Interdisciplinary Medicine and Health Sciences Concepts, Researches and Practice

Editors
Prof. Dr. A. Erdinç Yalın
Assoc. Prof. Dr. Meriç Eraslan

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PREFACE

The field of health and life sciences is constantly evolving and expanding, as we continue to learn more about the processing information and discoveries and breakthroughs being made on the related technologies. İn this book, we aim to provide a comprehensive overview of the latest research and developments in this interdisciplinary field, with a focus on the biological and physiological mechanisms underlying health and disease and latest developments in medical treatments and therapies.

The book is designed for students, professionals, and anyone with an interest in the medical and life sciences. It is written in a clear and concise manner, with an emphasis on providing practical information and real-world examples. We hope that this book will serve as a valuable resource for those looking to learn more about this exciting and constantly evolving field.

We would like to thank all of the contributors to this book, who have generously shared their expertise and insights. We would also like to thank the editorial and production teams who have worked tirelessly to bring this book to fruition.

Prof. Dr. A. Erdinç YALIN
Assoc. Prof. Dr. Meriç ERASLAN

CONTENTS

CHAPTER I

RECOGNIZING THE IMPORTANCE OF BIOCHEMISTRY IN FACILITATING TRANSDISCIPLINARY INTERACTIONS IN MEDICAL SCIENCES

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1. General information about Biochemistry and its branches on interdisciplinary approaches in life sciences

Biochemistry is the molecular arrangement of the atoms in a biomolecule. "Bios" means "life" in Greek. Thus, biochemistry is the study of the molecular foundation of life. Simple organic molecules, which are the elements of organisms, are unique to the living world and result from biological activity. These substances are biomolecules and act as building blocks in developing biological structures (1). They were chosen by evolution for their capacity to execute activities that are precisely specified in live cells. These molecules are comparable and interconnected in all living creatures, interacting and participating in energy transmission and material change processes. These biomolecules, for example, can be characterized in two ways: chemically and biologically, implying that biochemistry is a matter of super-chemistry organized perfectly, offering great possibilities in studying the secrets of living organisms, a chemical of the most perfectly organized matter (2). Biochemistry studies the chemical makeup of living creatures, the structure, and characteristics of component substances, and the transformations these substances undergo inside living organisms. These biomolecules are investigated in structural or descriptive biochemistry, a branch of organic chemistry. The conversion of biomolecules into plant and animal life is a topic in dynamic biochemistry or metabolism

(3). Biochemistry is closely related to life sciences such as agriculture, animal husbandry, and veterinary medicine. Understanding biochemical processes occurring at the level of plant and animal organisms is essential for influencing plant productivity and rational animal husbandry. Analytical biochemistry is the science of techniques for determining the qualitative and quantitative composition of substances or mixtures of substances or the science of methods for analyzing substances (2). The analysis is a technique of scientific investigation that is focused on the examination of each component. Analysis methods are generally categorized into biochemical, instrumental, and biological. Biochemical analysis studies the content, structure, characteristics, and methods of acquiring substances in biochemistry as a science. Biochemical analysis techniques rely on biochemical processes to produce a visual representation of the result of the study. Biochemical analysis approaches are qualitative, quantitative, structural, and systemic (4).

The qualitative biochemical analysis aims to determine what elements, groups of atoms, ions, or molecules make up the analyte or combination of substances. Next, the biochemical quantitative analysis-the quantitative composition of the biochemical studied is determined. Finally, the structural analysis seeks to determine the structure of substances, i.e., how the components in the substances are bound together (4).

Biochemistry, often known as biological chemistry or physiological chemistry, is the science that uses chemical techniques of analysis to investigate the chemical composition of living organisms and the chemical processes that occur in them. Biochemistry is a science that deals with life-long molecular research. It is a blend of chemistry, which investigates atomic interactions, and molecular biology, which analyzes the structure and relationships of the body. The initial ingredients of living matter are the atoms of H and O and the atom of C (5-7). The atom of N is an element since, together with C, H, and O, it is found in the composition of proteins, that is, compounds that reflect the molecular structures of critical concerns in living matter. Atoms of C, H, O, and N share ownership, and certain may form covalent connections by distributing electrons. Slightly abundant elements (P, S, Ca, Mg) form an element construction in the molecules' composition in forming live things. Cl, Na, and K are also vital to life and are termed biomolecules. They influence both the biochemical structure and function (8-10).

The organism's macromolecules are composed of very simple, low molecular weight environmental antecedents (CO2, H2O, NH3). These

environmental precursors are transformed by living organisms through metabolic mechanisms (with intermediates: pyruvate, citrate, malate, glyceraldehyde-3-phosphate) into organic compounds with a slightly higher molecular weight, also known as basic biomolecule constituent units: nucleotides, amino acids, monosaccharides, fatty acids, glycerol. Biomolecules from the base form covalent connections with one another, resulting in macromolecules unique to the living cell: nucleic acids, proteins, polysaccharides, and lipids; inorganic biomolecules: Inorganic molecules include water and mineral salts; organic biomolecules include proteins, lipids, carbohydrates, enzymes, hormones, and so on (11-15).

Buffers are solutions that maintain a nearly constant concentration of hydrogen ions after dilution and even when a strong acid or basic is added. When modest quantities of strong acid or strong base are added to these solutions, they include two compounds that oppose ambient pH changes. Precipitation bioreactions are used in the analysis to separate the ions into groups and subgroups (16-18).

The majority of bioreactions occur in the aqueous phase, which is a fundamental scientific consensus. At the same time, there are numerous identification indicators and methods, such as those based on the size, charge, color, and heat change of molecules or reactions, not to mention numerous spectroscopic methods based on the electromagnetic properties of molecules. Precipitation bioreactions are used in several identification bioreactions (19). A precipitation phenomenon is also used in several dosing methods (quantitative determination) (20). Precipitation is forming a new solid phase in a liquid system. Both biochemical and physical processes can cause it. For example, if alcohol is added to a salt solution, the liquid outflow may drop below the solid form.

2. Clinical Biochemistry for diagnosing and treatment of patients in Medical Sciences

Biochemistry is a fundamental discipline in the practice of clinical medicine. Many diseases have long been known to have a biochemical foundation, and biochemistry research increasingly gives molecular descriptions of pathological processes. Because of applying biochemical concepts and techniques to studying human fluids and tissues, clinicians now have access to a diverse and expanding spectrum of biochemical studies to help clinical decision-making. Such examinations can give critical information for diagnosing and managing

a wide range of illnesses, including those with a clear metabolic foundation (e.g., diabetes mellitus) and others in which metabolic changes emerge from the disease (e.g., renal failure). On the other hand, many disorders are effectively identified and treated without biochemical investigations. At the same time, there are other situations for which biochemical investigations would be helpful but for which relevant tests are not yet accessible (21,22).

The potential variety of studies available to assist clinicians is extensive, ranging from simple, low-cost urine dip-stick tests to magnetic resonance imaging employing costly equipment. Clinical biochemists naturally believe biochemical investigations are the most important of all studies. In some cases, they are; in others, they play no part, and in many others, their worth is substantially boosted when combined with the results of other studies, such as imaging. Therefore, the physician should know all possible investigations and recognize their distinct advantages and limits. The clinical biochemist must also be aware of the function of other investigations to evaluate biochemical tests in context and advise on their usefulness and interpretation in specific clinical situations.

3. Biochemical Tests for Diagnosing, Management, and Screening

Although not always necessary for individual patient care, it may be able to expand the clinical diagnosis by further inquiry to discover the pathophysiology of the ailment and, ultimately, its underlying cause. For instance, angiography might be performed to demonstrate coronary atherosclerosis before surgery or angioplasty; the discovery of hypercholesterolemia would indicate a causal component for atherosclerosis, and a family history of early heart disease would imply that hypercholesterolemia is the cause of atherosclerosis (23,24). Whatever function biochemical data are utilized for, they must be dependable and available promptly. Under certain conditions, it may be acceptable to compromise some quality to achieve a result quickly. However, in general, every effort should be taken to limit the impact of both analytical and preanalytical variables on data accuracy and precision.

Most biochemical studies are quantitative, and the more aberrant a result, the more probable a pathological disturbance is to blame. The degree to which a result is abnormal is frequently related to the severity of a problem, but this is not always the case. The diagnostic test may not reflect the essential aspect of the condition in terms of severity; for example, two patients with

hepatitis may have identically elevated plasma aminotransferase activities (reflecting tissue damage), but the condition will be judged more severe if one patient's prothrombin time is prolonged (reflecting impaired hepatic functional capacity) (25).

Biochemical test findings are often reliable predictors of prognosis. For example, in patients with primary biliary cirrhosis, plasma bilirubin concentration correlates well with prognosis; a high plasma concentration of a-fetoprotein in a patient with testicular teratoma is predictive, whereas paraprotein concentration in a patient with myeloma is not (26).

Serial measurements can be extremely useful in tracking the progression of an illness or its response to therapy. The greater the relationship between the variable being assessed and the underlying pathological process or functional impairment, the better it will be for this purpose. Biochemical data must always be interpreted in the light of clinical assessment and the results of other relevant investigations, not in isolation. Nevertheless, intervention may sometimes be appropriate based on a biochemical change alone if this has been shown reliably to predict a significant clinical change, for example, in hyperkalemia in a patient with renal failure (27). When repeated biochemical tests are used to track therapy response, the lack of an expected change to occur may indicate that the treatment is insufficient or unsuitable or that the diagnosis is erroneous. Biochemical parameters in therapeutic drug monitoring (TDM) may suggest a probable reason for non-response to therapy.

Screening for disease aims to identify illness before it becomes clinically obvious. Screening might entail clinical evaluation and lab tests. Some disorders (primarily hereditary metabolic diseases) may only require one biochemical test (28). The phrase is also used to describe the performance of a range of biochemical tests (sometimes coupled with other forms of research) in healthy persons to identify several diseases on the premise that 'normal' findings – results within the applicable reference limits – exclude these disorders. A set of 'normal' findings may appear comforting and rule out the existence of some diseases; nevertheless, they may also provide the wrong impression and potentially cause a delay in identifying a disease in its early stages. Because of the way that reference ranges are specified, the greater the number of tests that are carried out, the greater the likelihood that an 'abnormal' result will be generated that is not related to the presence of disease. This is because results that fall outside the reference limits are considered abnormal. Economic and logistic constraints limit whole screening

populations for diseases, but all adults (some say only men, others both) should be examined for hypercholesterolemia (29). In wealthy nations, selective biochemical screening for illness is widely used. Among the most well-known examples are screening programs for phenylketonuria, congenital hypothyroidism, sickle cell disease, and cystic fibrosis in newborns. Tandem mass spectrometry has made it feasible to test for a plethora of diseases with a minimal amount of blood and a manageable budget, including medium-chain fatty acid oxidation disorders, some organic acidaemias, and the most frequent form of congenital adrenal hyperplasia (30).

Conclusion

Biochemistry is a modern scientific discipline that studies biological matter and the specific processes governing its composition, type, molecular structure, assemblage, and correlation of component biomolecules. Additionally, biochemistry investigates the biosynthesis and biodegradation processes that govern how living things produce and consume the energy required for life. The compounds produced as a result of these iterative transformations fulfill various functions in plant life, including those of polymers, storage, active substances, and by-products. Understanding the mysteries of life in all its manifestations is the ultimate objective of biochemistry as a scientific discipline. In the field of medicine, biochemical data are put to practical use, both in the treatment of patients and in the conduct of research. However, before requesting an inquiry, one must always consider the reasons for testing. Although the cost of using automated analyzers to carry out several tests is relatively low compared to the overall cost of providing medical care, this does not mean it is free. The sufferer may also incur a financial burden. Repeated venepunctures to obtain blood for so-called “routine” tests are, at best, an annoyance and, at worst, can cause a significant drop in the hematocrit, which is especially likely to occur in very young children. The following guidance for junior medical staff used to be included in the laboratory handbook at one of the hospitals where the authors have acquaintances. It read, “If you need advice or time to think, ask for it; do not ask for a complete blood count and measurement of “urea and electrolytes. “ In the same way as with other kinds of investigations, biochemical investigations ought to be requested to find answers to particular questions; if there are no such questions, the results cannot give any.

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CHAPTER II

FREE RADICALS AND THEIR BIOLOGICAL RESOURCES

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1. Introduction

Free radicals are constantly produced by normal metabolic processes, but their production rate is increased in certain inflammatory or other disease conditions. Normally, the body is protected by a wide range of defense mechanisms against reactive oxygen metabolites and their toxic products. An imbalance between reactive oxygen metabolites and their safe disposal can initiate oxidative chain reactions and lipid peroxidation. Transport, which is one of the important activities of farm management, is one of the factors that affect animal welfare and create stress in animals. These molecules, which have a lot of destructive effects, can be at the starting point of human diseases. Therefore, it is important to talk about the molecular effects and structures of free radicals and to make suggestions primarily for the prevention of cancer and other chronic diseases.

2. Free Radicals

Electrons in atoms or molecules move around the nucleus in regions defined as orbitals. Each orbital has at most two electrons moving in opposite directions. An atom or molecule is called a free radical (SR) if it has one or more unpaired electrons in its outer orbitals. Such molecules are highly reactive due to their unpaired electrons (1, 2). The simplest free radical is the hydrogen atom, which has an electron and a proton.

Normally, the electron arrangement of molecules containing two or more chemically bonded electrons determines the stability of a molecule. If the electron in the structure of this molecule does not have a partner, the molecule behaves extremely reactively and tends to form a pair with an electron in order to become stable (3). Recent studies show that free radicals play a major role in the formation of many important diseases such as atherosclerosis, diabetes, rheumatoid arthritis, some aging-related diseases, autoimmune diseases, and various cancer types, especially neurodegenerative diseases such as Alzheimer's, Parkinson's, and hungtington's (4, 5).

The oxygen we need to live is also the source of free radicals. These molecules have extra energy and they discharge this energy to the cells in the body and change their normal functions. Free radicals are structures that have chemically lost an electron in their outermost electron orbit. The most important reason for its various harmful effects is that it tries to share the electrons of other atoms in order to close this electron gap. Studies have shown that free radicals interfere with the functioning of the tissue or molecule they affect. Depending on the biological significance of the affected tissue or molecule, it can cause a variety of significant or insignificant ailments. If free radicals of oxygen, hydrogen and hydroxyl type obtain the electron they want to take from antioxidants, it is prevented that they are expected to damage another structure.

In the scientific studies carried out on these molecules so far, it has been stated that free radicals can cause various diseases. The most important of them are; cancer, aging, heart attack, chronic fatigue. Due to various physical factors and chemical events, there is a constant formation of radicals in the environment and cellular conditions. Free radicals occur during the functioning of normal metabolic pathways in the organism or under the influence of various external factors such as environmental agents (pesticides, aromatic hydrocarbons, toxins, solvents, etc.), stress and radiation. Free radicals can be formed during the normal metabolic activities of our body (for example, after a meal). In addition, industrial wastes, sun rays, cosmic rays, ozone, gases especially from automobile exhausts, heavy metals, viruses, cigarettes, alcohol, stress, waste products formed as a result of fat metabolism in the body, various chemicals, water and air are environmental factors that create free radicals.

2.1. Formation Mechanisms of Free Radicals

In biological systems, free radicals are mostly formed as a result of electron transfer. Free radicals can be formed when the two electrons in the

bond structure remain on separate atoms during the breakage of the covalent bond in the molecule with the homolytic splitting of the covalent bonds in the molecules (1). If an unpaired electron remains in its outer orbital during the loss of electrons in a molecule without radical properties, a radical form is formed. For example, cellular antioxidants such as tocopherol and ascorbic acid donate a single electron to radical species and reduce radicals, while their own radical form is formed (6). If an unpaired electron is formed in its outer orbital with a single electron transfer to a non-radical molecule, such a reduction may lead to radical formation. For example, molecular oxygen is reduced with a single electron to form the radical form, superoxide (7).

In biological systems, free radicals can be positively charged, negatively charged, or neutral. Although the most important radicals are free oxygen radicals (SOR); There are also radicals and inorganic molecules that are derivatives of C, N, S. According to the definition of free radical, transition metals such as Cu^{2+}, Fe^{3+}, Mn^{2+}, Mo^{5+} are not considered free radicals even though they have unpaired electrons. However, since these ions catalyze reactions, they play an important role in the formation of free radicals (1, 2, 6).

2.2. Sources of Free Radicals

Free radicals are short-lived, reactive molecules that contain unpaired electrons in their outer orbitals. At the same time, reactive oxygen species are formed as a natural result of oxygen use in aerobic organisms (8). Free radicals and other reactive oxygen species are also produced during special metabolic events in the organism or they can be taken from the outside.

2.2.1. biological resources

Mature macrophages, neutrophils, eosinophils, and phagocytic leukocytes are cells that provide the reaction of various biological targets and initiate the body's cellular response to infections. During the phagocytic respiratory burst, free oxygen radicals such as H_2O_2, superoxide and hydroxyl radicals are formed. Phagocytized microorganisms and bacteria in the target environment are destroyed by the effect of these products. However, when these oxidant products exceed the antioxidant defense powers of the cells , they also damage normal host cells and play a role in the pathogenesis of various diseases. Radiation and environmental agents also trigger the formation of free radicals. Environmentally, air pollution, pesticides, cigarette smoke, solvents, anesthetics, aromatic hydrocarbons cause free radical formation. Antineoplastic agents such

as doxorubicin and adrioxmicine can also generate free radicals. For example, doxorubicin, an anticarcinogen agent, inhibits the cell's DNA replication, leading to the formation of H_2O_2. Thus, it leads to the initiation of lipid peroxidation (9, 10). Finally, neural stimuli increase the synthesis of catecholamines (11). Oxidation of catecholamines is also a cause of free radical formation.

2.2.2. Intracellular Resources

According to the acceptance of the scientific community, normally the largest source of free oxygen radicals in cells is leakage in the mitochondrial electron transport chain. Cells in living systems reduce most of the oxygen used (about 95%) to water by taking four electrons with the oxidative phosphorylation chain located in the inner mitochondrial membrane. Electron leakage in this system can convert 1-3% of oxygen to superoxide radical. Free radical generation in the endoplasmic reticulum and nuclear membranes may result from oxidation of membrane-bound cytochromes.

H_2O_2 may occur during the catalytic cycle of many enzymes (xanthine oxidase, aldehyde oxidase, flavoprotein dehydrogenase, amino acid oxidase) (11). Autooxidation of small molecules and some compounds such as thiols, catecholamine, tetrahydrofolate can produce superoxide radical. Especially transition metals such as iron and copper are involved in oxidation-reduction reactions under physiological conditions. Because of these properties, they act as catalysts that accelerate free radical reactions.

Iron and copper especially catalyze thiyl synthesis from thiols, H_2O_2, superoxide and hydroxyl radical synthesis. Arachidonic acid metabolism is also an important source of reactive oxygen metabolites. As a result of stimulation of phagocytic cells, arachidonic acid in the plasma membrane is liberated and various free radical intermediates may occur by enzymatic oxidation (6). In addition, toxic substances increase the production of free radicals in the cell with various effects. The toxin itself is a free radical, the toxin may be metabolized to a free radical, or a free oxygen radical may be formed as a result of the metabolism of the toxin.

2.3. Formation Forms in the Body

The main source of free radicals in mammals with aerobic metabolism in the body is reactive particles formed as a result of single electron transfers during the reduction of oxygen to water (12).

$O_2 + H^+ + e \rightarrow HO_2$ (hydroperoxyl radical)
$HO_2 \rightarrow ++ O_2^-$ (superoxide radical)
$O_2 + 2H^+ + e \rightarrow H_2O_2$ (hydrogen peroxide)
$H_2O_2 + e \rightarrow OH^- + OH$ (hydroxyl radical)
$OH + e + H \rightarrow H_2O$ (water)
Table 1. Free radical formation in mitochondria

These formation reactions are as follows (*Table 1*).

1. Superoxide radical; It is a strong reducing agent. It rapidly dismutates to form H_2O_2 and O_2. The protonated form, the hydroperoxyl radical (HO_2), is a stronger oxidant. H_2O_2 is a weak oxidoreductant. It is quite stable in the absence of transition metals (Fe, Cu) in the environment . It is broken down by catalase and glutathione peroxidase (GSHPL). The OH• radical is the main radical that stimulates lipid peroxidation and is formed from the superoxide radical or H_2O_2 with the effect of Fe ions (13).

2. Activated neutrophils; Hypochlorous acid is a powerful oxidant produced from activated neutrophils. Myeloperoxidase (MPO), an enzyme found in the phagocyte cytoplasm and containing HEM, catalyzes the formation of hypochlorous acid (HOCl) from H_2O_2 and Cl ions. HOCl reacts with superoxide radical or iron salts to form hydroxyl radical (14, 15).

3. Nitric oxide and nitrogen dioxide carry an odd number of electrons, so they are free radicals. Although nitric oxide itself is a weak reducing agent, it combines with endogenous free radicals to form the peroxynitrite radical. It is a strong oxidant and can easily form the hydroxyl radical.

4. Mitochondrial electron transport system; During the reduction of oxygen to water in the mitochondria, a superoxide radical is formed by the autoxidation of a part of the electron transport chain localized in the inner membrane (16).

5. Endoplasmic reticulum; these intracellular membranes contain the cytochrome P-450 and cytochrome b5 systems. These systems are involved in the oxidation of unsaturated fatty acids and xenobiotics. During the formation of these reactions, free radicals occur (17).

6. Peroxisomes; Since peroxisomes contain high levels of oxidase, they constitute a strong source of cellular H_2O_2. These peroxisomal enzymes include D-amino acid oxidase, urate oxidase, and acyl-CoA oxidase (18).

7. Plasma membranes; Plasma membrane enzymes that cause free radical production are lipoxygenase and cyclooxygenase. As a result of the reactions

catalyzed by these enzymes, prostaglandins, thromboxanes, leukotrienes and the slow-acting substance of anaphylaxis are synthesized (19).

2.4. Effects of Free Radicals

Free radicals, which are known to have strong reactive properties due to their tendency to react, can easily interact with all cell components. If they are not eliminated by the defense mechanisms of the cells responsible for the defense system, they may react with biological molecules and initiate a chain reaction in which new free radicals are formed (1).

Free radicals are highly reactive molecules. They react with many biomolecules to form various compounds. These compounds often have toxic properties. Membrane damage is one of the most harmful effects. The most damaged structures inactivate the enzymes in the membrane with the damage of free radicals to the membranes. Thus, they easily react with unsaturated bonds of fatty acids in membranes and cholesterol to form various peroxidation products. It has also been reported that they impair the structure, permeability and function of membranes (6).

3. conclusion

Free radicals can come from outside the body, or they can occur as a natural result of human metabolism. The endogenous production of free radicals occurs in different ways. In contrast, living organisms have various mechanisms to protect themselves against the potentially destructive effects of free radicals. Free radicals are molecules that need attention as they are extremely harmful compounds that can be seen as the first starting point of all diseases such as vagrant mines. For this reason, some warnings should be given to human populations and it should be recommended to stay away from negative effects that trigger free radicals such as external effects, smoking, polluted and poor quality air, stress, heavy working conditions, radiation, electromagnetic waves.

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CHAPTER III

EFFECTS OF ANTIOXIDANTS AND NUTRITION ON THE DEFENSE SYSTEM

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1. Introduction

Inflammations and chronic diseases, especially cancer. There are various defense mechanisms in our body to prevent diseases and cancer caused by these adverse conditions. These mechanisms are known as "antioxidant defense systems" or "antioxidants" for short. Antioxidants are of vital importance in the prevention and treatment of many diseases, as they are agents that remove free radicals, in other words, reactive oxygen species (ROS), and prevent the damage that can be caused by them. In every cell that makes up our body against radicals, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH- Px), glutathione There is a defense mechanism called the radical scavenging enzyme system consisting of enzymes such as reductase (GSSGR) and an auxiliary defense mechanism consisting of antioxidant vitamins such as A, E, C, and lipoic acid (1). Antioxidants effectively reduce these reactive oxygen and nitrogen species, which damage cells, into low- toxic or non- toxic products. The presence of these harmful compounds makes antioxidants an indispensable part of life for a healthy life (2).

2. Antioxidants

Antioxidants; As they are produced by cells, they can also be taken through food. The main natural antioxidants present in foods and destroy the human body from harmful free radicals; vitamins (vitamins A, E and C), flavonoids, carotenoids, and polyphenols. Many studies have shown an inverse relationship

between fruit and vegetable consumption and the occurrence of certain cancers and heart diseases (3). Medicinal plants, which started to be used in 3000 years, began to lose their popularity with the development of modern medicine in the 1900s. It largely left its place to chemical drugs and began to be called an alternative medicine branch.

2.1. Endogenous Antioxidants

The body's endogenous defense system should be supported by antioxidant compounds to be taken with a regular and balanced diet. Therefore, increasing dietary intake of antioxidants or foods enriched with antioxidants are becoming increasingly important. In the food industry, usually butylated for the preservation and shelf life of oils and other oil-containing products. hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) is used. However, the studies carried out mention the toxicity of these compounds, revealing the risk of their being carcinogenic (4). Endogenous antioxidants are divided into two classes, enzyme and non-enzyme (5).

2.2. Exogenous Antioxidants

Exogenous antioxidants as vitamins, drugs and food oxidants . α- tocopherol, β-carotene, ascorbic acid and folic acid (Vitamin B9) are exogenous antioxidants that are vitamins. Exogenous antioxidants; includes vitamins, foods, and drug forms (6). Exogenous antioxidants used as drugs; xanthine oxidase inhibitors (allopurinol, oxypurinol, folic acid), NADPH oxidase inhibitors (adenosine, local anesthetics, calcium channel blockers, non-steroids anti-inflammatory drugs), recombinant SOD, those that increase endogenous antioxidant activity (ebselen, acetyl cysteine), nonenzymatic radical scavengers (mannitol, albumin), cytokines, iron chelators, iron redox cycle inhibitors (desferrocamine , ceruloplasmin)(7). Iron chelators: They enter the cell and bind free iron, thereby inactivating it, thus inhibiting the Fenton reaction and ultimately the formation of hydroxyl radicals. Cytokines: They activate antioxidant enzymes, especially catalase. However, they can be harmful because they also activate proteolytic enzymes.

2.3. Natural Sources of Antioxidants

Some plants are a good source of natural antioxidants. Studies with antioxidant compounds found in fruits have reported significant amounts of

antioxidants in strawberries, cherries, citrus fruits, kiwi, prunes, and olives (5). Lemons and oranges have high concentrations of vitamin C and, due to these properties, have a good antioxidant capacity (8). Most of the vegetables, especially cocoa beans , potatoes, tomatoes, spinach, phaseolus The antioxidant potential was analyzed in vegetables such as lupine seeds such as vulgaris , buckwheat, sunflower or red pepper, and corn on the cob (5). Broccoli, which is among the most consumed vegetables especially in America and Europe, has vitamins (A, E, C) and flavonoids, and because it contains quercetin and kaempferol, which provide antioxidant properties, it has both immune-enhancing and antioxidant properties. Broccoli, which has a fibrous structure, ensures the removal of heavy metals from the intestines. In addition to this feature, broccoli has many benefits, especially prostate and breast cancer preventive power. Carotenes are an extremely colorful (red, orange, yellow) group of oil-soluble vegetable pigments. Carotenes, which play an important role in the human body, are converted to vitamin A in the body and have an antioxidant effect. These are called " provitamin A". Examples of this carotene group are lutein, lycopene , and zeaxanthin (9). Tomato, an annual plant native to Mexico and Peru, is considered a natural antioxidant due to its lycopene content. Lycopene is a pigment belonging to the carotene family found naturally in some vegetables and fruits. The human body cannot produce lycopene and must take this substance from outside.

In a study conducted to examine the relationship between carotenes and prostate cancer risk, it was explained that a carotene called lycopene has a protective feature against this cancer risk. It has been shown that men who receive high amounts of lycopene in their daily diet (6.5 mg/day or higher) have a 21% reduced risk of prostate cancer compared to those who receive less lycopene (10). In a study on whether pomegranate juice has an effect on antioxidant activity in liver and testis tissue of rats , it was determined that lipids in liver and testis tissues of rats given pomegranate juice. glutathione while peroxidation is reduced perosidase (GSH-Px) and catalase (CAT) were observed to increase (11). Green tea, polyphenol components camellia It is obtained from dehydration of sinensis leaves without oxidation (12). It has been shown that the flavonoids in the structure of tea have a very strong antioxidant effect and that these flavonoids protect cells from damage caused by free radicals (13). In addition to the enzymatic defense systems in the organism, there are also molecules with antioxidant properties that are formed endogenously or taken with food. These antioxidants can neutralize ROS directly .

2.4. Vitamin C (Ascorbic Acid)

Vitamin C is a water-soluble vitamin. It acts as a reducing agent for many compounds in the organism. It is also a powerful antioxidant due to its strong reducing activity. Ascorbate effectively scavenges H_2O_2, hypochlorite, superoxide, hydroxyl and peroxyl radicals and singlet oxygen. Converts all peroxyl radicals in the liquid phase to plasma lipids. keeps it without diffusing and in this way the lipid Inhibits the onset of peroxidation. It reacts with the α- tocopherol radical formed in the membranes to form α- tocopherol. Provides regeneration (6). Vitamin C has also been shown to be important for phagocytosis; It protects plasma lipids against peroxidation caused by activated neutrophils and is a potent hypochlorate scavenger (13). It provides protection against reactive oxygen species in cigarette smoke, and plasma vitamin C levels were found to be lower in smokers and passive smokers compared to non-smokers.

Nitrosamines found in some foods and cigarette smoke in various studies it has been shown to have an antitumorgenic role by inactivating it (14). It has been reported that ascorbic acid has antioxidant activity at high concentrations, as well as prooxidant activity at low concentrations. By reducing iron in the presence of transition metals, it contributes to the formation of $^{\bullet}OH$ radical with the Fenton reaction. Since transition metal ions are bound to proteins in healthy organisms, this situation is very limited in vivo and the antioxidant property of ascorbic acid is more dominant than the prooxidant property (15).

2.5. α- tocopherol

It is the main component of the vitamin E family, which is widely found in nature. Its antioxidant activity is due to the aromatic ring with the phenolic hydroxyl group in its structure (6). Lipid due to its lipophilic property. It is the most important chain breaking antioxidant of cell membranes and plasma lipoproteins against peroxidation. Lipid by removing peroxyl radicals peroxidation inhibits.

The α-tocopherol radical ($\alpha T^{\bullet}$) is a relatively stable radical with little reactivity. It can be conjugated with glucuronic acid and excreted in the bile. It can be re-reduced by ascorbic acid and glutathione after oxidation or before disposal. Thus, it is regenerated . Of in vivo and in vitro studies α- tocopherol and glutathione showed that peroxidase has complementary effects against free radicals. GSH- Px removes formed peroxides, while α- tocopherol inhibits the formation of peroxides (6).

In the development of atherosclerosis in recent years, lipid it is reported that peroxidation, especially LDL peroxidation, plays an effective and critical role. Lipid by α-tocopherol peroxidation is inhibited in the propagation step. It has been experimentally shown that the risk of coronary heart disease is reduced with the intake of α- tocopherol (16). α- tocopherol nitrites it acts as an anticarcinogen by inhibiting the conversion to nitrosamines, it is effective in preventing peroxidative damage associated with ischemia / reperfusion, increases immunity, essential for its stability. α-tocopherol also plays an important role in selenium metabolism by preventing the loss of selenium from the organism and keeping it active (16).

2.6. carotenoids

They are natural color pigments commonly found in plants. photooxidative Protects plants against processes. The most well-known is β-carotene, a precursor to vitamin A. Carotenoids are especially effective antioxidants that remove singlet oxygen ($^1O_2$) and peroxyl radicals. The most effective $^1O_2$ scavenger among carotenoids; It is lycopene , the open-chain analogue of β-carotene (17). LDL It also prevents the development of atherosclerosis and other coronary diseases by protecting against oxidative damage (17).

Lycopene photooxidative the process is an event that causes some diseases in tissues exposed to light such as eyes and skin and leads to the formation of ROS. In age-related macular damage that causes blindness, singlet oxygen-protecting pigments are particularly lutein and zeaxanthin. The pigment that protects against erythema and prevents photooxidative damage in sunburns is β- carotene. Due to their lipophilic properties, they play an important role in protecting cellular membranes and lipoproteins against oxidative damage. β-carotene exerts a synergistic effect with vitamins C and E in removing reactive nitrogen species (17, 18).

2.7. Flavonoids

They are yellow-white pigments found in high proportions in many fruits and vegetables. Secondary of plants metabolites are polyphenolic compounds. According to their ring structure, they are classified as flavonols, flavones, flavanones, anthocyanins, catechins and isoflavonoids. $O2^{\bullet-}$, lipid content of flavonoids and other plant phenolics alkoxyl ($RO^{\bullet}$), lipid scavenging peroxyl ($ROO^{\bullet}$) and $NO^{\bullet}$ radicals, chelating Fe and Cu, α- tocopherol It has also been

reported to participate in functions such as regeneration (19-21). This antioxidant, which is found in most of the plants, can be taken into the body in large amounts, especially with a fruit and vegetable-based diet, since it is found in much higher amounts than the antioxidant vitamins C and E.

2.8. Glutathione

Tripeptide it is a peptide containing three amino acids linked together by a peptide bond. It has three double bonds. Glutathione (γ-glutamyl-cysteine-glycine) is an antioxidant that protects cells from toxins such as free radicals. It plays a role in preventing the conversion of hemoglobin to methemoglobin by oxidation. It also keeps the sulfhydryl groups in proteins in a reduced state and protects these groups against oxidation . It is a powerful antioxidant and three effective anti- aging amino acids synthesized from L-cysteine, L-glutamic acid and glycine.

2.9. Melatonin

Melatonin is secreted from cells of the pineal gland called pineolacid. Biorhythm (circadian rhythm) determines or influences biorhythm. Pineolacite cells are light sensitive. As the electromagnetic wave intensity increases, melatonin secretion decreases. Melatonin is a type of ethanoamide . Melatonin is a hormone secreted between 23:00 and 05:00, although it varies from person to person. The main task of the hormone is to maintain the body's biological clock and regulate its rhythm. In addition, the strong secretion of melatonin has a protective effect against cancer. For this reason, it is requested that those who have leukemia and other cancers should be hospitalized in dark environments. According to recent studies, the hormone also has an anti-aging effect. Melatonin is a hormone that plays a role in many biological functions such as summer-winter, long-short day, regulation of the light-dark cycle (22). It is a very powerful antioxidant that eliminates the hydroxyl free radical, the most harmful free radical. It is considered to be the most powerful of the antioxidants known to date (23).

3. The Effect of Nutrition on the Antioxidant Defense System

The body's antioxidant balance is greatly affected by diet. Pathological conditions can occur when the body's defense mechanisms are destroyed due to nutritional deficiencies. The increase in reactive oxygen species and a deficiency

in the defense systems lead to the deterioration of the antioxidant balance in the body and the formation of "oxidative stress" conditions. The effectiveness of the antioxidant defense system; It depends on adequate intake of foods containing antioxidant vitamins such as vitamin E, vitamin C and carotenoids and essential trace minerals (24). These vitamins work together effectively to neutralize the effects of harmful reactive oxygen species that cause disease and damage. For example, there are studies investigating the effects of antioxidant vitamins on patients, such as studies evaluating vitamin B12 and folate levels (25). In some studies, in vitro antioxidant properties of polymer products synthesized by organic reactions were compared with synthetic antioxidants such as BHT and BHA, and new synthetic antioxidants could be used in drug production (26). It is possible to increase the number of these and similar examples.

Vitamin E (tocopherols), one of the fat-soluble antioxidants, are found in the membranes of all tissues and cells. Vitamin E inhibits degradation by its ability to combat the oxidation of polyunsaturated fatty acids, also known as fat degradation (27). It is reported that high vitamin E supplements strengthen the defense system by combating oxidative stress in the diet (28). Ascorbic acid is an important water-soluble antioxidant found in the body's extracellular fluids. Since it cannot be synthesized in the body, it must be taken from the outside with food. In addition to being a reducing agent of ascorbic acid, it also has the ability to regenerate vitamin E (27). Carotenoids are; They show their antioxidant activities by participating in free radical reactions and reducing the rate of formation of harmful hydrogen peroxides (29). β-carotene from important dietary carotenoids; in yellow, orange vegetables and fruits, green vegetables, lycopene; and lutein in tomatoes ; found in broccoli and fibrous green vegetables (27). Phenolic compounds, especially in plant foods, are considered among important antioxidants because they are reducing agents, hydrogen donors, singlet oxygen scavengers and metal chelators (30). Minerals such as selenium, copper, manganese and zinc are also required for the structures and catalytic activities of protective enzymes (27).

4. conclusion

The effect of nutrition on the antioxidant defense system shows its effects on the mechanisms of action of antioxidants as follows.

They act by trapping free oxygen radicals or converting them to weaker molecules. They act by adding a hydrogen to free oxygen radicals, reducing

their activity or making them inactive. They have the effect of breaking the chains of free oxygen radicals. Hemoglobin, ceruloplasmin and minerals show chain breaking effects. They have a repairing effect on the damage caused by free radicals. According to their ability to prevent cellular kinase losses, they stop oxidation reactions. They show their effects by increasing the synthesis of antioxidant enzymes such as SOD and non-enzymatic antioxidants.

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CHAPTER IV

HERBAL ANTIOXIDANTS: NARINGENIN, CURCUMIN AND ARONIA

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1. Introduction

Free radicals are defined as energy rich atoms or molecules that carry one or more unpaired electrons in their valance shell (1). Free radicals can easily react with other substances because they have unpaired electrons. Since atoms or molecules that have their electrons paired (conjugated) have a stable structure, their tendency to react with other molecules is not as great as that of free radicals. Therefore, molecules that are stable, do not have unpaired electrons, and react more weakly with other substances than radicals are defined as nonradicals (2). Free radicals can originate from oxygen and nitrogen. Radicals originating from molecular oxygen are called reactive oxygen species (ROS) and radicals originating from molecular nitrogen are called reactive nitrogen species (RNS) (3).

There are several defense mechanisms to keep the levels of reactive oxygen species in the body under control and prevent the damage they can cause (4,5). These substances that metabolize free radicals, prevent the formation of free radicals, or increase the scavenging ability of free radicals are called antioxidants. Antioxidants prevent lipid peroxidation by preventing the peroxidation chain reaction or scavenging reactive oxygen species. Antioxidant molecules donate electrons to free radicals to reduce their reactivity and maintain the cellular balance between pro-oxidants and antioxidants. There are many antioxidant systems in aerobic cells. These antioxidants are divided into two groups, endogenous and exogenous (5-8).

Based on their mechanism of action, endogenous antioxidants are most commonly classified as enzymatic and nonenzymatic antioxidants. Glutathione transferase (GST), glutathione reductase (GR), mitochondrial oxidase, glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) are considered as enzymatic antioxidants. Examples of non-enzymatic antioxidants are substances such as ceruloplasmin, glutathione, α-tocopherol, ferritin, albumin, bilirubin, ascorbic acid, transferrin, and uric acid. They form the earliest line of defense against oxygen radicals (5,9,10).

Allopurinol, folic acid, vitamin C, vitamin E, acetylcysteine, mannitol, adenosine, calcium channel blockers, nonsteroidal anti-inflammatory drugs, and iron chelators can be counted among exogenous antioxidants (5,6).

Antioxidants occur naturally in our body and in various foods. Antioxidants have a high affinity for free radicals and thus scavenge harmful molecules in our body to protect our health.

