery using resection or reduction. Advantages of reduction include preservation of bladder capacity and less ureteral injury. Laparoscopic techniques have been used frequently in recent years.

By using physical examination effectively, it will provide economic benefits to avoid unnecessary diagnostic methods and costs. USG can also be used and can distinguish the bladder from other intrascrotal conditions such as hydrocele, spermatocele, epididymal cyst, and abscess (Branchu, Renard, Larre and Leon, 2018).

Complications of inguinal bladder hernia include hydronephrosis and strangulation, which can result in vesicoureteric reflux, bladder rupture, ischemia, and bladder infarction. In addition, it has been shown that the incidence of genitourinary cancer is higher in patients with bladder hernia (Fisher et al., 20047, AlMohaya, Alabdrabalameer, AlAnazi, AlMuhsin and Eltomy, 2019).

The review of Oruç et al. reported 13 cases with malignancy out of 116 (11%) patients with inguinoscrotal bladder herniation (Oruç et al., 2004). Nine were reported as bladder cancer, 3 as prostate cancer and 1 as neoplasm. Another study showed that 11.2% of inguinal bladder hernias were associated with urological malignancies and 23.5% were associated with various complications (Yong et al., 2013).

CONCLUSION

Inguinoscrotal bladder hernias present with intermittent swelling of the inguinal/scrotum and prominent voiding findings. More advanced cases may be associated with 2 – stage urination or reduction in post-void scrotal size. A comprehensive physical examination supported by imaging techniques is extremely important for diagnosis and prognosis.

REFERENCES

AlMohaya N., Alabdrabalameer M.N.E., AlAnazi K., AlMuhsin A.M., Eltomy H., (2019). Bilateral inguinal bladder hernia following unilateral transabdominal preperitoneal repair. A case report and review of the literature. Ann Med Surg (Lond);46:23–6.

Branchu B., Renard Y., Larre S., Leon P., (2018) Diagnosis and treatment of inguinal hernia of the bladder: a systematic review of the past 10 years. Turk J Urol;44:384–8.

Calıskan S., Türkmen M., Sungur M., (2018) Inguinal bladder hernia in female patient, Iran. J. Med. Sci. 43 (6) 671–672.

Elkbuli A., Narvel R.I., McKenney M., Boneva D., (2019). Inguinal bladder hernia: a case report and literature review. Int J Surg Case Rep;58:208–11.

Fisher P.C., Hollenbeck B.K., Montgomery J.S., Underwood W., (2004). Inguinal bladder hernia masking bowel ischemia, Urology 63 (1) 175–176

Habib A., (2017). A rare case of inguinal hernia with complete bladder herniation, Case Rep. Surg. 2017: 4658169.

Hellerstein L.H., Sacks S.M., Hellerstein D.K., (2018). Obstructive uropathy from complete bladder and bilateral ureteral incarceration within an inguinal hernia. BMJ Case Rep. Feb 27;2018:bcr2017222637.

Khan K., Chaudhry A., Feinman M.B., (2016) Inguinoscrotal hernia containing theurinary bladder, BMJ Case Rep. Sep 1; 2016: bcr2016217408.

Kraft K.H., Sweeney S., Fink A.S., Ritenour C.W.M., Issa M.M., (2008). Inguinoscrotalbladder hernias: report of a series and review of the literature, Can. Urol. Assoc. J. 2 (6) 619–623.

Madani A.H., Nikouei H.M., Aval H.B., Enshaei A., Asadollahzade A., Esmaeili S., (2013). Scrotal herniation of bladder: a case report, Iran. J.Med. Sci. 38 (1) 62–64.

Mahadevappa B., Suresh S.C., Natarajan K., Thomas J., (2009). Cystogram with dumbbell shaped urinary bladder in a sliding inguinal hernia, J. Radiol. CaseRep. 3 (2) 7–9.

Moufid K., Touiti D., Mohamed L., (2013). Inguinal bladder hernia: four case analyses, Rev. Urol. 15 (1) 32–36.

Oruç M.T., Akbulut Z., Ozozan O., Coşkun F. (2004). Urological findings in inguinal hernias: A case report and review of the literature. Hernia. Feb;8(1):76-9.

Papatheofani V., Beaumont K., Nuessler N.C. (2020) . Inguinal hernia with complete urinary bladder herniation: a case report and review of the literature. J Surg Case Rep. Jan 2; (1):rjz321.

Sarr A., Ondo C.Z., Sow Y., Fall B., Thiam A., Sine B., et al., (2015). Inguinal hernia of the bladder: about 8 cases. Pan Afr Med J;22:7.

Taskovska M., Janeˇz J., (2017) Inguinal hernia containing urinary bladder—a casereport, Int. J. Surg. Case Rep. 40:36–38.

Ugur M., Atcı N., Oruc C., Akkucuk S., Aydogan A., (2016). Left inguinal bladder hernia that causes dilatation in the ureter, Arch. Iran. Med. 19 (5) 376–378

Wagner A.A., Arcand P., Bamberger M.H., (2004). Acute renal failure resulting fromhuge inguinal bladder hernia, Urology 64 (1). 156–157.

Wang P., Huang Y., Ye J., Gao G., Zhang F., Wu H. (2018). Large sliding inguino-scrotal hernia of the urinary bladder. Medicine (Baltimore); 97:e9998.

Yong G.L., Siaw M.Y., Yeoh A.J.L., Lee G.E.G., (2013). Inguinal bladder hernia: case report. Open Journal of Urology. 3:217-218.

CHAPTER 4

PAINLESS LABOUR AND DELIVERY

Ozlem SEN[1], Dr.

[1]Asst Prof, Kanuni Sultan Suleyman Training and Research Hospital, Department of Anesthesiology and Reanimation. e-mail: turanozlem1@yahoo.com.tr , ORCID ID: 0000-0002-1344-006X

INTRODUCTION

Since the birth of mankind, always all women experience the pain of delivery. On the other hand, every woman experiencing childbirth wants to get rid of labour pain as soon as possible. The actual perspective of painless labour became a current topic with the advancements over the last decades as the birth of obstetric anesthesia as a subspecialty, which gained a firm place in the field of obstetrics and analgesia.

Fear of childbirth and conflicting feelings about birth are a normal part of pregnancy (1). Delivery is always a physically and psychologically stressful process. Physiological response to delivery stress can lead to exhaustion of the mother's energy reserves and even fatal complications during puerperium. In this chapter, painless delivery and labour are discussed in light with the recent studies in the literature.

1. ANATOMICAL AND PHYSIOLOGICAL ASPECTS OF LABOUR PAIN

Labour pain is caused by numerous complex physiological and psychological interactions. The first stage of labor pain is due to dilation and stretching of the lower uterine segment and cervix (2, 3). During the second stage of labour, greater dilatation of the vagina and pressure on the perineum are responsible for more intense somatic pain. The 2nd, 3rd and 4th sacral spiral segments have pain impulse through pudendal nerve in this second stage. The chemical mediators including leukotrienes, prostaglandins, lactic acid and bradykinin are involved in this process.

Complete understanding of the relevant anatomical structure is essential in order to have knowledge about pathophysiological aspects of labour pain. First stage labour pain can be alleviated through paracervical plexus blockade, while 2nd stage pain can be inhibited by pudendal nerve. Advancements and developments in the field of obstetrical anesthesia have caused a shift from simply abolishing the labour pain to providing a quality labour analgesia with minimal side effects (4). The International Association for the Study of Pain (IASP) declared the year 2007-2008 as ‘Global year against pain in women, Real Women-Real Pain’. The focus is on contemporary obstetrical advancement for evidence based management of labour and thus, improving quality of life (QoL). Various factors affect the decrease or increase of severity of labour pain including maternal age, parity, condition of the cervix, maternal condition etc. The pain is more severe in elderly prini-gravida. Dysmenorrhea, psychological state of the mother, fear

of labour, and anxious attitude also are responsible for a lower threshold of labour pain (5).

1.1.Epidural Anesthesia

The painless delivery is one of the most important techniques of parturient delivery. The continuous epidural anesthesia method can improve the pain index, shorten the stages of labour and reduce labour pain (5-7). In painless delivery anesthesia, the continuous epidural technique help to shorten the first and second phases of delivery, promotes vaginal delivery and improves analgesic quality of the body.

Ma et al. reported that the uterus of a parturient would contract sharply so as to have severe pain (5). Furthermore, a large amount of catecholamines might be released, which can inhibit adequate contractşon of the uterus, prolonging the course of delivery and increasing the risk of fetal distress. Therefore, continuous epidural anesthesia should be ensured to optimize delivery technique and reduce labour pain.

Continuous epidural anesthesia technique involves full use of the epidural catheter equipment to allow entrance of the anesthetic to the body smoothly. With this method, by blocking the pain and nervous system, the muscle groups at the pelvic floor are always relaxed. Epidural anesthesia accelerates opening of the uterus and avoid the requirement for cesarean section (8). In addition, owing the reasonable use of fentanyl and ropivacaine, this anesthesia could avoid adverse symptoms and achieve analgesic efficacy without affecting uterus contractions, keeping the patrutient awake and shortening the course of delivery (9).

1.2.Epidural Analgesia in Painless Delivery

Labour pain is one of the most severe pains and its fear is one of the reasons women would not desire normal vaginal delivery. Research for effective techniques of pain relief during labour have been the focus of numerous studies in the literature. Since ages, scientific advancements have been carried out to alleviate labour pain, which is the most dreadful pain of women's life that they have to bear inevitably. Opioids like pethidine have been used traditionally for this pain, but they pose a very high risk of fetal depression (10). Tramadol has also been used to reduce labour pain, but it was not very effective in high pain scores. Epidural analgesia has been reported by a number of studies as an effective means of pain relief. Today, low concentration of newer anesthetics as discussed below have been introduced

with new techniques like patient controlled analgesia (11). Previously lidocaine, which had significant motor blocking properties was used as anesthetic in epidural anesthesia. But this side effect is less with ropivacaine.

Some controversial results have been stated regarding the first and second stages of the labour. Antonakou et al. reported that epidural analgesia prolongs both stage 1 and stage 2 and labour (12). On the contrary, Dipti Agarwal et al. reported that duration of the first stage was shorter than the second stage with epidural analgesia (13). In addition, the same authors compared VAS scores among the patients receiving epidural anesthesia and systemic opioids. Epidural analgesia was better than parenteral opioids in pain relief. The rate of achieving analgesia was shorter than 1 minute with epidural analgesia.

The effects of epidural analgesia on the fetus and neonate was the major concern. In a study by Chaurasia et al., there was slight reduction in fetal heart rate (FHR) epidural group, but the difference was not statistically significant (14). Hamza et al. found that there was a decrease in FHR variability in the epidural group, but they did not observe such an outcome with bupivacaine (15).

Epidural block is the most effective means of relieving pain during labour and is associated with better pain control compared to opioids (16). Visceral distention resulting from rhythmic uterine contractions and progressive cervical dilatation are the causes of much of the pain experienced during the first stage of labour. Afferent impıulses are transferred from the cervix and uterus to the spinal cord through T10-L1 segments. This usually causes pain in the lower back and the sacrum (17). In addition, pain caused by stretching of the birth canal and perineum in the second stage of labour involves the pudendal nerve, which derives from the sacral segments 2, 3 and 4. The second stage of labour is generally shorter than the first stage, although pain is more intense. Perineal pain because of stretching of the vagina, vulva and perineum are added on the pain of uterine contractions. Pain in the second stage is mainly somatic and is transferred via the spinal S2-4 segments.

Whereas some women experience unbearably severe pain during childbirth, other may have only mild pain. Several parameters may help clinicians predict which parturients are more likely to experience more severe pain and thus, are likely to benefit more from epidural block. Younger maternal age, low back pain during menstruation, nulliparity, i.v. induction of labor with oxytocin, increased maternal and fetal weight etc. have been reported to increase pain during labour and delivery. Studies have shown a

correlation between the use of epidural analgesia and a higher rate of cesarean section. On the other hand, women that select epidural analgesia are more frequently nulliparous, have slower cervical dilatation, come to hospital earlier, have smaller pelvic outlets and deliver heavier infants (18).

1.2.1. Ketamine Hydrochloride

More than one modality is available for painless labour. It can be accomplished by using epidural block, spinal block, combined spinal epidural block and general anesthesia. However, the technique using ketamine hydrochloride in low dose controlled i.v. drip is less morbid, easy to apply and safe.

Ketamine hydrochloride is an ideal analgesic agent due to its water-soluble property and ability to remain stable in solution for a long time. It can be administered as both intramuscularly or intravenously. Ketamine is an N-methyl-D-aspartate receptor antagonist, which shows excellent analgesic property even in sub anaesthetic doses. It is easily available and is being used even by non-anesthesiologists for providing sedation in minor procedures (19). Effective pain relief with ketamine hydrochloride facilitates enhanced cooperation of the mother. It also reduces stress induced elevation of catecholamine, allowing smooth cervical dilatation and making labour more tolerable (20). Unlike other anesthetics, ketamine has a potent analgesic property even at subanesthetic doses. Studies have indicated that analgesia produced by ketamine is mediated via opiate receptors and N-methyl-D aspartate receptors. Ketamine seems to offer a clear advantage over the narcotics in which major drawback is respiratory depression (21).

Havle et al. conducted an interventional study investigating the effects of ketamine on painless labour in 73 cases. Low dose ketamine caused neither any change in uterine contractions nor any untoward effect on cervical dilatation. Ketamine provided relief from labour pain during active labor in the majority of patients. Normal Apgar scores were found in the newborns. Slow intravenous infusion of low-dose ketamine was significantly effective, easy to apply and monitor, cost effective, and without significant maternal and fetal complications when used for painless labour (22).

2. INSIGHT INTO PAINLESS LABOUR AND DELIVERY

Some studies have reported the fact that the pregnant woman had a previous problematic birth experience or had an obstetric complication has an effect on the fear of childbirth. Severe pain can significantly affect mental

health of the partiruent, infant and relationship with her partner, leading to emotional disorders, depression and anxiety (Mi23ller). The first obstetric anesthesia application was performed in 1846 by James Young Simpson using ether in order to relieve labour pain (24).

American Society of Anesthesiologists (ASA) and American College of Obstetricians and Gynecologists (ACOG) adopted labour pain as an indication to treat labour pain (25). Relief pain may decrease maternal and fetal adverse effects. Vasomotor blockage effect increases intervillous blood flow in disorders that impair placental blood flow and function such as preeclampsia, hypertension and diabetes mellitus.

In a survey study by Aslan et al. from Turkey conducted in 2018, in order to evaluate knowledge, experience and awareness of obstetricians about painless labour approach. A total of 98 physicians participated in a survey study by Aslan et al.. The number of clinics where painless labour was reported as 44%ü while painless labour does not apply in 5 (34%) clinics. Twenty physicians preferred painless and 78 physiciants normal delivery. Of the participants, 77% thought that their patients had no sufficient knowledge about painless delivery. Thirty-three physicians reported that painless labour has negative effects on the fetus.