2. Polyphenols

Polyphenols are phytochemicals found primarily in tea, chocolate, coffee, fruits, legumes, vegetables, beverages, and grains. There are more than 8000 species of polyphenols found in nature. Since the main effect of polyphenols is antioxidant, they can assist in coping with free radical damage and ward off UV radiation or pathogen attack.

In recent years, the potential health benefits of dietary polyphenols as antioxidants have attracted more attention. Consumption of products such as grains, vegetables, legumes, and chocolate contributes to the absorption of polyphenols, thus protecting our bodies from chronic diseases like cancer, aging, cardiovascular, cerebrovascular, neurodegenerative diseases, and diabetes mellitus (11). Flavonoids, Stilbenes, Lignans, and Phenolic acids are four different classes polyphenols classified according to the number of phenolic groups and other structural components (12). There are two subgroups, hydroxy benzoic acids and hydroxy cinnamic acids.

Flavonoids

Studies have shown that diets rich in fruits and vegetables reduce the risk of several diseases, especially cardiovascular disease and some cancers (13). These positive health effects are largely due to vitamins and flavonoid components. The advantageous outcomes of flavonoids found in the plant kingdom in vivo

are suggested by their ability to scavenge oxygen-free radicals, reduce the activity of transition metals, and/or strengthen the endogenous antioxidant defense system (14). Flavonoids are classified into flavanones, anthocyanidins, catechins, flavonols, isoflavones, flavones, and chalcones (15).

Naringenin

Naringenin is one of the major naturally occurring flavonoids found in some edible fruits such as citrus fruits, tomatoes and also in figs of the genus Ficus carica (16,17). Naringenin has broad biological effects on human health. It reduces lipid peroxidation and protein carbonylation and supports carbohydrate metabolism, strengthens the antioxidant defense system, eliminates reactive oxygen species in the biological environment, regulates immune system activity, and also has antiatherogenic and anti-inflammatory effects (17).

Studies have shown that consumption of citrus plants, which are high in flavonoid antioxidants, has many beneficial properties such as anticarcinogenic, antiviral and anti-inflammatory effects (18). The flavanone naringenin and its glucosides are widely distributed in nature. Citrus juice [such as grapefruit] contains high amounts of naringenin (19).

In vitro studies on various cell models have shown that naringenin, the major flavanone of grapefruit (Citrus paradisi), is a potent antioxidant (20). In vivo studies, naringenin partially protected against oxidative stress induced by oxytetracycline in rat liver (21).

Flavonoids are structurally composed of 15 carbon atoms, 3 carbon chains, 2 benzene rings, and 3 chains. Naringenin is commonly found in the skin of grapes and tomatoes and in citrus fruits such as oranges and grapefruits. Naringenin is particularly responsible for the bitter taste of the fruit and is therefore abundant in grape fruit (21,22).

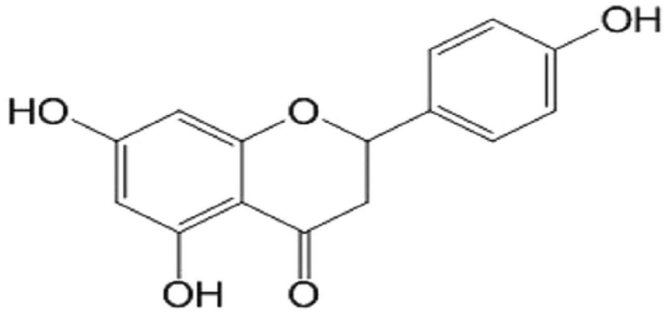

Figure 2.1. Structure of naringenin (21)

In studies on mice, naringenin has been shown to have an effect on obesity and hepatitis C in the liver and to reduce sugar-induced neuropathy in diabetes mellitus (23).

In a study conducted to examine the role of naringenin in glucose and lipid metabolism, one group was given a normal diet, while the other group was given a diet containing naringenin. It was determined that the postprandial glucose level and insulin level were lower in the naringenin group compared to the control group. At the same time, it was determined that intra-abdominal and subcutaneous adiposity and monocyte chemotactic protein-1 and IL-6 levels decreased and hepatic steatosis improved (24).

In an experimental research, naringenin administration was found to lower levels of conjugated dienes, lipid hydroperoxides, and thiobarbituric acid reactive substances (Chtourou et al., 2015). Additionally, it might increase the activity of antioxidant enzymes such glutathione reductase, catalase, superoxide dismutase, and glutathione peroxidase (25).

Yao et al. evaluated the safety and therapeutic efficacy of naringen in the treatment of pediatric bronchial pneumonia. According to the results of the study, they concluded that naringenin can shorten the time to disappearance of clinical symptoms, inhibit inflammation, decrease the incidence of bronchial pneumonia complications and connected adverse reactions, and enhance the patients' health (26).

Researchers studied the effects of naringenin treatment on cancer and discovered that it inhibits inflammatory and survival signaling pathways. In addition to suppressing tumor growth, naringenin was shown to reduce cancer metastasis. Naringenin may be used as a chemotherapeutic drug to treat prostate cancer, according to research by Lim et al. (27).

Some drugs may interact with naringenin during intestinal absorption to alter circulating drug levels, resulting in increased or decreased drug effects. Therefore, consumption of citrus fruits (especially grapefruit) and other fruit juices with drugs is not recommended to avoid interactions with drug absorption and drug metabolism (21-23).

Curcumin

Turmeric (Curcuma longa) is a spice of great interest to researchers in the medical world. Turmeric is a rhizomatous herb of the ginger family (28). The medicinal properties and benefits of turmeric containing curcumin have been

described previously (29). Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione], also called diferuloylmethane, is a natural polyphenolic constituent found in the rhizome of Curcuma longa. It is a tropical plant used as a spice and dye. The yellow pigments are obtained by grinding the roots of the plant.

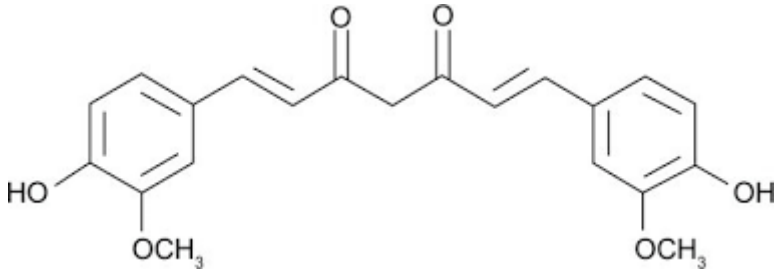

Figure 2.2. Structure of curcumin (30)

Curcuma longa is traditionally used as a medicinal plant in Asian countries for its anti-inflammatory, anticancer, antioxidant, antimicrobial, and antimutagenic properties (29). Polyphenol curcumin has been shown to have many health benefits as it targets multiple signaling molecules and is also active at the cellular level (31). It has been shown to help treat inflammatory diseases, metabolic syndrome, pain, and inflammatory and degenerative eye diseases (32). It has also been shown to have a beneficial effect on the kidneys (33). Many of the many benefits of curcumin are due to its antioxidant and anti-inflammatory effects (32).

Since curcumin has antioxidant and anti-inflammatory properties, it has several effects on the body (34). Curcumin is effective in reducing oxidative stress (35). Antioxidants such as superoxide dismutase (SOD) have been found to increase serum activities. Consumption of turmeric helps to reduce the risk of developing cancers and has a protective effect in humans (36).

Systematic research and meta-analysis studies on curcuminoids have shown a significant effect on serum lipid peroxides and glutathione peroxidase concentrations, including all oxidative stress parameters studied, including plasma activities of SOD and CAT (35). Since curcuminoids are poorly absorbed, a form of formulation was prepared to overcome the problems of bioavailability in meta-analysis studies. The effect of curcumin on free radicals is achieved by several mechanisms. It can scavenge various forms of free radicals such as ROS and RNS (37). It can modulate the activity of CAT, SOD, and GSH enzymes that are active in neutralizing free radicals. It can also inhibit ROS -producing

enzymes such as lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase (34). In addition, curcumin is a lipophilic compound that effectively scavenges peroxyl radicals, so curcumin is considered a chain-breaking antioxidant like vitamin E (38).

Curcumin is able to suppress the expression of various cell survival and proliferative genes involved in apoptosis, cellular proliferation and transformation by nuclear factor kappa B activity inhibition. Tumor cell proliferation has been suppressed in many species by inducing tumor suppressor genes such as p53, activating caspase, and reducing the expression of reactive oxygen compounds. It has limited the effect of human EGFR-2 (HER2) and TNF, a growth factor for tumor cells, which is closely associated with breast, lung, kidney and prostate cancers. Some experimental studies have demonstrated that curcumin may exert its efficacy in anti-inflammatory effect via peroxisome proliferator-activated receptor-c activation, a group of transcription factors that regulate gene expression (39).

In clinical studies conducted in recent years, the effectiveness of turmeric and its curcumin content against many diseases have been evaluated. Some promising effects have been observed in obesity, diabetes, depression, arthritis, skin diseases, inflammatory bowel disease, muscle lesions, premenstrual syndrome symptoms, gynecological diseases and inflammatory diseases (40).

In particular, several clinical studies have evaluated the efficacy of curcumin consumption in patients with obesity, metabolic syndrome, or diabetes. Based on the results of these studies, it was found that curcumin consumption has a positive effect on weight management, lowering blood lipids and improving glycemic control in obese individuals. These results are supported by a meta-analysis showing that curcumin supplementation can reduce plasma leptin levels, and a systematic review demonstrating that it reduces glycosylated hemoglobin levels A1c and fasting glucose levels (41,42). Curcumins have been reported to reduce serum total cholesterol, serum triglyceride and liver cholesterol levels. There is also evidence that it lowers LDL and raises HDL.

Aronia

Aronia is a shrub plant also known as chokeberry. It is also popularly known by various names like bird cherry or hunter's grape. Aronia; the Rosaceae family belongs to the genus Aronia. There are three known species in this genus. The most common Aronia cultivars in Europe include 'Aron' (Denmark), 'Viking'

(Finland), 'Nero' (Czech Republic), 'Kurkumachki' (Finland), 'Rubin' (Russia), 'Hugin' (Sweden) , 'Fertoedi' (Hungary) 'Albigowa', 'Dabrowice', 'Egerta', 'Kutno', 'Nova', 'Wies', 'Hakkija', 'Ahonnen', 'Serina' (Poland) (43-47).

The chokeberry has been used by Native Americans for centuries. Native Americans, whose homeland is North America, often use the aronia berry in their meals because of its powerful effects. They have benefited from this fruit to stay resistant to diseases and protect their immunity in harsh winter conditions. After 1900, aronia berries began to be cultivated in Europe and Russia from North America. In Eastern Europe, especially in Germany, it has been grown commercially since 1950. Most notably, since 2009, the Midwest Aronia Union has been established in the United States, organizing meetings and events every year to promote its cultivation. Thanks to scientific research in the last 15-20 years, the amazing ingredients of the chokeberry have been discovered and its popularity has increased thanks to its health benefits (43-53).

Aronia berry contains various complex chemicals, is rich in phenols, minerals and vitamins. The phenolic substances in chokeberry are very valuable for medicine. It contains a very high amount of anthocyanins, proanthocyanidins and phenolic acid. Anthocyanins make up 25% of the total content of phenolic substances. The most important anthocyanin is cyanidin-3-glycoside. Anthocyanins include 3-xyloside, cyanidin-3-glucoside, 3-arabinoside, and 3-galactoside. *Aronia melanocarpa* (Michx.) *Elliott's* (Aronia) contains significant amounts of hydroxycinnamic acids in addition to anthocyanins: chlorogenic acid and its isomer neochlorogenic acid (43-53). Aronia berries are also called chokeberry because they are rich in polyphenolic compounds such as anthocyanins, because the fruits gain astringent properties and create a puckering sensation in the mouth.

Fresh fruits have a dominant bitter almond odor because they contain a large amount of amygdalin. Amygdalin has a great effect on the healing of cancer cells and the prevention of some cancers, especially stomach, colon, liver and prostate. About 1 kg of chokeberry contains 20 g of polyphenols and 4-8.5 g of anthocyanins, quite high amounts of K, Zn, Na, Ca, Mg, Fe and vitamins A, C, E, K, B1, B2, B6, B12. has. The content of vitamins and minerals in processed fruit juice ranges from 300-600 mg per 100 ml. In addition, the juice is rich in potassium and zinc. Fresh fruit contains abundant B1, B2, B6, vitamin C and niacin. Aronia fruit contains large amounts of carotene, as well as vitamins and minerals (43-53). It contains all vitamins except vitamin D. That's why aronia is called Super Fruit.

Studies on the effects of chokeberry (*Aronia melanocarpa* (Michx) *Elliot*), which belongs to the berry fruits, on human health have shown that its fruits have a higher value than other berry fruits in terms of antioxidant capacity and anthocyanin content. Studies conducted in recent years have shown that the Oxygen Radicals Absorbance Capacity (ORAC) value of chokeberry is quite high compared to other products. Since aronia has a very high antioxidant capacity among berry-like fruits, it can be consumed as a fresh fruit in the world, as well as used in the pharmacy and food industry. It has been used as a medicine by the natives for centuries. They dried their fruits and used them as an addition to meals, and dried their leaves to make tea (43-53).

There are many studies in the literature regarding the health benefits of aronia. Aronia fruits have been found to be beneficial for many diseases and human health, especially for their anticancer properties. Indeed, many of the suggested health benefits of aronia are due to its polyphenol content. In the study of Tolic et al., it was determined that aronia has the highest polyphenol content among 143 plants (50). In the study on the effects of aronia fruit on chronic diseases, it was stated that the antioxidant activity of aronia fruit is high, its phenolic compounds are very valuable, and the importance of anthocyanins and procyanidins (54). In the study examining the chemical composition of aronia fruit, fruit juice and concentrate, aronia tea and dried fruit, it was reported that the total phenolic content of dried fruit was higher when compared to fresh fruit and fruit juice concentrate (55). It has been determined that the phenolic component content of aronia fruit varies depending on the maturity period of the fruit, variety and ecology.

Wang et al. examined the effects of *Aronia melanocarpa* (AM) on alcohol-induced liver injury in mice, and concluded that AM prevents alcohol-induced liver injury by suppressing oxidative stress through the Nrf2 signaling pathway. In a study evaluating the effects of aronia juice in rats with liver damage, it was found that the juice reduced the severity and symptoms of liver damage (43).

Omairi et al. determined that aronia application in A549 human lung cancer cell line showed apoptotic activity by down-regulating PARP-1, caspase-3 and Bcl-2 proteins, and they predicted that aronia can be used in the treatment of lung cancer due to its minimal side effects (56). Another study found that aronia extract helped reduce cell damage due to breast cancer. Researchers concluded that aronia extract has protective properties in people suffering from breast cancer. In a study investigating the effects of grape, aronia and blueberry

extracts on colon cancer, it was found that all extracts inhibited the growth of cancer cells, while aronia had the strongest effect (57).

It has been supported by some studies that aronia is also effective in preventing the development of diabetes and obesity, strengthening the immune system and reducing insulin resistance (46,58). In one study, it was thought that aronia might protect against coronary artery disease, protecting against plaque that develops inside the arteries. They discovered that aronia is effective in lowering blood pressure and can help fight high blood pressure in the arteries (59).

Regular consumption of this fruit has been found to protect against cardiovascular disease, digestive system diseases and some types of cancer. Aronia extracts can help lower serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol in patients with metabolic syndrome, as well as glucose levels in the blood. Aronia fruit extract has been found to reduce risk factors for insulin resistance by modulating several signaling pathways associated with insulin signaling, adipogenesis, and inflammation. Many in vitro studies and animal studies show the antiproliferative or protective effects of chokeberry fruits and extracts, especially in colon cancer. Numerous studies confirm the effects of consumption of *Aronia melanocarpa L.* varieties on hypertension, glucose metabolism disorders, dyslipidemia, proinflammatory conditions and reduction of metabolic syndrome risk factors (43-53).

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CHAPTER V

MECHANISM OF ANTIOXIDANT DEPLETION IN AMYOTROPHIC LATERAL SCLEROSIS

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1. Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a devasting progressive neurodegenerative disease that results in the death of motor neurons and skeletal muscle atrophy. Pathology of ALS includes oxidative stress, neuroinflammation, mitochondrial dysfunction, excitotoxicity, protein aggregation, and changes to axonal transport and structure. Neuronal cell death is a result of, and contributor to, this process in ALS. Mutations affecting endogenous antioxidant function, such as in ALS, an increase in reactive oxygen or nitrogen species (ROS/RNS) occurs. The presence of ROS/RNS can cause neuroinflammation which is characterized by activation of glial cells, including astrocytes, microglia, and resident macrophages, along with infiltrating immune cells, in the central nervous system (CNS). These cells, particularly astrocytes and microglia, release pro-inflammatory cytokines, chemokines, and oxidative species which further damage neurons and add to the oxidative burden. As a result, mitochondria experience damage to the electron transport chain (ETC) and energy metabolism which result in apoptosis. Physical and chemical damage from increased ROS/RNS generation by these dysfunctional mitochondria and neuroinflammatory glial cells can further contribute to excitotoxicity in ALS (1).

2. Amyotrophic lateral sclerosis

ALS is a relatively rare but incurable and relentlessly progressive neurodegenerative disease, characterized by motor neuron loss in the brain and spinal cord (2). ALS begins insidiously with local symptoms, but rapidly spreads to majority of muscles, which eventually leads to death commonly due to respiratory failure. Although around 10% of ALS patients survive 10 years or longer, vast majority of patients die within 3-5 years after symptom onset (3).

Motor neurons are grouped into corticospinal motor neurons in the motor cortex (upper motor neurons) and bulbar or spinal motor neurons (lower motor neurons). In a healthy person, the upper motor neurons make direct or indirect connections with the lower motor neurons, which subsequently innervate skeletal muscles and control movement. In ALS patients, however, the communication between brain and muscles is interrupted by the deficit of either the upper motor neurons (presenting as stiffness and spasticity) or lower motor neurons (presenting as fasciculation and amyotrophy), or both (3,4).

2.1. Oxidative Stress and Mitochondrial Dysfunction in ALS

Oxidative stress is the excessive production of ROS/RNS in the form of free radicals. Oxidative stress markers are elevated in ALS patients (5). In the case of sporadic ALS, excessive oxidative stress may be due to environmental factors such as toxins, pesticides, and heavy metals, along with lifestyle habits (5,6). Epidemiological and case control studies have linked pesticides, agricultural chemicals, and heavy metal exposure to the development of ALS (7-9). Lifestyle factors such as smoking, strenuous physical activity, and stress also may increase oxidative stress and contribute to the development of ALS (10-12). Lastly, the pathology from single or repeated head trauma, even of mild severity, results in overt oxidative stress and has been researched as a potential contributor to ALS in athletes and military personnel (12,13).

These factors, along with dysfunction in the mitochondrial ETC that comes with aging, likely contributes to oxidative stress in sporadic ALS cases (14). In fact, mitochondrial function deficits are present prior to symptom onset (15). In the aging individual, mitochondrial function is reduced, and ROS generation is increased as a byproduct of dysregulated ATP production. Furthermore, the number of damaged mitochondria with mutated DNA also increases with age and further contributes to ROS generation (16). Dysfunctional calcium regulation and ETC activity, along with morphological changes to mitochondria, have been observed in cell and animal models of ALS (17).

Familial ALS cases have been linked to mutations in chromosome 9 open reading frame 72 (C9orf72), TDP-43, fused in sarcoma (FUS), and SOD-1. Mutations in C9orf72 and SOD-1 account for the majority of familial ALS cases. Mutations to SOD-1 account for 20-25% of all familial cases, making it the second most common ALS mutation behind mutations to C9orf72 (18). Furthermore, recent data from an analysis of 22 countries has reported that, out of 2,876 prevalent mutant SOD-1 cases, 47% were familial and 53% were sporadic cases (19). Also, over 100 mutations in SOD-1 have been identified in patients with ALS (20).

Superoxide dismutase-1 is an endogenous antioxidant enzyme with a copper binding site that can be reduced by superoxide to form dioxygen. An additional molecule of superoxide oxidizes the reduced copper ion to form hydrogen peroxide (20). In ALS, further research is needed to understand how mutations in SOD-1 result in increased oxidative stress. Some studies have reported that SOD-1 loses its function and is unable to reduce superoxide resulting in increased levels of superoxide and peroxynitrite. Other studies have reported increased activity of SOD-1 resulting in increased production of hydrogen peroxide, hydroxyl radicals, and SOD-1 protein aggregation (21). Mutated SOD-1 may also render normal SOD-1 nonfunctional (14). Mutations in SOD-1 also contribute to mitochondrial dysfunction as mutant SOD-1 aggregates in the mitochondrial outer membrane (22).

Oxidative stress can result in DNA, lipid, and protein damage. Patients with ALS have increased levels of MDA, 4-HNE, 8-oxo-2'-deoxyguanosine, 3-NT, and protein carbonyls in blood, cerebrospinal fluid, spinal cord, and/or motor cortex (5,14). This damage contributes to the motor neuron death that is characteristic of ALS. Furthermore, there is crosstalk between oxidative stress and other pathological mechanisms present in ALS, such as those described below, which further contribute to motor neuron death.

2.2. Neuroinflammation in ALS

Inflammatory markers and cytokines released from glial and immune cells, such as IL-6, -8, and -1β, TNF-α, c-reactive protein, and interferon-γ (IFN- γ) have been found to be upregulated in ALS (23). This dissertation will focus on the role of activated astrocytes and microglia in ALS, termed neuroinflammation, as a potential therapeutic target.

In ALS, astrocytes become toxic, release pro-inflammatory cytokines, and are typically located near dying neurons (24). Astrocytes express mutated genes

that have implications in ALS, including mutant SOD- 1, and have been shown to cause neurotoxicity and increased levels of pro-inflammatory cytokines in culture which likely contributes to motor neuron death (25). Microglia are resident immune cells of the brain that sense the environment, exhibit macrophage activity, and respond to toxins. Like astrocytes, microglia also express ALS mutant genes, such as mutant SOD-1, and contribute to motor neuron death. Both microglia and astrocytes can adopt anti- and pro-inflammatory phenotypes. Classically, an M2 phenotype is anti-inflammatory and promotes cell and tissue regeneration while an M1 phenotype is pro-inflammatory and is characterized by the generation of ROS/RNS and pro-inflammatory cytokines (26). In a healthy individual, these phenotypes aren't polarized but astrocytes and microglia express these phenotypes on a spectrum. In ALS, it seems that initially, many microglia adopt an anti-inflammatory, M2 phenotype. However, as the disease progresses, microglia take on a pro-inflammatory M1 phenotype (27). Activated M1 microglia have been found in the motor cortex and spinal cord of ALS patients (28). Lastly, there is crosstalk between astrocytes and microglia. For example, a reduction in microgliosis has been shown to decrease the activation of astrocytes (29). Similarly, removal of astrocytes expressing mutant SOD-1 and replacement with wild-type astrocytes resulted in decreased microglial activation (25). Regardless of the precise hierarchy of glial involvement in ALS pathogenesis, it is clear that these non-cell autonomous mechanisms play an important role in disease progression.

Motor neurons also express mutant genes, such as SOD-1, however, this alone does not seem to cause motor neuron death (25). Furthermore, wild-type glial cells not expressing mutant ALS genes extend survival of motor neurons in animal models (30). These data indicate that glial cells such as astrocytes and microglia contribute to the death of motor neurons through the loss of necessary functions such as motor neuron support, homeostasis, and synapse support resulting in excitotoxicity and pro-inflammatory processes. Astrocytes and microglia also gain a toxic function through expression of mutant genes, pro-inflammatory activities, and generation of ROS/RNS, which further contribute to oxidative stress and motor neuron death.

2.3. Excitotoxicity in ALS

As mentioned above, healthy astrocytes have roles in synapse maintenance and neurotransmitter homeostasis. These processes include a dominant role in glutamate clearance from the synapse. In ALS, astrocytes have reduced

functionality in this role which leaves excessive glutamate in the synapse and causes ionotropic glutamate receptor activation on the post-synaptic neuron (31). Astrocytes have the EAAT2 transporter which is responsible for uptake of glutamate from the synapse, and this transporter displays decreased expression in ALS (32). Also mentioned prior, oxidative stress and mitochondrial dysfunction are both consequences and causes of excitotoxicity in ALS, as mitochondria have calcium buffering functions which become unregulated in ALS.

In general, motor neurons express a high number of AMPA receptors and exhibit an increased response to calcium influx (33). Motor neurons respond to glutamate as their primary excitatory neurotransmitter and are sensitive to excitotoxicity, particularly by AMPA ionotropic glutamate receptors. In the case of mutant SOD-1 ALS, aggregation of mutant SOD-1 causes death of motor neurons specifically. Calcium influx through AMPA receptors can further contribute to mutant SOD-1 aggregation and the death of motor neurons (34).

Furthermore, altered glutamate metabolism has been observed in ALS patients (35). There is sound evidence of excitotoxicity caused by serum, plasma, and cerebral spinal fluid (CSF) isolated from ALS patients. Plasma, serum, and CSF from ALS patients induces cytotoxicity in cultured neurons (36). The neurotoxicity of CSF from ALS patients was blocked by an AMPA receptor antagonist, and an NMDA receptor antagonist showed a milder protection which suggests excitotoxicity plays a role (37). Overall, it seems that glutamate excitotoxicity is an important underlying pathology observed in ALS (38). However, it remains unclear whether this is due to increased extracellular glutamate or to changes in glutamate transporters, and what effect it has on disease phenotype. Andreadou et al. (39) found increased plasma glutamate levels were correlated with longer disease duration in lower limb onset ALS but were not observed in bulbar onset ALS. Vesicular glutamate transporters, which package glutamate into vesicles for synaptic release, when reduced in expression in a mouse model of ALS, did protect motor neurons from death but ultimately had no effect on overall survival or disease duration (40).

2.4. Observed Protein Aggregation Contributes to ALS Pathology

Protein aggregation has been observed in both sporadic and familial cases of ALS. Proteins encoded by genes such as SOD-1, TDP-43, FUS, and C9orf72 (e.g., dipeptide repeat proteins) have been observed to aggregate in ALS patients. Cytoplasmic inclusions of these proteins, classified as lewy-body like or neurofilamentous, have been observed in motor neurons and glial

cells located in brain and spinal cord isolated from ALS patients (41). RNA binding proteins specifically, such as TDP-43, FUS, and heterogeneous nuclear ribonucleoproteins A1/A2 (hnRNP A1/2), can become mutated and/or drawn into RNA foci and/or stress granules, and cause mislocalization of the protein and impaired nuclear-cytoplasmic transport. Inclusions of these proteins have been observed in patients with ALS (42). Their functions and regulation of mRNA stability vary depending on the location, whether nucleus or cytoplasm, which further supports the idea that improper localization of RNA binding proteins may disrupt the regulation of mRNA and resulting in disease pathology (43).

Aggregation of mutant SOD-1 has been observed in sporadic and familial ALS cases. Although more research is needed, SOD-1 may have similar roles to RNA binding proteins and its aggregation could have implications in the processes described above (44). Mutant SOD-1 does localize to RNA rich structures, support RNA metabolism, and SOD-1 inclusions do contain RNA (45). Aggregation of SOD-1 has been directly linked to motor neuron and glial cell death (46).

There are a few hypotheses which help explain why protein aggregation contributes to ALS disease pathology. Many observed protein aggregates in ALS have functions as RNA binding proteins or support RNA metabolism and, when these proteins are mutated and not performing their functions, there is a loss of regulation of RNA metabolism and disrupted transport of RNA from nucleus/cytoplasm. The improper formation or disintegration of stress granules also seems to play a role in ALS. Stress granules control mRNA localization, stability, and translation (47). In response to stress signals, granules will sequester mRNA molecules, translation initiation factors, and RNA binding proteins such as those mentioned above. During stress, bulk translation is inhibited but when the stress signal dissipates, translation can be reinitiated, and the stress granules disassemble due to their dynamic nature (43). Mutated RNA binding proteins that localize to stress granules can prevent their disassembly or can lead to aberrant formation causing a disruption in these regulatory processes (48). The accumulation of mutant proteins may also act in a prion-like manner, causing the aggregation of other normal and mutated proteins such as SOD-1 and TDP-43 and encouraging misfolding of these proteins (49). Lastly, protein aggregates may also disrupt axonal transport of proteins, lipids, and mRNA which will be further described below, although the connection is not fully understood and conflicting evidence exists (50).

2.5. Dysfunctional Axonal Transport, Synaptic Failure, Motor Neuron Death, NMJ Deterioration, and Muscle Atrophy in ALS

Increased oxidative stress, mitochondrial dysfunction, neuroinflammation, excitotoxicity, and protein aggregation can all contribute to axonal transport deficits. Mutations to axonal transport machinery such as to dynein or kinesin, or that affect microtubule stability, have been observed in ALS (51). Excitotoxicity, oxidative stress, and mitochondrial damage are the cause and result of abnormal mitochondrial transport along axons (52).

Protein aggregates of TDP-43 and SOD-1 can cause aberrant activation of signaling pathways, such as activation of p38 mitogen-activated protein kinases or c-Jun kinases, which play roles in microtubule and kinesin transport (53). Deficits in anterograde and retrograde axonal transport which transport mRNA, proteins, lipids, vesicles, and mitochondria have been observed in in vivo models of ALS. Hyperphosphorylated neurofilament heavy polypeptide chain, a marker of axonal loss, abnormal accumulation of mitochondria and lysosomes, and axonal spheroids containing vesicles, lysosomes, mitochondria, and microtubules, have all been observed in patients with familial and sporadic forms of ALS (52).

Upper motor neurons have cell bodies in the cerebral (motor) cortex and lower/spinal motor neurons have cell bodies in the ventral horn of the spinal cord. Their axons allow for movement and reflex responses, with spinal motor neuron axons extending for several meters (54). The synthesis of proteins and lipids occurs in the cell body; therefore, the axon is also crucial for the transport of neurotrophic factors, proteins, and mitochondria (55). Furthermore, neurotransmitters in vesicles are transported along the axon for release at axon terminals. In the case of lower/spinal motor neurons, the neurotransmitter release of acetylcholine occurs at the NMJ, or the synapse between the motor neuron axon and the muscle it innervates. An early event in ALS pathogenesis appears to be the retraction of motor axons away from NMJs: this in turn, leads to loss of skeletal muscle innervation, muscle atrophy, a dying back process of motor neuron cell bodies, and resulting loss of movement. However, the precise order in which these events occur is subject to debate (56).

2.6. Endogenous Antioxidant Depletion Underlies ALS Pathology

All of the pathological mechanisms described above contribute to the overall antioxidant depletion observed in ALS patients (Figure 1). Much of

the pathology occurs prior to symptom onset and therefore goes undetected for prolonged periods. Long term oxidative stress, excitotoxicity, mitochondrial dysfunction, neuroinflammation, and accumulation of toxic protein aggregates deplete the endogenous antioxidant system (57,58).

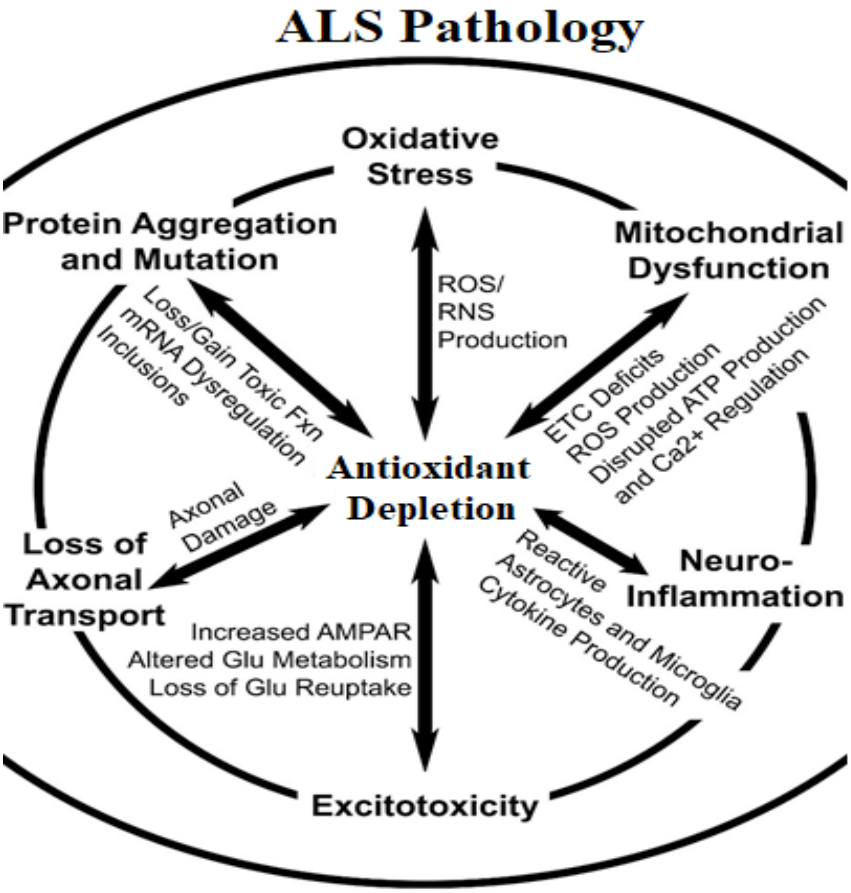

Figure 1. Antioxidant Depletion and ALS (57,58)

Depletions in antioxidant defense markers have been observed in ALS patients, although this is largely dependent on disease stage, progression, and duration of disease since diagnosis. As described in depth above, GSH is an endogenous antioxidant which has roles in reducing free radical oxygen. Glutathione levels, along with enzymes involved in GSH antioxidant activity such as catalase, GR, and glucose-6-phosphate dehydrogenase, have been shown to decrease with ALS disease progression and these deficiencies have been observed in both sporadic and familial forms of ALS (57-59).

SOD-1 is also a powerful cellular antioxidant. Copper and zinc binding to SOD-1 helps maintain copper homeostasis and facilitates the conversion between copper and cuperate, respectively. Many mutations to SOD-1 affect copper or zinc binding to SOD-1 rendering these processes dysfunctional in ALS (60). As described above, mutations to SOD-1 can result in loss of function, cause increased activity and overproduction of ROS, or may inhibit functional SOD-1 and/or induce its aggregation.

On a transcriptional level, both SOD-1 and GSH play roles in, and are affected by, activation of the Nrf2 (nuclear factor erythroid 2 [NF-E2]-related factor 2 [Nrf2])–Keap1 (Kelch-like erythroid cell-derived protein with CNC homology [ECH]-associated protein 1) signaling pathway. This pathway becomes activated in response to oxidative stress.

When this occurs, cysteine residues on Keap1 are modified which results in its degradation and disassociation from Nrf2, targeting Keap1 for degradation and eliminating the interaction with Nrf2. Once freed from Keap1, Nrf2 forms a complex on antioxidant response elements in gene promoters and regulates gene expression of NAD(P)H quinone oxidoreductase 1, GSH S-transferase, and glutamate-cysteine ligase (61). Nrf2 levels in SOD-1 mutant ALS have been found to be reduced in animal models and ALS patients (62).

Reduced response of this signaling pathway to oxidative stress helps explain why GSH and supporting enzymes are reduced in ALS patients (63). This indicates a loss of the endogenous antioxidant response to oxidative stress in ALS, although this is likely not the only pathway involved.

3. Conclusion

ALS is the most common adult motor neuron disease, and the incidence of ALS is increasing in both females and males worldwide. The median survival time of ALS patients is approximately three years after the first symptoms have started, and there is no fully effective treatment for ALS, only two approved drugs, riluzole, and edaravone, are used for the patients with ALS. These drugs are only effective at the early stages of ALS, however, the diagnosis of ALS is usually difficult and takes time, since there are no definitive prognostic biomarkers or pathognomonic tests are existing, also patients show significant variability in terms of progression and presentation of the disease symptoms. Studies have confirmed that there is a link between oxidative stress and the pathogenesis of ALS disease, that oxidative stress plays a role in the progression

of ALS disease, and therefore there is a need to develop specific peripheral biomarkers during the follow-up of the disease.

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CHAPTER VI

PEDIATRIC CONGENITAL DEFORMITIES

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The nervous system develops from neural plaque in the 3rd week(1). At the end of the 3rd week, the lateral edges of the neural plate rise, forming neural folds (2). This region between the folds is called the neural groove. Neural folds close along the line, approaching each other along the middle line. As a result of these events, the neural tube is formed (1). The cranial neuropore closes on the 24th day and the caudal neuropore closes on the 28th day. With the closure of the neuropores, neurulation is completed. The central nervous system has become a closed tubular structure consisting of a wide cephalic section with brain vesicles, a narrow caudal section with a spinal cord (1).

Neural tube defects are formed by factors affecting the embryo in the first four weeks of life (3). Closure defects of the anterior neuropore are formed into anencephaly and encephalocele. The closure defects of the posterior neuropore, on the other hand, cause spina bifida, meningocele and meningomyelocele (3).

If we look at the etiology, we can collect it into two main groups (4). The first group; is neural tube defects (NTDs) due to a specific etiology and usually accompanied by other malformations (10%) (4). These are divided into two groups themselves; Single gene mutations; Chromosomal abnormalities (Meckel-Gruber syndrome, Chemke syndrome, etc.); (Trisomy 18 and Trisomy 13, etc.), and pregnancy. Other special conditions such as amniotic rupture, amniotic band, which occurs before the week. The second group is isolated developmental NTDs due to multifactorial inheritance (90%)(4).

In the prenatal period in NTDs patients, alpha-fetoprotein is found in the maternal serum and fetal specific gamma-globulin is found high in the amniotic fluid.

1. Spina Bifida Occulta

It is the mildest form of NTDs. They usually do not give symptoms. The congenital spinous process and arcus vertebrae are not formed in one or rarely more than one vertebra. However, the spinal cord and spinal nerves are normal. In the meninges, there is no herniation. Chiari type II or hydrocephalus is not observed. There may be signs on the skin such as hairy structure, dermal sinus, dimple, hemangioma, lipoma (5)(Figure 1).

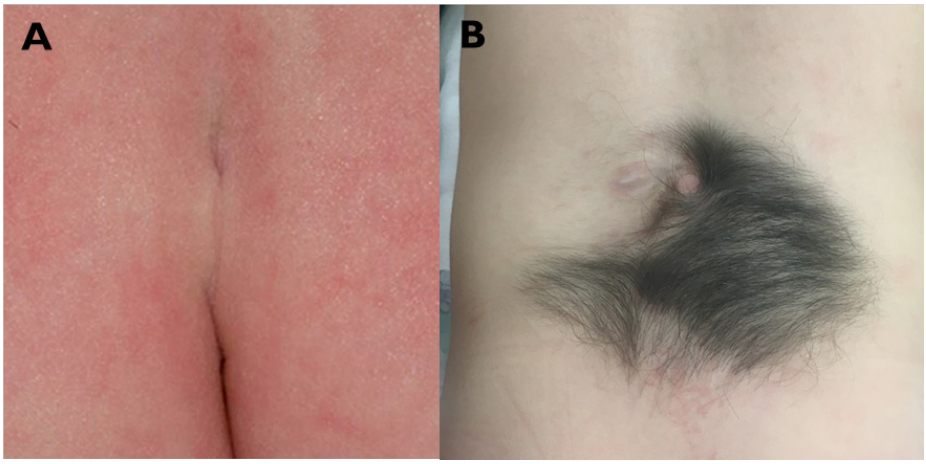

Figure 1: The images are taken from own clinical archive.
A: Sacral Dimple B: Hairy structure

2. Meningomyelocele

It is a neural tube defect in which the skin, vertebrae, nerve roots, spinal cord, and meninges are affected.(Figure 2)(6) The incidence is shown as 2-3/1000 live births. They are usually seen with lumbosacral or lumbar placement. The proportion of cervical and thoracic lesions is approximately 11%.(7)

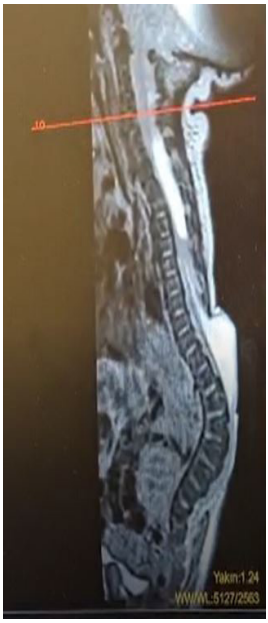

Figure 2: The image is taken from own clinical archive.