Contemporary pain treatment concept severe labour pain and its negative effects on the nother and uterus should be alleviated. Delivery analgesia has been shown to decrease clinical morbidity and mortality. Painless delivery is increasingly being performed in numerous clinics all around the world and offered as an alternative to normal vaginal delivery.

Labour is normal when occurs spontaneously at term with vertex presentation, without undue prolongation, and without any complication affecting the mother and her infant. A scientific definition of pain is “an unpleasant sensory and emotional experience associated with actual and potential tissue damage”. Labour pain has two elements as visceral and somatic. The visceral component of labour pain occurs during the first stage and is exerted on the cervix, causing its dilatation. The somatic component appears at the end of the first stage and pain is exerted on the vaginal part of the cervix, vagina and perineum (Hulsbosch). Pain relief have a crucial role in maternal satisfaction with their labour experience and may promote their well-being and their future mothership.

The methods of pain relief can be broadly examined in two categories: non-pharmacological and pharmacological. Non-pharmacological methods help to decrease pain without taking medicine, are easy to perform,

and do not cause side effects. These methods include emotional support, massage, aromatherapy, hydrotherapy, Transcutaneous Electrical Nerve Stimulation (TENS), acupuncture, acupressure and hypnosis (26). Pharmacological methods involve anesthetics and analgesics (Figure 1).

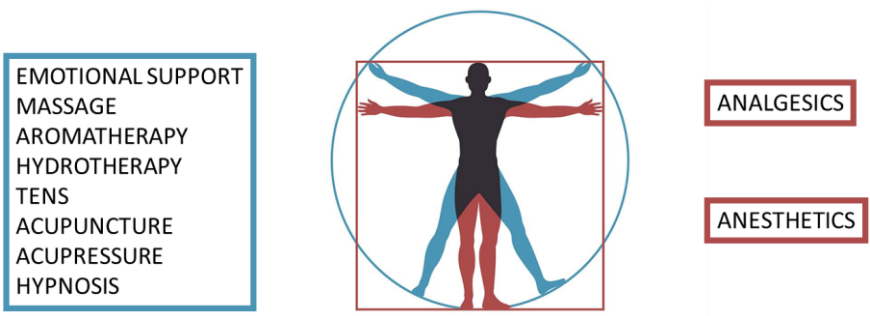


Figure 1. Non-pharmacological and pharmacological methods of relieving labour pain

Analgesics reduce pain without loss of consciousness, muscle functşon and feeling. Whereas anesthetic agents relieve pain by blocking most connected with the tension feeling, including pain. Pain relief medication can be systemic, regional and local. Systemic medications affect the whole body. Regional anesthetics affect a region in th body, while local anesthetics usually affect a small area of the body and are extremely safe, but do not relieve labour pain.

Labour pain management is now adopted by many countries in the world. Studies demonstrated that women with fear of labor require more use of pain-relieving methods in labor compared to women without fear. El-Nasr et al. measured knowledge and attitudes of 150 women regarding painless labour in Egypt. In this study, 60.7% of women participating in the study lived in rural areas. Less than two thirds of the women (65.3%) have secondary education. More than half (54.7%) of the participants had poor knowledge about painless delivery. Of the 150 women, 57.3% have a negative attitude regarding painless labour. The lack of knowledge and misunderstanding regarding safety, acceptability and availability of pain relief options are considered major factors of not receiving adequate labor pain relief (27). In another study, Thakur et al. reported that more than half of expectant mothers had below average level of knowledge regarding labor analgesia (28).

3. COMPARATIVE STUDIES REGARDING PAINLESS LABOUR AND DELIVERY

3.1.Epidural and i.v. Tramadol

It is not the fear of bringing a new life into the world that frightens a woman, but instead is the fear of pain she experiences. It is now well-established that the only consistently effective method of painless labour is epidural analgesia. It possesses a long record of safety and has few complications. On the other hand, tramadol, a centrally acting opioid analgesic is currently being assessed in women undergoing labour.

Jaitley et al. compared the efficacy of epidural vs i.v. tramadol. According to the results of this study, the mean operational time was significantly lower with epidural tramadol administration (10.83 vs 13.67 min). The mean duration of analgesia was statistically significantly lower in the epidural tramadol group than in i.v. tramadol group (3.37 h vs 3.07 h). In conclusion, epidural tramadol is a simple and effective method for safe and painless delivery. Analgesia produced is significantly more effective than i.v. tramadol. Maternal side effects are minor without any fetal respiratory depression (29).

CONCLUSION

Labour pain is one of the most common experiences faced by women. However, the fear of this pain can be relieved especially with the administration of analgesic and anesthetic medications. In this regard, labour analgesia should be popularized and delivered to every demanding mother who really wants a painless process of parturition. There is a statistically significant correlation between total knowledge and attitude scores regarding painless labour. Patient education programs for both healthcare providers and parturients would improve the attitudes and knowledge regarding labour pain and will increase well-being of the mother and the fetus. It is necessary for healthcare providers to clarify the influencing factors, to take effective measures to improve the psychological state of pregnant women and thus, promote the smooth delivery.

REFERENCES

Duman Z., Köken N.G., Şahin F.G., Coşar E., Arıöz D.T., Aral İ. (2007). Sağlık çalışanlarının normal doğum ve sezaryen ile ilgili düşünceleri. [TR] Perinatol Derg, 15:1-7.

Wong C.A. Obstetric Pain. In: Ballantyne JC, Rathmell JP, Fishman SM, eds. Bonica's Management of Pain. 4th ed. Philadelphia: Lippincott Williams & Wilkins; In press.

Pan P, Eisenach JC. The pain of childbirth and its effect on the mother and the fetus. In: Chestnut DH, Polley LS, Tsen LC, Wong CA, eds. Obstetric Anesthesia Principles and Practice. 4th ed. Philadelphia: Elsevier Mosby; 2009: 387- 404.

Lang, A. J., Sorrell, J. T., Rodgers, C. S., & Lebeck, M. M. (2006). Anxiety sensitivity as a predictor of labor pain. European journal of pain (London, England), 10(3), 263–270.

Ma J., Zhou J., Tian G. (2016) Effect of painless delivery with different anaesthetic methods on parturient lactation function and Apgar score of new-borns. J Hainan Med,27:2546-8.

Ding, Y., Zhang, C., Zhang, J., Zhang, N., Li, T., Fang, J., Zhang, Y., Zuo, F., Tao, Z., Tang, S., Zhu, W., Chen, H., & Sun, X. (2017). miR-145 inhibits proliferation and migration of breast cancer cells by directly or indirectly regulating TGF-β1 expression. International journal of oncology, 50(5), 1701–1710.

Ghasemi, A., Fallah, S., & Ansari, M. (2016). MiR-153 as a Tumor Suppressor in Glioblastoma Multiforme is Downregulated by DNA Methylation. Clinical laboratory, 62(4), 573–580.

Zhou, H., Wu, G., Ma, X., Xiao, J., Yu, G., Yang, C., Xu, N., Zhang, B., Zhou, J., Ye, Z., & Wang, Z. (2018). Attenuation of TGFBR2 expression and tumour progression in prostate cancer involve diverse hypoxia-regulated pathways. Journal of experimental & clinical cancer research : CR, 37(1), 89.

Wei, C. Y., Tan, Q. X., Zhu, X., Qin, Q. H., Zhu, F. B., Mo, Q. G., & Yang, W. P. (2015). Expression of CDKN1A/p21 and TGFBR2 in breast cancer and their prognostic significance. International journal of clinical and experimental pathology, 8(11), 14619–14629.

Sosa, C. G., Balaguer, E., Alonso, J. G., Panizza, R., Laborde, A., & Berrondo, C. (2004). Meperidine for dystocia during the first stage of

labor: A randomized controlled trial. American journal of obstetrics and gynecology, 191(4), 1212–1218..

Anim-Somuah, M., Smyth, R. M., Cyna, A. M., & Cuthbert, A. (2018). Epidural versus non-epidural or no analgesia for pain management in labour. The Cochrane database of systematic reviews, 5(5), CD000331.

Antonakou, A., & Papoutsis, D. (2016). The Effect of Epidural Analgesia on the Delivery Outcome of Induced Labour: A Retrospective Case Series. Obstetrics and gynecology international, 2016, 5740534.

Agrawal, D., Makhija, B., Arora, M., Haritwal, A., & Gurha, P. (2014). The effect of epidural analgesia on labour, mode of delivery and neonatal outcome in nullipara of India, 2011-2014. Journal of clinical and diagnostic research : JCDR, 8(10), OC03–OC6.

Koutsiana, E., Hadjileontiadis, L. J., Chouvarda, I., & Khandoker, A. H. (2017). Fetal Heart Sounds Detection Using Wavelet Transform and Fractal Dimension. Frontiers in bioengineering and biotechnology, 5, 49.

Hamza J. (1994). Retentissement de l'anesthésie péridurale sur le foetus et le nouveau-né [Effect of epidural anesthesia on the fetus and the neonate]. Cahiers d'anesthesiologie, 42(2), 265–273.

Capogna G. (2001). Effect of epidural analgesia on the fetal heart rate. European journal of obstetrics, gynecology, and reproductive biology, 98(2), 160–164.

Alexander, J. M., Sharma, S. K., McIntire, D. D., Wiley, J., & Leveno, K. J. (2001). Intensity of labor pain and cesarean delivery. Anesthesia and analgesia, 92(6), 1524–1528.

Chen, S. Y., Lin, P. L., Yang, Y. H., Yang, Y. M., Lee, C. N., Fan, S. Z., & Chen, L. K. (2014). The effects of different epidural analgesia formulas on labor and mode of delivery in nulliparous women. Taiwanese journal of obstetrics & gynecology, 53(1), 8–11.

Bautista, L., & George, R. B. (2020). Epidural analgesia in labour. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 192(19), E509.

Joel, S., Joselyn, A., Cherian, V. T., Nandhakumar, A., Raju, N., & Kaliaperumal, I. (2014). Low-dose ketamine infusion for labor analgesia: A double-blind, randomized, placebo controlled clinical trial. Saudi journal of anaesthesia, 8(1), 6–10.

Wang, J., Huang, J., Yang, S., Cui, C., Ye, L., Wang, S. Y., Yang, G. P., & Pei, Q. (2019). Pharmacokinetics and Safety of Esketamine in Chinese Patients Undergoing Painless Gastroscopy in Comparison with Ketamine: A Randomized, Open-Label Clinical Study. Drug design, development and therapy, 13, 4135–4144.

Boom, M., Niesters, M., Sarton, E., Aarts, L., Smith, T. W., & Dahan, A. (2012). Non-analgesic effects of opioids: opioid-induced respiratory depression. Current pharmaceutical design, 18(37), 5994–6004.

Havle P.A., Patange R.P., Havle P.A. et al. (2020). Ketamine Hydrochloride for Painless Labour - An Interventional Study. J Evolution Med Dent Sci, (9):3373-76.

Miller RD. Miller's anesthesia. 6th ed. Philadelphia, Pennsylvania: Elsevier Churchill Livingstone; 2005. p. 16.

Erdine, S. (2007) Ağrı. İstanbul:Nobel Tıp Kitabevleri [TR]; p. 142.

Aslan, D.D., Altunkaya, N., Ferlengez, A.G. (2019). Assesment of the Approach to the Painless Delivery: A Survey Study. Med Bull Haseki, 57:180-184.

Czech, I., Fuchs, P., Fuchs, A., Lorek, M., Tobolska-Lorek, D., Drosdzol-Cop, A., & Sikora, J. (2018). Pharmacological and Non-Pharmacological Methods of Labour Pain Relief-Establishment of Effectiveness and Comparison. International journal of environmental research and public health, 15(12), 2792.

Alsharidah, D. and Alhuzaimi, F. (2018). Knowledge and attitude of Saudi women toward painless labor and misconception about risks and complications. International Journal of Advanced Research; 6(2): 1440-1449

Thakur, M., Sagar, N., & Tandon, P. (2017). Knowledge and attitude regarding epidural analgesia among expectant mothers. International Journal of Reproduction, Contraception, Obstetrics and Gynecology; 6(5): p, 1930.

Jaitley, A., Singh, S., Srivastava, U., Nagrath, A., Prajapati, N.C., Singh, R.A. (2011) comparison between epidural and IV tramadol for painless labor and effect on perinatal outcome. J Obstet Gynaecol India, 61(1):42-47.

CHAPTER 5

PROTECTING FERTILITY IN THE CANCER PROCESS

Nurten ÖZÇALKAP[1], Professional Midwife

Emel GÜÇLÜ CİHAN[2], Professional Midwife

[1] İnönü University, Faculty of Health Sciences, Department of Doctorate in Midwifery, Malatya, Turkey, nur_dan044@hotmail.com, ORCİD ID: 000-000-032-440-968-X

[2] İnönü University, Faculty of Health Sciences, Department of Doctorate in Midwifery, Malatya, Turkey, emel_guclu4606@hotmail.com, ORCİD ID:000-000-025-016-591-7

INTRODUCTION

Cancer is malignant mesenchymal tumors. It is one of the most important health problems of our age. Every year, more people are diagnosed with cancer due to the increase in diagnosis and prolongation of life expectancy. According to the data of the Ministry of Health in our country, among the known causes of death, cancer has risen to the second place after cardiovascular diseases. Unhealthy diet, sedentary lifestyle, genetic characteristics, environmental factors and stress are the most important reasons for this increase (Karageyim-Karşıdağ et al. 2015). New applications brought by technology and developed treatment protocols, new antineoplastic and chemotherapy drugs produced have made it easier to diagnose and treat cancers at an early stage. Methods such as conservative surgery, chemotherapy (CT), radiotherapy (RT) and allogeneic bone marrow transplantation (BMT) applied for cancer treatment adversely affect the fertility of women. Radiotherapy and chemotherapy cause infertility and amenorrhoea, while radiation and alkylating agents cause premature ovarian failure. The increase in the survival rates of individuals, the necessity of maintaining the reproductive functions as well as the endocrine and sexual functions of gynecological organs, the advancement of women's maternal age and the increase in the incidence of cancers in the reproductive period have brought the protection of fertility, especially in young cancer patients, to the agenda. Fertility-preserving technique (FKT) methods have started to be applied for the most common gynecological cancers (Batu et al. 2013 & Koçak et al. 2017). A multidisciplinary team including gynecology, perinatology, endocrinology specialist, oncology, radiology and pathology specialists takes part in fertility-preserving treatment (FKT). In general, prognostic factors related to the disease (stage of the disease), grade of the disease, tumor-related factors, family history of the patient, age, fertility desire, obstetric and gynecological history should also be questioned in the selection of patients in FCT. The patient should be informed about the treatment methods that can be applied in FKT (TJOD 2015). In order to apply FKT, the patient's disease should be staged correctly and the risk group should be evaluated well. It is time for reasons such as the family and the patient do not have enough information about FKT, they are not interested in the proposed surgical method, the cancer patient and the family are worried about the outcome of the cancer treatment rather than their fertility desire, and they are not emotionally willing and ready to talk about

the possible benefits-risks of the treatment due to the diagnosis of cancer. Occasionally, suitable patients who may need PKT may be overlooked. Generally; FCT hormonal therapy in endometrial cancer, conization in the early stage in patients with cervical cancer, radical vaginal trachelectomy, ovarian transposition, and unilateral salpingo-oophorectomy (USO) treatment options in the early stage of ovarian cancer are recommended. However, depending on the characteristics of the patients and the disease, other fertility-preserving treatment methods such as ovarian tissue cryopreservation, ovarian transplantation, embryo cryopreservation and oocyte cryopreservation can be applied (Kocak et al. 2017).