MRI image of a meningomyelocele patient is shown.

It occurs when polygenic or environmental factors are affected together. Autosomal recessive inheritance can be a factor. Although it is most common in people of Scottish and Irish descent, the use of medications such as carbamazepine and valproic acid by the mother is highly effective in the formation of folic acid deficiency. It is more common in females (60-70%). In addition, the risk is 4-8% higher in those who have previously given birth to a baby with NTDs.

After childbirth, the thin membrane of the meningomyelocele often ruptures, and the cerebrospinal fluid flows out. In such a situation, urgent surgery is required. However, otherwise, surgery can be performed within the first 48 hours (Figure 3). The baby is laid flat/laterally. The lesion is covered with a wet dressing and soaked intermittently. To prevent meningitis, the combination of ampicillin + gentamicin is often started. 50% of these patients have a latex allergy. Therefore, the gloves used should be taken care of in this regard. If they are not treated, meningeal epithelization develops in the membrane and neural layer, the fibrous band can curl in the cord, create compression, and death can occur. In some cases, an epidermoid or dermoid cyst may develop because the skin cells pass into the scar over time.

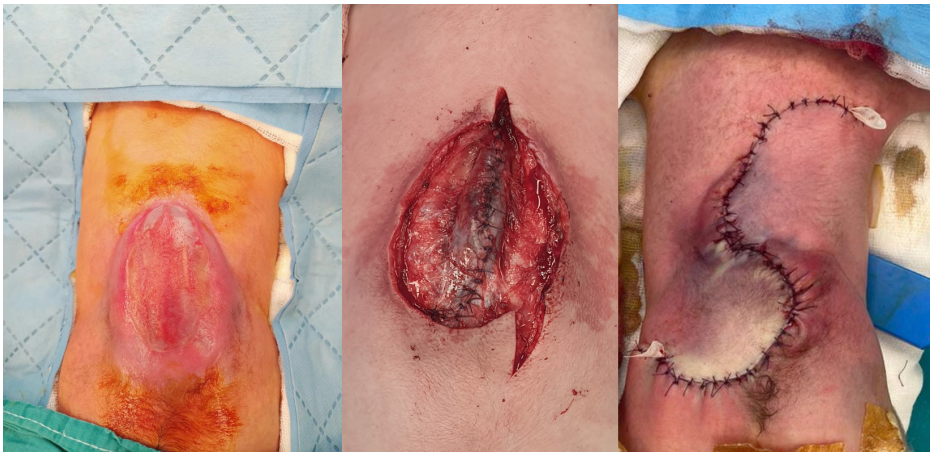

Figure 3: The images are taken from own clinical archive.

Steps of the surgical operation are shown.

It is accompanied by many deformities involving the brain(Chiari II malformation>90%, Hydrocephalus>90%, Syringomyelia 88%, Brain stem anomalies 75%, Cerebral ventricle anomalies >90%, Corpus callosum agenesis 12%, Polymicrogria 15-30%), intestinal, cardiac, esophageal, renal, urogenital, orthopedic systems. It is essential that these patients are managed with a multidisciplinary system.

3. Meningocele

It is the least common type of NTDs (8). The closure of the vertebral arch is incomplete. The nerve tissue is not bagged in the defect consisting of the meninges sac and the normal structure of the nerves is not disturbed. It often occurs in the lumbosacral region (9). There is usually no neurological deficit. If it is covered with skin, there is no problem, but if there is a thin membrane, there may be anomalies.

4. Dermal Sinus

Congenital dermal sinus is a type of closed spinal dysraphism and is the remains of an incomplete closed neural tube. Embryologically, they are formed as a result of an error in the separation of superficial ectoderm and dermal structures from the neuroectoderm (10). They are tracts whose inner surface is paved with flat epithelial cells, one end of which can reach the skin and the other end to the neural tissue (Figure 4). Approximately 60% of cases contain dermoid

or epidermoid cysts. Although dermal sinus tracts can form in any region along the neural axis, they are most often located in the lumbar and lumbosacral regions.

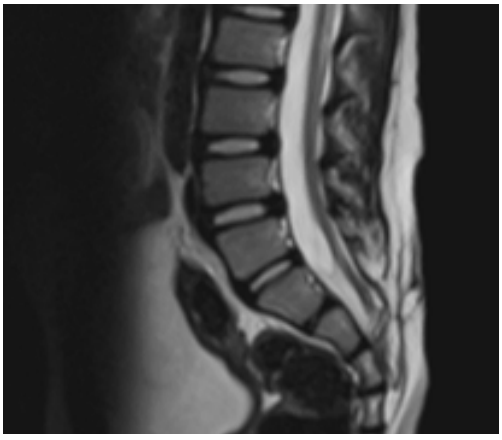

Figure 4: The image is taken from own clinical archive.

MRI image of a meningocel patient is shown.

5. Tethered Cord Syndrome

The spinal cord ends at the level of the S1 vertebra in the 6-month-old fetus, at the level of the L2-3 vertebra in the newborn and at the inferior border of the L1 vertebra in the adult. The most common causes of tethered cord are Diastemetamia, Short and thick phylum terminale, Intradural lipoma, Adhesions that develop after Lipomyelomeningocele and Meningomyelocele surgery, and conus medullaris is located below the level of the L2 vertebra (11). Skin symptoms (such as dimpling, hypertrichosis etc) , Motor deficits, Foot deformities, atrophy of the legs, Urological symptoms (incontinence etc), Progressive spinal deformities such as kyphosis, Scoliosis are common. In adults, perineal and perianal pain, urological symptoms and motor losses are in the foreground.

Radiologically, the presence of the subject medullaries in the L2 horse on MRI, the fact that the terminals of the anterior phylum are thicker than 2 mm makes the diagnosis (Figure 5). Cutting the short, thick phylum terminale with lower lumbar restricted laminectomy, if the tension is due to a lipoma, dissecting the lipoma from the neural elements and removing it constitutes the plan of surgical treatment (12).

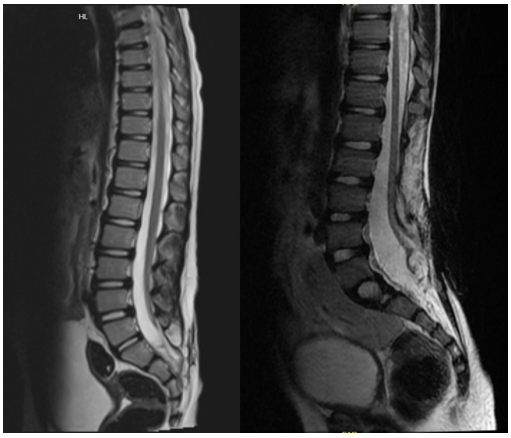

Figure 5: The images are taken from own clinical archive.

MRI images of a tethered cord are shown.

6. Diastematomyelia

Signs and symptoms of tethered cord syndrome are present. There are two types. Type I Diastematomyelia: There are two half-cords wrapped with a separate dural sheath along one or two segments of the medulla spinalis (13). There is a septum (spicule) between the two cords(Figure 6).

Type II split cord malformation (Diplomyelia): The presence of two half-cords in a single dural sheath along one or two segments of the medulla spinalis. Between the cords, there is a fibrous septum, which is not rigid (13).

Both types can be combined with thick phylum terminal.

This bone or fibrous septum, which causes tension in the cord, and the thick phylum, if present, are surgically eliminated to the terminale.

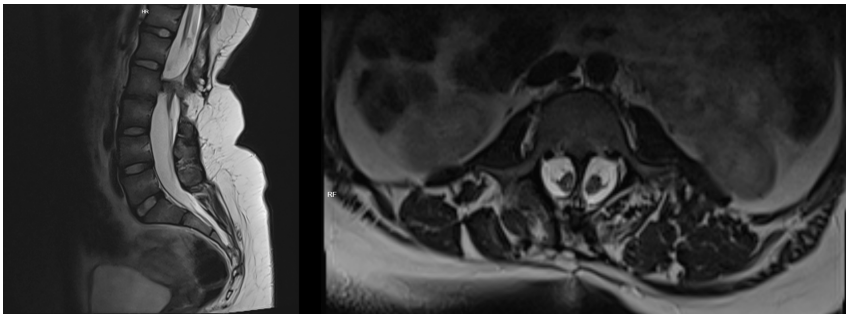

Figure 6: The images are taken from own clinical archive.

7. Cocktail Party Syndrome

It is a typical feature of children with spina bifida (14). Most of the children are happy, extroverted, smart, sweet child types. They answer the questions asked very well. Such children relate very well to their families and their environment (14). They become lively, intelligent, bright-witted, talkative, affectionate children.

8. Congenital Scoliosis-Kyphosis

Most often it develops after a defect at the embryological stage (15). This defect can be a segmentation or formation defect, but it is often a combination of the two. It is quite rare compared to idiopathic scoliosis (16). The ages at which the fastest spine growth occurs are between the ages of 0-5 years and 10-15 years-old. Increased hair growth at the waist, unsymmetrical skin folds on the back and waist, different skin color, vertebrae are not arranged properly when viewed from behind, protrusion (hump) forms on one side of the back when leaning forward, abnormal bone protrusion is seen in the back (Figure 7)(15).

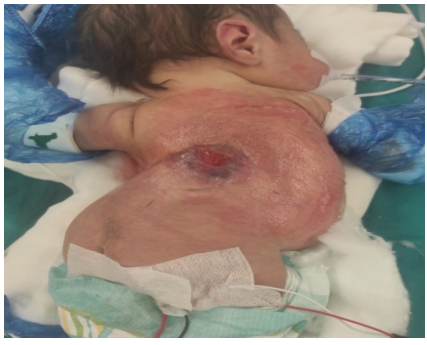

Figure 7: The images are taken from own clinical archive.

If the curvature of congenital scoliosis is a flexible obliquity that covers a long part of the spine, corset treatment may be useful. Surgical treatment is the only option in cases where there is a high probability of progression and progress is detected during the follow-up process. Surgical treatment is the most commonly applied treatment method because the obliquity increases in the majority of cases and corset treatment is not successful (16). The main purpose of treatment options; correction if the deformity is advanced, prevention of

development if the deformity is undeveloped, preservation and growth of the growth potential of the spine, development of the rib cage and protection of lung-heart functions, short stature, cosmetic and psychological problems can be listed as prevention (16).

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CHAPTER VII

GASTROINTESTINAL ATRESIAS IN NEWBORN

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1- Esophageal Atresia and Tracheoesophageal Fistula

It is a common birth defect among newborns. Esophageal Atresia (EA) and Tracheoesophageal Fistula (TEF) is caused by the incomplete separation of the tracheobronchial tree from the foregut during the intrauterine period. The incidence of EA/TEF is 2-4 per 10,000 individuals. It occurs in 2-4 of every 1,000 live births and is slightly more common in males. With the development of technology in anesthesia and neonatal intensive care, the survival rate for esophageal atresia and tracheoesophageal fistula has risen to over 95%. On prenatal ultrasonography, the presence of polyhydramnios and a small or absent stomach indicate esophageal atresia. These newborns experience respiratory distress, malnutrition, suffocation, and aspiration. The cardiovascular, digestive, urogenital, musculoskeletal, and central nervous systems are frequently affected in infants with esophageal atresia. 60-70 percent of cases exhibit additional anomalies.

Babies born with EA-TEF are more likely to be premature than healthy infants. These patients are also present in 15% of cases involving vertebral, anorectal, cardiac, tracheoesophageal, renal, and extremity anomalies, which are grouped under the name VACTERL. There are also urogenital, skeletal, anorectal, and gastrointestinal malformations (1-6).

Classıfıcatıon:

Various classifications have been proposed in the process (1-6).
Gros anatomical typing: 4

a. Isolated esophageal atresia (8%)
b. Proximal TEF + EA (0.6%)

c. EA+ Distal TEF (most common type (85%)
d. EA, proximal and distal TEF (1.4%)
e. H-type tracheoesophageal fistula - (4%)

Risk classification: Developments in neonatal intensive care, anesthesia techniques, and devices have now made the Waterston classification obsolete. In 1994 Spitz et al. proposed a new risk grading system based on birth weight and the presence or absence of congenital heart disease, which is widely practiced in the modern era.

Clinical presentation: Newborns with EA have saliva flowing from their mouths. Cough, choking and cyanosis attacks may occur shortly after birth. In every feeding attempt, the baby has respiratory distress (1-6).

Clinical findings can be summarized as follows (1-6):

1. Flowing, foaming of saliva from the mouth in a newborn baby
2. After feeding, vomiting, choking, coughing, cyanosis attacks are seen
3. Failure to insert the nasogastric tube into the stomach
4. Abdominal distension (in cases with fistula)
5. Aspiration of saliva and gastric juice
6. The diagnosis is made with the development of pneumonia and failure to advance the nasogastric tube to the stomach.

When 8 or 10 Fr feeding tubes are advanced orally or nasally into the stomach, a characteristic resistance is felt about 10 cm from the mouth, the tube is inserted and cannot enter the stomach.

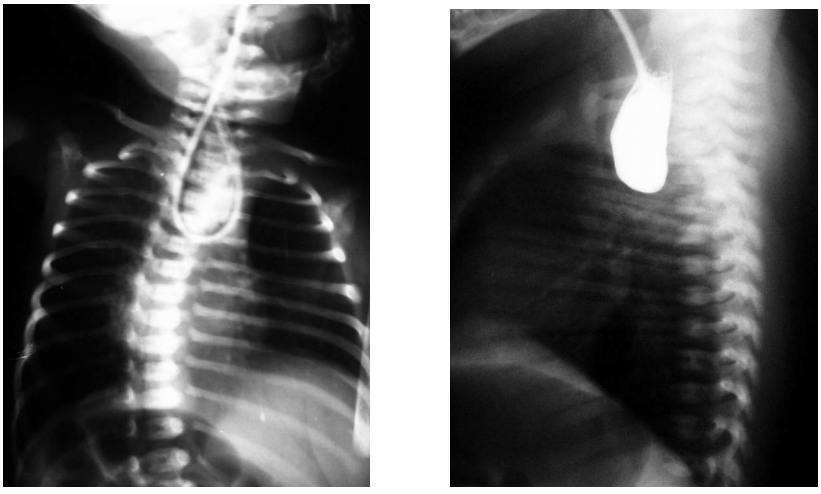

Fig 1: The tube is inserted and cannot enter the stomach and lateral pouch radiograph

The presence of gas in the abdomen indicates TEF. A gasless abdomen indicates isolated atresia or absence of distal fistula. The appearance of a double-bauble on a flat film of the abdomen indicates the presence of associated duodenal atresia. The features of EA without TEF are the non-flatulent abdomen on the film without swelling in the abdomen clinically (1-6).

EA surgery is performed in a stable newborn as a planned procedure. Emergency surgery is not required unless patients with severe respiratory distress due to gastric distension encounter symptoms of gastric perforation (1-6).

Stages of surgery: A skin incision is made in the 4th intercostal space just below the corner of the scapula, followed by a right posterolateral muscle-splitting thoracotomy. An extrapleural approach is preferred. The azygous vein may or may not be ligated. TEF ligation and separation are performed. The surgeon should identify the distal esophagus by following the vagus nerve. Upper esophageal mobilization helps in reducing the space between the ends for anastomosis. In most cases of EA with distal TEF, a primary anastomosis is performed, but sometimes there is a significant amount of tension to complete the repair. Requires long-term atresia-stage operation (1-6).

Long-gap EA with distal TEF: If primary anastomosis cannot be achieved, under these circumstances, cervical esophagostomy and gastrostomy may be performed. Then esophageal replacement surgery should be performed at a later date (1-6).

Long-gap EA: The upper sac should be fully mobilized proximally to the thoracic inlet. Gastric transposition and colon graft interposition are currently the most popular operations. Spitz initially recommended evaluating the length of the gap when performing a gastrostomy. If the gap length is >6 vertebral bodies (6 cm), replacement should be considered and cervical esophagostomy should be performed (1-6).

Postoperative complications: Common complications are anastomotic leakage, stenosis, recurrent TEF, gastroesophageal reflux (GER) and tracheomalacia. It is manifested by the development of pneumothorax and salivary drainage from the chest tube following the repair of EA and TEF. The esophagus usually heals with adequate drainage, broad-spectrum antibiotics, and total parenteral nutrition, but a prolonged period may be required with a chest tube. A large anastomotic leak may require reanastomosis or diversion procedure more often (1-6).

Anastomotic stenosis: The incidence of symptomatic strictures requiring therapeutic dilation following EA repair varies between 37-55%. Patients should be sought for symptoms of postprandial vomiting, prolonged feeding,

malnutrition, or related respiratory distress. Balloon dilatation is our preferred method in the treatment of symptomatic strictures. Some surgeons use steroid injections or mitomycin (1-6).

Tracheomalacia: Many patients with EA have different rates of tracheomalacia. It causes a characteristically loud "barking" cough. Infants with severe tracheomalacia show expiratory stridor that can cause episodes of desaturation, apnea, cyanosis, and bradycardia (usually feeding-related) (1-6).

Type H TEF: There is a history of coughing and choking attacks during feeding and recurrent pneumonia. Diagnosis can be made by contrast esophagography and bronchoscopy + esophagoscopy. The contrast esophagogram is performed in the prone position, with the tube gradually withdrawn as the contrast is injected into the distal esophagus through the feeding tube. Surgical treatment is usually performed with ligation and separation of the fistula, with a neck incision, or rarely with a thoracotomy (1-6).

Long-gap esophageal atresia: All isolated esophageal atresia without TEF is usually long-gap. When primary esophageal anastomosis is not possible even after intensive mobilization, it is considered long-range OA (1-6).

2- Pyloric atresias:

Apart from hypertrophic pyloric stenosis, gastric outlet diseases are rarely seen in children. It is seen at a rate of 1/100000. The most common prepyloric antral web is seen in this group. Other congenital anomalies are pyloric atresia, duplications, or ectopic pancreatic tissue. Vascularization accidents are considered in the etiology of pyloric atresia. In pyloric atresia that does not allow passage, non-bilious vomiting is observed from the first day. If there is an opening that allows passage, they pass distally to liquid foods and give symptoms later. Breastfeeders may not show symptoms until complementary foods are started. Symptoms vary according to the width of the opening (7,8).

In treatment: Nasogastric tube should be started with decompression. The liquid electrolyte should be regulated. In pyloric atresia, the transition should be relieved by performing pyloroplasty. If there is a mesenteric defect between the stomach and duodenum in pyloric atresia, gastrojejunostomy should be performed (7,8).

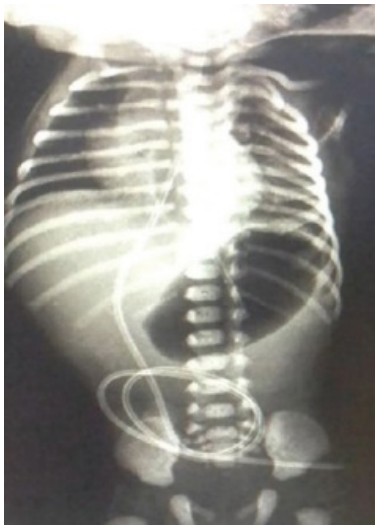

Fig 2: Pyloric atresia direct radiograph

3- Duodenal Atresia:

Congenital duodenal occlusions are the most common cause of intestinal obstruction in the neonatal period and are seen in one in 5000-10000 live births in various series. Intestinal atresia is the first anomaly that should be considered in newborns with complaints of vomiting, abdominal distention, and inability to remove meconium immediately after birth. The location of atresia can be estimated based on whether the distension is widespread or localized. Duodenal atresia is manifested by epigastric distension and collapse of other parts of the abdomen. The cause of duodenal obstruction may be intrinsic or extrinsic, or both. The obstruction may be complete or partial. Atresia and stenosis are the ones that come to mind when an intrinsic obstruction is mentioned (9-13).

Prenatal diagnosis: Prenatal diagnosis of duodenal atresia is possible and crucial. Prenatal recognition of atresia allows for an early treatment approach and prevents increased morbidity and mortality. Detection of polyhydramnios in prenatal ultrasonography (USG) does not specifically indicate an obstruction in the gastrointestinal tract, but suggests its possibility and requires detailed examination. With prenatal USG, the diagnosis can be made with the fluid-filled "Double Bubble" image of the stomach and duodenum part proximal to the atresia (9-13).

Postnatal diagnosis: "Duoble Bubble" image and the absence of air further distally in the direct abdominal X-ray taken after birth is characteristic of the diagnosis of duodenal obstruction. Occasionally, some gas may be seen in the distal intestines. This is usually seen in association with bile duct anomalies. If there is a Duoble Bubble image on the direct abdominal X-ray, no further imaging studies are required for the diagnosis of duodenal atresia (9-13).

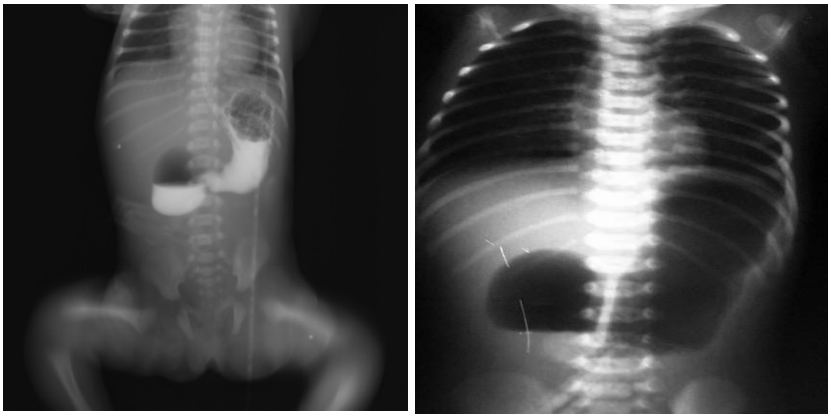

Fig 3: Double Bubble Appearance

Clinical presentation: About half of the patients are premature and have low birth weight. Vomiting with or without bile is observed according to the level of atresia on the first day after birth. Vomiting is usually bilious, as approximately 80% of atresia is distal to the ampulla of Vater. Vomiting is non-bilious in obstructions proximal to the ampulla of Vater. The newborn baby may expel some meconium, but then the meconium will not come out. Abdominal distension is not common and is only in the epigastric region. Duodenal occlusions can be complete or partial. In partial occlusions, the symptoms appear late, so the diagnosis may be delayed until childhood or even adolescence. Recurrent vomiting, aspiration problems, and growth retardation are seen in these patients. In these patients, serious complications such as dehydration and hypokalemic, hypochloremic metabolic alkalosis may develop due to delayed diagnosis, leading to increased morbidity and mortality. Therefore, the diagnosis should be made early and the metabolic status should be corrected before the operation (9-13).

Differential diagnosis: Duodenal atresia is the first thing that comes to mind in newborns with complaints such as biliary vomiting, epigastric distension, and inability to remove meconium. However, compression of Ladd

bands due to malrotation, annular pancreas, and, rarely, the preduodenal portal vein should also be considered in the differential diagnosis. Contrast radiographs of the upper gastrointestinal tract are helpful for differential diagnosis. Although some report that malrotation with duodenal atresia is a common anomaly and that mid-gut volvulus should be considered in the differential diagnosis, some claim that duodenal atresia prevents mid-gut volvulus (9-13).

It is to prevent the baby from vomiting by inserting a nasogastric tube in Duodenal Atresia and starting IV fluid replacement. It is important to maintain body temperature and protect the baby from hypoglycemia. At this time, the baby is evaluated in terms of fluid and electrolytes. If there is metabolic alkalosis, it is corrected. If the physical examination and laboratory findings show that the baby is not stressed and stable, the operation is performed under elective conditions. The treatment of duodenal atresia is surgery. Various techniques have been described for the repair, but mostly the chosen method is the "Diamond-Shaped" duodenoduodenostomy described by Kimura. Duodenoduodenostomy can be done end-to-end or end-to-side anastomosis. It has been reported that Diamond Shaped anastomosis provides early drainage, so enteral feeding is started early in patients and long-term results are good (9-13).

Late complications: Babies with duodenal atresia need long-term follow-up. Because megaduodenum, motility disorder, duodenogastric reflux, gastritis, peptic ulcer, gastroesophageal reflux, choledochal cyst, cholecystitis attacks, and cholelithiasis are complications that may occur in these patients in the late period. As a result, duodenal atresia and stenoses are recognized antenatally and give symptoms in the early postnatal period. Early diagnosis and appropriate treatment are required. The early postoperative recovery rate is 95%, and mortality is usually due to cardiac anomalies. Late complications are more than expected. To avoid late-term complications, meticulous attention should be paid to the operation and the patients should be followed closely for a long time (9-13).

4- Jejuno-ileal Atresia:

Intestinal atresia is one of the most common causes of intestinal obstruction in newborns. It constitutes approximately one-third of congenital intestinal obstructions, the incidence is 1/5,000.

Prenatal diagnosis: Antenatal detection of jejunoileal atresia has been reported in approximately half of the cases in some series. Multiple dilated

loops, cystic masses, ascites, or polyhydramnios may be seen. The combination of dilated bowel and polyhydramnios can give us an idea (14-17).

Clinical presentation: Atresia or severe stenosis of the small intestine presents clinically with bilious vomiting beginning in the first or second day of life. In general, the more proximal the level of obstruction, the sooner vomiting begins, whereas vomiting may be delayed in distal intestinal obstruction. Abdominal distention is often found in distal ileal intestinal atresia, distension is common, while proximal jejunal atresia is limited to the upper abdomen. When delayed diagnosis or perforation occurs, severe distention and respiratory distress may occur. Constipation is usually not absolute, and meconium passage can range from normal color to more common gray mucus plugs. Sometimes if ischemic bowel is present, as in type IIIb atresia, blood may pass through the rectal route. Dilated small bowel loops and air-fluid levels are seen on standing abdominal X-ray. The lower the obstruction, the larger the intestinal loops, and the more fluid levels are observed (14-17).

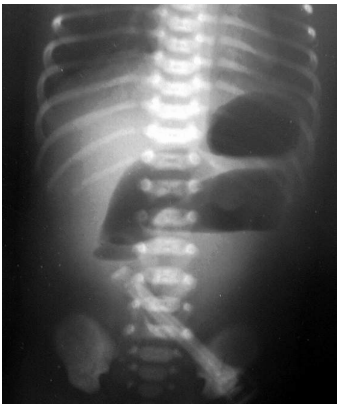

Fig 4: Jejunal atresia

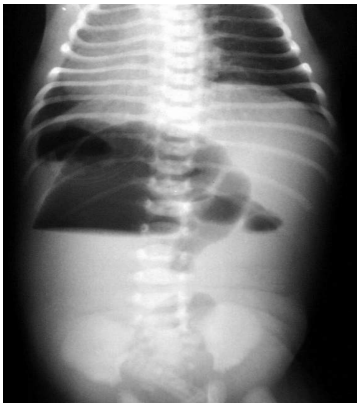

Fig 5: İleal atresia

Treatment: After a few hours of preoperative preparation, the newborn baby tolerates surgery better, especially if it is diagnosed late. In general, patients should not be overly delayed during this preparation because they run the risk of intestinal infarction, fluid and electrolyte disturbance, and infection. Special attention should be paid to hypothermia, hypoxia, hypovolemia, hypoglycemia, and hypoprothrombinemia. The surgical field can be covered with a sterile, transparent adhesive drape to keep it dry during surgery and stop heat loss. For both diagnosis and treatment, it can be inserted through right transverse incisions made 2-3 cm above the umbilicus during laparoscopy or open surgery (14-17).

To test for distal intestinal patency, warm saline is injected into the intestinal lumen. Malrotation, if any, is corrected. It is measured how long the small intestine is overall. The next step is anastomosis of the disproportionate proximal and distal blunt ends once it is known that the distal small intestine and colon are fully open. The end-to-end anastomosis can be done with 5-0 or 6-0 polydioxanone sutures if the ends are proportionate. If the intestines are too small and insufficient, plication or tapering can be used instead of removing the dilated proximal segment. Primary serial transverse enteroplasty (STEP) can be used if it is too short (14-17).

Postoperative care: Nasogastric decompression is usually required until proximal intestinal peristalsis has resolved. Proximal jejunal atresia may require prolonged decompression. If there is no abdominal distension with bile from the nasogastric tract and feeding is delayed until the baby makes meconium (14-17).

5-Colon atresia:

Colon atresia is one of the rare atresia in newborns. 10% of total atresia are colonic atresia. In its etiopathogenesis, there are causes such as vascular accidents and embolism showers. There are 3 types of colon atresia. The proximal dilated distal of atresia is thin and filled with mucus. These patients may have concomitant small bowel atresia, anorectal malformations, cardiac and other additional anomalies. In these, clinical symptoms, as in other atresia, have symptoms such as biliary vomiting, abdominal distension, and inability to remove meconium.

These patients have images such as air-fluid levels and dilated colon on standing direct abdominal radiographs. If there is perforation, subdiaphragmatic free air is seen.

In colon atresia, if the patient is stable, the problem should be corrected surgically in the early period (18-21).

Conclusion: Esophageal atresia should be suspected in case of foaming at the mouth and feeding on the first day. Pyloric atresia should be suspected in case of non-bilious vomiting and collapsed abdomen. Duodenal atresia is diagnosed in the early period and gives symptoms in the early period. Distal intestinal atresia should be investigated in case of abdominal distension and bilious vomiting in newborns.

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CHAPTER VIII

IODINE AND COGNITIVE DEVELOPMENT

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1. Introduction

Iodine is a trace element first discovered in 1811. (1) It is an essential nutrient required in small amounts in the human diet. The amount of iodine in foods depends on the iodine levels of the environment from which the food is derived. (2) The amount of iodine in the soil generally decreases due to rain and glaciation. This causes iodine to be low in the diet and then results in iodine deficiency in the population. (3) Iodine deficiency is one of the most common micronutrient deficiencies, but it is easy to prevent. (2)

Iodine deficiency causes problems such as goitre, hypothyroidism, spontaneous abortion, stillbirth, congenital anomalies, increased perinatal infant mortality, cretinism, mental dysfunction and delay in physical development. (3).

Iodine status can be monitored by determining the urine iodine concentration (UIC). UIC is a measure of the amount of iodine excreted in the urine and is an indirect indicator of dietary iodine intake. The median UIC is used to diagnose the iodine status of a population. (4) An iodine concentration of <20 mg/L in the urine indicates severe iodine deficiency, 20-49 mg/L moderate iodine deficiency, and 50-99 mg/L mild iodine deficiency. While an average value of UIC between 100-299 mg/L indicates adequate iodine status; a value over 300 mg/L is classified as excessive iodine intake (Table 1). (2)

Table 1. Evaluation of Iodine Intake by Mean Iodine Level in Children

Urine Iodine Level (mcg/L)	The Corresponding Approximate Iodine Intake (mcg / day)	Iodine Intake Status	Clinical Effect
< 20	<30	Insufficient	Severe Iodine Deficiency
20-49	30-74	Insufficient	Moderate Iodine Deficiency
50-99	75-149	Insufficient	Mild Iodine Deficiency
100-199	150-299	Sufficient	Optimal Iodine Intake
200-299	300-449	Over Intake	Hyperthyroidism may develop for 5-10 years in those with underlying thyroid disease.
>299	>449	Excessive	Serious side effects (autoimmune thyroid disease, hypothyroidism)

2. Effect of Iodine on Brain Development

According to the World Health Organization (WHO), 2.2 billion people worldwide are at risk of iodine deficiency, and iodine deficiency is the single most important preventable cause of brain damage worldwide. (2) Iodine is converted by the thyroid gland into thyroid hormones: thyroxine (T4) and triiodothyronine (T3). They are essential for metabolism in almost all body tissues. (5) But they are especially important for brain development. (6) The mechanisms by which thyroid hormones affect brain development are not fully understood. (7) However, research continues that less severe iodine deficiency (i.e., mild to moderate) may affect cognitive function. (3)

3. The Effect of Maternal Thyroid Hormones and Iodine on Foetal Brain Development

It is known that iodine deficiency can seriously impair brain structure and function. (8) The low serum iodine level of the mother during pregnancy causes many negative situations in the mother and baby. For the foetus; there are risks such as low birth weight, cretinism, microcephaly, stillbirth. For the mother; there are risks such as insufficient fertilization, preeclampsia, maternal anaemia. (8) Severe iodine deficiency during pregnancy can cause goitre as well as

spontaneous abortion, increased infant mortality, and congenital abnormalities, an irreversible state of mental retardation that presents with dwarfism, deaf-mutism, and spasticity. (9)

Hormonal changes and metabolic demands during pregnancy cause significant changes in thyroid function. During pregnancy, iodine requirement increases due to increased production of thyroid hormones, increased renal clearance of iodine, and foetal placental acquisition of maternal iodine and thyroid hormones. (10)

In the first half of pregnancy, T4 and T3 concentrations increase significantly to maintain maternal euthyroidism. At the beginning of the second trimester, T4 and T3 concentrations are 30-100% higher than before pregnancy. (11) The reasons for this increase are; increase in thyroxine-binding globulin (TBG) concentration, thyrotrophic effect of human chorionic gonadotropin (hCG) and increased activity of enzyme type 3 iodothyronine deiodinase (D3). (12)

In addition to increased thyroid hormone production, iodine losses during pregnancy are higher due to the increase in renal iodine clearance resulting from increased glomerular filtration rate due to hyperestrogenism. (13) Renal clearance of iodine begins to increase in the first week of pregnancy and continues until delivery. (14)

The increased iodine requirement during pregnancy can also be explained by the placental transfer of hormones from the mother to the foetus. (15) Thyroid development of the foetus begins at 10-12 weeks of gestation and T4 is secreted by the foetal thyroid gland from 18-20 weeks. (16) During this period, iodine transfer takes place from the mother to the foetal thyroid gland through the placenta. Iodine transfer allows the foetal thyroid gland to produce its own thyroid hormones. (12)

The foetal thyroid grows between 12 and 39 weeks. The most significant increase in maternal thyroid size occurs during the second trimester, when the foetal thyroid becomes functionally active. (17) At 10 weeks of gestation, nuclear T3 receptors can be identified in the foetal brain. T3 hormone is mainly used by the foetus. A maternal T4 level within the normal range is required to prevent T3 deficiency in the foetus. It has shown better neurological outcomes in the early treatment of new-borns with congenital hypothyroidism born to mothers with adequate T4 concentrations. (18) In contrast, normal T3 values associated with insufficient T4 did not show a protective effect against foetal brain disorder. (18) Thus, an adequate supply of maternal T4 is required to convert T4 to active T3 for the foetus. (19)

Maternal T4 transfer to the foetus is particularly important in early pregnancy because before 12-14 weeks of gestation the foetus cannot produce its own T3 and T4 and these hormones are necessary for normal foetal brain development. (20, 21) It is involved in various important processes of brain development through genomic and non-genomic actions in T3 and T4 glial cells and neurons. (21) Thyroid hormones play a role in neural migration, neural differentiation, myelination, synaptogenesis and neurotransmission. It is mainly involved in the development of neural processes in the cerebral cortex, cochlea and basal ganglia. Deficiency of thyroid hormones in the foetus can cause a decrease in the number and distribution of dendritic synapse in the auditory cortex. A similar effect was found in pyramidal cells of the visual cortex. (12)

Even in iodine-sufficient regions, iodine deficiency can be seen if the thyroid gland cannot meet the increased demand for thyroid hormone during pregnancy. (22) Even mild to moderate iodine deficiency during pregnancy can lead to maternal hypothyroxinaemia. Mild and subclinical cognitive and psychomotor impairments have been observed in neonates, infants and children in Europe, both in mildly iodine-deficient areas and when maternal T4 concentrations are low during pregnancy. (23) Currently, in some European countries, iodine intake during pregnancy is considered insufficient and iodine supplementation is recommended for pregnant women. (24) The period of iodine supplementation is also very important. In a study examining 18-month-old babies, the cognitive measurement score of the children of mothers who took iodine supplementation in the first trimester was found to be higher than the children of mothers who took supplements in the second and third trimesters. (25) A follow-up study of 122 mothers and children who received placebo, or 150 µg/day, or 300 µg/day of iodine from birth to 12 months examined the neurocognitive development of children. Cognitive scores were found to be higher in children whose mothers took 150 µg iodine/day compared to children whose mothers took placebo; no difference was found in language or motor development scores. There was also no evidence of improved or delayed neurodevelopmental outcomes in children whose mothers received 300 µg iodine/day. (26) According to the results of a meta-analysis study; the development of the verbal IQ of the foetus is affected by insufficient iodine concentration during early pregnancy until the onset of the second trimester. (27)

Iodine concentrations should be monitored periodically in pregnant women. (28) In a study conducted in Turkey, it was found that iodine deficiency

becomes more pronounced in pregnant women as the trimester progresses. (29) In a longitudinal study conducted in England, the iodine level of the mother was examined in the first trimester of pregnancy and the cognitive performance of the children of 646 mothers with iodine deficiency and 312 mothers with normal iodine levels were evaluated. Verbal intelligence, reading ability and reading comprehension levels of children born to mothers with mild and moderate iodine deficiency were lower than those born to mothers with normal iodine levels. (30) In a similar study, the grammar, writing, reading and literacy scores of children born to mothers with mild and moderate iodine deficiency were lower than those born to mothers with normal iodine levels. (31) In a study conducted in Finland, using WHO criteria, it was shown that 60-70% of mothers had iodine deficiency during and after pregnancy. In the same study, 29% of the infants of these mothers were found to have iodine deficiency (UIC < 100 ug/L) at 3 months of age. (32)

Cretinism results from severe, chronic iodine deficiency in the foetus and affects the foetus. Mental impairment is a condition characterized by severe stunting, delayed sexual maturation, motor spasticity, deaf-mutism, and other physical and neurological abnormalities. If iodine deficiency persists throughout the new-born, infant, and adult periods after the third trimester, the initial irreversible brain damage may further increase and turn into endemic cretinism. There are different types of cretinism. Sporadic and genetic cretinism are caused by abnormal development or function of the foetal thyroid gland. Because new-born screening tests are routinely performed and lifelong treatment with T4 is required, this type of cretinism has almost been eliminated in developed countries. Endemic cretinism is the most severe manifestation of iodine deficiency. It occurs when iodine intake is below the critical level of 25 μg per day. It usually affects populations living under conditions of severe iodine deficiency. (33)

In a study conducted in Japan, the neurodevelopment of infants from birth to one and three years of maternal iodine consumption during pregnancy was examined. 56.7% of pregnant women were found to have insufficient iodine intake. Low dietary iodine intake during pregnancy increased the risk of delayed child neurodevelopment at one and three years of age. In addition, the increased risk of neurodevelopmental delay in low iodine intake groups was found to be more pronounced at 3 years of age than at 1 year of age. (34)

4. The Effect of Iodine on Cognitive Development in Childhood

The global iodine nutrition scorecard was updated in 2021 by the Iodine Global Network. Considering the most recent median UIC data from 194 WHO member states, including Liechtenstein and Palestine, there is still an insufficient intake of iodine in school-aged children and women of reproductive age. (35) New evidence supports a lifetime perspective on childhood development with negative early experiences having long-term physiological and epigenetic effects on brain development and cognition. In this context, iodine greatly contributes to the postnatal development and plasticity of neural tissues. (36).