1. PRESERVATION OF FERTILITY IN THE PROCESS OF CANCER

1.1. Effect of chemotherapy on fertility

Because chemotherapeutic agents damage oocytes, they have devastating effects on a woman's future fertility potential. In chemotherapy, factors such as the type and dose of the drug and the age of the patient are effective. Alkylating agents such as cyclophosphamide increase the risk of toxic effects on the ovaries and the possibility of amenorrhea. These agents have been used more commonly in ovarian cancers in the past, but are now used more commonly in gestational trophoblastic diseases. When evaluating the patient's ovarian failure, the drug dose is also taken into account. In particular, high doses of high-risk drugs can cause more extensive damage to the ovaries. Even in young cancer patients, decreased ovarian reserve and increased follicular follicle stimulating hormone (FSH) levels indicate faster oocyte atresia and poor oocyte quality, and the reproductive rate is significantly reduced. Despite the toxic effects of chemotherapeutic agents on ovarian function, it has been reported that there is no difference in maternal morbidity rate after cancer treatment (Batu et al. 2013). Therefore, chemotherapy causes a decrease in the number of primordial follicles, a decrease in ovarian reserve, early menopause and infertility (TJOD 2015).

1.2. Effect of Radiation on Fertility

Compared to chemotherapy, radiation not only damages the ovaries, but also the hypothalamic-pituitary axes and the uterus. Total body, pelvic and abdominal irritation have detrimental effects on ovarian follicles depending on dosage. Radiation has a three-dimensional effect on the uterus.

First, it causes fetal growth restriction by reducing the fetoplacental circulation. Second, decreased uterine elasticity and volume subsequently causes preterm delivery. Third, radiotherapy can damage the endometrium. This causes the placenta to implant abnormally and placenta confinement becomes difficult (Batu et al. 2013).

1.3. Fertility Preserving Approach in Endometrial Cancer

Although endometrial cancer is mostly seen in the postmenopausal period, it has started to be seen more frequently and at earlier ages with the prolongation of human life. Standard treatment for endometrial cancer includes total abdominal hysterectomy (TAH), bilateral salpingoopherectomy (BSO), pelvic para-aortic lymph node sampling, surgical staging, and adjuvant chemotherapy. This has led to the demand to preserve the fertility of women of reproductive age. Treatments for endometrial cancer cause women to be infertile. Hormonal agents are used in FCT for patients with endometrial cancer. These; Progestins are tamoxifen and GnRH analogs. Thus, they reverse the dominant environment from the hormone estrogen. Tamoxifen prevents estrogen from binding to its receptors, increases the number of progesterone receptors and sensitivity to progestins (Koçak et al. 2017). Selection criteria for fertility-preserving treatment in endometrial cancer; It is the condition of under 40 years of age, Nulliparous, Fertility desire, Endometrioid type, Grade 1, No myometrial invasion, No extrauterine spread, Presence of progesterone receptor, Body mass index and Normal CA 125 level (Karateke et al. 2004).

1.4. Fertility Protective Approach in Cervical Cancer

The classical treatment of cervical carcinoma is radical hysterectomy and pelvic radiation. Hysterectomy, which includes resection of the uterus, cervix, upper part of the vagina and tissues surrounding the cervix, and pelvic lymph adenectomy, completely destroys the reproductive potential of women. Pelvic radiotherapy applied as a non-surgical treatment in cervical cancer negatively affects the gonads that are sensitive to ions and harms the fertility ability. The extent to which the fertility of the patient will be damaged increases in proportion to the dose received, the frequency of the fraction and the size of the radiation field, and the number of primordial follicles (ovarian reserve) decreases as the age of the woman and the time exposed to ions increase (Koçak et al. 2017).

FCT patient selection criteria for cervical cancer; The individual has a desire for fertility, The patient is under the age of 40, the stage of the disease, the volume of the tumor, the absence of distant organ metastasis and lymph node involvement, the limited endocervical involvement of the tumor, and the compliance with the follow-ups (Eskander et al. 2011).

1.5. Fertility Protective Approach in Ovarian Cancer

Classical treatment of ovarian cancer is hysterectomy, including bilateral salpingo-oophorectomy, omentectomy, pelvic and paraaortic lymph node dissection, peritoneal biopsy, and washing of the abdomen and pelvis. At least one ovary and uterus should be preserved in the FCT of a patient with ovarian cancer. FCT patient selection criteria for ovarian cancer; FCT is applied in low-risk groups such as stage IA, borderline tumor, intact ovarian integrity, the lesion being limited to one ovary, the absence of ascites, the tumor has not spread beyond the capsule, and the mass is mobile (Eskander et al 2011).

1.6. Fertilization presentation (FP) Approaches

Fertilization presentation (FP) approaches are gynecological oncology surgery, pelvic radiotherapy, cytotoxic therapy, fertility-sparing surgery, ovarian transposition and reproductive organ preservation, cryopreservation of embryo, oocyte or ovarian tissue (TRSM 2018).

1.6.1. Embryo and Oocyte Cryopreservation

Cryopreservation; It refers to the cooling of cells and tissues to temperatures below zero degrees, stopping all their biological activities and storing them for future use. In addition to turning to surgical modifications in protecting the organs necessary for fertility, there has been progress in assisted reproductive techniques in recent years. In this way, the hope of remaining fertile after treatment in women diagnosed with cancer has arisen. The two techniques that have achieved the highest success are; embryo and oocyte cryopreservation. Embryo freezing is the first choice for couples. Live birth success equates to fresh embryo transfer. With the oocyte cryopreservation technique, the first baby was born in 1986. Egg freezing, which was previously considered experimental, has been accepted as a method that should be offered routinely to patients with reproductive preservation indications, with the bulletin published by the American

Reproductive Association (ASRM) in 2013. In patients who want FP before cancer treatment, oocyte and/or embryo cryopreservation can be performed quickly. In general, the required time is between about 2-4 weeks. The reason why the duration is intermittent is that the patient's cycle is taken into account (Bastu et al. 2013).

1.6.2. Ovarian Protective Gonadotropin Releasing Hormone (Gnrh) Agonist

Some experts recommend the use of GnRH agonists. Variable doses of GnRH agonists are administered 1 month before chemotherapy treatment begins and may be more suitable for young girls with healthy ovarian reserve. Although there are many theories about how this treatment protects the ovary, the most accepted opinion belongs to Blumenfeld. According to this theory, gonadotoxic chemotherapy destroys ovarian follicles and raises FSH. Rising FSH leads to pick of primary follicles. These follicles re-enter the cell cycle and are destroyed by chemotherapy. GnRH agonists suppress endogenous FSH/pituitary secretion. In this way, they slow down the accelerated oocyte atresia after gonadotoxic treatment.(Blumenfeld 2008, & Uncu et al.2016).

1.6.2. Ovarian Tissue Freezing method

Ovarian tissue freezing method is offered to selected patients. It is the first choice especially for patients who are single or before puberty or who need to start treatment immediately. There are over 70 pregnancies reported to date (Brânnström et al.2014).

1.6.3. Ovarian and Uterine Transposition

It is especially used to protect the gonads from radiation therapy to the pelvis area. The ovaries are removed from the area. After the treatment, the ovaries are returned to their places (Uncu et al. 2016). Ovarian transplantation has been applied since the beginning of the 20th century and in recent years, it has been applied to 50 cases with premature ovarian failure, and pregnancy occurred in one third of them. Regarding uterine transplant; In 2013, at Gothenburg Hospital, Sweden, a 35-year-old woman with Rokitansky syndrome without a congenital uterus had a uterus transplant from a 61-year-old living postmenopausal woman who had given

two births. This is the first birth with a uterus transplant (Brânnström et al. 2014).

1.7. Surgery in Fertility Preserving

The aim of fertility-sparing surgery is to protect all genital organs, if not an ovary or uterus, if possible, and to provide fertility with natural or assisted reproductive techniques (TJOD 2015).

1.8. Protectıon Of Cancer And Fertılıty In Male Patıents

Sperm freezing is the basic fertility preservation approach for male patients. In postpubertal male cancer patients, semen should be frozen before chemotherapy or radiotherapy. If the sperm count is low or if there is not enough and healthy sperm in the semen, invasive methods can be used to obtain sperm from the testicular tissue and freeze it. In cases where sperm number, movement or quality is decreased due to cancer or the negative side effects of the disease, healthy sperm should be tried to be obtained by repeated semen analysis. Again, in cases where it cannot be found, sperm should be obtained and frozen for fertility preservation approaches with invasive methods. Patients diagnosed with testicular cancer should be referred to sperm freezing before orchiectomy. Patients with congenital growth retardation in one testis or testicular sizes smaller than normal due to previous infections should also be referred to sperm freezing method due to possible sperm deficiency in the future. The testicular tissue freezing method is a proven method in cases of obstructive and non-obstructive azoospermia (Uncu et al. 2016).

1.9. Indications for Reproductive Protection in Turkey

Conditions of medical necessity that require the storage of reproductive cells and gonad tissues in Turkey are stated in the 20th article of the ART of 30 September 2014 and numbered 29135; Indications for "Protection of Reproduction" in Turkey The medical imperatives that require the preservation of reproductive cells and gonad tissues in Turkey are stated in the 20th article of the ART of 30 September 2014 and numbered 29135; Indications for "Protection of Reproduction" in Turkey (TRSM 2018).

Indications for the preservation of reproduction in men; In the case of obtaining sperm by surgical methods, before the treatments that damage the gonad cells such as chemotherapy and radiotherapy, before the

operations that will lead to the loss of reproductive functions (removal of the testicles and the like), and the presence of very few sperm (cryptozoospermia). Indications for the preservation of fertility in women; These are the cases where there is a low ovarian reserve before the treatments that damage the gonad cells such as chemotherapy and radiotherapy, before the operations that will cause the loss of reproductive functions (operations such as removal of the ovaries), or the family history of early menopause is documented with a medical board report consisting of three specialist doctors (TRSM 2018, TJOD 2015).

In accordance with the regulation, reproductive cells and gonad tissues can be stored in people with diseases and risk factors (therefore, in people with the specified diseases or risk factors):

- Before chemotherapy drugs and targeted treatments in cancer patients (Lymphoma, Leukemia, etc.)
- Before radiotherapy
- In diseases that require high-dose chemotherapy and/or radiation for myeloablation for therapeutic bone marrow transplantation (Thalassemia Major, Fanconi Anemia, Sickle Cell Anemia, Aplastic Anemia, Hemochromatosis, Myeloproliferative Disease)
- Autoimmune and systemic diseases and cases requiring the use of cytotoxic agents for immunosuppression (SLE, Chronic Kidney Disease, etc.)
- In case of diseases requiring surgery or previous ovarian or testicular surgery (TJOD, 2015, TRSM 2018, & Batu et al. 2013).

CONCLUSION

As a result, cancer rates are increasing in the world. Treatments used in cancer adversely affect people physiologically and biologically. This situation negatively affects fertility by damaging the reproductive organs. Preservation of fertility with the developing technology gives hope to couples who are considering having a child in the future. Health professionals should have sufficient knowledge and equipment about the preservation of fertility in the period of cancer. It should inform patients who want fertility preservation and should help and guide them in choosing the appropriate fertility preservation methods.

REFERENCES

Bastu, E., & Baysal, B. (2013). Oncofertılıty: Fertılıty preservatıon ın cancer patıents. *J Turk Soc Obstet Gynecol.* 10(3): 173- 80.

Blumenfeld, Z., & Avivi, I., & et al. (2008). Gonadotropin-releasing hormone agonist decreases chemotherapy-induced gonadotoxicity and premature ovarian failure in young female patients with hodgkin lymphoma. *Fertil and Steril.* 89(1):166-73.

Brânnström, M., Johannesson, L., Bokström, H., et al. (2014). Livebirth after uterus transplantatioîL. *Lancet.* S0140-6736(14)61728-1.

Eskander, N. R., Randall, L. M., Berman , M. L., & et al. (2011). Fertility preserving options in patients with gynecologic malignancies. *J Obstet Gynecol.* 205(2):103–110.

Karateke, A., Çam Ç. (2004). Endometrium kanserinde fertiliteyi koruyucu yaklaşım *TJOD*. 1:17-22.

Karageyim-Karşıdağ, A.Y., Giray,B., Subaş, S., & et al. (2015). Maternal kanser tanısı olan gebelerin değerlendirilmesi. *Sakarya Med J*. 5(3):135-139.

Koçak, D., & Duman, N. (2017). Jinekolojik Kanserlerde Fertilite Koruyucu Yaklaşımlar. *Türkiye Klinikleri J Obstet Women Health Dis Nurs-Special Topics*. (3):1.

Türk Jinekoloji ve Obstetrik Derneği (TJOD). (2015). Kanserde organ koruyucu yaklaşım. Türk Jinekoloji ve Obstetrik Derneği. https://www.tjod.org/over-kanserinde-organ-koruyucu-yaklasim-tjod2015/ accessed: May 02 2022.

Üreme sağlığı ve infertilite derneği. TRSM (2018). thttps://www.tsrm.org.tr/pro accessed: May 02 2022.

Uncu, B., & Çil, A.P. (2016). "ÜREMENİN KORUNMASI. *Üreme sağlığı ve infertilite derneği.* chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/http://www.tsrm.org.tr/pro/konu/dosyalar/bultenler/tsrm_blast_5.pdf May 02 2022.

CHAPTER 6

FACTORS AFFECTING THE AVAILABLE CONTROL AND PREVENTION STRATEGIES OF TUBERCULOSIS IN UGANDA

Hadijah MBONDE [1]

Dilek ÖZTAŞ[2], Assoc. Prof. Dr.

Salih MOLLAHALİLOĞLU[3], Prof. Dr.

[1] Ankara Yıldırım Beyazıt University,Public Health Department ,Ankara Turkey. mbonde243@gmail.com ORCID ID 0000-0002-6693-811X

[2] Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Public health, Ankara, Turkey. doztas@hotmail.com ORCID ID 0000-0002-8687-7238

[3] Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Public health, Ankara, Turkey. smho@ybu.edu.tr ORCID ID0000-0001-7384-4106

INTRODUCTION

Tuberculosis (TB) is a condition due to Mycobacterium TB that invades the human lungs. Before the coronavirus outbreak at the end of 2019, TB is ranked as the ninth major infection causing death, high above HIV/AIDS as the cause of all deaths worldwide(World Health Organization(b), 2021). To make matters, TB exhibits drug-resistant forms that threaten struggling control efforts in many parts of the world. Patients with weakened immunity, such as HIV-positive individuals, malnourished or diabetic patients, and cigarette users, (Buregyeya et al., 2011), are more susceptible to this disastrous infection (World Health Organization(a), 2021).