A randomized, double-blind, placebo-controlled study in infants with direct and indirect iodine supplementation (providing iodine supplementation to nursing mothers); the effectiveness of iodine supplementation was compared and it was concluded that indirect iodine supplementation was more effective. (37) In areas with moderate to severe iodine deficiency without effective iodized salt programs, therefore, iodine supplementation should be considered for nursing mothers. (36)

Iodine deficiency in the new-born and childhood can cause irreversible cognitive damage. (33) The thyroid gland of a new-born baby contains 0.1 mg of iodine. For this reason, the baby should take iodine supplement after birth. Mothers whose iodine deficiency continues after birth cannot provide enough iodine to their babies with breast milk. As a result, neonatal hypothyrotrophinemia also increases. (38)

The mental retardation caused by the effects of iodine deficiency on the central nervous system during foetal development is irreversible once it is established. In contrast, additional impairment resulting from persistence of postpartum hypothyroidism and/or iodine deficiency may improve with appropriate thyroid hormone replacement and/or iodine supplementation. (39) In a trial of iodine supplementation and placebo in 310 children, iodine supplementation significantly improved thyroid function and performance on cognitive tests by reducing the prevalence of hypothyroxinaemia. (40)

In childhood and adolescence, iodine deficiency causes school failure together with mental function deficiency. (41) A meta-analysis study of children with insufficient iodine intake showed that intelligence scores were lower, from 6.9 to 10.2 points. (42) In a study conducted in Turkey in 2005 and investigating urinary iodine excretion in 299 children aged 6-15 years, it was found that 17.87% of children had moderate or mild iodine deficiency in terms of iodine intake. (43)

The cheapest and most useful method for the prevention of iodine deficiency is the iodization of salt. Significant progress has been made through national salt iodization programmes. (44) For the prevention of iodine deficiency diseases in Turkey, the "Prevention of Iodine Deficiency Diseases and Salt Iodization Program" has been carried out in cooperation with the Ministry of Health of the Republic of Turkey - UNICEF since 1994. (45) With the salt communiqué prepared in 1998, it was ensured that all table salts were enriched with iodine. According to this communiqué, table salt defines finely ground, iodine-enriched, refined or unrefined processed salt that is offered directly to the end consumer. It is mandatory to add potassium iodate at the rate of 25-40 mg/kg to salt. (45)

Ensuring adequate iodine intake is necessary to prevent problems caused by iodine deficiency. Adequate iodine intake recommendations according to WHO and IOM are shown in Table 2. (2, 46)

Table 2. Iodine Requirements by Age and Population Groups

	WHO Recommendations		IOM Recommendations
Up to 5 years old	90 mcg	1-8 years	90 mcg
6-12 years	120 mcg	9-13 years	120 mcg
12 years and older	150 mcg	Young adults and non-pregnant adults	150 mcg
Pregnancy	250 mcg	Pregnancy	220 mcg
Lactation	250 mcg	Lactation	290 mcg

While the prevalence of severe and moderate iodine deficiency in Turkey was 58% in 1997, it was found to be 28.2% in 2008. In this study including 30 provinces, iodine level is sufficient in 20 provinces. The frequency of household use of iodized salt was found to be 89.9% in urban areas and 71.5% in rural areas. Although iodine deficiency has decreased in the world and in Turkey, it is still an important public health problem. (47)

5. Conclusion

Iodine is an important micronutrient for the development and maintenance of brain structure and function through thyroid hormone. Iodine deficiency is a global public health problem, and tackling it should be focused on diagnosis and intervention at the community level rather than the individual. Ensuring adequate iodine intake in the population will eliminate the need for specific

supplementation during pregnancy and lactation. (7) However, there is no scientific evidence to support generalized iodine supplementation in mild to moderate deficiency in pregnant and lactating women, preterm/term infants and young children. (48) In addition, there are no randomized maternal iodine supplementation studies with long-term follow-up data on the psychomotor and mental development of infants in our country. In this way, it will be beneficial to plan and conduct epidemiological studies.

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CHAPTER IX

DIABETES AND COMPLICATIONS

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Diabetes mellitus is a disease that the body cannot balance the blood glucose level. Glucose provides the energy which needed for daily activities to human body. The glucose which is converted from the nutrients by the liver enters the bloodstream and in healthy people, blood glucose level is regulated by hormones, such as insulin. Insulin is produced by the pancreas which also secretes some enzymes that play a role in the digestion. Insulin allows glucose to get through the cells and used for energy. There are two scenarios for the diabetes. The people with type 1 diabetes cannot produce enough insulin. On the other hand, pancreatic b-cell dysfunction and varying degrees of insulin resistance occurs in type 2 diabetes. Glucose cannot pass into cells effectively and blood glucose level remains high as a result of diabetes. This situation causes energy loss of the cells and damages some organs and tissues (1-5).

1. Type 1 Diabetes (Diabetes Insipidus)

Type 1 diabetes is an autoimmune disease which pancreas B cells cannot produce enough insulin. Blockage of the glucose entrance causes to the increasing the blood glucose level. This may lead to life-threatening hypoglycemic and hyperglycemic results. Confusion, distraction, and coma may occur in the hypoglycemic conditions. Hyperglycemia and prolonged absence of insulin lead to ketoacidosis. In the absence of glucose, energy production is provided from fats. Ketones make the blood acidic and lead to a decline in body functions. The increase in ketones also causes ketoacidosis. Failure to improve this condition causes coma and ultimately death (6-11).

2. Type 2 Diabetes (Diabetes Mellitus)

Type 2 diabetes is a complex endocrine and metabolic disorder which is affected by the combination of genetic factors and environmental effects. Insulin resistance and glucose intolerance occurs as a result of dysfunction of pancreatic B cells and usually causes overweight and obesity (12-18). Overweight and obesity cause an imbalance in certain hormone concentrations (such as increased leptin, decreased adiponectin, and increased glucagon), leading to insulin resistance. Concentrations of cytokines such as TNF-a and IL-6 increase. When insulin secretion becomes insufficient to meet insulin resistance, glucose intolerance progresses to type 2 diabetes. Chronic hyperglycemia, chronic non-ester fatty acid accumulation, oxidative stress, inflammation and amyloid formation occurs with decreased pancreatic B cell functions (19-21).

Complications of the diabetes

1. Acute Complications

Diabetic ketoacidosis and hyperosmolar hyperglycemic state are the main acute complications. While diabetic ketoacidosis usually observed in type 1 diabetes, non-ketosis hyperosmolar state (hyperosmolar hyperglycemic state) observed in type 2 diabetes. Both disorders occur insulin absence. Glucagon, catecholamines, cortisol, and growth hormone levels increase in diabetic ketoacidosis and insulin deficiency. When glucagon levels compared to the decreased insulin levels lead to gluconeogenesis, glycogenolysis and ketone body conversion in the liver. Nausea and vomiting are common in diabetic ketoacidosis. Central nervous system depression and lethargy can be seen in the progression of the condition. The non-ketosis hyperosmolar state is mostly seen in adults with type 2 diabetes. Common symptoms such as polyuria, orthostatic hypotension, and various neurological symptoms may cause decreased mental status, lethargy, decreased level of consciousness, paralysis, and coma (10,12)

2. Chronic complications

Chronic complications are mainly divided into vascular and non-vascular. Vascular complications are also divided into two groups which are microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (coronary artery disease, peripheral vascular disorders and cerebrovascular diseases) complications. Non-vascular complications include gastroporesis, sexual dysfunction, and skin changes. Blindness, neuropathies that reduce mobility,

cerebral and vascular problems occur as a result of chronic complications. Treating these complications is much more difficult than controlling the disease. Intracellular hyperglycemia causes failure in blood flow and increases vascular permeability in the early stages of diabetes. The activity of vasodilators such as nitric oxide decreases as a conclusion. The effect of vasoconstrictors such as Angiotensin II and Endothelin-1 increases (22, 23).

2.1. Diabetic Retinopathy: Diabetic retinopathy occurs 75% of patients with diabetes for more than 15 years and causes blindness. Diabetic retinopathy is divided into two phases: non-proliferative and proliferative. The non-proliferative phase occurs in the first decade of the disease or early in the second decade. In the proliferative phase, neovascularization occurs in response to retinal hypoxia. New vascular structures can rupture easily, causing bleeding, fibrosis, and retinal detachments (23).

2.2. Neuropathy: Various degrees of neuropathy are seen in approximately half of diabetic patients. These can be in the form of polyneuropathy, mononeuropathy or autonomic neuropathy. In polyneuropathy; thickening of axons and reduction of microfilaments. Constriction occurs in capillary tubes containing small myelinated or unmyelinated C fibers. While these can be caused by direct damage to the nerve parenchyma due to hyperglycemia, they also occur due to neural ischemia caused by endothelial cell activation, basal cell thickening, and microvascular anomalies due to monocyte adhesion. Mononeuropathy is less common than polyneuropathy and includes dysfunction of isolated cranial or peripheral nerves. Autonomic neuropathy can affect multiple systems such as cardiovascular, gastrointestinal, genitourinary, sudomotor and metabolic systems (24).

2.3. Nephropathy: Glomerular hemodynamic abnormalities are seen as a result of glomerular hyperfiltration. Certain proteinuria, decreased glomerular filtration rate and causes renal disease. As a result of microalbuminuria, dysfunction occurs in the glomerular filtration apparatus. Another possible mechanism explaining the increase in glomerulus permeability is the elevation of renal VEGF level which is both an angiogenic and permeability factor (25).

2.4. Cardiovascular Morbidity and Mortality: There is an increase in various cardiovascular diseases in diabetes patients. These are peripheral vascular disease, congestive heart disease, coronary artery disease and myocardial infarction. Absence of chest pain is remarkable and is common in diabetic patients. Cardiac evaluation is indicated in patients undergoing major surgical procedures.

Despite evidence showing that microvascular complications are reduced with improved glycemic control, macrovascular complications may remain unchanged or even worsen. In addition to coronary artery disease, cerebrovascular diseases can be seen at a high rate in diabetes patients. There is an increased incidence of congestive heart failure in patients with DM (diabetic cardiomyopathy). The etiology of this abnormality is likely multifactorial and includes factors such as myocardial ischemia from atherosclerosis, hypertension, and myocardial cell dysfunction secondary to chronic hyperglycemia (26).

2.5. Hypertension: Hypertension accelerates the complications of Diabetes Mellitus, especially cardiovascular disease and nephropathy. Antihypertensive drugs; It should be applied by determining the advantages and disadvantages by considering the risk factor profile of the patient.

Conditions to consider associated with Diabetes Mellitus:

1) α -adrenal blockers significantly increase insulin resistance and positively affect the lipid profile. α-blockers and thiazide diuretics increase insulin resistance, negatively affect the lipid profile, and significantly increase the risk of type 2 diabetes.

2) B-blockers are effective agents because of their potential to mask hypoglycemic symptoms, and hypoglycemic events are rare when cardioselective B1 agents are used.

3) Central adrenergic antagonists and vasodilators exhibit neutral behavior on lipid and glucose.

4) Sympathetic inhibitors and α-adrenal blockers are associated with orthostatic hypertension in diabetic patients with autonomic neuropathy.

5) Calcium channel blockers have a neutral effect on lipid and can reduce cardiovascular morbidity and mortality in type 2 DM patients, especially in elderly patients with systolic hypertension (27).

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CHAPTER X

DIABETIC KETOACIDOSIS

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1. Introduction

Diabetic ketoacidosis (DKA) is one of the most serious acute complications of diabetes. DKA is the consequence of a total absence or relative (that is, levels insufficient to supress ketone production) lack of insulin and concomitant elevation of counter-regulatory hormones, usually resulting in the triad of hyperglycaemia, metabolic acidosis and ketosis, often accompanied by varying degrees of circulatory volume depletion.[1]

Although DKA is mostly seen in patients with type 1 diabetes (T1DM), it can also be seen in patients with type 2 diabetes (T2DM) or those with gestational diabetes.[2] DKA may be the first sign of the disease in 20-25% of adult patients with type 1 diabetes.[2]

1.1. Risk factors

A precipitating event can usually be identified in patients with diabetic ketoacidosis (DKA). The most common events are infection (often pneumonia or urinary tract infection) and discontinuation of or inadequate insulin therapy. Compromised water intake due to underlying medical conditions, particularly in older patients, can promote the development of severe dehydration. In addition, new-onset type 1 diabetes (20-25% in cases) may present with DKA. Less common causes include cerebrovascular events, alcohol, cocaine use, pancreatitis, myocardial infarction, trauma, burns, drugs that disrupt carbohydrate metabolism (corticosteroids, thiazide group diuretics, adrenergic agonists, antipsychotics, anticonvulsants, immune checkpoint inhibitors), eating disorders (fear of weight gain and hypoglycemia, especially in young

girls with type 1 diabetes with a history of recurrent DKA), endocrinological diseases distrupting carbohydrate metabolism (hyperthyroidism, acromegaly, pheochromocytoma, Cushing's syndrome).[2]

1.2. Pathophysiology

T1DM or T2DM, when there is absolute or relative insulin deficiency or in times of acute illness, which is associated with an increase in the counter-regulatory hormones. These alterations in hormone levels and the subsequent inflammatory response form the basis of the pathophysiological mechanisms involved in DKA. The changes in hormone concentrations lead to alterations in glucose production and disposal, as well as increased lipolysis and ketone body production. Intercurrent illness can lead to the production of counter regulatory hormones leading to hyperglycaemia and the pro-inflammatory state resulting from an infection precipitate DKA.[1]

1.3. Gluconeogenesis and hyperglycaemia

In diabetes mellitus, insulin deficiency leads to increased gluconeogenesis (hepatic glucose production), which is simultaneously accompanied by impaired glucose uptake and use in peripheral tissues[3,4] resulting in hyperglycaemia. In healthy individuals, ~20% of total endogenous glucose production also comes from the kidneys as a result of a combination of gluconeogenesis and glycogenolysis.[5] Endogenous renal glucose production has been speculated to be increased in DKA because data from the 1970's suggest that the presence of an acidosis increase renal glucose output, while impairing hepatic gluconeogenesis.[6] In T1DM and T2DM, increased hepatic gluconeogenesis results from the increased availability of gluconeogenic precursors such as lactate, glycerol and several gluconeogenic amino acids including alanine, glycine and serine. Furthermore, low insulin concentrations lead to catabolism of protein from muscles, liberating amino acids that are gluconeogenic and ketogenic such as tyrosine, isoleucine and phenylalanine, or purely ketogenic such as lysine and leucine. Catabolism of isoleucine, lysine and tryptophan lead to the formation of acetyl coenzyme A (acetyl CoA); catabolism of phenylalanine and tyrosine lead to the formation of acetoacetate; and leucine leads to the production of β-Hydroxy-β-methylglutaryl-CoA (HMG-CoA) — all of which feed into the production of ketone bodies. High glucagon, catecholamine and cortisol concentrations relative to insulin levels stimulate gluconeogenic enzyme activity, in particular

phosphoenol pyruvate carboxykinase, fructose-1,6-bisphosphatase and pyruvate carboxylase, all of which augment hyperglycaemia.[7–9]

1.4. Ketogenesis

The increase in counter-regulatory hormone concentrations associated with severe insulin deficiency activates hormone-sensitive lipase in adipose tissue. Lipolysis of endogenous triglycerides by this enzyme releases large quantities of free fatty acids (FFAs) and glycerol into the circulation.[10] These FFAs are oxidized to ketone bodies in the hepatic mitochondria, a process mediated by high glucagon concentrations. Glucagon reduces the hepatic concentrations of malonyl CoA, which is the first committed intermediate in the lipogenic pathway.[11] Malonyl CoA is also a potent inhibitor of fatty acid oxidation and inhibits the enzyme, carnitine palmitoyl transferase 1 (CPT1). CPT1 regulates the uptake of FFAs into the mitochondria for b-oxidation[12], causing an accumulation of acetyl CoA. Under normal circumstances, acetyl CoA enters the tricarboxylic acid (TCA) cycle (also known as Krebs cycle) and, subsequently, the mitochondrial electron transport chain to synthesize ATP. However, when acetyl CoA production exceeds the levels that can be metabolized by the TCA cycle, two molecules of acetyl CoA condense to form acetoacetyl-CoA, which can condense with another acetyl CoA molecule to form β-hydroxy-β-methylglutaryl-CoA (HMG-CoA). The enzyme HMG- CoA synthase is stimulated by glucagon and inhibited by insulin, therefore, in times of fasting or insulin deprivation, the enzyme actively produces HMG-CoA. HMG-CoA within the mitochondria is lysed to form acetoacetate (as opposed to in the cytosol, where it is involved in cholesterol synthesis), which can further spontaneously degrade to form acetone or be metabolized to b- hydroxybutyrate.[13]

The acetone, acetoacetate and b-hydroxybutyrate constitute the three ketone bodies produced by the liver. The exhaled acetone is what gives the classic ‘fruity’ breath in people presenting with DKA. Concurrent with increased ketone body production, the clearance of b-hydroxybutyrate and acetoacetate is reduced. Acidosis occurs due to the buffering of the protons produced by the dissociation of ketoacids that occurs at physiological pH. The reduced clearance of ketones contributes to the high concentration of anions in the circulation, which also contributes to the development of DKA.[14]

Accumulation of ketoacids leads to a decrease in serum bicarbonate concentration and retention of these ‘fixed acids’ leads to the development of high anion gap metabolic acidosis. The measure of acidity is important because as pH

falls <7.35, intracellular biological systems begin to fail, leading to irreversible damage at ~pH <6.8. This low pH can lead to neurological dysfunction, leading the coma, and if severe or prolonged enough, death.

2. Diagnosis, screening and prevention

2.1. Presentation

It is important to evaluate the symptoms and physical examination findings in the diagnosis. Table 1 shows the signs and symptoms of DKA. DKA frequently presents with a short history, with symptoms developing usually over a few hours. These include the classic symptoms of polyuria , polydipsia and, in those for whom DKA is the first presentation of diabetes, weight loss. Polyphagia has been reported in children, but remains rare in adults.[15] Gastrointestinal symptoms such as nausea, vomiting and generalized abdominal pain are reported in >60% of patients.[16,17] Abdominal pain, sometimes mimicking an acute abdomen, is especially common in children and in patients with severe metabolic acidosis. Abdominal pain typically resolves during the first 24 hours of treatment and lack of resolution of abdominal pain within this time frame should prompt a search for other causes.[17]

Physical examination usually reveals signs of circulatory volume depletion, including dry mucous membranes and tachycardia. Mental status on admission varies from full alertness to lethargy and stupor, with <20% of adults hospitalized showing loss of consciousness. As pH drops, respiratory compensation for the metabolic acidosis, that is, excreting acidic carbon dioxide in an attempt to maintain plasma pH, leads to Kussmaul respirations (a deep and laboured breathing pattern) in individuals with DKA and the breath might have a classic fruity odour owing to acetone exhalation.[18]

Table 1: Signs and symptoms of diabetic ketoacidosis[2]

Symptoms	Physical examination findings
Weakness	Tachycardia
Anorexia, nausea, vomiting	Dryness of mucous membranes, decreased skin turgor
Dry mouth, polydipsia, polyuria	Hot and dry skin
Abdominal pain, cramps	Dehydration, hypotension
Shortness of breath	Tachypnea, Kussmaul respiration
Weight loss	Abdominal tenderness
Clouding of consciousness	Ketone odor in the mouth
	Lethargy, mental blunting, coma

Despite infection, most cases do not develop fever due to vasodilation. Moreover, some patients with a poor prognosis are hypothermic.

In DKA, metabolic acidosis is often the major finding, while the serum glucose concentration is generally below 800 mg/dL and often in the 350 to 500 mg/dL range.[19–21] However, serum glucose concentrations may exceed 900 mg/dL in patients with DKA, most of whom are comatose[19,22], or may be normal or minimally elevated (<250 mg/dL) in patients with euglycemic DKA (which occurs more often in patients with poor oral intake, those treated with insulin prior to arrival in the emergency department, pregnant women, and those who use sodium-glucose co-transporter 2 [SGLT2] inhibitors).

2.2. Diagnosis

The diagnosis of DKA is based on the triad of hyperglycaemia, ketosis and metabolic acidosis.[23] In the course of DKA, metabolic acidosis with an increased anion gap is usually present and the serum bicarbonate level is moderately to severely decreased. In some cases, hyperchloremic metabolic acidosis may develop within the first 8 hours of treatment.

2.3. Clinical Follow-up

At hospital admission, immediate assessment of the haemodynamic state and level of consciousness, together with measurement of blood glucose, blood or urine ketones, serum electrolytes, venous blood gases and complete blood count should be performed. As part of the rapid assessment of the individual, precipitants for DKA should be sought, including an ECG to exclude acute coronary syndrome and repolarization abnormalities (that is, peaked T waves) due to hyperkalaemia.

The systemic effect of DKA in adults depends on the severity of the acidaemia and circulatory volume depletion. There is an average of 5-7 liters of fluid deficit in DKA. However, one of the drawbacks of the ADA classification is that the degree of acidaemia is imperfectly correlated with the patient's level of consciousness.[17]

Since the serum Na+ level may initially decrease as a result of the displacement of water into the extracellular space due to hyperglycemia, the corrected Na+ level should be considered in the treatment. In some cases, the serum Na+ level may be falsely low due to concomitant severe hypertriglyceridemia (pseudohyponatremia).

Intracellular potassium is decreased due to insulin deficiency. However, the serum potassium value can be measured as normal or high due to reasons such as leakage of potassium into the extracellular space, dehydration, hypertonicity, acidosis, and renal failure. A low or lower limit of the first measured potassium level should suggest a severe potassium deficiency. However, in a patient with DKA who has a normal initial serum potassium value, it should be estimated that intracellular potassium is low and will decrease further with insulin therapy and correction of hyperglycemia.

Calcium, phosphate, and magnesium deficiencies can also be seen in DKA. Although intracellular phosphate is low, phosphate level can be measured as normal or increased due to different reasons such as leakage into the extracellular fluid and dehydration.

During follow-up, serum electrolytes, glucose, urea, creatinine, osmolality and blood pH should be evaluated every 2-4 hours. It is recommended to measure arterial pH initially, but venous pH can be used for follow-up.

3. Treatment

Insulin therapy and fluid and electrolyte replacement are the cornerstones of DKA treatment. The aim is to correct acidaemia, restore normal circulatory volume and normalize blood glucose concentrations and acid-base disturbances to restore normal levels of inflammatory and oxidative stress markers[24,25] (Table 2).

Table 2. The goals of treatment in DKA/HHD

The goals of treatment in DKA/HHD
• regulating circulation volume and tissue perfusion
• bringing serum glucose and osmolality to normal limits
• clearing ketone bodies in urine and serum
• correcting electrolyte balance and to metabolic decompensation
• identifying and treating the causative factors

Once DKA is identified, management of the patient is twofold. The precipitating stressors must be identified and treated, as must the serum glucose level and the additional significant symptoms.

Careful monitoring of the patient's response to DKA treatment and appropriate adjustments in treatment based on this response are essential. Monitoring should include tracking of blood pressure, pulse and respiratory rate

as well as accurate documentation of fluid intake and output. For most patients, glucose levels should be monitored hourly and electrolytes (sodium, potassium, chloride and bicarbonate), urea nitrogen, creatinine and venous pH should be measured every 2–4 hours. Levels of phosphate, calcium and magnesium are measured less frequently (generally every 4–6 hours). There should be a low threshold for moving individuals presenting with altered cognitive status or severe metabolic derangement and those who fail to improve after initial treatment to an intermediate care unit (high dependency) or critical care unit in the hospital.[16,26]

Successful treatment of DKA is possible by maintaining fluid and electrolyte balance, correcting hyperglycemia, and treating comorbid conditions.

3.1. Volume correction

Administration of intravenous fluid is the key to intravascular volume correction, thereby improving renal perfusion. The concomitant decrease in circulating counter-regulatory hormone concentrations also reduces insulin resistance.[27] In adults with DKA, the ADA and UK guidelines recommend normal saline (0.9% sodium chloride solution, NS) for the initial fluid replacement.[18,28]

Patients with DKA typically need 3 to 6 L NS during the first few hours after onset.[29] The ADA recommends that 0.9% NS be administered intravenously at 15 to 20 mL/kg/hr for 60 to 90 minutes.[30] Others have recommended that 0.9% NS be given intravenously at 15 mL/kg/hr for one hour, then at 7.5 mL/kg/hr for 2 hours, then at 3.75mL/kg/hr for the next 24 to 36 hours as long as the corrected serum sodium isn't elevated.[31]

After restoration of intravascular volume, the serum sodium concentration and state of hydration assessed by blood pressure, heart rate and fluid balance should determine whether the rate of normal saline infusion can be reduced to 250 ml/hour or changed to 0.45% sodium chloride (250–500 ml/h).[21]

3.2. Insulin administration

In most adults with DKA, a continuous intravenous infusion of regular (soluble) insulin is the treatment of choice. Routine treatment includes administering regular insulin 0.1 to 0.15 units per kg IVP followed by a 0.1 unit/kg/hr IV infusion.[29]

If the glucose level doesn't drop by 50 to 100mg/dL every hour, the insulin infusion rate should be doubled.[32,33]

Once the hourly blood sugar measurements are less than 250 mg/dL, the hourly rate of intravenous insulin should be reduced by half. Start an infusion of 5% dextrose and 0.45% NS at 100to 150 mL/hr to replace the 0.9% (or 0.45%) NS infusion. The insulin infusion should continue until at least two of the following outcomes occur: the anion gap is less than 14 mEq/L, the venous pH is 7.3 or greater, or the bicarbonate level is greater than 18 mEq/L.[29,32,33]

3.4. Potassium replacement

At presentation, serum potassium concentrations are frequently normal or slightly elevated in spite of total body deficits. As insulin treatment starts, ketone production is suppressed, and the acidosis begins to resolve. In addition, insulin drives potassium back into the cell, and the individual can become profoundly hypokalaemic. Hypokalaemia occurs frequently despite aggressive potassium replacement[34,35] and frequent monitoring of potassium during the first few hours of treatment is an essential part of managing DKA.[21,36] Because of potentially rapid shifts in potassium and the possible risk of developing cardiac arrhythmias, continuous cardiac monitoring is recommended in all cases where potassium is being administered at >10mmol/hr. The development of severe hypokalaemia (<2.5 mmol/l) was associated with increased mortality (OR 3.17; 95% CI 1.49–6.76).[34] In patients who develop symptomatic hypokalaemia (muscle weakness and cardiac arrhythmia), potassium replacement should be started and insulin administration should be delayed until the potassium concentration has risen to >3.3mmol/l.

3.5. Bicarbonate administration

For treating acidosis, if the pH is less than 6.9, the ADA recommends adding 100 mmol of sodium bicarbonate (NaHCO3) to 400 mL sterile water and administering the solution over two hours. If the pH is 6.9 to 7.0, 50 mmol of NaHCO3 should be added to 200 mL sterile water and given over two hours. The venous pH should be reevaluated every two hours. When the pH reaches 7.0, no further NaHCO3 need be administered. Because bicarbonate therapy might increase the risk of hypokalaemia, slow the resolution of ketosis, cause paradoxical increases in cerebral acidaemia due to an increase in tissue pCO2 and increase the risk of cerebral injury.[37,38]

3.6. Phosphate replacement

Similar to potassium, serum phosphate concentrations are typically normal at presentation but intracellular depletion is present and serum concentrations

decline during DKA treatment. Phosphate replacement is necessary in those with serum phosphate concentration <1.0– 1.5mg/dl (0.3–0.5mmol/l).[21] Inclusion of phosphate in the infusion has been proposed to diminish the risk of hypophosphataemia, which has been associated with severe complications in some patients including rhabdomyolysis (breakdown of skeletal muscles), renal failure, respiratory failure, arrhythmias and haemolytic anaemia.[39–43]. Phosphate levels should be monitored during treatment at least every 4–6 hours, although more frequent monitoring (every 2–3 hours) is recommended for those not receiving phosphate replacement.

4. Conclusion

Complications due to uncontrolled diabetes cause a serious increase in health expenditures, the intensity of health services and significant labor losses. The first thing to do to deal with these complications is to inform people about the disease correctly and to ensure that they reach the right treatment. Education and the implementation of protocols might reduce lapses in treatment and are a cost-effective way to reduce future risk of hospitalization for hyperglycaemic emergencies.

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CHAPTER XI

GUILLAIN-BARRÉ SYNDROME

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1. Introduction

After the decreasing prevalence of poliomyelitis in the population, Guillain-Barré Syndrome (GBS) has become the most common cause of acute diffuse flaccid paralysis (incidence: 1-2/100.000)(1). GBS is a polyneuropathy characterized by rapidly developing diffuse weakness due to inflammatory damage of peripheral nerves. Inflammation is associated with a monophasic autoimmune process and leads to demyelination of nerves. Incidence is lower in childhood than in other age groups however there is a relative increase especially after 50 years of age(2). Unlike many other autoimmune diseases, GBS is 1.5 times more common in men than women(3). It usually occurs in people who do not have any other disease. But it could be associated with another systemic or autoimmune disease. There are some subgroups that differ from each other sometimes by clinical aspects and sometimes more by laboratory characteristics (Table 1). The term GBS is practically used for this most common classic inflammatory demyelinating form of the disease. Because this form is seen in approximately 90% of GBS cases in the Western countries(4). In this section, this form will be mentioned.

Table 1. . Classification of Guillain-Barré Syndromes((EP Bosch and BE Smith, modified)(5)

Acute inflammatory demyelinating polyradiculoneuropathy
Acute motor axonal neuropathy
Acute motor-sensory axonal neuropathy
Acute motor-sensory axonal neuropathy Pharyngeal-cervical-brachial Paraparetic Facial paralysis Pure oculomotor
Functional variants of GBS Acute pandysautonomia Pure sensorial GBS Ataxic GBS

2. Acute Inflammatory Demyelinating Polyradiculoneuropathy

2.1. Clinic

Most patients have an event such as an upper respiratory tract or gastrointestinal tract infection, surgery, or vaccination within 1-4 weeks before the onset of GBS symptoms. The target of immune attack in Acute Inflammatory Demyelinating Polyradiculoneuropathy (AIDP) is the myelin of the peripheral nerve.

GBS is classically monophasic and all symptoms usually resolve in 1-2 weeks. In about half of the cases, the weakness starts in the feet and legs and spreads to the trunk and arms in a few days (ascendant spread). Weakness begins simultaneously in the legs and arms in 1/3 of the cases, and firstly in the arms in 12% (6). Indeed few times, a muscle weakness that goes beyond the general distribution pattern defined for polyneuropathy syndrome and is evident in the proximal extremities is seen. The accompanying sensory complaints in approximately 80% of the patients are generally in the background and include paresthesia at the extremities or, rarely, back, waist and leg pains. Pain due to nerve root inflammation may be the first symptom of the disease and may begin before muscle weakness in 1/3 of cases.

Neurological examination reveals widespread loss or reduction of tendon reflexes, in addition to muscle weakness in the identified areas. However, rarely, tendon reflexes can be found to be normal at the beginning of the disease and especially only in motor and axonal forms. Objective sensory findings are mostly limited to mild superficial hypoesthesia at the extremities or reduced vibration and joint position sense.

Respiratory failure requiring mechanical ventilation develops in 10-30% of cases(7). Cranial nerve palsies occur in half of the patients, most commonly facial paralysis. Oropharyngeal muscle weakness develops in half of the cases, and eye movement paralysis develops in 10-20%. Uncommonly, papilledema can be seen and is associated with very increased CSF protein (>200 mg/dL). Autonomic nervous system disorders occur in 38 to 70 percent of patients and are more common in patients with severe motor weakness and respiratory failure (8,9). Autonomic signs and symptoms include ECG changes (T wave abnormalities, ST segment depression, QRS widening, QT prolongation and various heart blocks), cardiac arrhythmias (sinusal tachycardia, bradycardia, ventricular tachycardia, atrial flutter, atrial fibrillation and asystole), orthostatic hypotension and hypertensive crisis, less frequently transient urinary retention, sweating disorders, and paralytic ileus(8). Severe and persistent sphincter defect should be considered as a criterion that excludes the diagnosis of GBS. The factors that support and exclude the diagnosis of GBS are discussed in Table 2.

Table 2. Diagnosis of typical Guillain-Barré Syndrome (PA van Doorn et al, modified)(10)

Features required for diagnosis
Progressive weakness in both arms and legs (might start with weakness only in the legs)
Areflexia (or decreased tendon reflexes)
Features that strongly support diagnosis
Mild sensory symptoms or signs
Pain (often present)
Autonomic dysfunction
Cranial nerve involvement, especially bilateral weakness of facial muscles
Relative symmetry of symptoms
Typical electrodiagnostic features
High concentration of protein in CSF
Progression of symptoms over days to 4 weeks
Features that should raise doubt about the diagnosis
Persistent bladder or bowel dysfunction
Slow progression with limited weakness without respiratory involvement (consider subacute inflammatory demyelinating polyneuropathy or CIDP)
Bladder or bowel dysfunction at onset
Marked persistent asymmetry of weakness
Sharp sensory level
Severe pulmonary dysfunction with limited limb weakness at onset
Polymorphonuclear cells in CSF
Fever at onset
Increased number of mononuclear cells in CSF (>50×106 /L)
Severe sensory signs with limited weakness at onset

CSF: Cerebrospinal fluid, CIDP: Chronic inflammatory demyelinating polyradiculoneuropathy

Muscle weakness usually completes its progression in terms of severity and distribution within 1-3 weeks (50% of cases reach maximum muscle weakness in 1 week, 80% in 3 and 90% in 4 weeks)(11). Four to eight weeks of progression occasionally called subacute inflammatory demyelinating polyradiculoneuropathy (SIDP). Acquired demyelinating polyneuropathy cases that progress for more than two months should be considered as chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)(12). Fulminant form is observed in patients who progress to respiratory failure within 24-48 hours. After pause of progression, the disease passes into plateau phase of 2-4 weeks followed by a slow recovery period. Deterioration may occur during or shortly after treatment with intravenous immunoglobulin (IVIg) or plasmapheresis in approximately one-quarter of patients.

In 75% of cases with GBS, complete or almost complete improvement in weakness occurs within one year at the latest. Only pathological fatigue and mild weakness at the ends may remain as sequelae. In approximately 20% of the cases, severe neurological sequelae remain and approximately one out of every 6-7 cases cannot walk unaided(13). Despite the increase in intensive care facilities and new treatment methods, mortality rate of GBS is 3-7%(14). Mortality in patients who become ventilator dependent is approximately 20 percent. Death is often due to ventilation complications, cardiac arrhythmias, pulmonary embolism, and sepsis. Scales (Erasmus GBS Outcome Score- EGOS) are used to determine prognosis. Especially old age, a history of pre-diarrhea, and reaching the most severe level of weakness in less than 2 weeks were found to be poor prognostic signs**(Table 3).** Modified EGOS scale can be beneficial that determine the prognosis in the first week in cases with GBS(15).

Table 3. Poor prognostic factors in Guillain-Barré Syndromes (6,16)

1. Older age (>50–60)
2. Rapid onset before presentation (<7 days)
3. Ventilator dependency
4. Severely reduced distal CMAP amplitudes (<20% lower limit of normal)
5. Preceding infection with Cytomegalovirus
6. Preceding diarrheal illness or Campylobacter jejuni
7. Erasmus GBS outcome score at 2 weeks ≥ 5 a. Ventilator dependence, or b. Bedbound or chairbound and elderly (>60), or c. Bedbound or chairbound and preceding diarrheal illness

CMAP: Compound muscle action potential

2.2. Pre-disease events and Pathogenesis

As noted above, up to two-thirds of patients with GBS have a history of respiratory or gastrointestinal infection 1-3 weeks prior to the disease(17). The most common infectious agent is Campylobacter jejuni. Epstein-Barr virus, Cytomegalovirus (CMV), Haemophilus influenzae and Mycoplasma pneumoniae are known to be associated with GBS. GBS following C. jejuni infection mostly progresses with motor symptoms and axonal involvement. In addition, these cases show slower recovery and have a worse prognosis with increased disability rates(18). Although cases of GBS accompanied by coronavirus disease 2019 (COVID-19) and zika virus infections have been reported, the existence of a direct causal relationship between them has not yet been clarified(19). Other events reported to precede GBS are vaccinations (influenza, rabies, tetanus and diphtheria toxoids, oral poliomyelitis vaccine), surgical interventions, and the use of certain drugs (streptokinase, suramin, gangliosides, and heroin). Studies have shown that the risk of developing GBS associated with vaccination is not greater than the risk of developing GBS associated with influenza(20).

Pathological changes have many variable forms according to GBS subtype. In AIDP, the focal inflammatory response occurs against peripheral myelin or myelinating Schwann cells. The main histopathological findings in the form of AIDP are mononuclear inflammatory infiltration in the endoneurium and segmental demyelination in nerve fibers. Although these lesions are seen all over the peripheral nerves from nerve roots to distal intramuscular nerve branches, mostly motor roots and proximal plexus segments are involved.

It is accepted that the pathological processes leading to GBS are initiated by autoantibodies formed against antigens in the structure of peripheral nerves. Antiganglioside antibodies have been shown in the blood of patients with GBS at a very high rate (approximately 60%). Gangliosides are found in layers called “lipid rafts” in peripheral nerve membranes and play a role in maintaining membrane integrity. Some antibodies do not bind to individual gangliosides, but to new conformational epitopes formed by different ganglioside complexes.

2.3. Laboratory Findings

The clinical diagnosis of GBS is supported by the results of diagnostic tests such as cerebrospinal fluid (CSF) and electrodiagnostic studies(21). Lumbar puncture is performed for CSF evaluation in all patients. Electrodiagnostic studies and imaging are performed in patients with atypical symptoms and when initial CSF evaluation is not diagnostic. These diagnostic tests can also help rule out alternative diagnoses(22).

2.3.1. Routine Laboratory Examinations

Erythrocyte sedimentation rate, leukocyte count and CK level may be a slight increase. Hyponatremia due to inappropriate ADH release may occur.

2.3.2. Cerebrospinal Fluid

Protein level increased. In general, cell isn't observed (albuminocytological dissociation). Protein increase due to disruption of the blood- Cerebrospinal Fluid (CSF) barrier usually occurs after 48 hours (more specifically the first week) and peaks at 3-4 weeks. At the end of the first week, while 66% of the cases have high CSF protein, the CSF protein level never rises in about 10% (23). The number of cells in the CSF generally does not exceed 10 mononuclear cells per mm3 (rarely, 50/mm3 mononuclear cells can be seen). In a case of GBS with CSF pleocytosis, other diagnostic possibilities such as leptomeningeal malignant diseases, Lyme disease, HIV infection or poliomyelitis should be considered.

2.3.3. Electrophysiological Studies

Nerve conduction studies may be normal in the early days of GBS. In 87% of patients with AIDP, abnormal findings are found on electrophysiological examination in the first 5 weeks(5). Characteristic findings of demyelination are observed in motor nerve conduction examination of patients with AIDP long latency F response or failure to receive F response, prolongation of distal latency, slow conduction velocity and conduction blocks, and disperse response. Sural preservation in sensory conduction study (sural sensory conduction normal, median and ulnar sensory conduction abnormal) is an important finding in GBS electrophysiology. In axonal forms, reduction in CMAP and SAP amplitudes without demyelination findings and spontaneous activity in needle EMG in the advanced period are detected. A single ENMG examination couldn't distinguish the axonal and AIDP forms of GBS at an early stage. Therefore, it is recommended to repeat the electrophysiological examination within 1-2 weeks(24).

2.4. Treatment

2.4.1. Management

Patients with GBS should be followed in neurology intensive care units or intermediate intensive care units. It should be followed closely and, if necessary, monitored, especially in terms of respiration, bulbar functions and autonomic disorders. Pulmonary function assessment is priority. If vital

capacity decreases rapidly and/or falls below 15 ml/kg and/or negative inspiratory pressure falls below 60 cm H2O, the patient should be intubated immediately and mechanically ventilated before hypoxemia develops. Patients who cannot swallow their secretions and/or cough adequately due to bulbar weakness should also be evaluated for intubation. Patients with significant dysphagia should be fed with a nasogastric tube. Tracheostomy should be performed for intubation longer than two weeks. Although there is improvement in respiratory functions in an intubated patient, extubation should not be rushed and waited for a while. Autonomic functions, especially cardiac rhythm and TA should be closely monitored. When hypotension or hypertension develops, aggressive treatment should not be used, and long-acting drug use should be avoided.

Pain is a distressing symptom in patients with GBS and may be difficult to manage. Antidepressant and antiepileptic drugs, tramadol and mexiletine can be used for radicular and neuropathic pain that does not respond to NSAIDs. Deep vein thrombosis in the legs and associated pulmonary embolism is one of the important causes of mortality in patients with GBS. From the first moment, a protective dose of heparin or low molecular weight heparin should be given. Passive exercises from an early period may contribute clinical improvement.

2.4.2. Immunotherapy

Plasmapheresis (PLEX) or IVIG is used in the immunological treatment of GBS(1). Multicenter controlled studies have shown that if these treatments are applied within the first 2-4 weeks from the onset of the disease, they provide a significantly faster recovery than those who do not receive treatment, and there is no significant difference between the efficacy of these two treatment options (25). Earlier initiation of treatment (first 2 weeks) provides a higher effect. The choice between PLEX and IVIG depends on local availability, patient preference, risk factors, and contraindications (26).

Plasmapheresis is usually performed with the aim of changing a total of 5 plasma volumes in 5 sessions over a 2-week period. While 2 sessions of plasmapheresis are sufficient in mild cases, it has been shown that 4 sessions of plasmapheresis are required for moderate and severe patients, and that severe patients do not provide an additional benefit from 2 additional sessions of plasmapheresis (6 in total) (27). Plasmapheresis requires a good venous

access route (such as a central venous catheter) and is a treatment method that can only be applied in large medical centers. Patients with cardiovascular instability and autonomic dysfunction couldn't tolerate these treatment regymes, also these are very difficult to be aplied in children. Severe side effects such as sepsis are more common. For these reasons, IVIG, which is easier to apply, finds widespread use(28).

IVIG is administered by administering a total dose of 2 g/kg in daily doses of 0.4 g/kg over 5 days. Less than 10% of patients experience mild side effects such as flu symptoms, headache, chemical meningitis, nausea, and fatigue. These can be prevented by administering antiallergics such as diphenhydramine, paracetamol and low-dose corticosteroids before treatment. Serious side effects are very rare. These include anaphylactic reaction (mainly seen in patients with IgA deficiency, therefore immunoglobulin A levels should be checked before treatment), renal failure (more common in patients with renal disease, a pre-treatment evaluation of kidney function is appropriate) and thromboembolic complications(29). It has been shown that combining plasmapheresis and IVIG treatments has no superiority over their individual application(6). Corticosteroids are generally ineffective in GBS. The combined use of IVIG and corticosteroids was also not more beneficial than IVIG alone(30). A summary of the principles regarding the monitoring and treatment of cases with GBS is given in Table 4.

Table 4. GBS treatment (IR Bella, modified) (31)

Clinical Status	Follow-up and Treatment
Mild GBS	Hospitalisation
Walking independent, no respiratuar dysfunction	Observation Plasmapheresis or IVIG (consider).
Walking with support, no respiratuar dysfunction	If possible, take patient to the intensive care unit, otherwise keep in a place where VC can be measured frequently and access to intensive care facilities is relatively easy Follow-up ABG IVIG or plasmapheresis (4 session)
No walking, mild respiratuar dysfunction	Take patient to the intensive care unit Follow-up VC closely Follow-up ABG Entubation criteria: VC <15 ml/kg, or VC tends to decrease within 4-6 hours, Oropharyngeal paresis and aspiration Respiratuar distress findings and VC 15 ml/kg. IVIG or plasmapheresis (4 session)
No walking, respiratuar dysfunction needs mechanic ventilation	Take patient to the intensive care unit Autonomic nervous system dysfunction observation Fluid replacement for hypotension Short-acting beta-blockers can be used for resistant hypertension Frequently turn over in bed for decubitus ulcer and compression neuropathies, position changes Physical therapy IVIG or plasmapheresis (4 session)

ABG: arterial blood gas, IVIG: intravenuos immunoglobulin,
VC: vital capasity.

3. Conclusion

After the decreasing prevalence of poliomyelitis in the population, Guillain Barré Syndrome has become the most common cause of acute diffuse flaccid paralysis (incidence: 1-2/100.000)(1). In approximately 20% of the cases, severe neurological sequelae remain and approximately one out of every 6-7 cases can not walk unaided(13). Despite the increase in intensive care facilities and new treatment methods, mortality rate of GBS is 3-7%(14). Plasmapheresis or IVIG is used in the immunological treatment of GBS(1). Multicenter controlled studies have shown that if these treatments are applied within the first 2-4 weeks from the onset of the disease, they provide a significantly faster recovery than those who do not receive treatment, and there is no significant difference between the efficacy of these two treatment options. Earlier initiation of treatment (first 2 weeks) provides a higher effect(26).

In conclusion, although GBS is a rare disease in the community, it carries a substantial risk of disability and death. Considering that early initiation of treatment provides a higher effect, it is important that clinicians know the diagnosis, treatment and management of the Guillain-Barré syndrome.

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CHAPTER XII

SYNCOPE IN THE EMERGENCY DEPARTMENT

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1. Introduction

Syncope is a symptome characterised by sudden and transient loss of consciousness (TLOC) of relatively short duration with spontaneous complete recovery. (1) Syncope is caused by inadequate cerebral blood flow, most often the result of an abrupt drop of systemic blood pressure. TLOC is a broader definition that includes syncope. The term of syncope should not be used for clinical features of other non-syncope causes of loss of consciousness, such as seizure, head trauma, or apparent loss of consciousness (pseudosyncope). Syncope can be caused by benign or life-threatening conditions (Table 1.) and is a common reason for visiting the emergency depatment (ED). Often, the primary responsibility of the ED clinician is to identify which patients are at high risk for adverse outcomes. In most cases the underlying cause of the syncope episode may not be clearly identified in the ED. This chapter will discuss how to evaluate and manage patients presenting to the ED with syncope.

2. Epidemiology and frequency

The incidence of syncope depends on the population being evaluated. Due to the changes in the definition and because different patient populations were evaluated, the frequency of syncope in the literature is less than it is and a clear assessment could not be made. (2) Studies of syncope report prevalence rates estimated to be 32%-41% in the general populationas and 35% with recurrent syncope. (3-5) Syncope is more common in women. (6) The first syncope episode is common around twenties and a sharp increase in incidence after 70

years of age. (7) Older institutionalized patients have a 7% annual incidence of syncope, a 23% overall prevalence, and a 30% two-year recurrence rate. (8)

3. Pathophysiology

Syncope is defined as a temporary, self-limited loss of consciousness that usually leads to falling. The underlying mechanism is transient global cerebral hypoperfusion due to systemic arterial pressure. Decreased venous filling is usually the main factor contributing to decreased cardiac output. Other factors, such as bradyarrhythmias or tachyarrhythmias, can also cause impaired cardiac output and trigger syncope. This is more common in conditions such as left ventricular dysfunction, severe valvular disease, obstructive cardiomyopathy, volume depletion, and abnormal vascular reactivity. Inappropriate vasodilation and inadequate venous return are the main causes of reflex syncope. Decreased capacity to increase vascular resistance during the upright position is critical in orthostatic hypotension.

4. Differential Diagnosis

The primary responsibility of the emergency clinician is to assess whether a life-threatening cause of syncope exists and to ensure appropriate management and regulation. Cardiac syncope, acute hemorrhage, pulmonary embolism, and acute cerebrovascular events are the most important causes to consider. conditions that mimic TLOC/syncope like seizures, sleep disturbances, accidental falls, and some psychiatric conditions should be also considered.

4.1. Myocardial ischemia

Syncope could be the initial complaint in 5-12% of patients with acute myocardial infarction (MI) and is related to arrhythmia or pump failure. (9) Vasovagal reactions, bradyarrhythmia, and atrioventricular blocks can be caused by ischemia. Ventricular tachycardia (VT) and bradyarrhythmias secondary to myocardial ischemia are infrequent causes of syncope. (10) While polymorphic VT and VF are common during acute ischemic conditions, monomorphic VT is common for chronic ischemia.

4.2. Cardiac arrhythmias

Cardiac arrhythmias can cause syncope if the heart rate is too slow or too fast to allow for adequate cardiac output and maintenance of systemic arterial

pressure. Bradycardia from prolonged sinus pauses, high-grade atrioventricular (AV) block or termination of atrial tachyarrhythmia can cause syncope. Similarly, syncope or a near-syncopal condition may occur at the onset of a tachycardia episode in which the decrease in cardiac output is not adequately compensated by vascular constriction. The diagnosis of arrhythmic etiology for syncope is often difficult, as most are paroxysmal and infrequent. Long-term ambulatory ECG monitoring is usually required for diagnosis.

4.2.1. AV block: Complete or Mobitz type II second-degree AV block can trigger syncope. First-degree and Mobitz type I (Wenckebach) second-degree AV blocks are usually benign and not associated with syncope.

4.2.2. Cardiac pauses: Cardiac pauses may be caused by intrinsic or drug-induced sinus pauses or prolonged recovery times after spontaneous termination of an episode of fast tachycardia.

4.2.3. Ventricular tachyarrhythmias: Syncope from VT occurs most often in the case of structural heart disease, particularly coronary heart disease. Patients with cardiomyopathies (eg, hypertrophic and dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy (ARVC)) are also prone to VT occurring with syncope. Congenital or acquired long QT syndrome can cause torsades de pointes, an unusual form of VT. Rare genetic cardiac ion channel diseases such as Brugada syndrome and catecholaminergic polymorphic VT (CPVT) also should be considered as differential diagnoses of syncope. Syncope is a relatively common presentation in Brugada syndrome (BrS), an inheritable primary arrhythmia syndrome. Considered one of the J wave syndromes, BrS has several clinical and genetic similarities with early repolarization syndrome (ERS). (11)

4.2.4. Supraventricular tachyarrhythmias: Supraventricular tachyarrhythmias are rarely associated with syncope. Syncope may occur more often in relatively fast tachycardias or at the onset of tachycardia or during exercise.

4.3. Structural cardiac or cardiopulmonary disease: Heart failure with low ejection fraction, severe aortic stenosis, hypertrophic cardiomyopathy, atrial myxoma, pulmonary embolus, pulmonary hypertension, pericardial tamponade, and acute aortic dissection are common causes of syncope and emergency consults.

4.3.1. Aortic stenosis: Syncope in patients with aortic stenosis is often associated with exertion. Aortic stenosis presents with syncope if the valve is critically stenotic. (12). Although it is mostly seen in the elderly, it can also be seen in young people in the case of bicuspid aortic valve.

4.3.2. Hypertrophic cardiomyopathy: Syncope occurs due to dynamic left ventricular outflow tract (LVOT) obstruction. LVOT obstruction may be exacerbated by postural changes, hypovolemia, or medications. In these patients, the cause of syncope is often ventricular arrhythmias or decreased cardiac output.

4.3.3. Other causes: Pulmonary embolism, severe pulmonic stenosis, pulmonary arterial hypertension, and atrial myxomas are other rare causes of syncope due to obstruction of blood flow. (13)

4.4. Cerebrovascular disease: Atherosclerotic disease of the cerebral arteries is not a cause of true syncope, but stroke and episodes of transient ischemia cause focal neurological deficits that do not heal rapidly and completely.

4.5. Reflex syncope: Reflex syncope is a condition in which a reflex response causes vasodilatation and/or bradycardia leading to systemic hypotension and cerebral hypoperfusion with TLOC. Types of reflex syncope include vasovagal syncope, situational syncope, and carotid sinus syncope.

4.6. Orthostatic (postural) hypotension: Orthostatic syncope most commonly occurs following a transition from a lying or sitting position to a standing position. Orthostatic hypotension, defined as a decrease in systolic blood pressure of at least 20 mmHg or diastolic blood pressure of at least 10 mmHg in standing upright. The major causes of orthostatic hypotension associated with syncope are decreased intravascular volume due to inadequate fluid intake and excessive fluid loss. Drugs are other important cause of orthostatic syncope. Antihypertensive drugs like alfa and beta blockers, nitrates are often related with syncope especially in older patients.

4.7. Other conditions

4.7.1. Neurologic syncope: Syncope could be the only sign of some neurological disorders like subarachnoid hemorrhage, transient ischemic attack, subclavian steal syndrome, epilepsy, and complex migraine headache. a history of non-syncope episodes featuring neurologic deficits, possibly including diplopia, vertigo, focal weakness, or numbness is often. Syncope can be misinterpreted as a seizure because many patients with TLOC experience brief convulsive episodes secondary to cerebral hypoperfusion, especially if bystanders or objects are holding them upright. Syncope can usually be distinguished from a seizure by the shortness of the convulsions; epileptic aura, urinary or fecal incontinence, absence of tongue biting; and the absence of a true postictal phase (typically five minutes or longer)).

4.7.2. Psychiatric syncope: Anxiety and panic disorders can cause situational syncope ofttimes. Emergency clinicians must be cautious when attributing syncope to psychiatric causes. Patients with hypoxia, inadequate cerebral perfusion, or other medical conditions may appear confused or anxious. Patients with psychiatric syncope are generally young, without cardiac disease, and complain of multiple episodes. (14)

4.7.3. Drug-induced loss of consciousness: Drugs of abuse and alcohol can cause a temporary loss of consciousness, but often these patients show signs of toxicity and do not spontaneously return to normal neurological function soon after regaining consciousness. Alcohol can also impair vasoconstriction, causing symptomatic orthostasis. (14)

4.7.4. Metabolic: Metabolic causes of syncope include hypoglycemia and hypoxia. Electrolyte abnormalities due to kidney damage or other conditions can precipitate arrhythmic syncope.

5. Diagnosis of syncope

The first problem is determining whether the episode was due to syncope or some other cause of real or apparent TLOC. Detailed history and physical examination are very important for differential diagnoses. However, in emergency conditions, a definite diagnosis may not be revealed. The most critical issue to be decided in the emergency department should be risk stratification.

5.1. History: The clinical features characterizing TLOC are usually derived from history taking from patients and eyewitnesses. History and physical examination are sufficient to diagnose in approximately half of the patients. Specific signs and symptoms are important to determine high risk for serious outcomes, particularly sudden death. (16)

5.1.1. Age: Vasovagal syncope is more common in young people. However, if there is exertional syncope, and a family history of sudden death, arrhythmic causes should also be considered. Again, pathological physical examination findings should suggest a structural disorder. Risk of adverse outcomes incereases with age. (17) Coronary artery disease, aortic stenosis, pulmonary embolism is the most common causes in olders. Also autonomic dysfunction, orthostasis, and multiple medications are commom among older patients. Age is also important for risck stratification.

5.1.2. Associated symptoms and triggers: Associated symptoms can provide important diagnostic clues. Chest pain may indicate acute coronary

syndrome or pulmonary embolism. Dyspnea raises concern for pulmonary embolism or heart failure. Accompanying palpitations suggest arrhythmia. Headache and neurological symtopms may indicate a neurological cause. Vasovagal syncope generally precedes and often includes symptomes such as warmth, nausea, vomiting and diaphoresis, either just prior to or shortly after the event. It is also helpful to question vasovagal triggers. Triggers commonly associated with vasovagal syncope include strong physical or emotional stress, voiding, defecation, coughing, swallowing, and prolonged standing in a warm environment.

5.1.3. Position: Fainting associated with vasovagal syncope is often associated with prolonged standing. Although very rare, vasovagal syncope in the sitting or supine position can occur. Arrhythmic syncope can be considered in such situations.

5.1.4. Duration of syncope: Measuring the duration of a syncope event is often difficult because patients are unconscious and events are often unwitnessed. Reflex syncope attacks are of very short duration and resolve quickly when lying down, but syncope longer than four or five minutes should raise concern for other causes.

5.1.5. Exertional syncope: Syncope with exertion raises the possibility of arrhythmia or cardiac outflow obstruction (eg, aortic stenosis, hypertrophic cardiomyopathy, pulmonary embolisn or pericardial tamponade). Detailed physical examination and imaging should be considered.

5.1.6. Medications: A review of the patient's medications may reveal the cause of syncope. This is particularly important with older adult patients. Medications often implicated include calcium channel blockers, beta blockers, alpha blockers, nitrates, antiarrhythmics, diuretics (affecting volume status and electrolyte concentrations), and medications affecting the QTc interval (eg, antiarrhythmics, antipsychotics etc)

5.1.7. Prior episodes: A history of syncopal episodes may be of value. A single episode or multiple episodes over many years suggests a benign etiology. Several episodes over a short period of time in someone with no history of syncope suggest a more significant cause, such as dysrhythmia.

5.1.8. Family history: A family history of unexplained sudden death, dysrhythmia, or early cardiovascular disease places patients at increased risk for cardiac syncope

5.1.9. Associated injury: Acute loss of consciousness may result in significant events. like motor vehicle accidents, fractures, and hemorhage.

These conditions are observed more frequently in patients without prodrome symptoms.

6. Physical Examination: The examination should focus on vital signs; blood pressure measurements, pulses, cardiac, pulmonary, abdominal, rectal, and skin signs. Any new focal neurologic findings suggest a primary central nervous system lesion. Most patients with syncope will have normal physical examination findings. (18)

6.1. Vital signs: Transient bradycardia or hypotension occurs during most episodes of syncope. Abnormal vital signs usually return to normal during evaluation in the emergency department. Persistent abnormal vital signs are of concern and should be investigated. Upper extremity differences in heart rate or blood pressure may be the sign of aortic dissection. Low oxygen saturation or tachypnea are common with cardiac failure or pulmonary embolism. Orthostatic vital signs should be obtained. The patient should lie on their back for five minutes before the first set is achieved. After the patient has been standing for three minutes, vital signs are taken again and compared with the initial measurements. (19) A drop in systolic blood pressure of 20 mmHg or more or an increase in heart rate of 20 beats per minute or more are compatibble with orthostatic hypotension. If available, pacemakers or wearable devices can be used in diagnosis by evaluating the records of vital signs at the time of the event.

6.2. Cardiac examination: Auscultation of the heart may reveal a rate that is either abnormal or irregular. Murmurs and extra heart sounds may be diagnostic for structural heart diseases like aortic stenosis and HOCM. The presence of an implantable pacemaker should be noted as malfunction may lead to syncope.

6.3. Lung examination: Auscultation of the lungs may reveal abnormal sounds consistent with heart failure or other pathologies (eg, pulmonary embolism, cardiac ischemia).

6.4. Neurological examination: By definition, patients with syncope return to basic neurological function. A thorough examination should be performed to identify any subtle focal abnormalities suggestive of stroke.

6.5. Rectal examination: Rectal examination with the stool guaiac test can identify some patients with gastrointestinal bleeding who may present with syncope.

6.6. Intraoral examination: Tears on the lateral surface of the tongue suggest a seizure

7. Diagnostic Tests: After the initial evaluation by history and physical examination, some tests are required to confirm the diagnosis of syncope and exclusion of differential diagnosis.

7.1. Electrocardiogram: Practice guidelines suggest that all patients presenting with syncope should receive an electrocardiogram (ECG). (20). ECG should be evaluated immediately at admission to the emergency department. patients should be followed up continuously in order to record the arrhythmias that may recur during the follow-up. Any abnormalities in ECG suggests a cardiac problem, and further investigation is needed. evidence of cardiac arrhythmia or ischemia as the cause of syncope should be assessed with the ECG. A list of ECG features associated with arrhythmia is found in Table 4. (21) ECGs of patients with pacemakers should be evaluated by a cardiologist, and even the functions of the device should be controlled with a programmer.

Table 1. Clinical and electrocardiographic (ECG) features of patients with syncope at high risk of an arrhythmic cause

Persistent sinus bradycardia <40 beats per minute or sinus pauses >3 seconds in an awake patient
Third-degree (complete) AV block, Mobitz II second-degree AV block
Significant structural heart disease or CAD ()
Preexcited QRS complexes, suggesting Wolff-Parkinson-White syndrome
VT or paroxysmal supraventricular tachycardia with rapid ventricular rate
Long or short QT intervals
Right bundle branch block pattern with ST elevation in leads V1 to V3 (Brugada syndrome)
Pacemaker or implantable cardioverter-defibrillator malfunction with cardiac pauses
Alternating left and right bundle branch block
Negative T waves in right precordial leads and epsilon waves suggestive of arrhythmogenic right ventricular cardiomyopathy

7.2. Laboratory evaluation: There is no clear evidence for routine laboratory screening in patients with syncope. However, routine kidney and liver tests, blood count, electrolyte levels should be evaluated. For such conditions as myocardial infarction, pulmonary embolism, troponin and d- dimer should be performed.

7.3. Neurologic studies: Patients with a history of transient ischemic attack, stroke, or new-onset seizure, or suspected on physical examination, require further evaluation. Patients without historical or examination features suggestive of neurological disease do not need further neuroimaging. Despite the low diagnostic yield of brain imaging, clinicians continue to overuse head computed tomography and magnetic resonance imaging in the evaluation of patients with syncope. An electroencephalogram may be useful in some situations where distinguishing syncope from seizure is clinically difficult. (22)

7.4. Echocardiography: While not immediately available in most emergency departments, echocardiography is helpful in identifying the presence of structural heart disease and is being performed at the bedside by more emergency room physicians. For patients with suspected heart disease, echocardiography serves to confirm or refute the suspicions in equal proportions and plays an important role in risk stratification. Echocardiography identifies the cause of syncope in very few patients when no more tests are needed (i.e. severe aortic stenosis, obstructive cardiac tumours or thrombi, pericardial tamponade, or aortic dissection).

8. Management of syncope in the emergency department

The most important tasks for the emergency clinician faced with a syncope patient are to identify and manage life-threatening problems and to differentiate between patients safe for discharge and those who require immediate investigation and in-hospital management. The risk classification recommended by the ESC guideline is shown in the Table 2. The risk stratification flowchart is also shown in Figure 1. (21) Treatment is based upon the underlying cause. In most conditions treatment includes only prevention for recurrences, preventing falls and sometimes death.

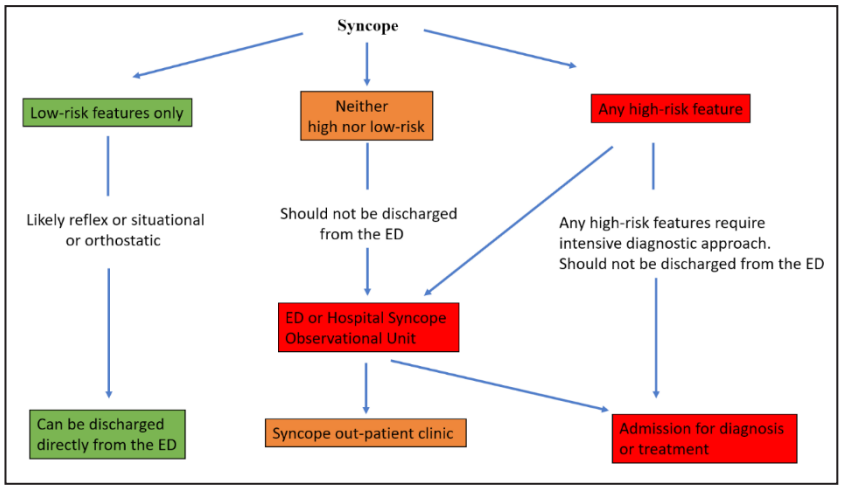
8.1. Immediate treatment

The immediate treatment of a patient with syncope includes; laying down the patient supine, with legs elevated if possible, assessing vital signs to distinguish cardiac arrest from syncope. For patients who are hypotensive but lack bradycardia, fluid resuscitation must be the first approach to subsequent treatment. Patients with symptomatic bradycardia or high-grade AV block atropine and if not sufficient followed by temporary cardiac pacing are the initial treatments. Dobutamine or isoproterenol infusion may help to increase heart rate if atropin is ineffective and temporary pacing is not available.

Table 2. High and low-risk features in patients with syncope at initial evaluation in the emergency department

Syncopal Event	High risk	Majör	Minor
		- New onset of chest discomfort, breathlessness, abdominal pain, or headache - Syncope during exertion or when supine -Sudden onset palpitation immediately followed by syncope	- No warning symptoms or short prodrome (<10 s) - Family history of SCD at young age - Syncope in the sitting position
	Low risk	- Associated with prodrome typical of reflex syncope - After sudden unexpected sight, sound, smell, or pain - After prolonged standing or crowded, hot places - During a meal or postprandial - Triggered by cough, defaecation, or micturition - With head rotation or pressure on carotid sinüs - Standing from supine/sitting position	
Medical History	High risk	- Severe structural or coronary artery disease	
	Low risk	- Long history of recurrent syncope with low-risk features with the same characteristics of current episode - Absence of structural heart disease	
Physical Examination	High risk	- Unexplained systolic BP in the ED<90 mmHg - Suggestion of gastrointestinal bleeding on rectal examination - Persistent bradycardia (<40 bpm) in awake state and in absence of physical training - Undiagnosed systolic murmur	
	Low risk	- Normal examination	
ECG	High risk	Major	Minor
		- ECG changes consistent with acute ischemia - Mobitz II second and third degree AV block - Slow AF - Persistent sinüs bradicardia or repetitive sinoatrial block or sinüs pauses >3 seconds in awake state and in the absance of physical training - Bundle branch block, intraventricular conduction disturbance, ventricular hypertrophy - Sustained and non-sustained VT - Dysfunction of an implantable cardiac device - Type 1 Brugada pattern - QTc>460 ms in repeated 12-lead ECGs indicating LQTS	- Mobitz type I second degree AV block and – First degree AV block with markedly prolonged PR interval - Asymptomatic inappropriate mild sinüs bradicardia or slow AF - Paroxysmal SVT or AF - Preexited QRS (WPW) - Short QTc interval (<340 ms) - Atypical Brugada patterns - Negative T waves in right precordial leads, epsilon waves suggestive of ARVC
	Low risk	-Normal ECG	

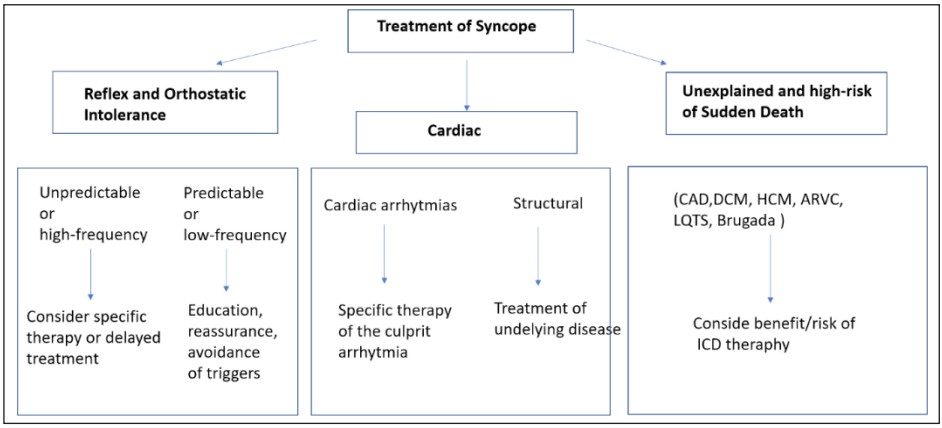
Figure 1. Emergency department risk stratification flow chart



8.2. Treatment of recurrences: Patient education is important to prevent recurrent syncope and to reduce the risk of traumatic injury and even death. If possible, triggers should be addressed directly, such as cough uppression in cough syncope, micturition in the sitting position, etc. Increased intake of oral fluids is also advised. Prolonged standing should be avoided. Patients should be educated to recognize early symptoms and take action to avert syncope and reduce the risk of injury. If the symptoms are mild, the patient may perform a physical counterpressure maneuver while moving safely to a seated or supine position, which should terminate the episode. If the symptoms are severe, the patient should move directly to a supine position. In either case, they should remain in a safe, gravitationally neutral position long enough to be sure that all of the warning symptoms have subsided. Patients with vasovagal syncope and prodromal symptoms should be taught how to perform physical counterpressure maneuvers. The rationale for these maneuvers is to reduce venous pooling and thus improve cardiac output. Some examples of these maneuvers are leg-crossing, handgrip, and arm tensing. Careful avoidance of agents that lower BP, i.e. any antihypertensive agents, nitrates, diuretics, neuroleptic antidepressants, or dopaminergic drugs, is key in the prevention of recurrence of syncope.

Additional treatment may be necessary in patients with severe forms when very frequent syncope alters quality of life; when recurrent syncope without, or with a very short, prodrome exposes the patient to a risk of trauma; and when syncope occurs during a high-risk activity. The general framework of treatment is based on risk stratification and the identification of specific mechanisms when possible (Figure 2).

Figure 2. The general framework of treatment



9. Prognosis: In most cases the prognosis of the patient with syncope is directly related to the underlying etiology and the underlying comorbidities. Those with an underlying cardiovascular cause are at higher risk for sudden death and all-cause total mortality rates than those with a noncardiovascular cause. Overall mortality in the cardiovascular group after five years of follow-up has been reported to approach 50 percent, with a 30 percent incidence of death in the first year. (25) A major problem in determining the true mortality rate in patients with syncope is that most individuals with TLOC do not seek medical advice. It is suspected that most individuals have a low recurrence rate of syncope and an excellent long-term survival in the absence of underlying cardiac disease or channelopathy (eg, long QT syndrome, Brugada syndrome). (26) Underlying cardiac disease substantially increases mortality risk. Outpatients never admitted for their episodes may be at lesser risk for recurrence and have a more benign long-term prognosis than those requiring hospitalization.

10. Conclusion: Syncope is a common clinical problem, which is one of the many causes of transient loss of consciousness (TLOC). Syncope patients often present to emergency department first. The most important step of the first evaluation here is the risk classification. The highest mortality risk occur when syncope is associated with underlying cardiac disease. Syncope in the ED should remain an area of focus given the high cost associated with ED visits, and the impact that ED clinicians have on decisions related to hospital admissions.

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CHAPTER XIII

THE STATE OF ANXIETY-LIKE BEHAVIORS IN ANIMALS WITH METABOLIC SYNDROME

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Metabolic syndrome (MetS), abdominal obesity starting with insulin resistance, glucose intolerance and It is a fatal endocrinopathy combined with systemic disorders such as diabetes mellitus, dyslipidemia, hypertension and coronary artery disease (1). Instead of metabolic syndrome, insulin resistance syndrome, syndrome X, poly metabolic syndrome, fatal quartet, and civilization syndrome is used also medical terminology (2). Long-term (one year) multidisciplinary therapy via physicians, nutritionist, physiologists, psychologist showed that beneficial effect on metabolic disorders (3).

Anxiety disorders are one of the most common mental illnesses (4). Anxiety Disorder Is associated with metabolic Syndrome in some studies (5, 6). While anxiety was not observed in patients with metabolic syndrome in some clinical studies, depression was observed to be significant (7). Some authors argue that anxiety occurs as a by-product of coexistence with depression (8). According to a systematic review and meta-analysis, there is an association between anxiety and metabolic syndrome (9).

Some of the different applications used to trigger off MetS in animals include dietary regimes, genetic modification and drugs administration (10). Anxiety-like behaviors are conditional or unconditional examined by animal

anxiety tests (11). It is the most frequently preferred anxiety test to induce natural and unconditional anxiety in rodents (12). Because of the variety of metabolic syndrome models in this study, anxiety-like behaviors were only examined in the elevated plus maze test. Conditional or unconditional anxiety tests applied other than the elevated plus maze test were excluded. The changes in anxiety-like behavior in the generated tables only include the results from the elevated plus maze.

The new environment triggers anxiety-like behaviors in the subject who is taken from the cage and placed in the new environment. The height in the test setup is the second factor affecting anxiety-like behaviors. Subject exposed to height exhibits sheltering behavior from the open arms of the maze to the closed arm. Therefore, the time spent in the open arm and the number of entries into the open arm are reduced. An increase in stretch-attend posture posture due to stress can also be observed in the open arm (11). Furhermore head dipping behavior (Exploratory behavior) decrease adversly in the open arm (13).

An increase in anxiety like behavior was clearly observed in some of the analyzed studies (14, 15).In some studies, some behavioral patterns were negatively affected, while others were not (16 ,17, 18).

There are also studies that show no change in the elevated plus maze (19, 20). Rebolledo-Solleiro et al reported that metabolic rats displayed anxiety like behaviors in the open field test and marble burying test in same study (19). According to Santos et al, 2017, anxiety lile behaviors were increased in metabolic syndrome rats under restraint stress but non- stressed metabolic syndrome's rat not (20).

Metabolic syndrome can also be seen in polycystic ovary syndrome patients. One of the polycystic ovary syndrome model is dehydroepiandrosterone (DHEA)-induced rodent model (21). Polycystic female rats induced by 5α-dihydrotestosterone exhibited anxiety like behavior in elevated plus maze (22).

Although the general result of the studies is towards an increase in anxiety-like behaviors, the underlying mechanisms have not been fully elucidated. More preclinical studies are needed regarding anxiety and metabolic syndrome.

Table 1: Elevated plus maze parameters

Behavior patterns and unit	Describtion
Open arm time (time or %)	Risky area and trigger off anxiety like behavior.
Open arm entrance (number or %)	Risky area and trigger off anxiety like behavior.
Head dipping (number)	exploratory behavior
Stretcg-attend- postüre (number)	Stress inudced posture

Table 2: Metabolic Syndrome Model Results in the Elevated Plus Maze

Study	Metabolic Syndrome Model	Animal	Behavior parameters	Result
Dinel et al, 2011 (15)	Genetic	*db/db* mice	Open arm time (%)↓, Open arm entrance ↓	ALB ↑
Ressler et al, 2015 (17)	High Fat Diet+ dihydrotestosterone	female Long-Evans rats	Open arm time (%)↓, Open arm entrance ↔	ALB ↔, ↑
Rebolledo-Solleiroet al, 2017 (19)	High Sucrose Diet	Male rats	Open arm time ↔, Open arm entrance (%)↔	ALB↔
Santos et al, 2018 (20)	high refined carbohydrate-containing diet	Male BALB/c mice	Open arm time(%)↔, Open arm entrance (%)↔	ALB↔
Ribeiro et al, 2020 (18)	High Fructose Diet	Male Wistar Rat	Open arm time↔ Open arm entrance↓ Stretch-attend posture↑	ALB↔, ↑
Coêlho et al, 2021 (16)	Monosodium L-glutamate (MSG)	Male swiss mice	Open arm entrance ↔ Rearing number ↓	ALB↔, ↑
Bayram et al, 2022 (14)	High Sucrose Diet	Male Wistar Albino rat	Open arm time↓ Open arm entrance↓ Stretc-attend posture↑, head dipping behavior ↓	ALB ↑

ALB: Anxiety-like behavior, ↑: increase, ↓: decrease, ↔: not change

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CHAPTER XIV

EYE ANATOMY AND PECTEN OCULI

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1. Introduction

The eye, which is the least growing organ after birth, is called oculus in Latin and ophthalmus in Greek. A pair of eyes has about 70% of the receptors in the whole body. In addition, 1/3 of all afferent nerve fibers going to the central nervous system come out of the eye. In birds, the eye is highly developed according to their lifestyle, habitat and physical activity. Their visual acuity is excellent and color vision is well developed. It is believed that most birds, with the exception of birds that hunt at night, can detect light in the ultraviolet spectrum. The visual organ consists of the eyeball (bulbus oculi), located in the orbit, and the accessory structures of the eyeball such as extraocular muscles, orbital fascia, eyelids, eyelashes, eyebrows, orbital fat tissue, and tear system (1,2).

2. Anatomy of the Eye

The eyeball (Bulbus oculi) is classified into three different types according to its shape in poultry. The eye is “flat” in flat-headed diurnal birds such as domestic chickens, “spherical” in sparrows and wider-headed diurnal birds hunting during the day, and “tubular” in nocturnal birds hunting at night (3). It consists of two parts, formed by the intertwining of two spheres of different diameters, the smaller part of which remains outside. The anterior part of the small sphere, called the cornea, is transparent and forms 1/6 of the bulbus oculi. The posterior part, which forms 5/6 of it, belongs to the large sphere and is not transparent. The anterior most prominent point of the eyeball is called the polus

anterior. This forms the central part of the cornea. The most protruding part at the back is called the pole posterior. The straight line connecting the outer part of these two protruding parts is called axis bulbi externus, and the straight line connecting their inner faces is called axis bulbi internus. The line starting from the pole anterior and ending in the fovea centralis of the retina is called the axis opticus (4,5).

2.1. Eye Layers

The bulbus oculi is composed of three layers: tunica fibrosa bulbi, tunica vasculosa bulbi and tunica interna bulbi. Tunica fibrosa bulbi consists of two parts, the cornea anteriorly and the sclera posteriorly. The sclera is made of fibrous tissue with a hard structure and is not transparent. It forms 5/6 of the eyeball and maintains the shape of the eyeball and its volume by resisting intraocular pressure (6). The thickness of the sclera becomes thinner from the posterior to the equator. In front of the equator, the thickness of the repeat increases due to the adhesion of the tendons of the muscles that move the eye. The anterior part of the sclera, that is, the part covered by the eyelids, is covered by the conjunctiva. The outer surface of its posterior part is in contact with the inner surface of the vagina bulbi (Tanon capsule), which separates the bulbus oculi from the orbita. There is a loose connective tissue called the lamina episcleralis within this range. In poultry, the distal part of the sclera bordering the cornea contains bone plates called anulus ossicularis sclerae, which are located in about 14 scleral rings like fish scale. This structure strengthens the bulbus oculi and forms the origin of the striated muscles that provide accommodation. The inner surface of sclera is brown and corrugated. Cilar vessels and nerves are located in these grooves (1,3). The inner face is attached to the lamina suprachoroidea, the outermost layer of the choroidea. On its posterior side, there is a perforated area called the lamina cribrosa sclerae, through which the fibers of the n. opticus pass. This is its weakest area, and the nerve fibers forming the n. opticus, a. and v. centralis retina pass from here. Where N. opticus emerges from the sclera, this layer extends over the nerve, forming a fibrous sheath, and then continues as the dura mater. The sclera continues anteriorly with the cornea, and at the junction of these two structures there is an outer groove, sulcus sclerae. In cross section of the eye, the anterior margin of the sclera appears as two leaves, between which the thin edge of the cornea is inserted. In the inner part of this fork, there is lig. pectinatum anguli iridocornealis, which is made of connective tissue to which m. ciliaris is attached with lig. pectinatum. This structure separates the camera

anterior from the sinus venosus sclera (schlemm's canal), which carries the humor aquosus in the camera anterior bulbi into the venous circulation (4,5,7).