World Health Organization's Global Tuberculosis Report,2021, shows that the illness is both treatable and avoidable, with roughly 85 percent of TB cases effectively treated with a 6- 8month treatment regimen. Universal health coverage is, however, required to ensure that all infected people have access to these therapies. (World Health Organization(b), 2021). By 2035, the World Health Organization has a set target of putting an end to the global TB epidemic. However, attaining this wonderful goal requires sufficient financing and high-quality data assessment to identify what is working and what actions are a must (World Health Organization(c), 2014).

Unfortunately, Uganda is ranked among the top 30 countries with the greatest TB burden (WHO global lists, 2021) with 86,000 cases in 2019 plus a sixty five percent TB coverage rate and a seventy two percent treatment rate of success ,both well below the eighty five percent government objective for 2019/20(Kola Oyediran, 2020). The ministry of health in Uganda, however, started working towards achieving an 85% tuberculosis incidence by 2019/20. (Kola Oyediran, 2020) . However, ever since the advent of COVID-19, there is a great decline in tuberculosis detection case rate as the pandemic has generated public fear, preventing individuals from seeking medical assistance (Bell et al., 2020; Ducarme, 2020). The purpose of this study is to examine the current TB situation in Uganda, as well as the existing techniques and factors affecting TB control and prevention. This is a narrative review that was carried out in a given situation. The goal of this review is to investigate the factors that hinder tuberculosis control and prevention in Uganda. Databases, as well as Google Scholar scientific search engine and PubMed, were utilized to collect data, and 126 articles were found to be suitable. While comparing, the studies

were classified and their management systems examined, and discrepancies and similarities were discovered.

1. AVAILABLE TUBERCULOSIS STRATEGIES IN UGANDA

1.1. Passive Case-Finding Technique

Earlier detections as well as treatment commencement have proven to be effective ways to combat TB (World Health Organization, 2010). Most national TB control programs (NTPs), including Uganda's, use passive case-finding as their primary strategy. (World Health Organization, 2013). The word "passive" suggests that TB case detection is totally dependent on persons reporting voluntarily to medical services with TB signs. (World Health Organization, 2010). Suspects are supposed to know and recognize TB symptoms and must be positive regarding TB medical services. However, in many poor and middle income nations, passive process is not as effective as recommended (Li et al., 2013).

1.2. Strategy for Active Case-Finding

A household contact investigation, on the other hand, is the best neighbourhood active case-finding technique, in which medical workers visit homes of newly diagnosed TB patients to screen close contacts and refer identified as high risk individuals to clinics for examination and treatment (Fox et al., 2013) . In 2012, the World Health Organization explicitly recommended household contact investigations be done on a regular basis in high-burden nations like Uganda(World Health Organization, 2012). Contact tracing patients have a higher curative rate and a reduced follow-up failure (Oliveira et al., 2017). However, few countries have not systematically implemented this intervention (Hwang et al., 2011)because it is a resource intensive exercise (Kazibwe et al., 2021).

1.3. Directly Observed Treatment, Short-Course

Directly Observed Treatment, Short-Course (DOTS) is the most cost-effective and best remedial technique to stem the tide of TB in populations. Its main goal is being able to monitor the use of medicines by patients, boost adherence to treatment, and prevent MDR patients(WHO report, 2003; WHO Report, 2006). It is a therapy procedure in which the patient swallows each and every dose of drugs under the observation of a

service provider. Several studies, however, have demonstrated that delays in TB case detection are linked to a negative view of medical services (P Godfrey-Faussett, 2002; Yimer et al., 2005), stigmatisation, (Kola Oyediran, 2020), low levels of knowledge about TB and traditional beliefs (N Shetty, 2004) One study found that TB is viewed as a penalty for disobeying cultural traditions that necessitate sexual abstinence following the death of a family member. (Edginton M E, 2002). The foregoing, as well as other delays in TB case diagnosis, cause infectious individuals to stay in the community, deteriorating personal outcomes and continuing the TB cycle of airborne transmission (Abebe et al., 2012; Murray CJL, 1998).

1.4. National Tuberculosis and Leprosy Programme

The Ministry of Health was given a mandate by the Ugandan government to bring the illness under control by providing high-quality diagnosis, preventive and treatment services to vulnerable Ugandans through the National Tuberculosis and Leprosy Program (NTLP) (Kola Oyediran, 2020). In addition, the NTLP attempts to address Uganda's ongoing TB concerns with the new National Strategic Plan (NSP) 2020/2021-2024/2025, which has a goal of reducing TB incidence by 20%. The NTLP will work to strengthen public systems, with a focus on attaining maximum people and scaling TB preventative therapy (TPT); improve diagnostic and treatment services, including the adoption of new technology and medications; as well as more (USAID,2021).

1.5. Community Health Campaigns

Community Health Campaigns (CHCs) and public awareness of tuberculosis have shown to be effective in the screening and treatment of the disease. This is accomplished through community-based screening campaigns for significant diseases like tuberculosis (TB), HIV, malaria, and cancer, undertaken out with the assistance of local leaders and the Ugandan government (Davis et al., 2019). TB screening together with mobile HIV testing campaigns in rural Uganda allows to improve tuberculosis case detections (Chamie et al., 2012, 2014; Ssemmondo et al., 2016) . On ground testing procedures are acknowledged as a useful technique for detecting infected patients since earlier detection of illnesses is always important to optimize the importance of proper treatments for both individual health and transmission prevention (Suthar et al., 2013) .

1.6. Mobile Health

Active tuberculosis is treated with a combination of drugs. Antibiotics are administered under the supervision of physicians or other healthcare experts, depending on state or municipal public health requirements. It is a beneficial programme since it keeps the TB patient under surveillance thus improving the treatment results (Michael Iseman, 2013). However, in low-resource situations like Uganda, DOT is challenging to apply. TB stigma, patients forgetful to meet with TB physicians, the discomfort of daily face-to-face encounters, expensive travel costs, poor capacity of the public health workforce, and long patient waiting periods at healthcare facilities are all major impediments to DOT's efficacy (Karumbi & Garner, 2015; Volmink & Garner, 2007).

Mobile health (m Health) tools, on the other hand, have showed promise as a replacement to in-person DOT programs (Cook et al., 2019). In Uganda , SMS text messages and real-time monitors have been utilized to increase adherence of antiretroviral treatment (Haberer et al., 2016). The utilization of a smartphone app to record drug swallowing videos as part of video directly observed therapy (VDOT) is a unique technique to remotely monitor adherence and circumvent patient treatment constraints (Friedena & Sbarbarob, 2007; Garfein et al., 2015; Mirsaeidi et al., 2015; Sekandi et al., 2020; Sinkou et al., 2017; Story et al., 2016). Therefore, without having to meet in person, VDOT allows patients to submit recordings of daily medicine intake for observation by TB care providers. However, fear of stigmatization of TB patients has been identified as a greater barrier to the VDOT in Uganda (Sekandi et al., 2020).

2.FACTORS AFFECTING TUBERCULOSIS PREVENTION AND CONTROL IN UGANDA

These factors are examined using Wilber’s integral model. This model enables the investigation of factors under four major sub-groups; internal individual factors, external individual factors, internal social factors as well as external social factor.

A summary of Wilber's model

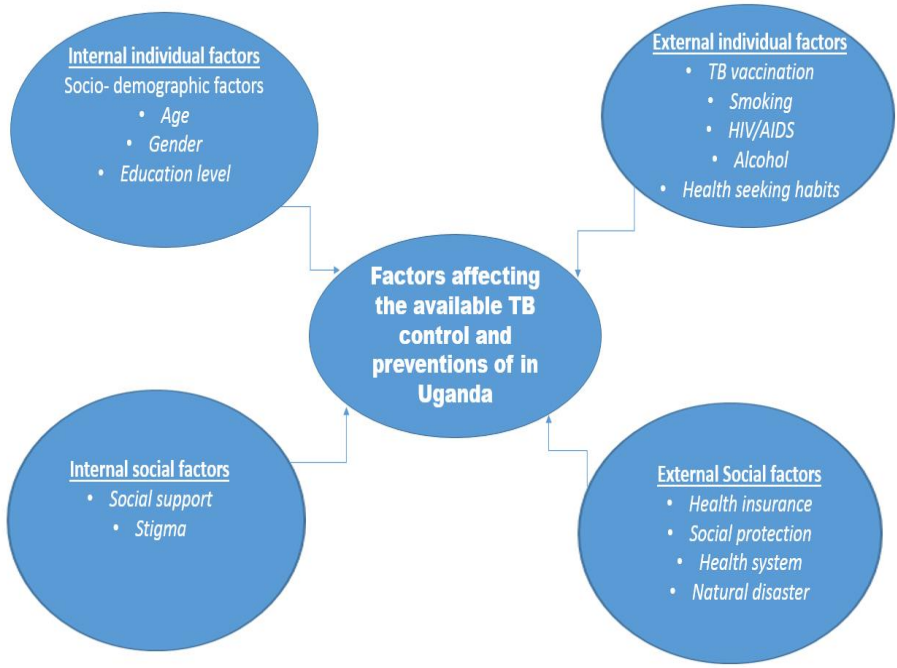


Figure 1: Summary of Wilber's model

2.1. Internal Individual Factors,

2.1.1. Socio-demographic factors

a. **Age:** Old age has less negative force on TB treatment results. In other studies, increased age-related immunosuppressive illnesses such as diabetes mellitus induce medication, resistance, death, and recurring TB in old people (Ncube et al., 2017; Oshi et al., 2014). Patients aged 20–39, on the other hand, had a higher likelihood of missing a dosage and a low risk of treatment failure than those aged 60 and up, according to a qualitative and quantitative analysis (Zhang et al., 2022).

b. **Gender:** In another study conducted in Bangladesh, males exhibited more positive behaviour in utilization of TB services than females (Ehsanul Huq et al., 2018)**.**

c. **Education level:** Individuals with low literacy level were likely to engage in non-self-report adherence behaviours (Gebremariam et al., 2010; Yasin Mohammed, 2014). However, in another study the risk was lower (Zhang et al., 2022). Lack of enough knowledge about tuberculosis was a determining factor for adherence behaviours, as reported in various previous studies (Chang et al., 2004; Demissie & Kebede, 1994; Fekadu et al., 2020; Michael et al., 2004; Muture et al., 2011; Tekle B, 2002). In another study, high tuberculosis knowledge meant lower tuberculosis risk. Therefore, health literacy should be emphasized for a better TB prevention and control plan (Zhang et al., 2022). According to the findings of the study from Uganda, a large proportion of pastoral community members are well-versed in mycobacterial infections, particularly tuberculosis, however social discrimination was a great barrier (Kankya, 2011).

2.2. External Individual Factors

2.2.1. TB vaccination

Vaccination is one of the most important factors in preventing and controlling the spread of tuberculosis. BCG vaccine is the most rampantly used vaccine for tuberculosis prevention since 1921. Evidence suggests that BCG has a 75 percent defensive efficacy in preventing some serious types of TB in children (Bannon, 1999). BCG is currently administered to new-borns in high-risk populations such as Uganda (Helen McShane, 2009). It protects against childhood tuberculosis though ineffective against adult pulmonary tuberculosis. As a result of extensive research into TB vaccine production, many new vaccine nominees are in drug trials, with many more in pre-clinical trials, with the goal of replacing or supplementing the BCG vaccine. A cohort study in Uganda, on the other hand, found that children aged 1 month to 5 years who received BCG exhibited a significantly reduced mortality rate than those who never received (Nankabirwa V, Tumwine JK, Mugaba PM, Tylleskar T, Sommerfelt H, 2015).

2.2.2. Smoking

Tobacco usage is linked to poor TB treatment results, particularly therapy failures and premature deaths. Tobacco use raises the risk of pulmonary tuberculosis infection, advancement, and premature death, and is linked to multi-drug resistant TB. (Bates et al., 2007; Chang et al., 2004; Jeyashree et al., 2016; Zvolska et al., 2020) Raising awareness of the health risks of smoking in TB patients and health providers, as well as routinely offering tobacco-dependence remedy in defined TB treatment systems in tobacco-free healthcare facilities, may contribute greatly to boosting the performance of abstinence in TB patients (Zvolska et al., 2020). From a mathematical model, if current smoking trends continue, tobacco smoking will be accountable for 18 million and 40 million TB cases and deaths respectively over the next 40 years (Basu et al., 2011). Cigarette smoking was linked to a reduced likelihood of treatment success in a Ugandan study (Baluku, Namiiro, et al., 2021). In addition, a Turkish study found that smoking had significant independent effects on TB patients' treatment adherence (Annakkaya et al., 2005).

2.2.3. HIV /AIDS

Considering TB therapy is prolonged and encompasses an amount of drugs, adverse effects are so rampant, patients feeling well prior to medication completion, adherence to it is particularly difficulty (M McLean, 2003). In order to treat patients with tuberculosis, they must stick to a regimen of at least 90% of the time (Awofeso, 2008). The additional burden of taking HIV-drugs, as well as probable drug interactions with undesirable consequences, may harm the patients' TB therapeutic outcomes (Ncube et al., 2017) . Tuberculosis and HIV diagnosis have also been related to stigma, which cause negative consequences for TB treatment outcomes (Mohammedhussein et al., 2020) . HIV has been attributed to poor TB treatment outcomes in several prior studies (Ali et al., 2016; Engelbrecht et al., 2017; Katana et al., 2022). The gradual drop in incidence rate in Uganda is attributed to an increase in new TB cases among HIV-uninfected people, despite a decrease in HIV co-infected TB cases (Cha et al., 2020). Treatment success rates for TB patients with and without HIV differ significantly across the state (Berger et al., 2020).

2.2.4. Alcohol

Alcohol is associated with a greater prevalence of HIV, tuberculosis, and pneumonia, as well as worse treatment outcomes, through behavioural and molecular mechanisms. According to preliminary research, heavy alcohol drinkers and people with alcoholic disorders are more likely to contract COVID-19 and develop serious diseases (Morojele et al., 2021). In Ugandan TB cases, alcohol consumption was linked to non-adherence (Amuha et al., 2009).

2.2.5. Health seeking habits

Analysing the health-care seeking tendencies of TB patients enables the National tuberculosis program boost its management, which as a result assist patients to obtain adequate treatment sooner (Ehsanul Huq et al., 2018). As shown in a Ugandan study, TB patients are reported to seek treatment late or avoid it altogether. It was also customary to combine therapies from both herbalists and the biomedical services (Buregyeya et al., 2011).

2.3. Internal Social Factors

2.3.1. Social support

Previous research found that patients with support from friends and family were more motivated to swallow their medication on a regular basis; additionally, economical and nourishment support also importantly ranked among the factors specifically mentioned (Michael et al., 2004; Mindachew et al., 2014; Tadele Eticha and Eden Kassa, 2014; Tekle B, 2002) , and family support was a safeguard for DOTs (Tekle B, 2002). In Uganda, however, VDOT was aided by a lack of stigma from friends and family (Sekandi et al., 2020) . Psychosocial support has been found to be the most important component associated with self-reported adherence practices in a more recent study (Zhang et al., 2022). Behavioural patterns were improved in patients who received psychological support from their families. According to studies, psychological support from trusted members, such as family or friends, can help people believe in the cure for tuberculosis.(Michael et al., 2004; Mindachew et al., 2014).

2.3.2. Stigmatization

Stigma is a significant influence of health that describes how TB patients perceive, anticipate, and/or experience negative societal judgment (Craig et al., 2017). Several victims who were concerned about the prognosis of tuberculosis faced stigma and discrimination from others (Gugssa Boru et al., 2017; Mesfin et al., 2009). Many patients who were concerned about the curability of tuberculosis faced stigma and discrimination from others. HIV has been linked to poor TB treatment outcomes in a series of consecutive studies (Ali et al., 2016; Engelbrecht et al., 2017). TB and HIV diagnosis have also been linked to stigma, which may have negative consequences for TB therapeutic efficacy (Mohammedhussein et al., 2020). As a result, it is critical for family members to support and encourage patients to recognize TB, as well as to assist patients in sticking to TB therapy.

2.4. External Social Factors

2.4.1. Health insurance

Patients with MDR-TB, in particular, face significant costs for diagnosis and therapies, which may be minimized by a well-designed and executed public health insurance framework (Kundu et al., 2015). More focus should be placed on novel patient support mechanisms, which also include, among other things, the prevention of devastating health expenditures due to resistant TB through health protection mechanisms (Kundu et al., 2018). Enhancing health insurance coverage and boosting payment rates for TB outpatient care appear to be major drivers in lowering total costs and financial burdens for patients (Mao et al., 2019) thus TB prevention and control are improved.

2.4.2. Social protection

Monetary transfer programs, insurance, for the unemployed, education and transportation incentives have all been found to lower the prevalence of TB and promote adherence to therapies, efficacy, and recovery (de Andrade et al., 2018; Oliosi et al., 2019). Furthermore, monetary rewards are more effective as well as preferred by TB patients over non-monetary bonuses (Lutge et al., 2015; W M Jakubowiak, 2007). According to reports, the amount of conditional monetary incentives utilized in trials to boost TB outcomes ranged from 193 to 858 US dollars (Richterman et al., 2018). However, without external support, these conditional monetary incentives

are too pricey for low-income countries to implement into routine programme TB care, despite the fact that they are extremely effective in improving TB treatment results in the least resourced populations, such as Uganda. A one-dollar incentive, for instance, was linked to a better rate of TB cure and in a study conducted in rural Uganda, there was less destruction among TB patients (Baluku et al., 2021).

2.4.3. Health system

Numerous researchers have found that the health system has an important impact on tuberculosis prevention and control because it affects the attitude of TB patients, case management and satisfaction with healthcare services. (Alene et al., 2020; Gugssa Boru et al., 2017; Mesfin et al., 2009; Nezenega et al., 2013) . The time it takes to diagnose TB and start treatment is still too long, especially in high-burden nations. (Finnie et al., 2011; Yimer et al., 2005) . Patient, health-care system, and diagnosis delays are all examples of delays. (Getnet et al., 2017; Mercaldo et al., 2021; Yimer et al., 2005). However, the health system in Uganda is to blame for the majority of the delays (Buregyeya et al., 2014). Delays in diagnosis can result in the progress of the disease, overall mortality, and the spread of community TB. (Uys et al., 2007). As a consequence, earlier detections of tuberculosis are critical for TB control. According to Ugandan and Tanzanian studies, average diagnosis delays vary from eight to thirteen weeks. (Kiwuwa et al., 2005; Sendagire et al., 2010).

2.4.4. Natural disasters such covid-19

As per Quality of TB Services Assessment Report-Uganda, 2020, availability to laboratory services and crucial TB-related services was considerable at most categories of health facilities in Uganda, with nearly all facilities providing screening, diagnosis, and treatment programs.(Kola Oyediran, 2020). But nevertheless, due to COVID-19, there's been a decrease in access to healthcare services worldwide, which has contributed in setbacks in TB assessment and treatment, contributing to the rise in TB deaths. Recent projections about HIV-negative TB deaths are; 1.3 million and 1.2 million for 2020 and 2019 respectively and for HIV-positive TB deaths are; 214 000 and 209 000 for 2020 and 2019 respectively(World Health Organization(b), 2021).

CONCLUSION

United States and Turkey, high and middle high developed countries respectively, are much more effective when it comes to tuberculosis control and prevention. Furthermore, countries that obey the World Health Organization's strategic recommendations are doing well in this fight. In order for Uganda to reach her target of eliminating TB by 85%, she has to strive at least to:

- ✓ Enhance community systems with a focus on reaching vulnerable populations
- ✓ Expand tuberculosis prevention and treatment through strengthening private-public teamwork;
- ✓ Enhance diagnostic and treatment services
- ✓ Improve information management, including digital technologies
- ✓ Intensify TB management, accountability, cross-sector collaborative effort, and resource mobilization.

REFERENCES

Abebe, M., Doherty, M., Wassie, L., Demissie, A., Mihret, A., Engers, H., & Aseffa, A. (2012). TB case detection: can we remain passive while the process is active? *The Pan African Medical Journal*, *11*, 50. /pmc/articles/PMC3343678/

Alene, M., Assemie, M. A., Yismaw, L., Gedif, G., Ketema, D. B., Gietaneh, W., & Chekol, T. D. (2020). Patient delay in the diagnosis of tuberculosis in Ethiopia: a systematic review and meta-analysis. *BMC Infectious Diseases*, *20*(1). https://doi.org/10.1186/s12879-020-05524-3

Ali, S. A., Mavundla, T. R., Fantu, R., & Awoke, T. (2016). Outcomes of TB treatment in HIV co-infected TB patients in Ethiopia: a cross-sectional analytic study. *BMC Infectious Diseases*, *16*(1). https://doi.org/10.1186/S12879-016-1967-3

Amuha, M. G., Kutyabami, P., Kitutu, F. E., Odoi-Adome, R., & Kalyango, J. N. (2009). Non-adherence to anti-TB drugs among TB/HIV co-infected patients in Mbarara Hospital Uganda: Prevalence and associated factors. *African Health Sciences*, *9*(Suppl 1), S8. /pmc/articles/PMC2890989/

Annakkaya, A. N., Arbak, P., & Bilgin, C. (2005). *Respiratory symptoms in adults living in pesticides applied fields View project A NEW SYSTEM FOR DIAGNOSING OBSTRUCTIVE SLEEP APNEA SYNDROME BY AUTOMATIC SLEEP STAGING USING PHOTOPLETHYSMOGRAPHY (PPG) SIGNALS AND BREATHING SCORING View project*. https://www.researchgate.net/publication/7770588

Awofeso, N. (2008). Anti-tuberculosis medication side-effects constitute major factor for poor adherence to tuberculosis treatment. *Bulletin of the World Health Organization*, *86*(3), B. https://doi.org/10.2471/BLT.07.043802

Baluku, J. B., Nakazibwe, B., Twinomugisha, B., Najjuuko, R., Isabella, N., Nassozi, S., Namiiro, S., Katagira, W., Byonanebye, D. M., Sekaggya-Wiltshire, C., Muchiri, J., Ndungu, E., Anguzu, G., Mayanja-Kizza, H., & Andia-Biraro, I. (2021). One dollar incentive improves tuberculosis treatment outcomes in programmatic settings in rural Uganda. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-98770-7

Bannon, M. J. (1999). BCG and tuberculosis. *Archives of Disease in Childhood*, *80*(1), 80–83. https://doi.org/10.1136/ADC.80.1.80

Basu, S., Stuckler, D., Bitton, A., & Glantz, S. A. (2011). Projected effects

of tobacco smoking on worldwide tuberculosis control: mathematical modelling analysis. *BMJ*, *343*(7826). https://doi.org/10.1136/BMJ.D5506

Bates, M. N., Khalakdina, A., Pai, M., Chang, L., Lessa, F., & Smith, K. R. (2007). Risk of tuberculosis from exposure to tobacco smoke: a systematic review and meta-analysis. *Archives of Internal Medicine*, *167*(4), 335–342. https://doi.org/10.1001/ARCHINTE.167.4.335

Bell, D., Hansen, K. S., Kiragga, A. N., Kambugu, A., Kissa, J., & Mbonye, A. K. (2020). Predicting the impact of COVID-19 and the potential impact of the public health response on disease burden in Uganda. *American Journal of Tropical Medicine and Hygiene*, *103*(3), 1191–1197. https://doi.org/10.4269/ajtmh.20-0546

Berger, C. A., Kityamuwesi, A., Crowder, R., Lamunu, M., Kunihira Tinka, L., Ggita, J., Sanyu Nakate, A., Namale, C., Oyuku, D., Chen, K., Turyahabwe, S., Cattamanchi, A., & Katamba, A. (2020). Variation in tuberculosis treatment outcomes and treatment supervision practices in Uganda. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, *21*, 100184. https://doi.org/10.1016/J.JCTUBE.2020.100184

Buregyeya, E., Criel, B., Nuwaha, F., & Colebunders, R. (2014). Delays in diagnosis and treatment of pulmonary tuberculosis in Wakiso and Mukono districts, Uganda. *BMC Public Health*, *14*(1), 586. https://doi.org/10.1186/1471-2458-14-586

Buregyeya, E., Kulane, A., Colebunders, R., Wajja, A., Kiguli, J., Mayanja, H., Musoke, P., Pariyo, G., & Mitchell, E. M. H. (2011). Tuberculosis knowledge, attitudes and health-seeking behaviour in rural Uganda. *The International Journal of Tuberculosis and Lung Disease : The Official Journal of the International Union against Tuberculosis and Lung Disease*, *15*(7), 938–942. https://doi.org/10.5588/IJTLD.10.0211

Cha, J., Thwaites, G. E., & Ashton, P. M. (2020). An Evaluation of the 2035 WHO End TB Targets in 40 High Burden Countries. *MedRxiv*, 2020.10.02.20175307. https://doi.org/10.1101/2020.10.02.20175307

Chamie, G., Kwarisiima, D., Clark, T. D., Kabami, J., Jain, V., Geng, E., Balzer, L. B., Petersen, M. L., Thirumurthy, H., Charlebois, E. D., Kamya, M. R., & Havlir, D. V. (2014). Uptake of community-based HIV testing during a multi-disease health campaign in rural Uganda. *PloS One*, *9*(1). https://doi.org/10.1371/JOURNAL.PONE.0084317

Chamie, G., Kwarisiima, D., Clark, T. D., Kabami, J., Jain, V., Geng, E., Petersen, M. L., Thirumurthy, H., Kamya, M. R., Havlir, D. V., & Charlebois, E. D. (2012). Leveraging Rapid Community-Based HIV Testing Campaigns for Non-Communicable Diseases in Rural

Uganda. *PLoS ONE*, *7*(8). https://doi.org/10.1371/JOURNAL.PONE.0043400

Chang, K. C., Leung, C. C., & Tam, C. M. (2004). Risk factors for defaulting from anti-tuberculosis treatment under directly observed treatment in Hong Kong. *International Journal of Tuberculosis and Lung Disease*, *8*(12), 1492–1498.

Cook, R., Lamont, T., & Martin, R. (2019). Smartphones can improve adherence rates for TB treatment. *BMJ (Clinical Research Ed.)*, *366*. https://doi.org/10.1136/BMJ.L4920

Craig, G. M., Daftary, A., Engel, N., O'Driscoll, S., & Ioannaki, A. (2017). Tuberculosis stigma as a social determinant of health: a systematic mapping review of research in low incidence countries. *International Journal of Infectious Diseases : IJID : Official Publication of the International Society for Infectious Diseases*, *56*, 90–100. https://doi.org/10.1016/J.IJID.2016.10.011

Davis, J. L., Turimumahoro, P., Meyer, A. J., Ayakaka, I., Ochom, E., Ggita, J., Mark, D., Babirye, D., Okello, D. A., Mugabe, F., Fair, E., Vittinghoff, E., Armstrong-Hough, M., Dowdy, D., Cattamanchi, A., Haberer, J. E., & Katamba, A. (2019). Home-based tuberculosis contact investigation in Uganda: a household randomised trial. *ERJ Open Research*, *5*(3). https://doi.org/10.1183/23120541.00112-2019

de Andrade, K. V. F., Nery, J. S., de Souza, R. A., & Pereira, S. M. (2018). Effects of social protection on tuberculosis treatment outcomes in low or middle-income and in high-burden countries: systematic review and meta-analysis. *Cadernos de Saude Publica*, *34*(1). https://doi.org/10.1590/0102-311X00153116

Demissie, M., & Kebede, D. (1994). Defaulting from tuberculosis treatment at the Addis Abeba tuberculosis centre and factors associated with it. *Ethiopian Medical Journal*, *32*(2), 97–106.

Ducarme, T. (2020). What will be the impact of the Covid-19 pandemic on healthcare systems? *Deloitte*, *June*, 1–3. https://www.mentalhealth.org.uk/statistics/%0Ahttps://www2.deloitte.com/fr/fr/pages/covid-insights/articles/impact-covid19-healthcare-systems.html

Edginton M E, S. C. S. G. S. J. (2002). *Patients' beliefs: do they affect tuberculosis control? A study in a rural district of South Africa - PubMed*. https://pubmed.ncbi.nlm.nih.gov/12546115/

Ehsanul Huq, K. A. T. M., Moriyama, M., Zaman, K., Chisti, M. J., Long, J., Islam, A., Hossain, S., Shirin, H., Raihan, M. J., Chowdhury, S., & Rahman, M. M. (2018). Health seeking behaviour and delayed management of tuberculosis patients in rural Bangladesh. *BMC Infectious Diseases*, *18*(1). https://doi.org/10.1186/S12879-018-