The cornea is the transparent part of the outer layer of the eye and forms the anterior 1/6 of the eyeball. It has no blood vessels except its periphery. Its nutrition is by diffusion from the tissue fluid circulating in the spaces between the lamellae. It has many free nerve endings. If the ratio of the surface area of the cornea to that of the sclera increases, there will be more light transmission (1,5). Nocturnal (night active) animals have larger cornea than diurnal (day active) animals. The most protruding anterior part of the convex anterior surface (facies anterior), which is in contact with the eyelids, is called vertex cotneae. Its concave posterior surface (facies posterior) limits the camera anterior. The thin peripheral edge of the cornea is called the limbus corneae. This place inserts into the groove formed by the anterior edge of the sclera. When viewed from the front, the cornea is slightly flattened from above to the back (8).

Tunica vasculosa bulbi is a layer with a lot of vascularization and loose connective tissue rich in fibroblasts, macrophages, mast cells, plasma cells, collagen and elastic fibers between blood vessels and consists of three parts (7). These are choroidea, corpus ciliare and iris. Choroidea, a thin layer very rich in vessels, lies in the 5/6th of the bulbus oculi and extends to the ora serrata by laying the inner surface of the sclera. Although the dark brown choroidea adheres more firmly to the sclera in the part where the n. opticus enters, it is loosely attached in the remainder. Between the outer surface and the sclera is a space called spatium perichoroideale. This range contains connective tissue, nerves, and vessels that are loosely attached to the sclera. The inner surface is firmly attached to the retina. In the posterior outer and outer part of this face, just above the discus nervi optici, there is a bright and colorful region called the tapetum lucidum. Since this formation reflects the light falling on it, it gives the animal the opportunity to see better in the dark (2,5). Tapetum lucidum cells reflect the incoming light, allowing the receptor cells to be stimulated a second time with the same light. This gives a very good view even in minimal light. The reflected light moves forward and passes out through the pupil. Reflected light is the reason why animals' eyes shine when light hits them at night. Choroidea consists of four layers from outside to inside. These are lamina suprachoroidea, lamina vasculosa, lamina choroidocapillaris, lamina basalis (1,9).

The corpus ciliare is the part of the middle layer that extends from the ora serrata to the outer edge of the iris. Its main structure is m. ciliaris and connective tissue. The peripheral ends of the fibrae zonulare, which hang the lens, attach

to the corpus ciliare and cover the thin pigment layer of the retina (pars ciliaris retinae) on its inner surface. On the inner surface of the corpus ciliare, there are plicae ciliares starting from the ora serrata and extending radially towards the iris. A few of these folds combine to form thicker projections called the processus ciliaris, which run in the same direction. The ring formed by arranging the plica ciliaris side by side is called orbicularis ciliaris. The ring formed by the processus ciliaris in a radial fashion is called corono ciliaris. Fibers called fibrae zonulares begin from the processus ciliaris, extending towards the lens and holding it in place. These fibers are involved in the accommodation of the eye with the m. ciliaris (10,11). Processus ciliaris are structures rich in blood vessels. The humor secreted from here flows into the camera posterior bulbi between the anterior surface of the aquosus lens and the posterior surface of the iris, and passes from the pupil to the camera anterior bulbi. Then it comes to the angulus iridocornealis, which is the junction of the iris and cornea, and passes through the trabecular tissue in the sclera and pours into the sinus venosus sclerae (schlemm's canal). M. ciliaris consists of smooth muscle fibers contained within the corpus ciliaris. In mammals, it starts from the reticulum trabeculare and extends in four different directions. These fibers are named fibrae meridionale (brücke muscle), fibra longitudinales, fibra radiales and fibra circulares (müller muscle) according to their directions. In domestic birds, mm. ciliares are transversely striped, consisting of two or three segments. The outer part is the Crampton's muscle. This muscle originates from the connective tissue covering the bony layers of the anulus ossicularis sclerae. Behind this muscle is the Brücke muscle (5,12).

The iris, meaning rainbow, is located behind the cornea as a circular formation. The color of the iris in poultry is dependent on breeding and age. In the middle is a round hole called the pupil. It is one of the three parts of the tunica vasvasculosa bulbi and is located between the cornea and the lens or in front of the lens. It has two edges, an outer and an inner. Its outer edge attaches to the corpus ciliare. This edge is called the margo ciliaris. Its inner edge delimits a hole in the middle of the organ, called the pupil, which varies in shape according to the species. This edge, which limits the pupil, is called the margo pupillaris (4,13). The iris has two faces, one anterior and the other posterior. Its anterior surface is called facies anterior, and this face facing the cornea also forms the posterior wall of the camera anterior bulbi. On this face, there is a narrow ring near the pupil, called the anulus iridis minor. On the outside of this narrow ring, there is another wider light colored ring, which is called the anulus iridis major.

The folds on the same face, close to and parallel to the margo pupillaris, are also called plicae iridis. The posterior aspect of the iris is called facies posterior. It is the inner face facing the lens and is concave (1,2,8).

Tunica interna bulbi is the innermost light-sensitive layer of the eyeball. This layer, also called the retina, thins towards the ora serrata. Its outer surface is in contact with the choroidea and its inner surface with the vitreous membrane, and it is also thin in the discus nervi optici and macula. The retina consists of two layers, the stratum pigmentosum and the stratum nervosum (2,14). The pars pigmentosa is a pigment layer that covers the entire inner surface of the tunica vasculosa. The pars nervosa extends from the discus nervi optici to the ora serrata on the inner surface of the pars pigmentosa. The part of the retina from discus nervi optici to ora serrata is called pars optica retinae. This is the part of the eye that actually sees. The invisible part in front of the ora serrata, between the processus ciliaris and the posterior aspect of the iris, is called the pars ceca (caeca) retinae. The part of the pars ceca retinae that covers the corpus ciliare is called the pars ciliaris retinae, and the part that covers the posterior surface of the iris is called the pars iridica retinae. Unlike mammals, poultry has pecten oculi, which originates from the retina due to its development and cellular structure (5,12,15).

The retina is soft, translucent and has a purplish color in vivo because of the rhodopsin. A round, yellow colored area is seen in the posterior part of the retina. This area that receives the best light is called macula lutea in humans and area centralis rotunda in animals. The depression in the middle of the macula is called the fovea centralis. In addition, discus nervi optici is seen in the part where the n. opticus pierces the retina. This part differs from one species to another (1,2). The depression in the middle of the discus nervi optici is called excavatio disci. A. and v. centralis retinae passes through this part. It is also known as the blind spot because there are no photosensitive receptors in this part. The retina is the only place where the artery can be seen directly. Pars optica retiae contains bacillus and cone cells, bipolar, multipolar ganglion and amacrine cells from outside to inside. The retina of domestic mammals is mostly rod-shaped, while the retina of domestic birds mostly has cone-shaped cells. Rods are the photoreceptor cells responsible for black and white vision, and cones are responsible for color vision. Rods are highly sensitive to light and are used for night vision, while cones provide the best daytime vision. The axons of the multipolar ganglion cells also extend towards the papilla nervi optici and form the n. opticus. The retina consists of

two layers, the outer stratum pigmentosum and the inner stratum nervosum. Histological sections from the choroidea to the corpus vitreum show that it consists of ten layers (2,16).

2.2. Camera bulbi

There are three cameras in the eye: camera anterior bulbi, camera posterior bulbi, and camera vitrae bulbi. The camera anterior bulbi is bounded anteriorly by the posterior surface of the cornea, posteriorly by the anterior surface of the iris, and where the pupil is located, by the middle part of the anterior surface of the lens. It is filled with humor aquosus. The part where it joins the corpus ciliares and iris is called the angulus iridocornealis. On the outer wall of this corner is the sinus venosus sclerae (schlemm's canal) (1,17). The camera posterior bulbi is located between the iris, lens, corpus vitreum, and corpus ciliare. Since the middle part of the anterior surface of the lens is in contact with the pupil and its surroundings, the camera posterior bulbi is a narrow gap between the peripheral parts that are not in contact. The lens, corpus vitreum, processus ciliaris and attached fibra zonularis are located posterior to the camera posterior bulbi. On the anterior side, it limits the peripheral part of the posterior surface of the iris that does not come into contact with the lens. The pupil connects the camera anterior et posterior bulbi to each other [4,5]. The camera vitrae bulbi is the part behind the retina, corpus ciliare and lens and forms the internal cavity in the bulbus oculi. It is filled with a substance called the corpus vitreum. The corpus vitreum is the largest of the refracting structures and fills the camera vitrae bulbi behind the ora serrata. The semi-gelatinous corpus vitreum is transparent. The depression in front of it is called fossa hyaloidea and the lens fits here. In the middle of the corpus vitreum is a canal extending from the discus nervi optici to the middle of the posterior surface of the lens. This canal is called canalis hyaloideus. In the fetus, a. hyaloidea passes through this channel. The gelatinous fluid inside the corpus vitreum is called the humor vitreus, and the surrounding membrane is called the membrana vitrae. There are no blood vessels in the corpus vitreum. For this reason, its nutrition is supplied by the vessels of its neighbor retina and processus ciliaris (2,16). It is a colorless liquid mostly water secreted by the humor aquosus processus ciliaris. It first fills the camra posterior bulbi, then passes through the pupil to the camera anterior bulbi. Finally, it passes through the angulus iridocornealis and is absorbed by the villi pectinati and empties into the sinus venosus sclerae (schlemm canal). Therefore, it mixes with the venous circulation system (3,13,18).

1.3. Lens

The lens, which is in the form of a convex lens, is located between the iris and the corpus vitreum. The anterior aspect of the lens is called facies anterior and the posterior aspect is called facies posterior. The edge joining these convex faces is called the equator. The posterior face is more convex than the anterior (1,2,14). The most prominent points on the anterior and posterior face are called the polus anterior and pollus posterior. The imaginary line connecting both poles is called the axis. The lens is surrounded by a transparent and flexible membrane. This capsule is called capsula lentis. The lens tissue that completely fills it is called substnatia lentis. The lens is suspended on the processus ciliaris via the fibra zonulares (5,17). Although the capsula lentis is tearable, it is quite flexible. When it is torn for any reason, it immediately folds on itself with its inner side facing out. The posterior surface of the lens sits on the fossa hyaloidea on the anterior surface of the corpus vitreum. The anterior surface, on the other hand, touches the edges of the pupil in the middle part, but does not contact the periphery, and the camera posterior bulbi is located between the iris and the iris (8,16).

The central part of the lens of the eye is composed of the nucleus lentis. The outer part is surrounded by cortex lentis and is harder than the inner part. The anterior surface of the lens is covered with a single cubical epithelium. These epithelial cells retain their shape only on the anterior surface of the lens and grow lengthwise as they approach the equator. In the sun, they take the form of a thin, long fiber. These fibers, called Fibrae lentis, go around the equator and dive deep into the opposite face and lose their nuclei. As a result of new fibers replacing old ones and wrapping them from the outside, lamellae called radii lentis are formed. The spaces between these fibers that keep the lens in its normal position are called spatia zonularia (5,14,18).

3. Pecten oculi

The pecten oculi is a highly vascular and pigmented structure unique to the bird's eye, not found in other vertebral species except birds (19). It originates above the papilla n. optici and has a pincer-like shape. The pecten oculi humour enters the vitreous in a convoluted fashion, but does not extend to the lens. The glial cell network is covered by a comb and contains numerous blood vessels. The pecten oculi is thought to supply the retina, which is devoid of vessels, with oxygen and other nutrients (14,20). It is assumed to have a primary function

in maintaining intraocular pressure (21). It plays a role in reducing intraocular glare, adjusting the blood and retinal fluid barrier, providing vascular circulation in the retina, light absorption, bird direction assurance, warming the bird's eye in cold weather and stabilizing the vitreous body (22).

Three morphological species of pecten oculi are recognized as conical, winged and pleated types. Conical type has been reported only in brown kiwifruit (Apteryxmantelli). It is found in winged ostriches (Struthio camelus) and rhea (Rhea americana). The pleated form is common in most birds such as quail, black kite, galah, vulture, wild duck, pigeon and forest crow (14,20). The size of the pecten depends on the visual requirements of the bird, so diurnal active bird species have a relatively large and highly complex pecten oculi with many folds, whereas nocturnal active bird species have a relatively small, simple, and low pile number pecten oculi (15).

4. Conclusion

Although the eye, as an organ of vision, has similar structures in all living things with its general anatomy, it shows species-specific differences. Birds have one of the most detailed visual systems of any animal species. Differences in eye axis are observed in birds, especially depending on whether they are nocturnal or diurnal. In this section, the general anatomy of the eye and pecten oculi, which is found only in vertebrate animals, are mentioned.

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CHAPTER XV

RECENT DRUG DEVELOPMENT STUDIES ON NOVEL AND SELECTIVE COX-2 INHIBITORS

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most popular medications sold in pharmacies. They are used for acute or chronic inflammation including arthritis, rheumatoid arthritis, osteoarthritis, muscle, and joint injuries, post-operative pain, and headache. (1, 2) Considering that some NSAIDs, such as ibuprofen and aspirin, which are considered safe, are available over-the-counter in most countries, it is clear that their use is high in the world.(3)

NSAIDs work by suppressing the prostaglandin production process, which is believed to be critical in the development of inflammation, pain, and fever. NSAIDs inhibit prostaglandin synthesis from arachidonic acid and exert their effects through the cyclooxygenase (COX) enzyme inhibition pathway.(4) COX-1 and COX-2 enzymes provide the synthesis of prostaglandins in tissues. NSAIDs inhibit the formation of prostaglandins from arachidonic acid *via* COXs pathway.(5, 6) In addition, a new type of COX-3 enzyme, which is the molecular

target of Paracetamol was reported and it has catalytic and structural features of COX-1 and COX-2. COX-3 is presents in large amounts in the cerebral cortex and heart and is affected by Paracetamol and similar drugs.(7)

The regions of enzymes in cells are different from each other. While COX-1 enzyme is present in large of tissues, COX-2 enzyme is synthesized in the injured tissues by stimulation of inflammatory cytokines. COX-1 enzyme has a role in platelet function, coagulation, setting of renal blood flow, protection of gastric mucosa, bone metabolism, etc.(2, 8, 9) The main COX enzymes differ from each other in structure. While COX-1 has Ile523, COX-2 has Val523 in the active site of the enzyme. Val523 creates a cavity in the wall of COX-2 enzyme's channel that leads to forming a side pocket affecting the binding site of many selective drugs compared to COX-1 enzyme.(10-12) Several NSAIDs in the clinic are given in Figure 1. It is necessary to develop selective COX-2 enzyme inhibitors with reduced side effects including gastrointestinal and cardiovascular adverse effects.

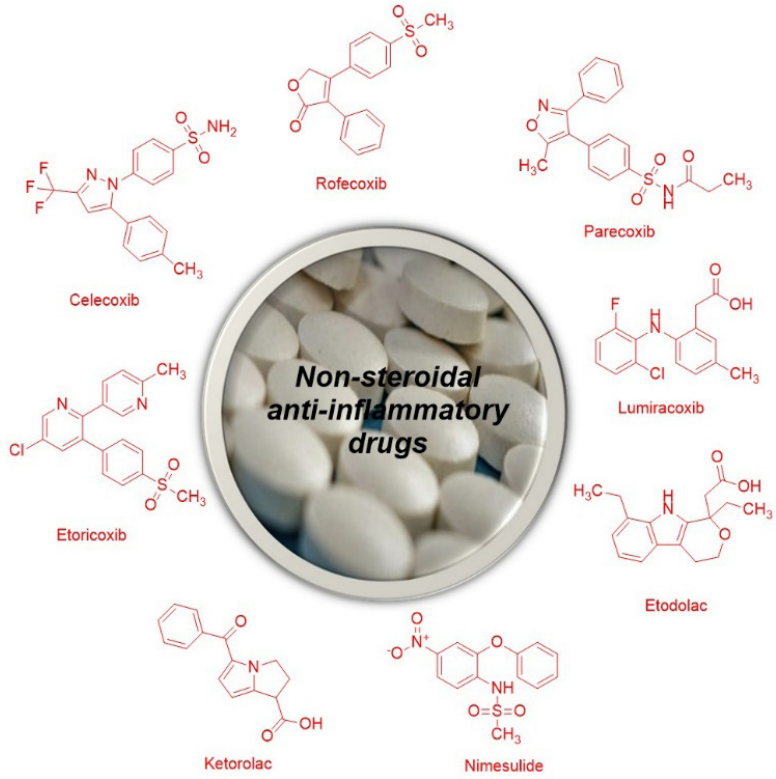


Figure 1. The formula of the well-known NSAIDs

1.1. COX-2 enzyme as a potential target for different diseases

COX-2 inhibitors as anticancer agents: It has been reported that chronic inflammation mediated by prostaglandin E2 may be a reason for cancer, since elevated COX-2 levels may promote the carcinogenesis process. Prostaglandin E2 has also the capacity to depress the immune system by inhibiting natural killer cells.(13-15) As a result, particular COX-2 inhibitors can be considered as possible anticancer drug candidates.

COX-2 inhibitors in anti-Alzheimer's disease: Clinical trials have shown that the level of COX-2 in the Alzheimer's patient is significantly higher compared to the control group. Additionally, it has been claimed that consistent NSAID use halts the progression of Alzheimer's disease.(16, 17)

COX-2 inhibitors in schizophrenia and depression: Celecoxib and risperidone, which were used in a double-blind trial, were found to offer symptomatic improvement for schizophrenia.(18) *In vivo* and clinical trials have shown that COX-2 inhibitors are effective in treating schizophrenia in its early stages. Studies on rats have demonstrated that serotonin levels in the frontal and temporoparietal cortex rise after receiving COX-2 enzyme inhibitors.(19-21) Therefore, it is thought to be favorable to utilize COX-2 inhibitors in the study of neurological diseases.

2. Current Studies on COX-2 Enzyme Inhibitors

Zarghi et al. designed and reported COX-2 inhibition of ketoprofen analogs. Potent compounds 8a and 8b with the azido pharmacophore group (Figure 2) exhibited promising inhibition potency compared to celecoxib. IC_{50} values of compound 8a were 65.55 μM (COX-1) and 0.057 μM (COX-2) while compound 8b had IC_{50} values >100 μM and 0.077 μM, respectively. IC_{50} values of Celecoxib were 24.3 μM and 0.06 μM. According to enzyme-ligand interaction studies of 8a and 8b with COX-2, the azido group bonded strongly to the active site of the enzyme by its interactions with Arg-513. The findings indicate that 2-aryl-4-carboxyl quinoline is a pharmacophore structure for the development of potent COX-2 inhibitors.(22)

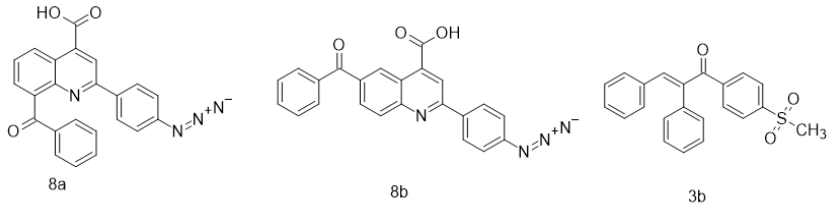


Figure 2. Chemical structures of the most potent compounds 8a, 8b, 3b

Arfaie et al. designed 1,2,3-triaryl-2-propen-1-one derivatives. Compound 3b (Figure 2) showed the remarkable inhibition effect and selectivity on COX-2 (IC_{50} = 0.07 μM) and COX-1 (IC_{50} = 14.27 μM). IC_{50} values of celecoxib were 0.06 μM (COX-2) and 24.3 μM (COX-1). According to the results, the form of propenone and its substituents affected COX-2 inhibition.(23)

Eren et al. reported *in vitro* COX inhibitory activities of 2-oxo-5*H*-furan, 2-oxo-3*H*-1,3-oxazole, and 1*H*-pyrazole structures (Figure 3). Compound 6b has been reported as a potent COX-1 enzyme inhibitor with high selectivity against COX-1 enzyme (IC_{50} = 0.325 μM for COX-2; IC_{50} = 0.061 μM for COX-1). Reference drug indomethacin's IC_{50} values were IC_{50} = 0.537 μM (COX-2) and IC_{50} = 0.069 μM (COX-1). Moreover, compound 11c potently and selectively inhibited COX-2 enzyme with an IC_{50} = 0.011 μM. According to molecular docking findings, a useful scaffold for the subsequent development of strong and selective COX-2 inhibitors has been presented by appropriately substituting *1H*-pyrazole with the benzoxazole structure.(24)

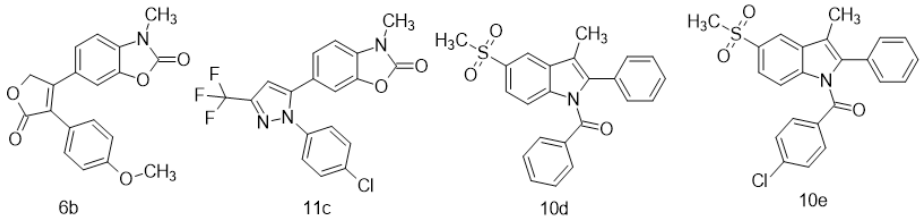


Figure 3. Chemical structures of compounds 6b, 11c, 10d, 10e

COXs inhibitory properties of compounds derived from 3-methyl-2-phenyl-1 substituted indoles were described by Abdellatif et al. They investigated the methanesulfonyl derivatives compound 10d and compound 10e (Figure 3) which showed promising biological effects on COXs. 10d inhibited COX-2 (IC_{50} = 7.98±1.91 μM) and COX-1 (IC_{50} = 32.23 ± 2.36 μM). 10e inhibited COX-2

(IC_{50} = 1.65 ± 1.02 μM) and COX-1 (IC_{50} = 42.36 ± 2.45 μM). The reference drug indomethacin inhibited COX-2 (IC_{50} = 11.36±1.6 μM) and COX-1 (IC_{50} = 0.63 ± 0.02 μM). Compounds 10d and 10e were revealed to have IC_{50} values that made them highly selective COX-2 enzyme inhibitors than the standard indomethacin. The molecular docking and bioassays demonstrated that the compounds bearing a methyl sulfonyl group conferred selectivity for COX-2. The selectivity may have been enhanced by three hydrogen bond interactions between this group and COX-2.(25)

Sabakhi et al. reported the chemical synthesis of 1,4-dihydropyridine derivatives. Compound 5e (Figure 4) had the highest selectivity index. 5e inhibited COX-2 (IC_{50} = 0.59 μM) and COX-1 (IC_{50} = 30.7 μM) while celecoxib had IC_{50} = 0.06 μM (COX-2) and IC_{50} = 24.3 μM (COX-1). According to the molecular docking results, the methyl and ethoxycarbonyl groups interact more strongly with COX-2 enzyme's secondary binding site (Arg513, Phe518, Gly519, and His90), providing selective inhibition of COX-2 enzyme.(26)

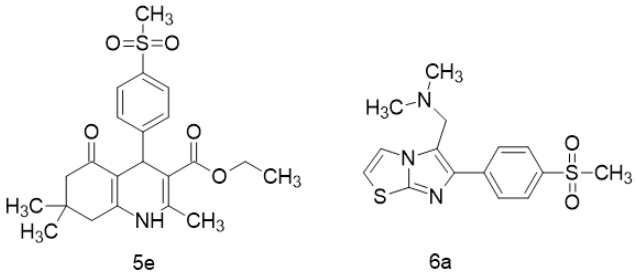


Figure 4. Chemical structures of compounds 5e and 6a

Shahrasbi et al. studied imidazo[2,1-b]thiazole-bearing compounds as selective and strong COX-2 inhibitors. Compound 6a (Figure 4) was identified as a remarkable COX-2 inhibitor. 6a inhibited COX-2 (IC_{50} = 0.08 μM) and COX-1 (IC_{50} = >100 μM). Celecoxib inhibited COX-2 with an IC_{50} = 0.06 μM and COX-1 with an IC_{50} = 24.3 μM. According to molecular docking experiments, the size and type of the amine on the imidazo[2,1-b]thiazole had a positive impact on the potency and selectivity against COX-2.(27)

El-Sayed et al. reported new arylhydrazone derivatives and 1,5-diphenyl pyrazoles with anti-inflammatory properties and COX inhibitory. Compounds 2f, 5, 6a, and 6d (Figure 5) attracted attention with the effects on COX-2 enzyme. 2f inhibited COX-2 (IC_{50} = 0.45 μM) and COX-1 (IC_{50} = >50 μM). Compound 5 inhibited COX-2 (IC_{50} = 0.55 μM) and COX-1 (IC_{50} = >50 μM). Compound 6a

inhibited COX-2 (IC_{50} = 0.45 μM) and COX-1 (IC_{50} = >50 μM). Compound 6d inhibited COX-2 (IC_{50} = 0.45 μM) and COX-1 (IC_{50} = >50 μM). The selectivity index was found between 90-110 against COX-2. The side pocket selectivity in COX-2 enzyme's active site was increased by the aryl hydrazide structure, according to the molecular docking data. Pyrazoles with the proper substitutions can interact with polar areas including Gln192 and Arg513 in the side pocket, increasing their selectivity for COX-2 enzyme.(28)

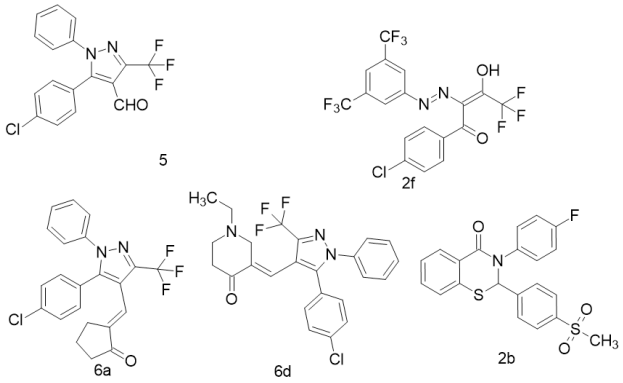


Figure 5. Chemical structures of compounds 5, 6a, 6d, 2f, 2b

Zarghi et al. were designed 1,3-benzdiazinan-4-one derivatives. Compound 2b (Figure 5) inhibited COX-2 (IC_{50} = 0.07 μM) and COX-1 (IC_{50} = 40.1 μM) while celecoxib inhibited COX-2 with an IC_{50} = 0.06 μM and COX-1 enzyme with an IC_{50} = 24.30 μM. Although compound 2b was weaker than celecoxib, its selectivity was higher. Compound 2b's selectivity index was 572.8, while celecoxib's was 405. The enzyme inhibitory activity are both influenced by the structure and size of the substitution made at the C-2 or N-3 locations of the 1,3-benzdiazinan-4-one structure according to molecular docking studies. Furthermore, substances with a methyl sulfonyl group in the para position of the C-2 phenyl ring were more specific for COX-2. In addition, potency and selectivity were improved by substituting appropriate groups like -F and -OMe at the *para* position of the N-3-phenyl ring.(29)

Zargh et al. designed 5,5-diarylhydantoin derivatives as COXs inhibitors. Compound 4 (Figure 6) inhibited COX-2 (IC_{50} = 0.077 μM) and COX-1 (IC_{50} = >100 μM. Celecoxib inhibited COX-2 with an IC_{50} = 0.06 μM and COX-1 with an IC_{50} = 24.30 μM. Compound 4's selectivity was >1298 while reference drug celecoxib's was 405. Molecular docking studies revealed that the *p*-methyl sulfonyl group on the C-5 phenyl ring was directed to the COX-2 enzyme's side pocket and increased selectivity.(30)

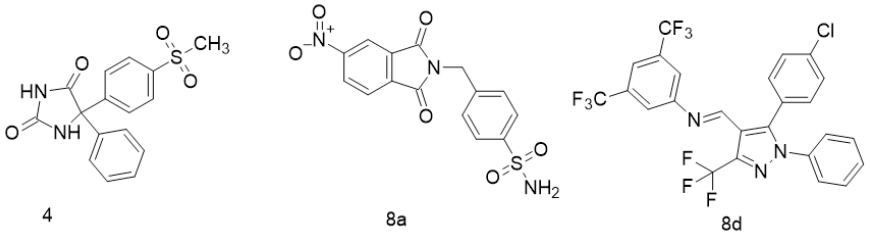


Figure 6. Chemical structures of compounds 4, 8d and 8a

El-Sayed et al. reported COXs inhibitory potencies of pyrazole and pyrazolines. Compound 8d (Figure 6) has been reported to be a promising COX-2 inhibitor. Compound 8d inhibited COX-2 (IC_{50} = 0.26 μM) and COX-1 (IC_{50} = >50 μM). Diclofenac also inhibited COX-2 with an IC_{50} = 1.1 μM and COX-1 with an IC_{50} = 0.22 μM while celecoxib inhibited COX-2 with an IC_{50} = 0.28 μM and COX-1 with an IC_{50} = >50 μM. The selectivity of compound 8d was found higher than the reference celecoxib. Similar to celecoxib, the molecular interactions of 8d demonstrated their effect by binding to the side pocket of the COX-2 enzyme. The trifluoromethyl moieties of compound 8d were discovered to penetrate deeper into the side pocket of the COX-2 enzyme, forming strong hydrogen bonds with Gln192 and Arg513. This study found that appropriately substituted triarylpyrazole-type compounds inhibited COX-2 and had a high anti-inflammatory activity.(31)

Al-Suwaidan et al. synthesized benzenesulfonamide derivatives. Compound 8a (Figure 6) showed strong COX-2 inhibition and high anti-inflammatory effect. 8a inhibited COX-2 (IC_{50} = 0.10 μM) and COX-1 (IC_{50} = >100 μM). Diclofenac inhibited COX-2 (IC_{50} = 4.2 μM) and COX-1 (IC_{50} = 0.25 μM) while celecoxib inhibited COX-2 with an IC_{50} = 0.26 μM and COX-1 with an IC_{50} = >100 μM. In comparison to celecoxib and diclofenac, compound 8a has been found to have better anti-inflammatory efficacy and higher COX-2 enzyme inhibition. Investigations of compound 8a's interactions with the enzyme concerning COX-2 were conducted. It was revealed that the sulfonamide group in compound 8a's structure entered the COX-2 enzyme's side pocket deeply and made hydrogen bonds with the amino acids Gln192, Phe518, and Arg513. According to reports, compound 8a and celecoxib had similar binding properties.(32)

Kim et al. reported *1H*-methyl-pyrrole-2,5-dione derivatives as COX inhibitors. The most potent compound 9d (Figure 7) inhibited COX-2 with an IC_{50} = 0.006 μM and COX1 with an IC_{50} = >1.0 μM. IC_{50} values of reference

celecoxib were IC_{50} = 0.072 μM (COX-2) and IC_{50} = >1.0 μM (COX-1) while ibuprofen had IC_{50} = 30 μM (COX-2) and IC_{50} = 26 μM (COX-1). The findings show that compound 9d had a stronger COX-2 inhibition than the standard celecoxib. Compound 9d improved COX-2 side pocket contact by establishing hydrogen bonds with the sulfonamide group, Arg499, Phe504, Ile503, Gln178, and Leu338 proteins.(33)

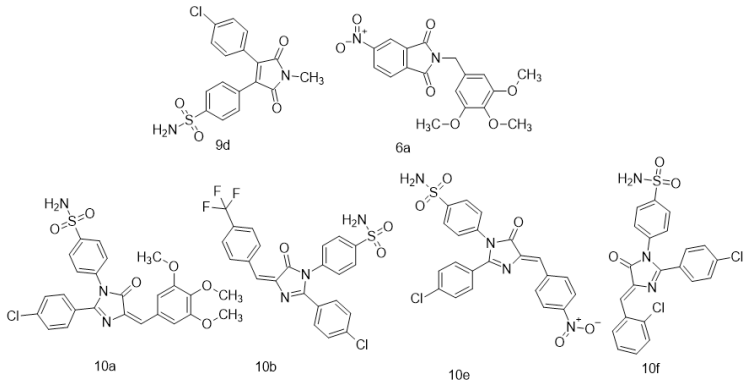


Figure 7. Chemical structures of compounds 9d, 6a, 10a, 10b, 10e, 10f

Alanazi et al. reported cyclic imide compounds with their COXs inhibition and other bioactivities. Compound 6a (Figure 7) was identified as COX-2 inhibitor. IC_{50} values of 6a were IC_{50} = 0.18 μM (COX-2) and IC_{50} = 120.3 μM (COX-1). Celecoxib had IC_{50} = 0.26 μM (COX-2) and IC_{50} = >100 μM (COX-1) while diclofenac had IC_{50} = 1.1 μM (COX-2) and IC_{50} = 4 μM (COX-1). Compound 6a's selectivity index was 668.3 while celecoxib's index was 384.6. Investigations into the molecular interactions of 6a with the COX-2 enzyme were investigated. According to report, the methoxy groups of chemical 6a form bonds with the amino acids His90, Arg513, and Gln192 and settle deep inside the side pocket of the COX-2 enzyme.(34)

Abdellatif et al. reported anti-inflammatory, ulcerogenic, and COXs inhibition of 1,2-diaryl-4-substituted-benzylidene-5-*4H*-imidazolone derivatives. Compounds 10a, 10b, 10e, and 10f (Figure 7) had higher selectivity towards COX-2 than celecoxib. Compound 10a inhibited COX-2 (IC_{50} = 0.42 μM) and COX-1 (IC_{50} = 4.52 μM), compound 10b inhibited COX-2 (IC_{50} = 0.62 μM) and COX-1 (IC_{50} = 6.74 μM). Compound 10e inhibited COX-2 (IC_{50} = 0.52 μM) and COX-1 (IC_{50} = 4.52 μM). Compound 10f inhibited COX-2 with an IC_{50} = 0.86 μM and COX-1 with an IC_{50}= 7.86 μM. IC_{50} values of celecoxib

were 0.84 μM (COX-2) and IC_{50} = 7.23 μM (COX-1). Comparing the four substances to ibuprofen and celecoxib, it was discovered that they were much less ulcerogenic.(35)

Mohamed et al. reported COXs inhibition studies of oxazolone and triazinon derivatives. Compounds 3c (IC_{50} = 0.024 μM), compound 3e (IC_{50} = 0.019 μM), compound 4d (IC_{50} = 0.011 μM), compound 4g (IC_{50} = 0.014 μM) were found as COX-2 inhibitors. IC_{50} of celecoxib was IC_{50}=0.05μM. Compounds 3c, 3e, 4d, and 4g (Figure 8) showed remarkable enzyme inhibition potency than the reference drug.(36)

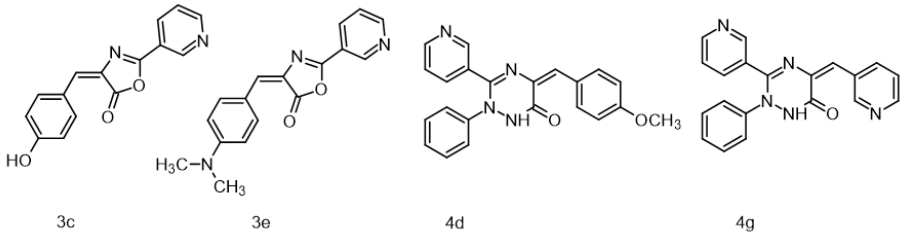


Figure 8. Chemical structures of compounds 3c, 3e, 4d and 4g

Abdelgawad et al. evaluated pyrazole-hydrazones as COX and lipoxygenase (LOX) inhibitors. Compounds 4a and 4b (Figure 9) selectively inhibited COX-2 enzyme. Compound 4a had IC_{50} = 0.67 μM (COX-2) and IC_{50} = 5.64 μM (COX-1) values. Compound 4b had IC_{50} = 0.58 μM (COX-2) and IC_{50} = 6.12 μM (COX-1) values. Reference drug celecoxib inhibited COX-2 enzyme at IC_{50} = 0.87 μM and COX-1 enzyme at IC_{50} = 7.7 μM. In addition, compounds 4a and 4b exhibited higher LOX enzyme inhibitory properties than zileuton. The interactions of 4a and 4b with the enzyme were investigated. According to the findings, pyrazole groups formed H-bonds in the active site of COX-2 receptor, which helped to contribute to the selective inhibition of COX-2 enzyme.(37)

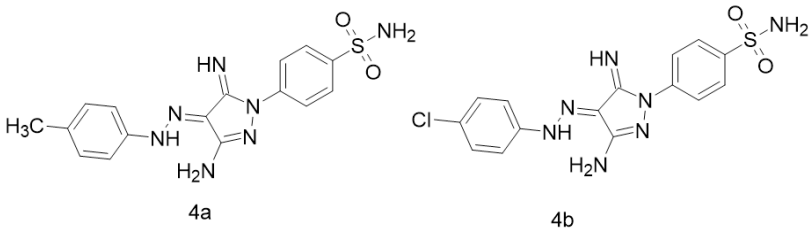


Figure 9. Chemical structures of compounds 4a and 4b

Moussa et al. designed a series of thioquinazolinone series as COXs inhibitors. Compounds 3h (Figure 10) was the most potent COX-2 inhibitor (IC_{50} = 0.11 μM). Favorable interactions between compound 3h and COX-2 enzyme's side pocket were observed.(38)

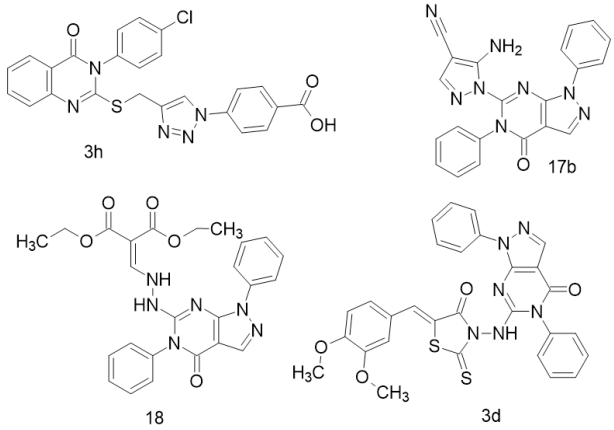
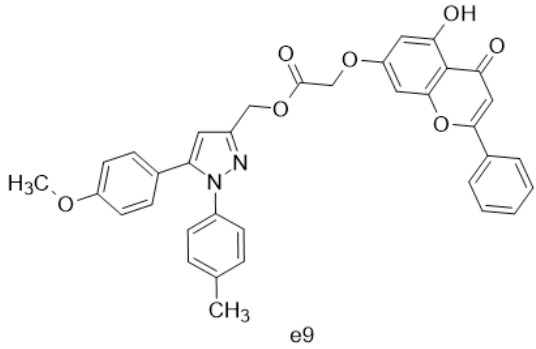


Figure 10. Chemical structures of compounds 3h, 17b, 18 and 3d

Tageldin et al. evaluated COXs inhibition effects of pyrazolo[3,4-d] pyrimidines. Compounds 17b and 18 (Figure 10) showed promising inhibition effects. 17b inhibited COX-2 (IC_{50} = 0.22 μM) and COX-1 (IC_{50} = 2.74 μM). Compound 18 inhibited COX-2 with an IC_{50} = 0.69 μM and COX-1 with an IC_{50} = 5.88 μM. IC_{50} values of celecoxib were IC_{50} = 0.78 μM (COX-2) and IC_{50} = 5.46 μM (COX-1). The compounds 17b and 18 demonstrated tolerable gastrointestinal safety.(39) In the next study, they reported different pyrazolo[3,4-d]pyrimidines with their bioactivities. Compound 3d (Figure 10) was found as a potent and selective COX-2 inhibitor with an IC_{50} = 0.11 μM. Additionally, it has been demonstrated that this class of drugs has a favorable gastrointestinal profile and potent anti-inflammatory effects.(40)