3430-0
Engelbrecht, M. C., Kigozi, N. G., Chikobvu, P., Botha, S., & Van Rensburg, H. C. J. (2017). Unsuccessful TB treatment outcomes with a focus on HIV co-infected cases: a cross-sectional retrospective record review in a high-burdened province of South Africa. *BMC Health Services Research*, *17*(1). https://doi.org/10.1186/S12913-017-2406-X
Fekadu, G., Bekele, F., Bekele, K., Girma, T., Mosisa, G., Gebre, M., Alemu, T., Tekle, T., Gamachu, B., & Diriba, A. (2020). Adherence to Anti-Tuberculosis Treatment Among Pediatric Patients at Nekemte Specialized Hospital, Western Ethiopia. *Patient Preference and Adherence*, *14*, 1259–1265. https://doi.org/10.2147/PPA.S258292
Finnie, R. K. C., Khoza, L. B., van den Borne, B., Mabunda, T., Abotchie, P., & Mullen, P. D. (2011). Factors associated with patient and health care system delay in diagnosis and treatment for TB in sub-Saharan African countries with high burdens of TB and HIV. *Tropical Medicine & International Health : TM & IH*, *16*(4), 394–411. https://doi.org/10.1111/J.1365-3156.2010.02718.X
Fox, G. J., Barry, S. E., Britton, W. J., & Marks, G. B. (2013). Contact investigation for tuberculosis: a systematic review and meta-analysis. *The European Respiratory Journal*, *41*(1), 140. https://doi.org/10.1183/09031936.00070812
Friedena, T. R., & Sbarbarob, J. A. (2007). Promoting adherence to treatment for tuberculosis: the importance of direct observation. *Bulletin of the World Health Organization*, *85*(5), 407–409. https://doi.org/10.2471/blt.06.038927
Garfein, R. S., Collins, K., Muñoz, F., Moser, K., Cerecer-Callu, P., Raab, F., Rios, P., Flick, A., Zúñiga, M. L., Cuevas-Mota, J., Liang, K., Rangel, G., Burgos, J. L., Rodwell, T. C., & Patrick, K. (2015). Feasibility of tuberculosis treatment monitoring by video directly observed therapy: a binational pilot study. *Int J Tuberc Lung Dis*, *19*(9), 1057–1064. https://doi.org/10.5588/ijtld.14.0923
Gebremariam, M. K., Bjune, G. A., & Frich, J. C. (2010). Barriers and facilitators of adherence to TB treatment in patients on concomitant TB and HIV treatment: a qualitative study. *BMC Public Health*, *10*. https://doi.org/10.1186/1471-2458-10-651
Getnet, F., Demissie, M., Assefa, N., Mengistie, B., & Worku, A. (2017). Delay in diagnosis of pulmonary tuberculosis in low-and middle-income settings: systematic review and meta-analysis. *BMC Pulmonary Medicine*, *17*(1). https://doi.org/10.1186/S12890-017-0551-Y

Gugssa Boru, C., Shimels, T., & Bilal, A. I. (2017). Factors contributing to non-adherence with treatment among TB patients in Sodo Woreda, Gurage Zone, Southern Ethiopia: A qualitative study. *Journal of Infection and Public Health*, *10*(5), 527–533. https://doi.org/10.1016/J.JIPH.2016.11.018

Haberer, J. E., Musiimenta, A., Atukunda, E. C., Musinguzi, N., Wyatt, M. A., Ware, N. C., & Bangsberg, D. R. (2016). Short message service (SMS) reminders and real-time adherence monitoring improve antiretroviral therapy adherence in rural Uganda. *AIDS (London, England)*, *30*(8), 1295–1299. https://doi.org/10.1097/QAD.0000000000001021

Helen McShane. (2009). *Vaccine strategies against tuberculosis - PubMed*. https://pubmed.ncbi.nlm.nih.gov/19152149/

Hwang, T. J., Ottmani, S., & Uplekar, M. (2011). A rapid assessment of prevailing policies on tuberculosis contact investigation. *International Journal of Tuberculosis and Lung Disease*, *15*(12), 1620–1622. https://doi.org/10.5588/IJTLD.11.0222

Jeyashree, K., Kathirvel, S., Shewade, H. D., Kaur, H., & Goel, S. (2016). Smoking cessation interventions for pulmonary tuberculosis treatment outcomes. *Cochrane Database of Systematic Reviews*, *2016*(1). https://doi.org/10.1002/14651858.CD011125.PUB2

Kankya, C. M. A. (ed.). M. A. (2011). *Myths, perceptions knowledge, attitudes, and practices (KAP) linked to mycobacterial infection management among the pastoralist communities of Uganda*. https://nru.uncst.go.ug/handle/123456789/1919

Karumbi, J., & Garner, P. (2015). Directly observed therapy for treating tuberculosis. *The Cochrane Database of Systematic Reviews*, *2015*(5). https://doi.org/10.1002/14651858.CD003343.PUB4

Katana, G. G., Ngari, M., Maina, T., Sanga, D., & Abdullahi, O. A. (2022). Tuberculosis poor treatment outcomes and its determinants in Kilifi County, Kenya: a retrospective cohort study from 2012 to 2019. *Archives of Public Health*, *80*(1), 48. https://doi.org/10.1186/S13690-022-00807-4

Kazibwe, A., Twinomugisha, F., Musaazi, J., Nakaggwa, F., Lukanga, D., Aleu, P., Kiyemba, T., Nkolo, A., Kirirabwa, N. S., Freitas Lopez, D. B., Birabwa, E., Dejene, S., & Zawedde-Muyanja, S. (2021). Comparative yield of different active TB case finding interventions in a large urban TB project in central Uganda: a descriptive study. *African Health Sciences*, *21*(3), 975. https://doi.org/10.4314/AHS.V21I3.3

Kiwuwa, M. S., Charles, K., & Harriet, M. K. (2005). Patient and health service delay in pulmonary tuberculosis patients attending a referral

hospital: a cross-sectional study. *BMC Public Health*, *5*, 122. https://doi.org/10.1186/1471-2458-5-122

Kola Oyediran, B. K. S. T. N. D. J. C. W. M. A. M. H. M. (2020). *Quality of Tuberculosis Services Assessment in Uganda: Report — MEASURE Evaluation*. https://www.measureevaluation.org/resources/publications/tr-20-398.html

Kundu, D., Katre, V., Singh, K., Deshpande, M., Nayak, P., Khaparde, K., Moitra, A., Nair, S., & Parmar, M. (2015). Innovative social protection mechanism for alleviating catastrophic expenses on multidrug-resistant tuberculosis patients in Chhattisgarh, India. *WHO South-East Asia Journal of Public Health*, *4*(1), 69. https://doi.org/10.4103/2224-3151.206624

Kundu, D., Sharma, N., Chadha, S., Laokri, S., Awungafac, G., Jiang, L., & Asaria, M. (2018). Analysis of multi drug resistant tuberculosis (MDR-TB) financial protection policy: MDR-TB health insurance schemes, in Chhattisgarh state, India. *Health Economics Review*, *8*(1), 3. https://doi.org/10.1186/s13561-018-0187-5

Li, Y., Ehiri, J., Tang, S., Li, D., Bian, Y., Lin, H., Marshall, C., & Cao, J. (2013). Factors associated with patient, and diagnostic delays in Chinese TB patients: a systematic review and meta-analysis. *BMC Medicine*, *11*(1). https://doi.org/10.1186/1741-7015-11-156

Lutge, E. E., Wiysonge, C. S., Knight, S. E., Sinclair, D., & Volmink, J. (2015). Incentives and enablers to improve adherence in tuberculosis. *The Cochrane Database of Systematic Reviews*, *2015*(9). https://doi.org/10.1002/14651858.CD007952.PUB3

M McLean. (2003). *Guidelines for TB control in New Zealand .Wellington , Ministry of Health 2003.* https://scholar.google.com/scholar_lookup?title=Guidelines+for+TB+control+in+New+Zealand&author=M+McLean&publication_year=2003&

Mao, W., Jiang, W., Hamilton, C., Zhang, H., Huang, F., Lucas, H., Huan, S., & Tang, S. (2019). Over- and under-treatment of TB patients in Eastern China: an analysis based on health insurance claims data. *Tropical Medicine & International Health*, *24*(9), 1078. https://doi.org/10.1111/TMI.13287

Mercaldo, R., Whalen, C., Kakaire, R., Nakkonde, D., Handel, A., & Sekandi, J. N. (2021). Community drivers of tuberculosis diagnostic delay in Kampala, Uganda: a retrospective cohort study. *BMC Infectious Diseases*, *21*(1). https://doi.org/10.1186/s12879-021-06352-9

Mesfin, M. M., Newell, J. N., Walley, J. D., Gessessew, A., Tesfaye, T.,

Lemma, F., & Madeley, R. J. (2009). Quality of tuberculosis care and its association with patient adherence to treatment in eight Ethiopian districts. *Health Policy and Planning*, *24*(6), 457–466. https://doi.org/10.1093/HEAPOL/CZP030

Michael Iseman. (2013). *Types of Tuberculosis (TB);National Jewish Health*. https://www.nationaljewish.org/conditions/tuberculosis-tb/types

Michael, K. W., Belachew, T., & Jira, C. (2004). Tuberculosis defaulters from the "DOTS" regimen in Jimma zone, southwest Ethiopia. *Ethiopian Medical Journal*, *42*(4), 247–253.

Mindachew, M., Deribew, A., Memiah, P., & Biadgilign, S. (2014). Perceived barriers to the implementation of Isoniazid preventive therapy for people living with HIV in resource constrained settings: a qualitative study. *The Pan African Medical Journal*, *17*. https://doi.org/10.11604/PAMJ.2014.17.26.2641

Mirsaeidi, M., Farshidpour, M., Banks-Tripp, D., Hashmi, S., Kujoth, C., & Schraufnagel, D. (2015). Video directly observed therapy for treatment of tuberculosis is patient-oriented and cost-effective. *Eur Respir J*, *46*(3), 871–874. https://doi.org/10.1183/09031936.00011015

Mohammedhussein, M., Hajure, M., Shifa, J. E., & Hassen, T. A. (2020). Perceived stigma among patient with pulmonary tuberculosis at public health facilities in southwest Ethiopia: A cross-sectional study. *PloS One*, *15*(12). https://doi.org/10.1371/JOURNAL.PONE.0243433

Morojele, N. K., Shenoi, S. V., Shuper, P. A., Braithwaite, R. S., & Rehm, J. (2021). Alcohol Use and the Risk of Communicable Diseases. *Nutrients*, *13*(10). https://doi.org/10.3390/NU13103317

Murray CJL, S. J. (1998). *Expanding the WHO tuberculosis control strategy: rethinking the role of active case-finding - PubMed*. https://pubmed.ncbi.nlm.nih.gov/9755959/

Muture, B. N., Keraka, M. N., Kimuu, P. K., Kabiru, E. W., Ombeka, V. O., & Oguya, F. (2011). Factors associated with default from treatment among tuberculosis patients in nairobi province, Kenya: A case control study. *BMC Public Health*, *11*. https://doi.org/10.1186/1471-2458-11-696

N Shetty, M. S. A. A. (2004). *Knowledge, attitudes and practices regarding tuberculosis among immigrants of Somalian ethnic origin in London: a cross-sectional study - PubMed*. https://pubmed.ncbi.nlm.nih.gov/15137287/

Nankabirwa V, Tumwine JK, Mugaba PM, Tylleskar T, Sommerfelt H, G. P.-E. (2015). Child survival and BCG vaccination:a community

based prospective cohort study in Uganda. BMC Public Health. 2015;15:175. *PubMed*.

Ncube, R. T., Takarinda, K. C., Zishiri, C., van den Boogaard, W., Mlilo, N., Chiteve, C., Siziba, N., Trinchán, F., & Sandy, C. (2017). Age-stratified tuberculosis treatment outcomes in Zimbabwe: are we paying attention to the most vulnerable? *Public Health Action*, *7*(3), 212–217. https://doi.org/10.5588/PHA.17.0024

Nezenega, Z. S., Gacho, Y. H. M., & Tafere, T. E. (2013). Patient satisfaction on tuberculosis treatment service and adherence to treatment in public health facilities of Sidama zone, South Ethiopia. *BMC Health Services Research*, *13*(1). https://doi.org/10.1186/1472-6963-13-110

Oliosi, J. G. N., Reis-Santos, B., Locatelli, R. L., Sales, C. M. M., da Silva Filho, W. G., da Silva, K. C., Sanchez, M. N., de Andrade, K. V. F., de Araújo, G. S., Shete, P. B., Pereira, S. M., Riley, L. W., Lienhardt, C., & Maciel, E. L. N. (2019). Effect of the Bolsa Familia Programme on the outcome of tuberculosis treatment: a prospective cohort study. *The Lancet. Global Health*, *7*(2), e219–e226. https://doi.org/10.1016/S2214-109X(18)30478-9

Oliveira, S. P. de, Carvalho, M. D. de B., Pelloso, S. M., Caleffi-Ferracioli, K. R., Siqueira, V. L. D., Scodro, R. B. de L., & Cardoso, R. F. (2017). Influence of the identification of contacts on the adherence of index tuberculosis cases to treatment in a high incidence country. *International Journal of Infectious Diseases*, *65*, 57–62. https://doi.org/10.1016/J.IJID.2017.09.005

Oshi, D. C., Oshi, S. N., Alobu, I., & Ukwaja, K. N. (2014). Profile and treatment outcomes of tuberculosis in the elderly in southeastern Nigeria, 2011-2012. *PloS One*, *9*(11). https://doi.org/10.1371/JOURNAL.PONE.0111910

P Godfrey-Faussett, H. K. J. K. S. van B. M. van C. R. K.-P. V. T. (2002). *Why do patients with a cough delay seeking care at Lusaka urban health centres? A health systems research approach - PubMed*. https://pubmed.ncbi.nlm.nih.gov/12234135/

Richterman, A., Steer-Massaro, J., Jarolimova, J., Nguyen, L. B. L., Werdenberg, J., & Ivers, L. C. (2018). Cash interventions to improve clinical outcomes for pulmonary tuberculosis: systematic review and meta-analysis. *Bulletin of the World Health Organization*, *96*(7), 471–483. https://doi.org/10.2471/BLT.18.208959

Sekandi, J. N., Buregyeya, E., Zalwango, S., Dobbin, K. K., Atuyambe, L., Nakkonde, D., Turinawe, J., Tucker, E. G., Olowookere, S., Turyahabwe, S., & Garfein, R. S. (2020). Video directly observed therapy for supporting and monitoring adherence to tuberculosis

treatment in Uganda: a pilot cohort study. *ERJ Open Research*, *6*(1). https://doi.org/10.1183/23120541.00175-2019

Sendagire, I., Van der Loeff, M. S., Mubiru, M., Konde-Lule, J., & Cobelens, F. (2010). Long Delays and Missed Opportunities in Diagnosing Smear-Positive Pulmonary Tuberculosis in Kampala, Uganda: A Cross-Sectional Study. *PLoS ONE*, *5*(12). https://doi.org/10.1371/JOURNAL.PONE.0014459

Sinkou, H., Hurevich, H., Rusovich, V., Zhylevich, L., Falzon, D., De Colombani, P., Dadu, A., Dara, M., Story, A., & Skrahina, A. (2017). Video-observed treatment for tuberculosis patients in Belarus: findings from the first programmatic experience. *European Respiratory Journal*, *49*(3), 1602049–1602049. https://doi.org/10.1183/13993003.02049-2016

Ssemmondo, E., Mwangwa, F., Kironde, J. L., Kwarisiima, D., Clark, T. D., Marquez, C., Charlebois, E. D., Petersen, M. L., Kamya, M. R., Havlir, D. V., & Chamie, G. (2016). Population-based active TB case finding during large-scale mobile HIV testing campaigns in rural Uganda. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *73*(3), e46. https://doi.org/10.1097/QAI.0000000000001142

Story, A., Garfein, R. S., Hayward, A., Rusovich, V., Dadu, A., Soltan, V., Oprunenco, A., Collins, K., Sarin, R., Quraishi, S., Sharma, M., Migliori, G. B., Varadarajan, M., & Falzon, D. (2016). Monitoring therapy compliance of tuberculosis patients by using video-enabled electronic devices. *Emerging Infect Dis*, *22*(3), 538–540. https://doi.org/10.3201/eid2203.151620

Suthar, A. B., Ford, N., Bachanas, P. J., Wong, V. J., Rajan, J. S., Saltzman, A. K., Ajose, O., Fakoya, A. O., Granich, R. M., Negussie, E. K., & Baggaley, R. C. (2013). Towards universal voluntary HIV testing and counselling: a systematic review and meta-analysis of community-based approaches. *PLoS Medicine*, *10*(8). https://doi.org/10.1371/JOURNAL.PMED.1001496

Tadele Eticha and Eden Kassa. (2014). *Non-Adherence to Anti-TB Drugs and Its Predictors among TB/HIV Co- Infected Patients in Mekelle, Ethiopia*. https://doi.org/10.4172/1948-593X.1000113

Tekle B, M. D. A. A. (2002). *Defaulting from DOTS and its determinants in three districts of Arsi Zone in Ethiopia - PubMed*. https://pubmed.ncbi.nlm.nih.gov/12102295/

UNAIDS. (2017). *90–90–90 - An ambitious treatment target to help end the AIDS epidemic*. https://www.unaids.org/en/resources/documents/2017/90-90-90

Uys, P. W., Warren, R. M., & van Helden, P. D. (2007). A Threshold Value for the Time Delay to TB Diagnosis. *PLoS ONE*, *2*(8).

https://doi.org/10.1371/JOURNAL.PONE.0000757
Volmink, J., & Garner, P. (2007). Directly observed therapy for treating tuberculosis. *Cochrane Database of Systematic Reviews*, *4*. https://doi.org/10.1002/14651858.CD003343.pub3
W M Jakubowiak, E. M. B. S. E. B. I. D. D. O. B. L. E. V. K. (2007). *Social support and incentives programme for patients with tuberculosis: experience from the Russian Federation - PubMed*. https://pubmed.ncbi.nlm.nih.gov/17958983/
WHO global lists. (2021). *WHO global lists of high burden countries for tuberculosis (TB), TB/HIV and multidrug/rifampicin-resistant TB (MDR/RR-TB), 2021-2025 Background document*. http://apps.who.int/bookorders.
WHO report. (2003). *Global tuberculosis control*. https://apps.who.int/iris/handle/10665/63835
WHO Report. (2006). *Global Tuberculosis Control: Surveillance, Planning, Financing : WHO Report 2006 - World Health Organization - Google Books*. https://books.google.com.tr/books?id=CDyzDwAAQBAJ&pg=PR2&lpg=PR2&dq=World+Health+Organization.+Global+tuberculosis+control:+surveillance,+planning,+financing.+WHO/HTM/TB/2006.362.+Geneva,+Switzerland:+WHO,+2006.&source=bl&ots=-pwm9cvjOE&sig=ACfU3U3Cb2MYROg8BO80druQjNDxbsBpwQ&hl=en&sa=X&ved=2ahUKEwias6in-vX2AhWdSPEDHa7yA6cQ6AF6BAgCEAM#v=onepage&q=World Health Organization. Global tuberculosis control%3A surveillance%2Cplanning%2Cfinancing.WHO%2FHTM%2FTB%2F2006.362. Geneva%2C Switzerland%3A WHO%2C 2006.&f=false
World Health Organization(a). (2021). *Tuberculosis*. https://www.who.int/news-room/fact-sheets/detail/tuberculosis
World Health Organization(b). (2021). *Global Tuberculosis Programme*. https://www.who.int/teams/global-tuberculosis-programme/tb-reports
World Health Organization(c). (2014). *Implementing the End TB Strategy*. https://www.who.int/westernpacific/activities/implementing-the-end-tb-strategy
World Health Organization. (2010). *The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis*. https://apps.who.int/iris/handle/10665/44437?locale-attribute=es&
World Health Organization. (2012). Recommendations for investigating contacts of persons with infectious tuberculosis in low- and middle-income countries. *World Health Organization*, 28–41.
World Health Organization. (2013). *Systematic screening for active*

tuberculosis: an operational guide.

Yasin Mohammed. (2014). *Treatment adherence among tuberculosis... - Google Scholar*. https://scholar.google.com/scholar_lookup?journal=Am+J+Public+Health+Res&title=Treatment+adherence+among+tuberculosis+and+human+immuno+defificiency+virus+coinfected+patients+in+ginnir+referral+hospital&author=A+Yasin+Mohammed&author=M+Kaso+Adem&volume=2&publication_year=2014&pages=239-243&

Yimer, S., Bjune, G., & Alene, G. (2005). Diagnostic and treatment delay among pulmonary tuberculosis patients in Ethiopia: a cross sectional study. *BMC Infectious Diseases*, *5*, 112. https://doi.org/10.1186/1471-2334-5-112

Zhang, R., Pu, J., Zhou, J., Wang, Q., Zhang, T., Liu, S., Wang, G., Chen, Y., Liu, J., Hu, D., & Li, Y. (2022). Factors predicting self-report adherence (SRA) behaviours among DS-TB patients under the "Integrated model": a survey in Southwest China. *BMC Infectious Diseases*, *22*(1), 201. https://doi.org/10.1186/S12879-022-07208-6

Zvolska, K., Pankova, A., Nohavova, I., Huque, R., Elsey, H., Boeckmann, M., Sheikh, A., Siddiqi, K., & Kralikova, E. (2020). A narrative review of facilitators and barriers to smoking cessation and tobacco-dependence treatment in patients with tuberculosis in low- and middle-income countries. *Tobacco Induced Diseases*, *18*. https://doi.org/10.18332/TID/125195

CHAPTER 7

THE VARIANT OF CONCERN: OMICRON

Ayse CETIN[1], MD. Serkan ALTUNTAS[2], MD.

[1] MD, Altinbas University, Bahcelievler Medical Park Hospital, Department of Emergency Medicine, e-mail: mdcetin.ayse@gmail.com **ORCID**: 0000-0002-2754-7175
[2] MD, Altinbas University, Bahcelievler Medical Park Hospital, Department of Emergency Medicine, e-mail: dr.serkanaltuntas@hotmail.com **ORCID**: 0000-0002-1352-0035

INTRODUCTION

Since it was first reported by South Africa, a novel variant, the Omicron has raised great concerns and unknowns among people and authorities all over the world with expected new waves and surges of the disease. In a short time, this new variant was declared as the "Variant of Concern" by the WHO. In the beginning, it showed a high number of newly diagnosed cases with a high transmission rate and increased hospitalization. This has raised the question of how limited health care resources will respond to this surge. However, as the scientific evidence accumulated, it has been reported that although transmissibility of this new variant is greater than the previous variants, the disease has a milder course in people infected with the Omicron. Today, the Omicron is the predominant variant in almost all countries in the world. While some countries began to lift the restrictions and bans, others are continuing to implement strict measures. During this period, the importance of a booster dose vaccination has been well-understood once again. This chapter of the book begins with the emergence of Omicron, epidemiology and mutations it harbors. Then it continued with a comparison of Omicron and Delta variants.

1. EMERGENCE OF THE OMICRON

A novel variant of SARS-CoV-2 named Omicron (B.1.1.529) was reported to the WHO by South Africa on November 24, 2021. Omicron was detected in South Africa in specimens collected on November 9, 2021. COVID-19 cases rapidly increased in South Africa especially in Johannesburg at the same period of time. The virus rapidly spread first to neighboring countries and subsequently all over the world. The new variant was seen in several countries including Hong Kong, Israel and Belgium almost at the same time (Torjesen, 2021). The first Omicron case in the USA was identified on December 1, 2021 in a person who returned from South Africa.

This variant was noticed to have a higher number of mutations and unique characteristics compared to the other variants (Meo et al., 2021). Omicron carries 32 mutations on the spike (S) protein, which is the main target of antibodies produced by vaccination or infections. The new variant was termed "COVID 3.0" in order to be understood better by the new generation (Queen, 2022). Omicron was declared by the WHO (2021) as "variant of concern" (VOC) on November 26, 2021. On November 29, 2021

the WHO stated that "Omicron might pose a 'very high' global risk and might be a more transmissible variant that could lead to infection surges" (Vaughan, 2022).

2. EPIDEMIOLOGY

SARS-Cov-2 has continued to affect the global population through its new variants through mutations. It seems that Omicron replaces the Delta variant in terms of infectivity and transmissibility. Omicron has rapidly become the dominant variant globally (Dejnirattisai, et al, 2022). As of the daily report by the WHO as of February 6, 2022, over 392 million confirmed cases and 5.7 million deaths have been reported globally (WHO, 2022). Worldwide travels from South Africa to the other regions in the world seem to be the major determinants of epidemiological characteristics of the new variant. In addition, the rapid transmissibility rate is another factor contributing to the spread of Omicron, which has created a highly challenging situation all over the world. The outbreak of this new variant is primarily seen in the Fall period (Meo et al., 2021).

Many countries have enacted travel restrictions to and from South Africa in an attempt to prevent rapid spread of Omicron. In this context many countries have imposed a travel ban on African countries despite the fact that Omicron has been detected in numerous European countries at the same time, suggesting that this variant was widespread even before it was identified (Petersen et al,. 2021). In addition, anti-vaxxer have contributed to this new form of COVID-19 outbreak. It has been reported that Omicron variant is an emerging threat to public health and may undermine the effort to eradicate the virus and control COVID-19 pandemic.

3. MUTATIONS

Omicron-specific mutations in both S protein and other sites of the virus appear to be co-evolved. Based on these mutations, the Omicron variant has been reported to be closely related to the previous Gamma variant (Kannan et al., 2022). The Omicron lineage harbors 59 mutations with 36 in the spike (S) protein including 15 within the receptor binding domain (RBD) region (Garcia-Beltran, et al., 2021). Studies on the previous variants have demonstrated that mutations within the RBD region can mediate escape from neutralizing antibodies that are induced by vaccines (Zhou et al., 2021). Mutations within the RBD region can affect the viral

infectivity and efficacy of the existing vaccines and medications. The 15 mutations in the RBD region of the Omicron are shown in Figure 1.

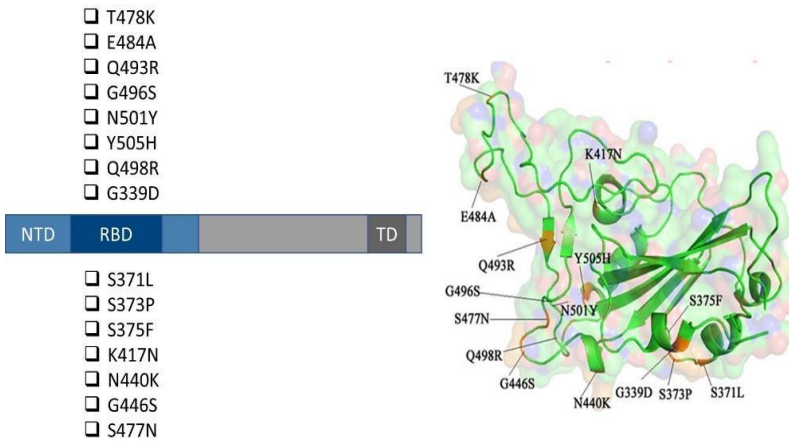


Figure 1. Mutations in the receptor binding domain (RBD) of S protein in the Omicron variant.

It has been reported that contagiousness of the Omicron variant is ten times higher compared to the original SARS-CoV-2 virus and twice compared to the Delta variant, mainly due to the mutations T478K, N440K and N501Y in the RBD region. In addition, The Omicron has a high potential to disrupt binding of 132 antibodies with the S protein owing to the mutations E484A, Y505H and K417N, suggesting that it has a stronger ability of vaccine breakthrough (Chen et al., 2021).

When the virus infects host cells, particularly 10 mutations in the receptor-binding motif (RBM) can interact with the angiotensin-converting enzyme 2 (ACE2) entry on membranes of the host (Wan et al., 2020). Therefore, it is claimed that the Omicron variant may significantly affect the effectiveness of currently used prophylactic and therapeutic medications and affinity for binding to ACE2.

Bloom et al. demonstrated that of the 15 Omicron RBD mutations, 9 single mutations (E484A, G446S, G496S, K417N, S371L, S373P, S375F, Q498R and Y505H) decrease the affinity to binding to ACE2, while 6 mutations (G339D, N440K, N501Y, Q493K, S477N and T478K) increase

the affinity (Starr et al., 2020). However, higher number of mutations in the Omicron variant makes prediction of ACE2 binding and immune evasion difficult compared to the other variants, especially because of the interactions of 10 mutations clustering in the RBM region.

4. OMICRON VS DELTA

During the ongoing pandemic, several variants of SARS-CoV-2 that pose a risk of infection in immunocompromised individuals have been identified (Corey et al., 2021). Some of these variants have been defined as "variant of concern". A variant of concern refers to viral variants in which mutations in the RBD region of the spike (S) protein dramatically increase binding affinity to the ACE2 receptor, resulting in rapid transmission in humans (Baj et al., 2021). Various approaches are currently used for the comparison of Delta and Omicron variants of the virus, both of which have been declared as the "variant of concern" by the WHO.

In terms of the mutations, numerous mutations in the RBD region of the spike protein in the Omicron variant compared to the Delta variant suggest that the Omicron may be immunologically resistant to antibody-induced protection. Some physical characteristics of the Delta and Omicron variants compared to the wild-type are presented in Table 1.

Table 1. Some physical characteristics of the Delta and Omicron variants

	DELTA	**OMICRON**	**WILD-TYPE**
Mutations	10	36	-
Amino acids	1271	1270	1273
pl (pH)	6.78	7.14	6.24
Molecular weight	140,986.31	141,328.11	141,178.47

Molecular weight and pl (pH) value of the Omicron variant is higher compared to the Delta variant and the original virus. In general, distinct features of the Omicron compared to the Delta variant have been gathered under three titles as replication, immune evasion and disease severity.

Omicron shows a higher rate of replication, escape from immunity and milder disease.

5. REPLICATION

The comprehensive amino acid substitutions in the S protein of the Omicron variant are likely to have major implications on replication. However, it should be remembered that there is still limited data on the biological and virological features of this new variant (Cele et al., 2021). In an *in vitro* study by Hui et al., (2022) viral replication rate was compared between the Omicron and Delta variants in an experimental setting. Omicron variant replicated to significantly higher titers compared to the Delta variant with a significant difference of more than 70-fold. According to the results of this study, the Omicron variant had a significantly higher replication competence in human bronchus compared to the Delta variant. However, its replication rate in the lung was found to be lower than the bronchus (Hui et al., 2022). There are also studies reporting higher viral replication rates with Omicron in human nasal epithelial cultures in vitro (Peacock et al., 2021).

6. IMMUNE EVASION

Antibodies produced after the natural response have a lower titer, resulting in decreased immunity over time. According to population-based studies from South Africa, Omicron reinfection/primary infection rate was 2.9, suggesting a potential for escape from immunity gained from primary infections (Pulliam et al., 2021; Poudel et al., 2022). Analyses of antibody titers of sera collected from vaccinated individuals reported lower neutralization capacity with Omicron compared to the Delta variant (Ahmed et al., 2022, Rössler et al., 2022). Andrews et al. (2021) reported that the effectiveness of vaccines was increased between 75-80% after a booster dose. This rate is lower than that of the Delta variant, but it is promising in terms of unknowns with the Omicron variant.