Ren et al. reported enzyme inhibitory and cytotoxic effects of diaryl pyrazoles. Compound e9 (Figure 11) was distinguished among them due to its remarkable bioactivities. e9 inhibited COX-2 (IC_{50} = 0.23 ± 0.02 μM) and COX-1 (IC_{50} = 47.48 ± 2.73 μM) similarly to reference drug celecoxib. The chemical interactions between e9 and the enzyme COX-2 were investigated. By using Van der Waals contacts, compound e9 strongly bound to the side pocket of the COX-2 enzyme than celecoxib. The findings suggest that compound e9's selective COX-2 enzyme inhibition and antiproliferative properties may make it a suitable candidate for cancer treatment.(41)



e9

Figure 11. Chemical structure of compound e9

Labib et al. revealed isoindoline hybrids as a promising class of compounds. In comparison to celecoxib (IC_{50} = 0.09 μM), compounds 10b, 10c, 11a, 11d, 13, and 14 (Figure 12) demonstrated COX-2 inhibition in the range of IC_{50} = 0.11-0.18 μM. The majority of the compounds exhibited notable central and/or peripheral analgesic efficacy. Investigations were done into how the produced substances interacted with the amino acid residues in the COX-2 active region. While the presence of a hydrogen acceptor group mimicking the aminosulfonyl pharmacophore, such as the methoxy group at 11d and the acetyl group at 13, improved the compounds' ability to inhibit enzymes, the aminosulfonyl group at the *para* position of the phenyl ring also contributed to the activity for compounds (10c, 14).(42)

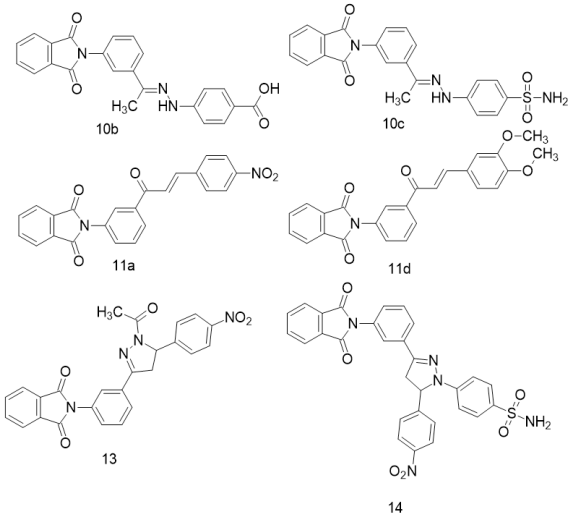


Figure 12. Chemical structure of compounds 10b, 10c, 11a, 11d, 13 and 14

Kaur et al. investigated inhibitory effects of benzamides towards COXs. Compound 3b (Figure 13) was discovered to be the most effective COX-2 inhibitor. IC_{50} values of 3b were IC_{50} = 0.14 µM (COX-2) and IC_{50} = 4.40 µM (COX-1). IC_{50} values of ibuprofen were IC_{50} = 1.8 µM (COX-2) and IC_{50} = 1.42 µM (COX-1) while celecoxib had IC_{50} = 0.15 µM (COX-2) and IC_{50} = 6.20 µM (COX-1) values. In addition, compound 3b showed higher anti-inflammatory activity than ibuprofen. Studies have shown that the ulcerogenic activity of compound 3b is lower than that of ibuprofen. The molecular interactions of compound 3b were investigated against COX-2 enzyme. The interaction with the COX-2 enzyme involved the crucial function of the benzoxazole ring. Furthermore, the insertion of electron-withdrawing groups to the *ortho* and *para* locations of the phenyl ring improved the compounds' activity.(43)

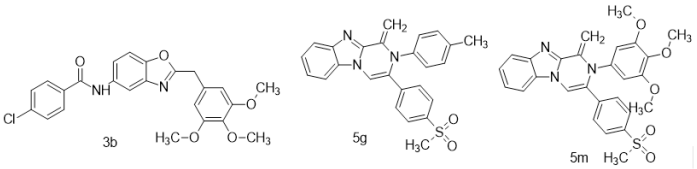


Figure 13. Chemical structure of compounds 3b, 5g, and 5m

Movahed et al. evaluated pyrazino[1,2-a]benzimidazole derivatives in terms of COX-2 enzyme inhibition, anticancer, and antiplatelet aggregation activities. Compound 5g (Figure 13) showed the strongest COX-2 enzyme inhibition. IC_{50} values of compound 5g were IC_{50} = 0.07 µM (COX-2) and IC_{50} = 55.7 µM (COX-1) (Selectivity index = 795.7). Celecoxib inhibited COX-2 with an IC_{50} = 0.06 µM and COX-1 with an IC_{50} = 24.3 µM (Selectivity index = 405). When compared to the reference drug, the compound 5g demonstrated effective and selective COX-2 inhibition. In addition, compound 5m (Figure 13) against COX-2 enzyme was the strongest compound in terms of selectivity index > 909. The reference drug cisplatin inhibited MCF-7 cancer cells by 76.2% at a dose of 10 µM, whereas the compound 5m inhibited MCF-7 cancer cells by 74.8% at the same concentration. These findings showed that compounds that selectively inhibit the COX-2 enzyme may exhibit strong cytotoxicity.(44)

The COX-1/COX-2 enzyme inhibition, anti-inflammatory, and ulcerogenic activities of pyrazolo-pyrimidinones and pyrazolo-triazolo-pyrimidinones were assessed by Tageldin et al. The most potent compounds 2c, 6a, 8, and 12 (Figure 14) had IC_{50} in the range of 0.29-0.74 µM. The anti-inflammatory activity of these substances was also greater than celecoxib's.(45)

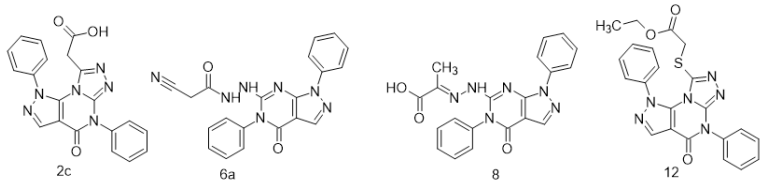


Figure 14. Chemical structure of compounds 2c, 6a, 8 and 12

Ahmed et al. evaluated pyridazinone and pyridazine derivatives in terms of COX enzyme inhibitory activity. Compound 3g (Figure 15) inhibited COX-2 with an IC_{50} = 43.84 ± 1.1 µM and COX-1 enzyme with an IC_{50} = 505.01 ± 16.5 µM. The reference drug celecoxib inhibited COX-2 enzyme with an IC_{50} = 73.53 ± 2.59 µM, and COX-1 enzyme with an IC_{50} = 873.44 ± 19.47 µM. In comparison to celecoxib and indomethacin, compound 3g displayed a better GI safety profile. Studies using molecular docking have demonstrated that compound 3g interacts more strongly than celecoxib with the binding site pocket of the COX-2 enzyme. Compound 3g's sulfonamide group established hydrogen bonds with the amino acids His 90 and Arg 513 in the COX-2 side pocket, and further hydrogen bonds formed with Ser 530 and Tyr 385 over two methoxy groups.(46)

Health et al. evaluated thiazolylhydrazine-methyl sulfonyl derivative compounds in terms of COX inhibition. Compound 3a (Figure 15) had an IC_{50} = 0.140 ± 0.006 µM (Selectivity index = > 714.286) towards COX-2. IC_{50} values of references were as follows; celecoxib IC_{50} = 0.132 ± 0.005 µM, ibuprofen IC_{50} = 5.326 ± 0.218 µM, nimesulide IC_{50} = 1.684 ± 0.079 µM towards COX-2. Investigations on compound 3a's interactions with COX-2 were conducted. Studies using molecular modeling have revealed that compound 3a interacted with COX-2 enzyme similarly to celecoxib.(47)

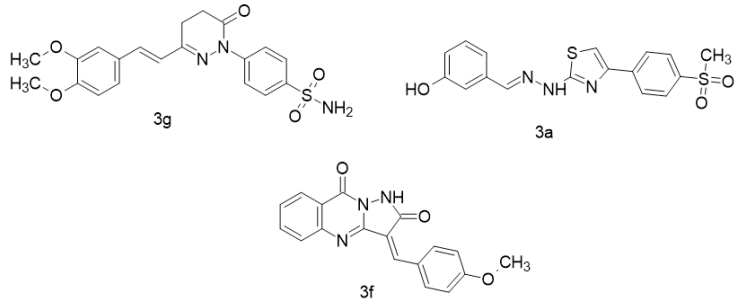


Figure 15. Chemical structure of compounds 3g, 3a and 3f

Shaaban et al. reported a group of pyrazoloquinazoline derivatives as COX and 5-LOX inhibitors. IC_{50} values of compound 3f (Figure 15) were IC_{50} = 488.2 ± 21.2 μM (COX-2), IC_{50} = 1.485 ± 34.8 μM (COX-1), and IC_{50} = 0.6 ± 0.02 μM (5-LOX). Celecoxib inhibited COX-2 enzyme with an IC_{50} = 95.02 ± 3.8 μM, COX-1 enzyme with an IC_{50} = 187.8 ± 13.2 μM. Zileuton inhibited 5-LOX enzyme at IC_{50} = 0.8 ± 0.03 μM. Compound 3f had higher COX-2 enzyme selectivity than celecoxib. The interactions of compound 3f with the enzyme and bioassay results showed that compound 3f could be a leading structure for designing novel agents.(48)

Alfayomy et al. evaluated pyrimidine-5-carbonitriles as COXs inhibitors. 10c, 10j, and 14e (Figure 16) had enzyme inhibitory potency in the range of IC_{50} values 0.042-0.081 μM. When the ulcerogenic potential of these substances was examined, compound 10j displayed superior safety to celecoxib, but compounds 10c and 14 exhibited minor lesions. Compared to rofecoxib and celecoxib, these substances demonstrated a comparable interaction with the hydrophobic side pocket of the COX-2 enzyme. In addition to interacting with Arg513 in the side pocket, the 4-chlorophenyl of compound 10c and the 2,6-dichlorophenyl of compound 10j also contributed to interactions with the proteins Ala516, Ile 517, Phe518, and Gln192.(49)

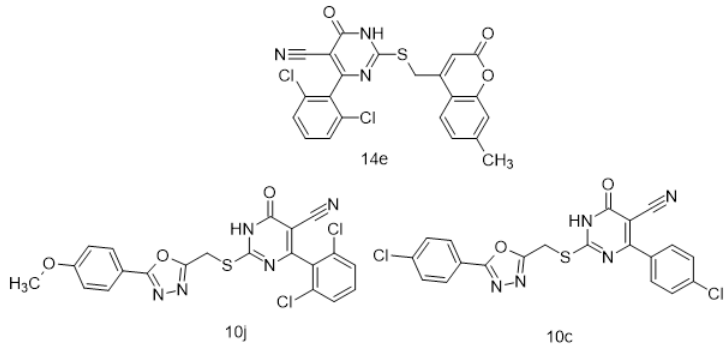


Figure 16. Chemical structure of compounds 14e, 10j and 10c

Sakr et al. reported quinazolinone-based compounds and their ulcerogenic, anti-inflammatory, anticancer, and COXs enzyme inhibitions. Compounds 4a (IC_{50} = 12.68 ± 0.11 μM for COX-1 and IC_{50} = 0.04 ± 0.08 μM for COX-2) and 7c (IC_{50} = 14.73 ± 0.13 μM for COX-1 and IC_{50} = 0.037 ± 0.20 μM for COX-2) (Figure 17) showed similar anti-inflammatory activity compared to ibuprofen and celecoxib, but more potent than indomethacin. The mean edema inhibition

was 45.37% for compound 7c and 47.18% and 47.60% for the reference drugs ibuprofen and celecoxib, respectively. When tested on HT29 cells that express COX-2, compounds 4a and 7c showed partial cytotoxic activity. Against the enzyme COX-2, interactions between compound 7c and compound 4a were examined. In the COX-2 enzyme's active region, compound 4a created an extra hydrogen bond with Ala527, and compound 7c created two hydrogen bonds with Val523 and Arg120. Compounds 4a and 7c may be candidates for further studies.(50)

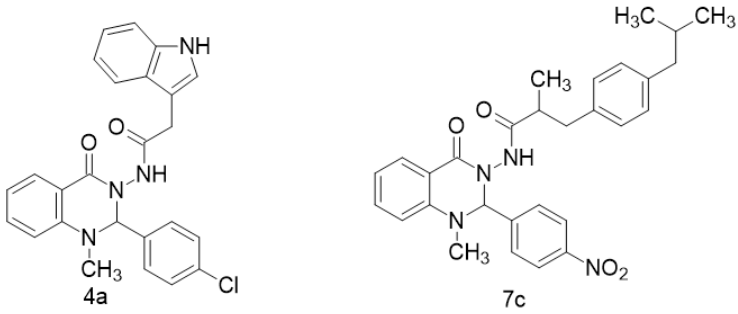


Figure 17. Chemical structure of compounds 4a and 7c

Abdellatif et al. reported indomethacin analogs with their ulcerogenic effects and COXs inhibitions. It has been reported that compound 4b (Figure 18) was a potent and selective COX-2 enzyme inhibitor with IC_{50} values IC_{50} = 0.09 μM (COX-2) and IC_{50} = 0.57 μM (COX-1). Celecoxib had IC_{50} = 0.89 μM (COX-2) and IC_{50} = 3.14 μM (COX-1) values. However, histopathological studies showed that compound 5 (Figure 18) was a promising candidate because compound 4b induced moderate lesions in the stomach. Results were also corroborated by interactions between compounds and the COX-2 enzyme.(51)

Osmaniye et al. reported COX-2 enzyme inhibitors having N-acyl hydrazone structure. IC_{50} of compound 3j was 0.143 ± 0.006 μM while celecoxib had IC_{50} = 0.132 ± 0.005 μM towards COX-2. Compound 3j (Figure 18) had similar COX-2 inhibition as the reference drug. Investigations of the COX-2 enzyme's interactions with 3j were also conducted. It has been demonstrated that the polar side pocket of the COX-2 enzyme interacts with the methyl sulfonyl group linked to the N-acyl hydrazone group. Additionally, the Gln512 amino acid in the side pocket of the COX-2 enzyme formed a second interaction (halogen bond) with the chlorine atom in the second position on the phenyl ring, strengthening the binding.(52)

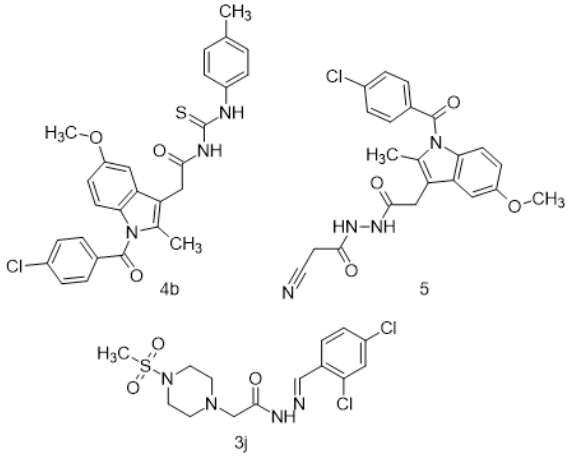


Figure 18. Chemical structures of compounds 4b, 5, 3j

Abdellatif et al. reported 1,2,4 triazoles as selective COX-2 inhibitors. Compounds 4a, 6a, and 7a (Figure 19) had remarkable activity compared to the reference drug celecoxib. In addition to having edema inhibition capability that was superior to celecoxib and indomethacin. According to the molecular docking findings, these substances had a stronger binding affinity for the proteins in the COX-2 enzyme's side pocket and improve selectivity.(53)

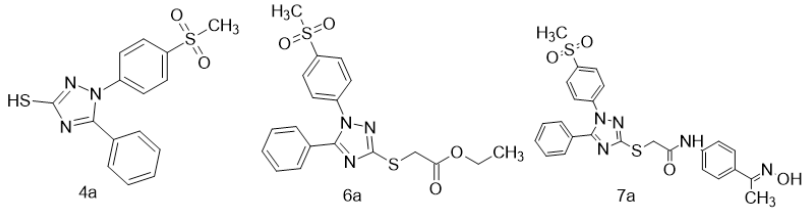


Figure 19. Chemical structures of compounds 4a, 6a and 7a

3. Conclusion

Prostaglandins are produced in the body when there is an infection, and they can lead to several unpleasant conditions like pain, fever, and inflammation. Prostaglandins are often produced *via* the COX enzyme pathway in the body, which indicates that we can eliminate prostaglandin's negative effects by blocking this mechanism. Drugs that inhibit the COX enzyme have been used in the clinic for many years. It has been established that there are many COX enzyme types with distinctive functions. Particularly in the stomach mucosa,

the COX-1 enzyme has a protective function. Because of this, excessive COX-1 inhibition is undesirable, and it also has side effects on the gastrointestinal system. Selective COX-2 inhibition is required to prevent these adverse effects. COX-1 and COX-2 have some structural variations. Compared to COX-1, COX-2 has a bigger side pocket. This side pocket enables the creation of specific drugs that target the COX-2 enzyme. COX-2 has been regarded as the primary target enzyme in the prevention of inflammation.

This review states that research on selective COX-2 enzyme inhibitors is ongoing due to the severe gastrointestinal and cardiovascular side effects of currently available COX inhibitors. The compounds described here, which have a variety of heterocyclic systems and functional groups, may be used to develop novel COX-2 enzyme inhibitors as potential non-steroidal anti-inflammatory pharmaceutical candidates. Additionally, numerous studies have mainly focused on the part COX-2 plays in the development of several illnesses, including cancer, Alzheimer's disease, and other neurological disorders.

Authors' contributions

This review was prepared within the scope of the graduation report prepared Onur Baltacıoğlu under the supervision of Assoc. Prof. Dr. Cem Yamalı.

CY (Supervisor): Design and organization of the study. Manuscript design, preparation, editing.

OB: Collecting data, searching literatures.

SD: Preparing figures, editing references, formatting.

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CHAPTER XVI

DRUG REPURPOSING STUDY FOR TRIAZOLE RING BEARING AZOLE ANTIFUNGALS AS CHEI CANDIDATES

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1. Introduction

Drug repurposing (drug repositioning, reprofiling or re tasking) is done for the discovery of new indications by repositioning the drug. This method is used to ensure that FDA-approved clinically used drug molecules or unlicensed drugs are used as new therapeutic agents. The aim is to adapt or expand the indication of the drug during the drug development phase (1). The main advantage of this method is that the risk of failure is lower; less likely to fail safety for new use as existing drug is already known to be safe (2). In addition, new drug candidates must go through many different stages, including disease state and target identification, preclinical study, toxicity study, formulation, clinical trial, approval process, and marketing. This method is becoming more and more attractive because it takes a long time to develop new drugs, is expensive, and allows drugs to be reused (3).

Azoles are the most widely used antifungals in clinical practice. Azole antifungals exert their effects by inhibiting the synthesis of fungus ergosterol (4). The first approved antifungal drugs were compounds bearing the imidazole ring. These drugs have serious side effects such as poor oral absorption, inability to cross the blood-brain barrier, and liver and gastrointestinal system

complications. For these reasons, new compounds have been developed using the triazole ring instead of imidazole as the azole. Fluconazole was introduced in 1990 and provided many advantages over imidazole derivatives. Subsequently, many drugs have been developed that contain a triazole ring in their structure (5-7). Currently, there are about 40 azole-containing drugs and drug candidates that can be classified (6, 8, 9).

Alzheimer's disease (AD) is a neurodegenerative disease whose prevalence and incidence increase with advancing age. It is expected to increase more in the coming years in the population aged 65 and over worldwide (10-12). Cholinesterase inhibition is a hypothesis of great importance for symptomatic treatment in Alzheimer's disease. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), known as cholinesterase enzymes, are enzymes responsible for the hydrolysis of acetylcholine in the synaptic cleft. The purpose of cholinergic inhibition is to prevent hydrolysis of acetylcholine and to ensure that it is present in the synaptic gap at an adequate level. There are cholinesterase inhibitor drugs used in the clinic for this purpose (13-16). One of the drugs approved for the treatment of AD, Donepezil has aroused great interest for researchers.

Due to the insufficient activity of the cholinergic system, Donepezil inhibits cholinesterase and may alleviate neuronal degeneration (17, 18).

In this study, triazole ring structure azole antifungal drugs used in the treatment of systemic fungal infections, different indication studies for Alzheimer's disease, which still has no definitive treatment, were carried out in silico methods. In a literature study, an azole library was evaluated against acetylcholinesterase and butyrylcholinesterase using in vitro and in silico methods (19). Fluconazole, Itraconazole, Voriconazole, Ravuconazole, Posaconazole, Eficonazole and Isavuconazole drug active ingredients, which are in triazole antifungal structure, were used in our study. The interactions of these drugs with AChE enzyme by in silico methods were investigated and compared with Donepezil drug active ingredient.

2. Methods

In this study, molecular docking studies were applied with in-silico approaches. Donepezil, which is effective in AChE and BChE enzymes, was taken as a reference compound and drugs containing azoles with triazole ring structure (Fluconazole, Itraconazole, Voriconazole, Ravuconazole, Posaconazole, Eficonazole and Isavuconazole) were examined.

2.1. Molecular Docking Studies

Schrödinger 2021-2 software (Schrödinger Release 2021-2: Glide, LLC New York, USA) (20) was used in molecular docking studies. Molecular docking examinations were applied according to the order in the specified steps.

➢ **Preparation of ligands:** Donepezil, Fluconazole, Itraconazole, Voriconazole, Ravoconazole, Posaconazole Eficonazole and Isavuconazole compounds were optimized using the LigPrep wizard (Schrödinger Release 2021-2: LigPrep) (21) utility of the Schrödinger 2021-2 software (22, 23).

➢ **Preparation of proteins:** Crystal structures of proteins with which compounds will interact were obtained from the Protein Data Bank (https://www.rcsb.org/). PDB ID:4EY7 (24) was used for AChE crystal structure and 4BDS was used for BChE crystal structure. The crystal structures obtained from the protein database were prepared separately with the "Protein Preparation Wizard" (25) module of the Schrödinger 2021-2 software, respectively.

➢ **Ligand-target interaction *via in silico* approaches:** After ligands and proteins were prepared one by one with intermediate modules, both docking score values and binding sites were determined by interacting with the ligand placement wizard. The method used in molecular docking was also applied as in previous studies (23, 26).

2.2. MM-GBSA

Molecular mechanical energies combined with the Poisson-Boltzmann or generalized Born and surface area continuous solving (MM/GBSA) method are popular approaches to estimate the free energy of attachment of small ligands to biological macromolecules (27). According to the MM-GBSA method, the binding value of the complex can be determined by calculating the free binding energy (ΔG_{Bind}) between the ligand and the target.

The OPLS-2005 force field and VSGB solvent model of Prime MM/GBSA (Schrödinger Release 2021-2: Prime) (28) were used to calculate ΔG_{Bind} energy values. MM-GBSA analysis was applied to calculate the free binding energies of the indicated compounds with both AChE and BChE enzymes, respectively.

3. Results and Discussion

3.1. Molecular Docking Studies

Molecular docking studies were performed to determine the binding parameters of Donepezil, Fluconazole, Itraconazole, Voriconazole,

Ravuconazole, Posaconazole, Eficonazole and Isavuconazole compounds. The best results determined for compounds in molecular docking studies were obtained with in silico approaches. Crystal structures PDB ID:4EY7 (24) for AChE and PDB ID:4BDS (29) for BChE were used.

The binding parameter values as a result of the binding interactions of these compounds with the AChE and BChE crystal structures are presented in Table 1.

First of all, the binding parameter values of Donepezil, which is used as the reference compound in Table 1, obtained in silico were examined. Docking score, XP GScore and Glide energy values of Donepezil interacted with AChE crystal structure are -15.837, -15.840, -49.514, respectively. Likewise, the docking score, XP GScore and Glide energy values of Donepezil in BChE were determined as -6.820, -6.824, and -44.119, respectively. When these values are taken as a reference, it can be said that Eficonazole has the closest values for AChE. Molecular docking result parameter values of Eficonazole compound were calculated as Docking Score, XP Gscore, Glide energy values -11,707, -11.737, -48.868 for AChE. For the BChE crystal, Posaconazole, which has better binding values than the reference drug Donepezil, was found remarkable in Table 1. Docking Score, XP Gscore and Glide energy values of the Posaconazole compound were calculated as -7.189, -7.207, -62.656, respectively, by molecular docking method.

Table 1. Binding parameter values of compounds interacting with AChE and BChE crystal structures in molecular docking.

	PDB ID: 4EY7			**PDB ID:4BDS**		
Compounds	**Docking Score**	**XP Gscore**	**Glide energy**	**Docking Score**	**XP Gscore**	**Glide energy**
Donepezil	**-15.837**	**-15.840**	**-49.514**	**-6.820**	**-6.824**	**-44.119**
Fluconazole	-7.534	-7.534	-66.524	-4.913	-4.913	-36.071
Itraconazole	-7.911	-7.933	-64.732	-4.646	-6.618	-55.530
Voriconazole	-8.670	-8.670	-46.734	-6.174	-6.174	-34.700
Ravuconazole	-8.432	-8.432	-58.117	-1.532	-1.532	-48.065
Posaconazole	-8.171	-8.188	-66.856	**-7.189**	**-7.207**	**-62.656**
Eficonazole	**-11.707**	**-11.737**	**-48.868**	-3.719	-5.496	-37.800
Isavuconazole	-9.944	-9.944	-55.424	-4.310	-4.310	-46.907

When the binding parameter values of the compounds examined in Table 1 were not sufficient, they were compared with Donepezil by examining the amino acids in the binding site.

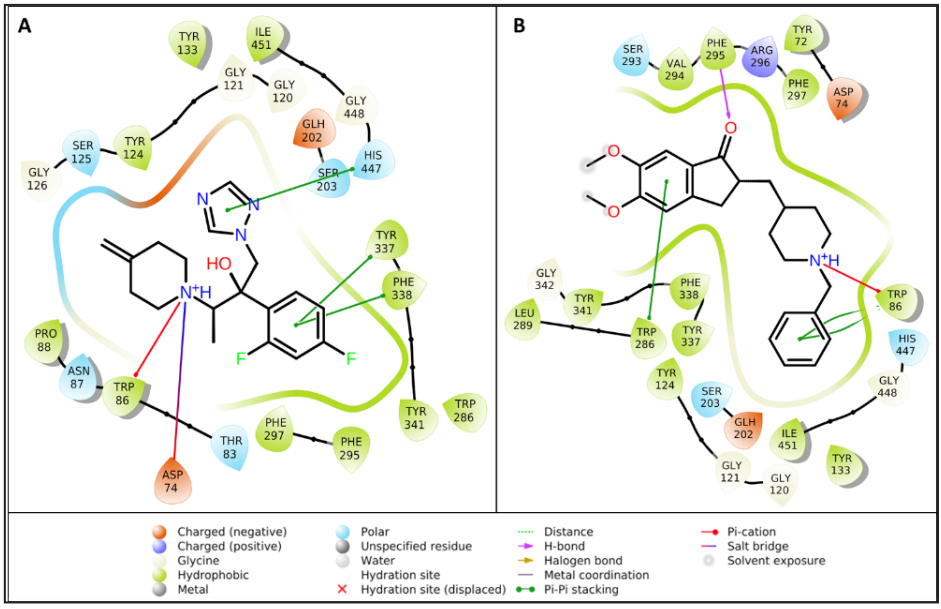

Figure 1. (A) 2D interaction diagram of Eficonazole compound with AChE (PDB ID:4EY7 (24)) crystal structure. (B) 2D interaction diagram of Donepezil reference compound with AChE (PDB ID:4EY7 (24)) crystal structure.

When the 2D interaction diagram of donepezil reference compound in Figure 1(B) was examined, it was determined that Phe 295 had hydrogen bonding, Trp 286 amino acid had π-π bond, and Trp86 amino acid had π-π bond interaction. In Figure 1 (A), it is seen that the Eficonazole compound is located right inside the AChE crystal structure and interacts with important amino acids. It is presented in Figure 1(A) that Phe337, Phe 338, Trp86 and Eficonazole, which are important amino acids for AChE crystal structure, make π-π interaction and also π-cation interaction with Asp74.

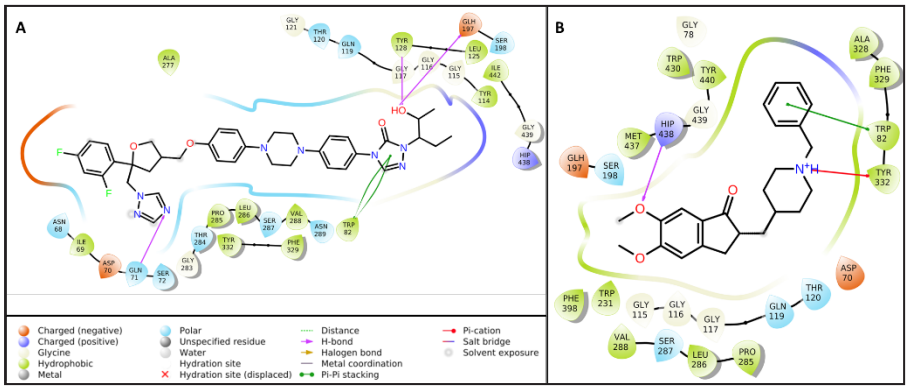

Figure 2. (A) 2D interaction diagram of Posaconazole compound with BChE (PDB ID:4BDS (29)) crystal structure. (B) 2D interaction diagram of Donepezil reference compound with BChE (PDB ID:4BDS (29)) crystal structure.

In Figure 2, 2D interaction diagrams of Posaconazole and Donepezil compounds interacted with the BChE crystal structure are presented. In Figure 2(B), it was determined that Donepezil had hydrogen bond interactions with the amino acid Hip438, π-cation interactions with Tyr332 and π- π interactions with Trp82. According to Tables 1 and 2, when the interactions of Posaconazole, which is the most active in BChE, were examined in Figure 2(B), it was determined that it had π- π interaction with Trp82, hydrogen bond interaction with Gln71, and hydrogen bond with Tyr128 and Glh197 amino acids.

3.2. MM-GBSA

Both the binding parameter values, and the binding sites of the compounds examined by in silico approaches were investigated. In addition, GBind and complex energy values with the MM-GBSA method are shown in Table 2. Considering the ΔG_{Bind}, complex energy values of donepezil, which is active in AChE and BChE, respectively, in Table 2, it was determined that -86.28, -22656.367 and -60.32 -22909.067 kcal/mol were calculated.

Table 2. ΔG_{Bind} and complex energy values of compounds interacting with AChE and BChE crystal structures in MM-GBSA method.

	PDB ID: 4EY7		PDB ID:4BDS	
Compounds	**ΔG_{Bind}**	**Complex Energy**	**ΔG_{Bind}**	**Complex Energy**
Donepezil	**-86.28**	**-22656.367**	**-60.32**	**-22909.067**
Fluconazole	-50.43	-22630.248	-34.43	-22901.055
Itraconazole	-87.16	-22620.762	-68.05	-22894.522
Voriconazole	-42.56	-22696.744	-33.39	-22962.041
Ravuconazole	-75.12	-22754.721	-50.50	-23005.466
Posaconazole	-71.63	-22598.932	**-69.14**	**-22882.798**
Eficonazole	**-68.79**	**-22660.836**	-40.49	-22881.916
Isavuconazole	-57.95	-22694.322	-38.05	-22910.690

When the other compounds discussed in Table 2 are examined, the ΔG_{Bind} and complex energy values for the Eficonazole compound for the AChE crystal structure are -68.79, -22660.836, respectively. It was determined that the Posaconazole compound in the BChE crystal structure had a better ΔG_{Bind} and complex energy values (-69.14, -22882.798) than Donepezil.

4. Conclusion

In this study, the efficacy of azole antifungal derivatives carrying triazole ring against cholinesterase enzymes was investigated by in silico approaches. Inhibitory properties against AChE and BCh were compared with the drug Donepezil as a reference. While it was determined that Eficonazole could be effective for AChE, it was determined that Posaconazole compound had better binding parameter values for BChE than Donepezil.

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CHAPTER XVII

VACCINE HESITANCY: A GLOBAL THREAT

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Vaccine hesitancy is people's reluctance to accept a proven safe and effective vaccine available to them to protection against an infectious disease (1). This hesitation has existed since vaccines were first developed and poses an ongoing threat to global public health. Historically, anti-vaccine discourse has a long history dating back to the first smallpox vaccination (2). So that, anti-vaccine articles on vaccination studies on smallpox which is the most common and crucial disease at that time, increased the hesitations about vaccination even more (Figure 1).

Prejudices against vaccination have contributed to the re-emergence of previously eradicated diseases such as polio and measles and increase vaccine preventable deaths. Vaccine hesitancy is increasingly becoming a global challenge in threatening public health. The World Health Organization (WHO) declared this situation as one of the top 10 threats to global health in 2019 (3-5).

While the concerns and factors driving vaccine hesitance have changed over time, many are similar to those in the past. Among them; vaccines are ineffective or cause disease, vaccines are used by companies to make a profit, vaccines contain dangerous substances, the harms of vaccines are hidden by the authorities, natural immunity is better than vaccine-induced immunity, etc. statements are often included. Vaccine hesitancy is generally complex and context-specific. In other words, this hesitation varies according to time, place and vaccines. Other factors associated with vaccination hesitancy include living

in a rural area, lower income, female gender, lower education, and vaccine costs (1, 6, 7).

Figure 1: A cartoon illustrated by English caricaturist James Gillray in 1802. In this cartoon, showing that cowpox pustules used to vaccinate against smallpox will cause cow-like appendages from different parts of people's bodies (James Gillray - British Cartoon Prints Collection - Library of Congress)

However, in 1998 Lancet report by Andrew Wakefield, a British physician, and his team of 12 colleagues suggested a link between the measles, mumps, and rubella (MMR) vaccine and the development of inflammatory bowel disease and autism (The Lancet retracted the Wakefield et al. paper in 2010) has opened the doors of fear of vaccines to the fullest. Over the next 20 years, epidemiological studies have consistently found no evidence of a link between MMR vaccine and autism, but it has further reinforced human vaccine concerns. In addition, although there is epidemiological research evidence that there is no association between childhood vaccines containing thimerosal and autism, concerns continue about pediatric mercury exposure (8-10).

With the increase in internet use, online platforms and social media in our digital world have become a source where health-related information can be easily reached by everyone. This has also allowed widespread access to misinformation. With the founding of Google in 1998 and the subsequent launch of other social media platforms such as Facebook, Twitter, YouTube, and

Instagram, the spread of true or false information about vaccines accelerated (Figure 2). Especially during pandemics, there has been an abundance of information from many sources, including social media, called an infodemic (9, 11, 12).

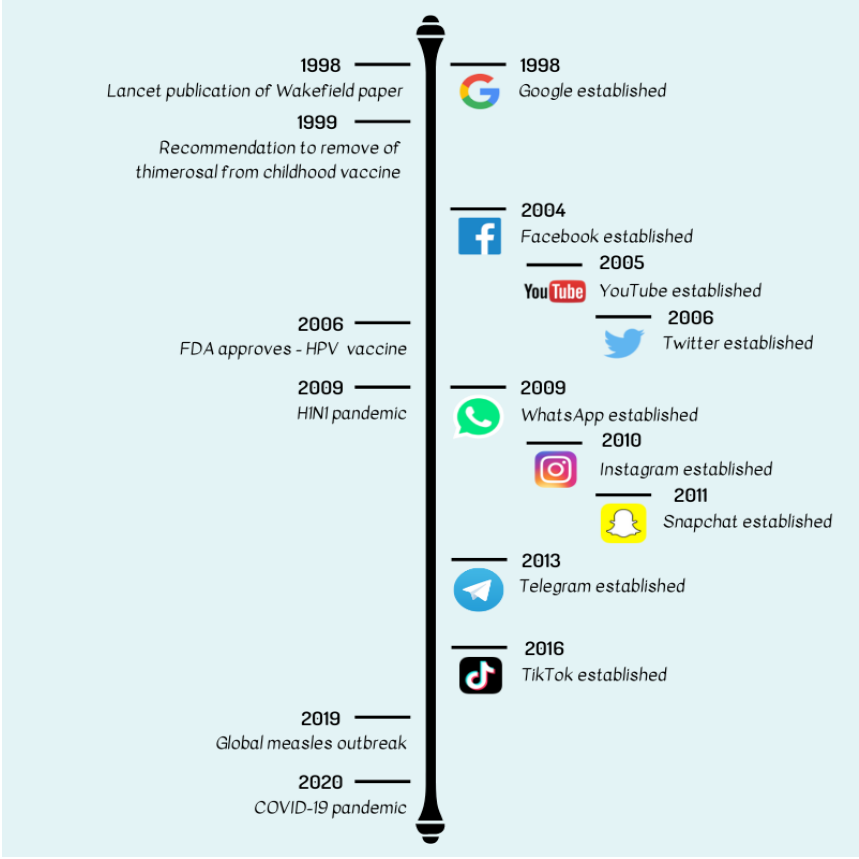

Figure 2: Timeline of Key Events Prompting Vaccine Hesitancy and Milestones in the Expansion of Social Media and Digital Technology (adapted from Larson *et al*., 2022)

Without a doubt, one of the most effective strategies to contain the COVID-19 pandemic is vaccination. Considering the dynamic and changing nature of vaccine hesitancy, it can be said that this rate has increased especially in the context of the COVID-19 pandemic. As a result, the post-pandemic world today is more challenging and more effort is needed to build confidence in vaccination programs. Apart from this, it is also very important to inform the public about the importance of vaccines, to disseminate the correct information to all communities and to prevent the spread of false information.

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CHAPTER XVIII

SOME EXAMPLES OF PSYCHOACTIVE PLANT SPECIES

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1. Introduction

Psychoactive plants; In the human mind, perception can be defined as plants with simple or complex structures that affect the living mind and consciousness that produce mood-state changes. Hundreds of psychoactive plants are known to exist. (1) These plants act by affecting neurons, putting pressure on the sympathetic and parasympathetic nervous system or hormones. The use of these herbs in small amounts can be stimulating, mood-altering, euphoria, and the use of high doses can lead to serious consequences up to death. (2) Most psychoactive plants have been used in cultural and religious rituals and daily routines for therapeutic purposes and to create a fun and joyful environment, albeit temporary.