7. DISEASE SEVERITY

In a study by Wolter et al. from South Africa, more than 11,000 individuals with COVID-19 were examined and the rate of hospitalization was found to be significantly reduced with the Omicron variant compared to the other variants including Delta (Wolter et al., 2022). According to the results of this study, the Omicron variant might lead to milder disease courses, resulting in reduced hospitalization and related use of limited

resources. In another study from England, people infected with the Omicron variant were found to be 15-20% less likely to attend hospital in general and 40-45% less likely to be hospitalized for a night compared to people infected with the Delta variant (Christie, 2021).

8. CLINICAL PRESENTATION

Since the emergence of the Omicron variant, scientists have tried to learn more about its properties with the concerns of uncertainty. In a research at Hong Kong University, the Omicron variant was found to replicate faster up to 70 times in the human bronchi compared to the Delta variant and the original Wuhan virus. However, the Omicron variant exhibited significantly lower viral replication in the lungs (Hui et al., 2022). Similarly, in another study from the United Kingdom, the Omicron variant showed a lower replication compared to the Delta and original variants (Willet et al., 2022). These findings indicate a reduction in severe clinical presentation in patients infected with the Omicron variant.

Currently, it is not clear whether the mild clinical symptoms and different symptom description are resulting from existing immunity or altered clinical features of the Omicron. In addition, it has been reported that one of the mutations of the Omicron variant leads to failure of S gene target, which is one of the several areas targeted by PCR testing, raising concerns about false negative test results (Torjesen, 2021). Fortunately the WHO (2021) reported that this new variant can be picked up by PCR testing sufficiently.

Early reports from South Africa have indicated that the sharpest rise in the newly diagnosed cases was among the school age young people (high school, university) who were relatively less affected by the previous variants of the SARS-CoV-2 (Callaway, 2021). Clinical characteristics of the Omicron include severe fatigue, mild body ache, headache, fever, generalized myalgia, malaise and muscle age. Pulmonary characteristics include cough, scratchy throat, shortness of breath and possible pneumonia, while extrapulmonary characteristics include (bot not necessarily) abdominal pain, nausea/vomiting and diarrhea (Meo et al., 2021).

Clinical presentations of the Omicron variant still have numerous questions to be answered and as the reports around the world are combined

and data accumulated for certain patient groups such as high risk, unvaccinated, previously infected and boosted patients.

9. ANTIBODY RESISTANCE

The S protein of the Omicron variant evades neutralization mediated by antibodies gained with infections or vaccinations, with higher efficiency compared to the other variant of concerns (VOCs). Omicron spike facilitates entry into several cell lines and robust binding to human ACE2, suggesting that the variant can easily infect human cells. In a study by Liu et al., (2021) sera collected from convalescent patients and vaccines exhibited significantly decreased neutralization mediated by antibodies against the Omicron variant. On the other hand, it was reported that soluble ACE2 comparably inhibited entry driven by the Omicron spikes, suggesting that soluble ACE2 can be a treatment option for patients infected by the Omicron variant (Hoffmann et al., 2022).

Various recombinant monoclonal antibodies have been identified that inhibit SARS-CoV-2. Antibody cocktails of imdevimab and casirivimab (Weinreich et al., 2021), and bamlanivimab and etesevimab (Dougan et al., 2021) are currently used for the treatment of COVID-19. However, entry driven by the Omicron was highly resistant against imdevimab, etesevimab and bamlanivimab and largely resistant against casirivimab (Hoffmann et al., 2022).

CONCLUSION

There is still much to know and answers to be questioned about the Omicron variant of SARS-CoV-2. Despite its escape from immunity and high transmission rates, mild disease courses reported from all over the world are promising for the future. However, the importance of booster dose vaccination is obvious in most reports. As the new reports are combined and data accumulate regarding special patient groups such as age groups, immunocompromised persons, vaccinated persons and boostered patients, we will gain more insight about the natural course of this new variant. Today, we are speaking about new sub-variants of the Omicron and it is still not clear what the future will bring us.

REFERENCES

Ahmed, S. F., Quadeer, A. A., & McKay, M. R. (2022). SARS-CoV-2 T Cell Responses Elicited by COVID-19 Vaccines or Infection Are Expected to Remain Robust against Omicron. Viruses, 14(1), 79.

Andrews, N., Stowe, J., Kirsebom, F., Toffa, S., Rickeard, T., Gallagher, E., Gower, C., Kall, M., Groves, N., O'Connell, A. M., Simons, D., Blomquist, P. B., Zaidi, A., Nash, S., Iwani Binti Abdul Aziz, N., Thelwall, S., Dabrera, G., Myers, R., Amirthalingam, G., Gharbia, S., … Lopez Bernal, J. (2022). Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. The New England journal of medicine, 386(16), 1532–1546.

Baj, A., Novazzi, F., Drago Ferrante, F., Genoni, A., Tettamanzi, E., Catanoso, G., Dalla Gasperina, D., Dentali, F., Focosi, D., & Maggi, F. (2021). Spike protein evolution in the SARS-CoV-2 Delta variant of concern: a case series from Northern Lombardy. Emerging microbes & infections, 10(1), 2010–2015.

Callaway E. (2021). Heavily mutated Omicron variant puts scientists on alert. Nature, 600(7887), 21.

Cele, S., Jackson, L., Khoury, D. S., Khan, K., Moyo-Gwete, T., Tegally, H., San, J. E., Cromer, D., Scheepers, C., Amoako, D., Karim, F., Bernstein, M., Lustig, G., Archary, D., Smith, M., Ganga, Y., Jule, Z., Reedoy, K., Hwa, S. H., Giandhari, J., … Sigal, A. (2021). SARS-CoV-2 Omicron has extensive but incomplete escape of Pfizer BNT162b2 elicited neutralization and requires ACE2 for infection. medRxiv : the preprint server for health sciences, 2021.12.08.21267417.

Chen, J., Wang, R., Gilby, N. B., & Wei, G. W. (2021). Omicron (B.1.1.529): Infectivity, vaccine breakthrough, and antibody resistance. ArXiv, arXiv:2112.01318v1.

Christie B. (2021). Covid-19: Early studies give hope omicron is milder than other variants. BMJ (Clinical research ed.), 375, n3144.

Corey, L., Beyrer, C., Cohen, M. S., Michael, N. L., Bedford, T., & Rolland, M. (2021). SARS-CoV-2 Variants in Patients with Immunosuppression. The New England journal of medicine, 385(6), 562–566.

Dejnirattisai, W., Huo, J., Zhou, D., Zahradník, J., Supasa, P., Liu, C., Duyvesteyn, H., Ginn, H. M., Mentzer, A. J., Tuekprakhon, A., Nutalai, R., Wang, B., Dijokaite, A., Khan, S., Avinoam, O., Bahar, M., Skelly, D., Adele, S., Johnson, S. A., Amini, A., … Screaton, G. R. (2022). SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. Cell, 185(3), 467–484.e15.

Dougan, M., Nirula, A., Azizad, M., Mocherla, B., Gottlieb, R. L., Chen, P., Hebert, C., Perry, R., Boscia, J., Heller, B., Morris, J., Crystal, C., Igbinadolor, A., Huhn, G., Cardona, J., Shawa, I., Kumar, P., Adams, A. C., Van Naarden, J., Custer, K. L., … BLAZE-1 Investigators (2021). Bamlanivimab plus Etesevimab in Mild or Moderate Covid-19. The New England journal of medicine, 385(15), 1382–1392.

Garcia-Beltran, W. F., St Denis, K. J., Hoelzemer, A., Lam, E. C., Nitido, A. D., Sheehan, M. L., Berrios, C., Ofoman, O., Chang, C. C., Hauser, B. M., Feldman, J., Gregory, D. J., Poznansky, M. C., Schmidt, A. G., Iafrate, A. J., Naranbhai, V., & Balazs, A. B. (2021). mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. medRxiv : the preprint server for health sciences, 2021.12.14.21267755.

Hui, K., Ho, J., Cheung, M. C., Ng, K. C., Ching, R., Lai, K. L., Kam, T. T., Gu, H., Sit, K. Y., Hsin, M., Au, T., Poon, L., Peiris, M., Nicholls, J. M., & Chan, M. (2022). SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo. Nature, 603(7902), 715–720.

Hoffmann, M., Krüger, N., Schulz, S., Cossmann, A., Rocha, C., Kempf, A., Nehlmeier, I., Graichen, L., Moldenhauer, A. S., Winkler, M. S., Lier, M., Dopfer-Jablonka, A., Jäck, H. M., Behrens, G., & Pöhlmann, S. (2022). The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. Cell, 185(3), 447–456.e11.

Kannan, S. R., Spratt, A. N., Sharma, K., Chand, H. S., Byrareddy, S. N., & Singh, K. (2022). Omicron SARS-CoV-2 variant: Unique features and their impact on pre-existing antibodies. Journal of autoimmunity, 126, 102779.

Liu, L., Iketani, S., Guo, Y., Chan, J. F., Wang, M., Liu, L., Luo, Y., Chu, H., Huang, Y., Nair, M. S., Yu, J., Chik, K. K., Yuen, T. T., Yoon,

C., To, K. K., Chen, H., Yin, M. T., Sobieszczyk, M. E., Huang, Y., Wang, H. H., … Ho, D. D. (2022). Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. Nature, 602(7898), 676–681.

Meo, S. A., Meo, A. S., Al-Jassir, F. F., & Klonoff, D. C. (2021). Omicron SARS-CoV-2 new variant: global prevalence and biological and clinical characteristics. European review for medical and pharmacological sciences, 25(24), 8012–8018.

Petersen, E., Ntoumi, F., Hui, D. S., Abubakar, A., Kramer, L. D., Obiero, C., Tambyah, P. A., Blumberg, L., Yapi, R., Al-Abri, S., Pinto, T., Yeboah-Manu, D., Haider, N., Asogun, D., Velavan, T. P., Kapata, N., Bates, M., Ansumana, R., Montaldo, C., Mucheleng'anga, L., … Zumla, A. (2022). Emergence of new SARS-CoV-2 Variant of Concern Omicron (B.1.1.529) - highlights Africa's research capabilities, but exposes major knowledge gaps, inequities of vaccine distribution, inadequacies in global COVID-19 response and control efforts. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases, 114, 268–272.

Peacock, Thomas & Brown, Jonathan & Zhou, Jie & Thakur, Nazia & Newman, Joseph & Kugathasan, Ruthiran & Sukhova, Ksenia & Kaforou, Myrsini & Bailey, Dalan & Barclay, Wendy. (2022). The SARS-CoV-2 variant, Omicron, shows rapid replication in human primary nasal epithelial cultures and efficiently uses the endosomal route of entry. 10.1101/2021.12.31.474653.

Poudel, S., Ishak, A., Perez-Fernandez, J., Garcia, E., León-Figueroa, D. A., Romaní, L., Bonilla-Aldana, D. K., & Rodriguez-Morales, A. J. (2022). Highly mutated SARS-CoV-2 Omicron variant sparks significant concern among global experts - What is known so far?. Travel medicine and infectious disease, 45, 102234.

Pulliam, J., van Schalkwyk, C., Govender, N., von Gottberg, A., Cohen, C., Groome, M. J., Dushoff, J., Mlisana, K., & Moultrie, H. (2022). Increased risk of SARS-CoV-2 reinfection associated with emergence of Omicron in South Africa. Science (New York, N.Y.), 376(6593), eabn4947.

Rössler, A., Riepler, L., Bante, D., von Laer, D., & Kimpel, J. (2022). SARS-CoV-2 Omicron Variant Neutralization in Serum from

Vaccinated and Convalescent Persons. The New England journal of medicine, 386(7), 698–700.

Queen D. (2022). Another year another variant: COVID 3.0-Omicron. International wound journal, 19(1), 5.

Starr, T. N., Greaney, A. J., Hilton, S. K., Ellis, D., Crawford, K., Dingens, A. S., Navarro, M. J., Bowen, J. E., Tortorici, M. A., Walls, A. C., King, N. P., Veesler, D., & Bloom, J. D. (2020). Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. Cell, 182(5), 1295–1310.e20.Torjesen I. (2021). Covid-19: Omicron may be more transmissible than other variants and partly resistant to existing vaccines, scientists fear. BMJ (Clinical research ed.), 375, n2943.

Vaughan A. (2021). Omicron emerges. New scientist (1971), 252(3363), 7.

Wan, Y., Shang, J., Graham, R., Baric, R. S., & Li, F. (2020). Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. Journal of virology, 94(7), e00127-20.

Weekly epidemiological update on COVID-19 - 8 February 2022 (who.int) https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---8-february-2022

Weinreich, D. M., Sivapalasingam, S., Norton, T., Ali, S., Gao, H., Bhore, R., Musser, B. J., Soo, Y., Rofail, D., Im, J., Perry, C., Pan, C., Hosain, R., Mahmood, A., Davis, J. D., Turner, K. C., Hooper, A. T., Hamilton, J. D., Baum, A., Kyratsous, C. A., … Trial Investigators (2021). REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. The New England journal of medicine, 384(3), 238–251.

WHO. Classification of Omicron (B.1.1.529): SARSCoV-2 variant of concern https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern

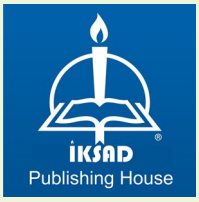
Willett, Brian & Grove, Joe & MacLean, Oscar & Wilkie, Craig & Logan, Nicola & Lorenzo, Giuditta & Furnon, Wilhelm & Scott, Sam & Manali, Maria & Szemiel, Agnieszka & Ashraf, Shirin & Vink, Elen & Harvey, Will & Davis, Christopher & Orton, Richard & Hughes, Joseph & Holland, Poppy & Silva, Sofia & Pascall, David & Thomson, Emma. (2022). The hyper-transmissible SARS-CoV-2 Omicron variant exhibits significant antigenic change, vaccine

escape and a switch in cell entry mechanism. 10.1101/2022.01.03.21268111.

Wolter, N., Jassat, W., Walaza, S., Welch, R., Moultrie, H., Groome, M., Amoako, D. G., Everatt, J., Bhiman, J. N., Scheepers, C., Tebeila, N., Chiwandire, N., du Plessis, M., Govender, N., Ismail, A., Glass, A., Mlisana, K., Stevens, W., Treurnicht, F. K., Makatini, Z., … Cohen, C. (2022). Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. Lancet (London, England), 399(10323), 437–446.

World Health Organization. Update on Omimcron Available from: https://www.who.int/news/ item/28-11-2021-update-on-omicron. .

Zhou, D., Dejnirattisai, W., Supasa, P., Liu, C., Mentzer, A. J., Ginn, H. M., Zhao, Y., Duyvesteyn, H., Tuekprakhon, A., Nutalai, R., Wang, B., Paesen, G. C., Lopez-Camacho, C., Slon-Campos, J., Hallis, B., Coombes, N., Bewley, K., Charlton, S., Walter, T. S., Skelly, D., … Screaton, G. R. (2021). Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell, 184(9), 2348–2361.e6.



ISBN: 978-625-8323-47-4