2. Classification of Psychoactive Plants According to the Effects on the Living Body

Psychoactive plants; It is classified in many ways according to its chemical structure, the type of secondary metabolite contained in it, and the effects it creates on the living body. These plants can cause pharmacological and psychological effects in different ways. In general, psychoactive plants

are classified as anxiolytics, euphoriates, stimulants, antidepressants, and hallucinogens according to the effect they have on the living body. A plant can also be in more than one class. (3)

3. The Most Used Psychoactive Plant Species

1.1. Banisteriopsis caapi (Spruce ex Griseb.) Morton

The species *Banisteriopsis caapi* is the main component of a drink called Ayahuasca, which is used by the indigenous people of the Amazon basin in both social and religious ceremonies. (4) The leaves of the *Psychotria viridis* bush, which contain N,N-dimethyltryptamine (DMT), and the roots of the *Banisteriopsis caapi* vine, which are abundant in beta-carbolin harmala alkaloids, are typically used to make this psychedelic beverage. (5)

Ayahuasca users have reportedly experienced experiences including inner tranquility, joy, interaction with plant and animal spirits, and even communion with a higher power. (6, 7) The perception of time alters, causing users to feel as though time is moving faster or slower or that they have traveled through time. Temporary side effects of ayahuasca that are not entogenic can include tremors, nausea, vomiting, diarrhea, autonomic imbalance, hyperthermia, sweating, motor dysfunction, drowsiness, relaxation, vertigo, dizziness, and muscular spasms, depending on the dose. (5)

1.2. Tabernanthe iboga Baill.

The main active ingredient of *Tabernanthe iboga* plant is ibogaine. Ibogaine was first isolated from the West African shrub and root bark that grew in the Congo and Angola. (8) This plant, which is about 3 or 4 m high, is found in the wild and is also grown around its native huts. Locals have discovered that root bark has a powerful stimulant and aphrodisiac effect, increases the strength of muscles and improves sexual abilities. (9) The aphrodisiac effect of ibogaine lasts for 2 days. (3) Traditionally, hallucinogen has been used by locals to suppress hunger and fatigue. This herb in high doses can cause convulsions and paralysis. (10)

Examples of the autonomic nervous system effects of ibogaine on the living body are the formation of visual hallucinations in person, insomnia, dry mouth, too much sweating, increased pulse, tremor, enlargement of the pupils. (3)

1.3. Lophophora williamsii (Lem. ex Salm-Dyck) J.M.Coult. (Peyote Cactus)

Mescaline (3,4,5-trimethoxyphenethylamine), the main component of the species *Lophophora williamsii*, has been used for thousands of years in religious rituals and for medicinal purposes by North American natives due to its psychedelic properties. It is a naturally occurring alkaloid. (11) It is still legally used today by the Indian Church in religious ceremonies traditionally held at night and lasting about 12 hours. (12) In addition, both cactus and mainly mescaline are consumed illegally. (13)

Although they exhibit different chemical properties, all hallucinogens usually produce similar psychological effects. However, mescaline and peyote have some distinctive features. Shortly after application, hallucinations and excessive sensitivity to sound appear. (14) Light and colors are pronounced, appear bright and intense. Typically, hallucinations can last longer than 10-12 hours. (15) Symptoms of mescaline poisoning include hyperreflexia, tachycardia, agitation, muscle stiffness, ataxia, seizures, midria fog, sialoure, hyperthermia. (13, 16)

1.4. Psilocybe mexicana Heim

In 1957, psilocybin was isolated from the fungus *Psilocybe mexicana*. In the examinations carried out from those years to the present day, it has been determined that psilocybin and psilocin are present in the composition of more than 75 different mushroom species. These mushrooms are also called magic mushrooms. (17)

Psilocybin was first synthesized by Hofmann. Clinical studies in the 1960s and 1970s showed that psilocybin produced an altered mood. Psilocybin and psilocin are known to cause changes in thought, time, space and self-concepts, along with subjective symptoms such as marked changes in perception and mood. (18) Many effects can be observed in individuals using psilocybin from laughing crises to the emergence of deep philosophical thoughts, from the increase in artistic abilities such as painting and music to the change in the flow of time. (3)

Table 1: The physiological, visual, cognitive, auditory, multi-sensory and transpersonal effects that magic mushrooms have on the living body (19)

PHYSIOLOGICAL EFFECTS	Mild sedation, physical euphoria, runny nose, mydriasis, hypersalivation, increase in body temperature
VISUAL EFFECTS	Color saturation, sharpness of vision, perspective distortion, perception of brightly colored shapes, and both closed and open-eyed figures and eyes
COGNITIVE EFFECTS	Increased empathy, simultaneous different emotional states, loss of ego, lack of time consciousness
AUDITORY EFFECTS	Enhancement or distortion of the sound
MULTIPLE SENSORY EFFECTS	Synesthesia
TRANSPERSONAL EFFECTS	An increase in emotion and spirituality

1.5. Cannabis sativa L.

Cannabis sativa L. (Cannabaceae) can grow in different habitats and altitudes. Cannabis has a rich history of medical use dating back to fairly ancient times. The cultivation and use of hemp dates back to 5000 to 6000 years ago. (20) Secondary substances known as "cannabinoids" or, more precisely, "phytocannabinoids" are a characteristic of all cannabis plants. Trichomes, which are primarily made from female hemp, yield more than 100 distinct phytocannabinoids. (21)

Because of its multi-faceted effect on humans, different forms of use of cannabis, such as marijuana (tobacco-like), have been used for recreational, religious, spiritual, and medical purposes for thousands of years. For example, cannabis has been used in the Middle East to treat epilepsy. There are also records of its use during funeral rites in western China BC. Patients who have multiple sclerosis, cancer, or AIDS can utilize products containing THC to relieve their pain, nausea, and vomiting. *Cannabis* has the potential to treat a wide range of illnesses, including Tourette's syndrome, post-traumatic stress disorder, and sleep difficulties. For this reason, it is removed from the list of prohibited substances in many countries and its use for medical and recreational purposes is tried to be legalized. (21)

Table 2: Known medicinal uses of cannabis (22, 23)

Amyotrophic lateral sclerosis	Glaucoma	Multiple sclerosis
Alzheimer	Hepatit C	Osteoporosis
Diabetes	HIV	Itch
Dystonia	Hypertension	Rheumatoid Arthritis
Fibromyalgia	Incontinence	Sleep apnea
Disorders of the Gastro-intestinal tract	MRSA	Tourette's syndrome

People who use cannabis have many physical and mental changes. Examples include;

- Altered senses (for example, seeing brighter colors)
- Altered perception of time
- Changes in mood
- Abnormal bodily movement
- Challenges in reasoning and solving problems
- Poor memory
- Hallucinations (when taken in high doses)
- Delusions (when taken in high doses)
- Psychosis (the risk is highest with regular use of high-potency cannabis)
- Breathing problems
- Tachycardia
- Child development issues both during and after pregnancy
- Temporary hallucinations
- Temporary paranoia. (24)

1.6. Papaver somniferum L.

Opium (*Papaver somniferum* L.) is a CNS depressant and narcotic analgesic. It is not hallucinogenic. It exerts its effects through specific opiate receptors mu, delta, kappa, nosiseptin receptors. Opium contains morphine, codeine and tebain, as well as papaverine and noskapin (isoquinolins). (25, 26) There are many examples of the use of opium in history. Examples include evidence that its seeds were consumed at the end of the Stone Age, doctors in Egypt and Mesopotamia used poppy water with prayers, spells, talismans and religious rituals, Helen of Troy added opium to wine to cheer up her guests, and

the poppy fields in Turkey during the Ottoman Empire spread over hundreds of kilometers. (27)

Milk and latex obtained from the opium plant contain 12% morphine alkaloid. This resulting morphine has been used for centuries as a poppy or poppy tincture. Another derivative of opium alkaloids is heroin. Heroin is used as an analgesic. (3)

Opium smoking causes many physiological and pharmacological effects. Sedation, nauseousness, vertigo, vomiting, constipation, physical dependency, tolerance, and respiratory depression are a few of these. Constipation and nausea are the most frequent ones, and there is no tolerance building up to them. (28) Although it does not cause sharp hallucinations, it has analgesic, hypotensive and antidiarrheal effects. It can also cause a dreamlike condition. (25, 26) Opium and some alkaloids have the potential to be seriously addictive. (3)

1.7. Erythroxylum coca Lam. *and Erythroxylum novogranatense* (Morris) Hieron.

Cocaine is a tropane alkaloid derived from the coca plant (*Erythroxylum coca* Lam. ve *Erythroxylum novogranatense* (Morris) Hieron.) native to Western South America. The coca plant has been cultivated for thousands of years, and 2000-year-old mummies have been found in Nazca, Peru, with small bags used for coca leaves in their hands. Coca leaves have shown interest in Europe due to their psychoactive properties, and with the discovery of methods of extracting and concentrating the alkaloid cocaine, cocaine use became more common in Europe towards the 19th century. (29) In the 19th century, cocaine was incorporated into a number of pharmaceutical and consumer products, such as Coca-Cola and cigarettes. (30)

The cocaine plant is an elongated shrub form that can grow up to 2-3 meters in height. The leaves are laid out in thin layers and dried in the sun. Then they are packed in sacks for transportation. To maintain the quality of cocaine, the leaves should be kept dry. Depending on the species and location grown, the leaf contains about 0.3-1.5% cocaine.

While a loud or abrupt change in emotion is what is meant by cocaine intoxication, there are other side effects as well, such as strong euphoria, a quick heartbeat, elevated blood pressure, and more active motor and speech functions. Additionally, there is an increase in attentiveness, hostility, anxiety, aggressiveness, alertness, poor judgment, and emotional instability. Greater behavioral complexity or repetitive habits (such as touching one’s nose or

forehead) are seen in heavy users. In any acute consumption, a high dosage of cocaine that results in an overdose can be linked to medical issues such cardiac arrhythmia-related mortality, seizures, hyperthermia, dehydration, and local vasoconstriction that causes myocardial infarction or paralysis. Physical examination results are less frequent or diagnostic in cocaine users. Tachycardia, elevated blood pressure, and motor agitation are seen in acute users. Regular nosebleeds and/or an eroded nasal septum can occur in heavy intranasal users. The typical signs of chronic needle usage in the arms, legs, or neck are present in injection users. (31)

3.8. Mandragora officinarum L.

Mandragora officinarum L., is a perennial plant that is a member of the Solanaceae family and is widely found in the Mediterranean region, including Greece. The species, also called mandrake grass, has oval leaves, a thick and erect root, bell-shaped flowers, and yellow or orange fruits. (32) It is even called "man's grass" because the appearance of plant roots resembles a human being.

Mandragora species have been one of the most famous medicinal plants in western culture throughout written history. This view has been clearly expressed by several authors in such statements as "Of all the medicinal herbs used in the ancient and medieval world, none of them has been looked upon with as much fear and amazement as mandrake." (33)

The unique names of mandrake give away the particular medical use of this plant. The Greek names for this plant are fistulóriza and fistulóchorto, which translate to "hemostatic-acting root" and "healing root," respectively. (34) The stem has a human-like shape (anthropomorphic). It alludes to sexuality and reproduction symbolically. (35, 36)

Among the main tropane alkaloids in mandrake are hyocyanamine and scopolamine (also known as hyosine). These tropane alkaloids have been shown to exhibit psychedelic and hallucinogenic effects and to be muscarinic receptor antagonists, which results in a parasympatholytic effect. Chronic spasms, a rapid pulse, tachycardia, enlarging of the pupil, suppression of saliva production, respiratory arrest, and coma can all result with larger dosages of these drugs. Mandrake is therefore regarded as being exceedingly harmful and at the level of a chemical that alters consciousness. (37)

Mandrake is said to have aphrodisiac properties. At least 136 chemical compounds were discovered in Mandrake fruit in one research, and it was hypothesized that the primary components, which were only discovered in

mature fruits and were absent from unripe fruits, may be to blame for the fruit's distinctive flavor, odor, and aphrodisiac effects. (38) Mandrake has never been pharmacologically confirmed to have aphrodisiac effects, yet it is nevertheless used for this purpose in many nations today, particularly in the Balkans and the Southeast Mediterranean. (39)

Mandrake is probably the most famous of the so-called "magical" plant species in history, and that is why it appears in many areas of literature. (40) The magical powers attributed to Mandrake, both good and bad, have made him a formidable object. Among the people, Mandrake was considered an entity with open ties to the forces of the underworld. (41, 42)

3.9. Peganum harmala L.

The plant is widely found in Central Asia, North Africa and the Middle East and is used as a medicinal plant. (43, 44) The dried capsules are mixed with other ingredients and burned as a talisman against the "evil eye" among Iranians. (43)

The alkaloids found in *P. harmala* also have some role in the pharmacological effects of the plant. (45) Harmaline was first isolated by Goebel from the seeds and roots of the plant. (46) Harmaline is found in many plants such as *Banisteriopsis caapi* (Malpighiaceae) in addition to *P. harmala.* It is also one of the components of Ayahuasca, a hallucinogenic drink drunk by Amazonian tribes in rituals. (47) This plant contains harmaline alkaloid as well as harmine alkaloid. Harmine and harmaline alkaloids contain monoamine oxidase inhibitors. (3)

3.10. Artemisia absinthium L.

It is a shrub-like perennial plant, growing up to 80 cm in height. In some habitats it even reaches a height of 1.5 m. The whole plant contains a large number of feathers and has an intense, pungent odour. (48) *A. absinthium* leaves contain feathers/glandular trichomes that secrete essential oil and hairs that have a protective function. These protect the plant against high temperatures and prolonged drought. (49)

A. absinthium is a species that is currently grown in Brazil, the USA, and Southern Europe. The start of the harvest season coincides with the emergence of the first blossoms. Cut leafy branches and base leaves are left with woody remnants. Many times a year, harvesting can be done. The essential oil of

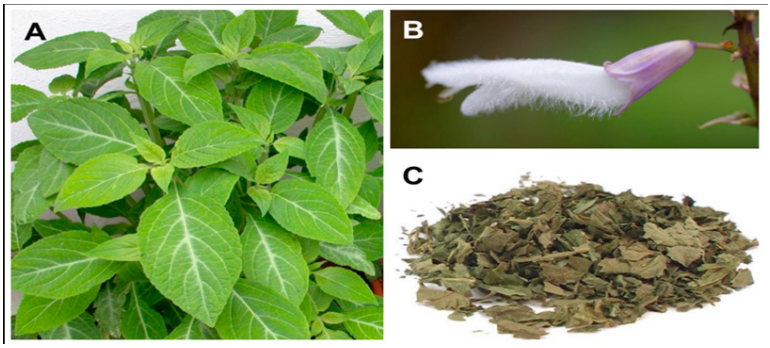
A. absinthium is significantly impacted by the drying process. It has been shown that even a small amount of air heating can alter the oil's organoleptic characteristics. The harvested plant shouldn't also be spread out in a thick layer because it will cause it to dry out much more slowly. Drying should be done at room temperature in drying rooms or airy, shaded areas. (50)

Wormwood has long been known for its very bitter flavor. The expression "bitter as wormwood" is used in Polish. This species was characterized by Dioscorides (1st century AD) and Theophrastus (4th–3rd century BC) as "ápsinthos," which is Greek for "unpleasant" or "ápinthos," unfit for drinking. (51) In addition to its distinctive bitterness, wormwood also has a central nervous system stimulant effect that, depending on the amount, can even result in epileptic convulsions and hallucinations. This feature has been known about since ancient times. (52) Wormwood, which is often used as a tincture, is mentioned in Dioscorides' book 'De Materia Medica' as having warming, astringent, and invigorating qualities as well as being useful against poisons. (53) Pliny (1st century AD) also recommended *Absinthium* as a hypnotic, laxative, menstruation trigger, healer of "fistulas in the eyes", and even cosmetically. He also stated that his ash, combined with a rose ointment, darkened the hair. (54) This plant's ritual use in funeral rituals and the exorcism of bad spirits is due to the same strong fragrance it emits when burned. (55) According to the recommended traditional use, the leaves are used to lower fever, while the flowers help with diseases of the stomach and helminthiasis. Tincture of *A. absinthium* has been evaluated as a tonic and digestive aid. (48)

To support the long-standing usage of *A. absinthium* for the treatment of memory problems and poor focus, a research was done. The study looked at the chemicals isolated from *A. absinthium*'s ability to bind to muscarinic and nicotinic receptors because it is known that cholinergic receptors are involved in cognitive processes. The efficacy of two sets of harvested plants' ethanol extracts to remove [3H]-(N)-nicotine and [3H]-(N)-scopolamine from receptors was tested using a homogenate of human cortical brain cells. According to the study, *A. absinthium* has a significant affinity for muscarinic and nicotinic receptors, which means that products derived from the plant may have beneficial effects. (56)

3.11. Salvia divinorum Epling & Jativa-M.

Figure 1 : (A) *Salvia divinorum* plant (B) Characteristic flower structure (C) Dry leaf specimens (57)



Salvia divinorum, a plant belonging to the Labiatae family, is indigenous to a tiny area of Oaxaca, Mexico. *S. divinorum* may be cultivated indoors or in any humid, semi-tropical climate, just like cannabis. The herb has been utilized for generations by the shamans of the Mazatec Indians of Oaxaca to treat conditions including diarrhea, migraines, rheumatism, and anemia as well as for prophetic and religious purposes. The panzón de Borrego sickness, which is semi-magical, was also treated with it. Fresh *S. divinorum* leaves are either chewed or crushed and ingested in a combination for ethnomedical purposes. (58) Due to its distinct psychomimetic properties, *S. divinorum*, sometimes known as *Salvia*, has recently attracted more interest for its recreational usage. *S. divinorum* is frequently marketed as a risk-free, accepted substitute for hallucinogens like cannabis, LSD, and mescaline. (58, 59)

The main active ingredient of *S. divinorum* is the neoclerodan diterpene Salvinorin A. (58) The reported Salvinorin A content in the dried leaf samples was found to be 0.89-3.70 mg. The concentration of Salvinorin A in leaves collected from individual plants can vary significantly. (60)

In vitro and *in vivo* studies have shown that Salvinorin A is a selective and potent agonist of k-opioid receptors (KOR), more effective than the two prototype KOR agonists, U69,593 or U50,488. Salvinorin A is the only KOR agonist found in *S. divinorum* and has no structural similarity to any known hallucinogen. (58, 61)

Sublingual doses of Salvinorin A have not been found to be psychoactive in humans. (62) On the other hand, when inhaled, Salvinorin A shows psychoactive effects in just minutes. (58, 63)

Inhalation of evaporated smoke of Salvinorin A is considered to be the most effective method for causing psychoactive effects in humans. It gives people a cannabis-like experience. In people, visual hallucinations, changes in bodily form in objects, time and space perception disorders, intense but short-term psychedelic-like effects, altered state of consciousness, auditory hallucination, dream-like experience, increase in sensory and aesthetic appreciation, spiritual experiences, acute psychosis and paranoid, temporary language disorder, psychomotor agitation can occur. (63)

3. Conclusion

Psychoactive plants can be classified in many ways according to their chemical structure, the type of secondary metabolites contained in them, and the effects they have on the living body. Psychoactive plants can cause pharmacological and psychological effects in different ways.

Psychoactive compounds mimic neurotransmitters that are responsible for chemical communication across synapses between neurons. (64) Many psychoactive compounds or foreign substances entering the body can easily bind to receptors thanks to their complex and three-dimensional structures and cause a number of changes in the body. (3) Although these compounds are not exactly the same, due to their compatible chemical structure, they can bind to specific neurotransmitter receptor sites, increase their activity, suppress or otherwise alter them. For example, mescaline, the active alkaloid of peyote cactus, is structurally similar to the neurotransmitter noradrenaline (also known as norepinephrine). Since both are derivatives of the phenylethylamine compound, mescaline can mimic noradrenaline by binding instead of noradrenaline. (64)

In general, psychoactive plants, which are included in the human mind as plants used for pleasure with their narcotic and hallucinogenic effects, are used for medicinal purposes with many secondary metabolites they contain.

In this study, briefly, information about the effects of psychoactive plants on the living body and the most commonly used psychoactive plant species, the active substances of these species and the mechanisms of action of these active substances are reviewed. In conclusion, it should be noted that these plant species, which are first thought to be used in shamanic ceremonies, have many active components that need to be identified, and these components may have many therapeutic aspects that need to be discovered in the scientific world.

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CHAPTER XIX

CHEESE, HISTORY AND DIVERSITY IN TURKISH CULTURE

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1. Introduction

One of the fundamental principles of leading a healthy life is adequate and balanced nutrition. Foods that meet the energy, protein, vitamin and mineral needs are divided into four main groups as foods providing this balance. It is extremely important for living beings to consume healthy foods to grow, develop, sustain their life; protect, improve and develop their health and increase their quality of life. The place and importance of milk and dairy products both in the food pyramid and in leading a healthy lifestyle is beyond doubt. Milk is an important source of animal protein that contains almost all of the essential nutrients required for growth and development. However, since raw milk has a short shelf life, it should be subjected to heat treatment at appropriate temperatures and time periods as soon as possible or converted into more durable products. Cheese is a dairy product that has quite an important place among dairy products and has a wide variety. The main reasons for this are that most of the nutrients in milk are concentrated in cheese, cheese has a long shelf life, and the milk can also be transformed into cheese in a short time period with different processing methods in seasons and regions where raw milk is abundant (1).

Cheese is a dairy product consumed fresh or after ripening, obtained by coagulating whole milk, cream, partially or completely skimmed milk, buttermilk or some or all of these with appropriate proteolytic enzymes known as rennet and/or harmless organic acids, separating the whey, shaping the clot and salting it (2). The Food and Agriculture Organization's (3) Codex of General Standards for Cheese (4) defines cheese as "a semi-soft, hard or extra-hard product obtained from milk, ripened or unripened, semi-soft, hard or extra-hard, which can be coated and where the whey does not exceed the amount of milk". According to the Turkish Language Association (5), cheese is defined as "a food made from milk by solidifying with yeast and has many types". Cheese, which is obtained from butchery mammals whose milk is consumed, is a popular foodstuff in our country. When cheese is produced from raw milk, it contains phosphorus, calcium, high quality protein, riboflavin (vitamin B2) and vitamin A. Cheese also contains essential fatty acids (linoleic, linolenic and arachidonic acid) and essential amino acids in sufficient and balanced amounts (6). As the amount of fat in cheese increases, the amount of aroma increases as a result of the separation of fat into its components. Therefore, the higher the fat content of the cheese, the higher the aroma will be. In addition, since fat-soluble vitamins such as A, D, E and K do not undergo any change during cheese making, they increase in direct proportion to the fat content of the cheese. The higher the fat content, the higher the amount of carotene. Water-soluble vitamins are partly in cheese and partly in whey. Vitamin C, which is present in small amounts in milk, passes completely into the whey during cheese making. Therefore, its amount in cheese is quite low. The lactose content in cheese is also very low. Calcium and phosphorus ratio in cheese is at the desired level (7).

Another important fact about the cheese in terms of nutrition is that it has a high biological value but a low marketing price. Another feature that increases the value of cheese is that the yeast (pepsin) used in the production process continues its activities in the stomach and therefore it is easily digestible and can help digest other foods as well (8, 9).

2. History of the Word "Peynir" (Cheese)

When we look at its etymological origin, the word "peynir" (cheese) has passed into modern Turkish from the Persian word "panīr", which means made from milk. It came into English from the Latin "caseus". It is thought that the origin of this word comes from the root "kwat" in Indo-European languages, which means fermenting-sourdough (10). Cheese comes from the

Latin word “formaticus” meaning “made in a mold”. The Italians used the word formaggio, the French first used formage, formaige, fourmage, frommaige and finally fromage. The words “cheese” in English, “käse” in German, “kaas” in Dutch, “questo” in Spanish, “queijo” in Portuguese, “formatge” in Catalan originate from the Latin word “coagulum” meaning coagulation and “caseus” meaning cheese (10, 11). Cheese, which is also defined by various names in other countries, is known to be called “chiz”, which is known as “excellent thing” in Urdu, the common language in the geography where many languages are spoken when the Indian Peninsula was under the sovereignty of the Great Mongol-Turkish Empire. It is called “sir” in Russia, “sirene” in Bulgaria, “sajt” in Hungary, “ser” in Poland, “brinza” in Romania, “ost” in Sweden, “juusto” in Finland, “tiri” in Greece, “cebbene” in Arabia (2, 4, 11-14).

When researching the information on the use of the word “peynir” in pure Turkish, we can see that it was first used by the Uyghur Turks (750 A.D.). The cheese obtained by leaving the milk to its own state and developing its acidity is called “eksimik” or “kesik” in the folk language. In the *Divanu Lügati't-Türk* written by Kashgarli Mahmud between 1072-1074 (1072 A.D.), it is pointed out that “milk is creamed”. In this work, it is known that fresh cheese is called “udma” or “udhıtmanın” (derived from the word udhıt, which means to put to sleep, to solidify, to keep waiting with yeast). “Udh” means cattle, oxen. It is also stated that a type of cheese made from sour milk was called “sogut” in the Karluk dialect and some Turkmen tribes migrating to Anatolia still use the same name. The word peynir entered Turkish from Persian during the migration from Central Asia. Among the words “benir”, “penir”, “beynir”, which first appeared in the Turkish vocabulary of the Egyptian Mamluks, “penir” was used by Turkmens when they passed through Iran on their westward march or when they settled in Anatolia. “Nan-ü penîr” means bread and cheese, “penîr’i gercek” means fatty cheese and “penîr-i teras” means grated cheese. In the language spoken by the Kipchaks (Kumans), a Turkish tribe that lived in the Ukrainian steppes in the twelfth century, it is also expressed as “benir”. In Chagatai, the written language of Eastern Turkic in the fifteenth century, the word “pendir” is used. Like Chagatais, Azerbaijanis also frequently use the word “pendir” or “penir”. In addition, the word peynir is used in Dede Korkut tales (such as tası peynir gibi ditti / südi peyniri bol gibi), which are considered to be 12th and 13th century works (2, 14-17).

During the Seljuk period, cheese was known as “udıtma” and/or “udhıtma”. The Uyghur term “udhıtmak” is still used today in some villages in Ankara

province as a term expressing the coagulation of milk. In addition, the oldest word for cheese is known as "bıslak". Originating from the Turkish "pis-, bis-", this word is still used in Anatolian folk language (Karachay tribe around Konya and Afyon Emirdağ). This word was also used by the Mongols to refer to cheese. This word was first found in the Turkish dictionaries of Mamluk Turks (Ibni Mühenna Lügat) and in ancient Anatolian inscriptions. In this dictionary, there are some Turkish words such as "ciet, cıkıt, irimcik" meaning cheese. The word "Irimcik" was used in Mamluk period for milk that coagulates during the heat treatment applied to make yogurt, while in Central Asian dialects this word means cheese. In addition to these, the fact that the Central Asian Turks use the words "agırımsık" or "akermisik" for white cheese is an indication that it means cheese and that our history of white cheese dates back to very ancient times (14, 16, 18).

In Yusuf Has Hacip's work "Kutadgu Bilig" written in the eleventh century, there is also information about the food culture of the Turks. Dry yogurt (kurut) and cheese types are also mentioned in the said work. In particular, it is known that they call the type of cheese obtained by draining and drying the degreased yogurt "kurut". This type of cheese, brought to Anatolia by the Turks of Central Asia, was called "black kurut", while in Iran it was called "kashk black" or "tarf black". Today, in many regions of Anatolia, this cheese made from skim milk or yogurt is called "kesk", "kesük", "quiche", "kes" or "cökelek» (14, 19-20).

3. History of Cheese Making

There is no precise information on how, where and when cheese was first made. According to many rumors, there are different opinions on this subject. According to R.W. Menges, the first cheese production was obtained by an Arab traveler by chance while carrying milk in a sheepskin bag made of sheep's stomach, while some historians such as Herodotus, Hippocrates and Strabo state that cheese was first made by the Scythian Turks (600-200 B.C. Southern Russia) from mare's milk, presumably after souring it. It has been reported that Scythians lived in the steppes north of the Black Sea (VII century BC-IV century BC) and that traces of cheese were found in bags made of goat or sheep skins encountered in ice-covered graves in these regions. According to Kosikowski, communities known as Turkish and Mongolian ancestors produced fermented products from goat milk during their migration from Asia to Europe (14, 21-22). Although there is no concrete evidence other than this information, it is reported

that cheese was first seen 8000-8500 years ago in lower Mesopotamia, that is, in the Neolithic Period (Polished Stone Age), when some plants and animals were domesticated, in the "Fertile Crescent" between the Euphrates and Tigris rivers (today's Iraq), or that it was accidentally found by shepherds in the Indus valley (today around Karachi) as a result of milk souring and coagulating while being transported in bags made of goat skin. Figures on a stone relief dating back to 3500-3100 B.C. in Mesopotamia, now on display in the Baghdad Museum, show that the Sumerians (4000 B.C.) were well acquainted with milk technology. It is believed that Akkadians and Sumerians used nearly 200 types of cheese (2, 14, 19, 23-24).

In the Torah and ancient Hebrew, the importance of cheese is emphasized by stating that the place known as the running field in Jerusalem was established near the "Plain of the Cheese Makers". It is stated that the raw milk to be processed into cheese was filled into a bag said to be made of goat skin, a piece of rumen was left in it, which curdled the milk, after the milk in the bag coagulated, they broke up the clot by hand, left these curdled pieces of milk in other bags, then dried the pieces taken out of the bag in the open air, crushed them well in a bowl, mixed them with salt and ate them. Although the Torah states that it is forbidden to leave organ parts in goat's milk, it says that it's not considered "makruh" to eat cheese made by this way. Therefore, the Jews named the edible cheese "kosher", which means "permissible". It is thought that the name of kashar cheese may have originated from this Hebrew word (13, 14, 17).

Although the Greeks and Romans (1000 BC) also made different types of cheese, there is not much information about them. They only mentioned these cheeses as sheep or goat cheeses. Cheese was not only the food of the lower and middle classes in the Greeks and Romans, but also the food of the noble class. It is reported that the noble class consumed cheese made from sheep's milk. It is said that cheese was among the gifts that the Hellenes offered to their gods on Mount Olympus. It is also mentioned that in the Olympics organized by the Greek site states, athletes ate cheese to strengthen themselves (13, 19).

In the Middle Ages, monasteries and feudal principalities made important contributions to the development of cheese technology and cheese variety. It is alleged that the name of the cheese variety called "Munster" comes from the monastery. Cheese was also used as medicine in the treatment of many diseases during this period (26).

Cheese first became known in the American geography with the immigration from Europe in the early seventeenth century. Since the immigrants settled only

on the Atlantic coast during this period, cheese was known as a food specific to this region. Due to the economic crisis that broke out in Switzerland in the early nineteenth century, the poor and unemployed Swiss people migrated to the state of Wisconsin, and cheese making became widespread in the interior regions of the American geography. The first cheese factory was established in the USA in 1851 and the second in England in 1899 (13, 14, 25-26).

4. History of Cheese Variety

It is mentioned that some of the types of cheese consumed today were already produced during the period of the principalities. In the "Tabiat-name" written by Tutmacı, a Turkish poet on behalf of Aydınoğlu Umur Bey in the fourteenth century, it is stated that there are fresh cheese and deleme (teleme) cheese types. In Evliya Celebi's Seyahatname, which is an important source about the Ottoman world in the seventeenth century, it is written that there are 400 artisans producing cheese in Istanbul and cheese types such as Kaskaval Cheese, Kesme Cheese and Teleme Cheese (22).

It is known that Turks were dealing with cheese before their migration to Anatolia. The fact that cheese was the main food of the soldiers of Attila the Hun, who was at war with the Romans, is an indication that cheese making was widely known among the Turks in those years. In addition, the stories of Dede Korkut that mention the cheese and the document called "Bostan, written by Sadi of Shiraz, who lived in the twelfth century, described cheese as "a sacred food" are among the important documents. It is also stated that Karacaoğlan, one of our folk poets, used the word cheese a lot in his poems (2, 14, 16). It is stated that in the foundation times of the Ottoman Empire, cheese was among the gifts that Osman Gazi Bey's tribes presented to the Bilecik beys who kept the remaining belongings while returning from the plateau. It is known that there were many types of cheese (fresh lor, fresh dil, fresh cayir, Mudurnu, Sumu, Karaman, Sofya, Esme, Midilli, Teleme, white cheese, Cimi Tulum, İzmir Tulum, Rumeli Tulum, fresh Kashkaval, Balkan Kashkaval) brought to Istanbul in 1502 during the reign of Beyazıt II (16).

5. Types of Cheese Produced in Turkey and Registered with Geographical Indication

It is stated that, while the milk was being transported in the skin bag, cheese formation took place with the development of acidity under the influence of

temperature and separation of the curd from the water during agitation. Today, cheese production has gained diversity with the combination of technology and modernization of traditional methods. In many countries, as in Turkey, there are still types of cheese that are traditionally produced and offered for sale in the world market. Cheese diversity depends on type of animal (cow, sheep, goat's milk cheese), clotting method (acid, yeast cheeses), heat treatment of milk (raw, pasteurized cheese), fat content (full fat, fatty, low fat, fat-free cheese), structure (very hard, hard, soft cheeses), salt ratio (salty, unsalted cheeses), additives (various herbs and spices, melting salts, cheeses made by supporting mold growth), ripening time (fresh, semi-ripened, ripened cheeses) (27). The socio-cultural development and change levels of societies are among the important factors affecting diversity. Cheese is a part of the culture of the society in which it belongs. In Turkey, it is stated that there are more than 25 local and regional cheese varieties depending on the breed of animal from which milk is obtained, regional and climatic differences and traditional and technological processes applied (28).

Cheese is the most diverse and globally recognized dairy product. It is a known fact that there is an absolute link between the number of cheese types and the consumption habits of nations. There are more than 4000 types of cheese worldwide and more than 150 types of cheese in Turkey. The production of cheese varies from year to year, and according to the data of the Turkish Statistical Institute (TÜİK) for 2021, a total of 763,266 tons of cheese was produced (29). The fact that cheese, whose raw material is only milk, has so many varieties is due to many differences such as the type of milk used, the type of yeast used, the way of processing the clot, the processing method, the fat ratio, the ripening process and conditions, and the salting method (30).

The variety of cheeses may vary according to countries and even regions within countries. For example; Brie, Camembert and Maroilles in France; Cheddar cheese in Cheddar Village in England; Edam and Gouda cheeses in the Netherlands; Gamalost cheese in Norway; Emmental cheese in Switzerland are among the world cheeses (2). The most produced cheese types in terms of tonnage in our country are white, kashar, tulum, gruyere, mihalic, lor and cökelek cheeses. In addition to these, there are about 20 local cheese types produced regionally to meet family needs and produced with primitive methods. Traditional cheese types produced in our country are shown in Table 1 (13, 17, 27, 31-33).

Tablo 1. Types of cheese produced in Turkey

Traditional Cheese Type	**Production Province / Region**
Abaza cheese	Hendek, Bilecik, Bursa, Düzce, Adapazarı, Kocaeli, Bolu, Sinop, Canakkale
Acı cheese	Giresun
Adıyaman Pazarcık Köy cheese	Adıyaman
Adıyaman Basma cheese	Adıyaman
Afyon Tulum cheese	Afyonkarahisar
Aho cheese	Araklı, Sürmene (Trabzon), Cimen, Balağot highlands (Gümüshane), Bayburt
Akcakatık cheese	Isparta and surrounding areas
Aladağ Köy cheese	Ağrı
Antep Sıkma cheese	Gaziantep and Kahramanmaras surrounding areas
Armola cheese	Seferihisar (İzmir)
Ayas Ovma cheese / Ayas Basma cheese	Ayas (Ankara)
Ayran Kırması cheese	Doğu Karadeniz Bölgesi (Eastern Black Sea Region)
Ayran cheese	Rize
Balkabağı Küp cheese	Adapazarı, Doğancay, Hendek, Arifiye
Basma cheese	Adıyaman
Batman Kozluk Kok cheese	Batman
White cheese	Edirne
Bez Kashar cheese	Mut
Bez Tulum cheese	Konya (Ereğli)
Biberli Cökelek	Akdağmadeni (Yozgat)
Cabalti Cökeleği	İnebolu (Kastamonu)
Cacık (Otlu Cökelek) cheese	Siirt, Hakkâri, Van, Bitlis
Cami Boğazı	Trabzon
Carra cheese	Hatay
Camur cheese	İzmir-Tire
Canak (Testi) cheese	Yozgat (Sorgun, Boğazlayan, Sefaatli, Yerköy)
Cayır cheese	Manisa
Cepni Tulum cheese	Akcabelen (Konya),
Cerkes cheese	Sinop, Bolu, Düzce, Canakkale (Biga), Balıkesir (Gönen), Hendek, Adapazarı, Bursa
Ciğleme cheese	Tokat, Kayseri

Traditional Cheese Type	Production Province / Region
Cihanbeyli Küflü cheese	Konya (Cihanbeyli, Karapınar, Ereğli)
Cimi cheese	Antalya, Akseki, Serik, Manavgat
Cimi Tulum cheese	Antalya (Akseki, Serik, Manavgat)
Civil (Tel) cheese	Erzurum, Ağrı, Kars, Ardahan, Mus
Coban cheese	Yenisehir (Bursa)
Cömlek cheese	Kayseri, Kırsehir, Nevsehir, Cankırı, Aksaray
Cürük cheese	Artvin
Deve Dili cheese	Kars
Dil cheese	İc Anadolu Bölgesi'nde (The İnterior Anatolia Region), Hatay
Divle Tulum cheese	Divle (Karaman), Konya-Ereğli
Dolaz (Tort) cheese	Isparta and surrounding areas
Dövme cheese	Hakkâri
Eğirdir Taze Kelle cheese	Isparta and Eğirdir
Eksi (Siyah) cheese	Cankırı
Eksimik cheese	Ordu, Samsun, Giresun (Tüm Karadeniz)
Eridik cheese	Yusufeli (Artvin)
Ereğli Bez Tulumu	Ereğli (Konya)
Eritme cheese	Endüstri
Erzincan (Savak)Tulum cheese	Erzincan, Tunceli, Bingöl, Elâzığ
Erzurum Civil cheese	Erzurum
Erzurum Kerti cheese	Erzurum
Erzurum Tortum Pismis cheese	Erzurum
Erzurum Sünme cheese	Erzurum
Ezme cheese	Hatay
Gaziantep Tulum cheese	Gaziantep
Giresun Acı cheese	Giresun
Gorcola cheese	Artvin (Savsat), Ardahan (Posof)
Gödelek cheese	Niğde
Gölbası Tulum cheese	Gölbası (Ankara)
Gravyer cheese	Kars
Ham Cökelek	Silifke
Hellim cheese	Kıbrıs
Herki cheese	Hakkari (Semdinli)
Hınıs cheese	Erzurum
İmansız cheese	Trabzon

Traditional Cheese Type	Production Province / Region
İspir Kuru cheese	Erzurum
İspir Kurun cheese	Erzurum
İvriz Tulum cheese	Konya Ereğlisi
İzmir Tulum cheese	İzmir
Kadina (Kadine) cheese	Camlıhemsin, Yukarıkavrun, Cat, Elevit, Cicekli, Ayder Yaylaları (Rize)
Karabük cheese	Safranbolu, Yenice, Eskipazar (Karabük)
Karaman Tulumu	Karaman (Ermenek)
Karasar Tulumu	Beypazarı (Ankara)
Kargı Tulum cheese	Karadeniz Bölgesi
Karın Kaymağı cheese	Pasinler, Aybastı, Gümüshane (some villages), Siirt, Sarıkamıs
Kars Kashar	Kars, Ardahan
Kars Cecil cheese	Kars
Kartal cheese	İslâhiye
Kaynamıs cheese	Hatay
Kayseri Cömlek cheese	Kayseri
Kazıklı cheese	Milas
Keci sepet cheese	Karaburun
Kelle cheese	Kahramanmaras province and in its district
Kesmük cheese	Cankırı and surrounding areas
Kes	Ordu, Giresun, Burdur
Kırklareli cheese	Kırklareli
Kırktokmak cheese	Milas
Kirlihanım	Ayvalık
Kolete (koleti, goloti, kolot, kolo) cheese	Artvin, Trabzon, Rize, Bayburt
Konya Küflü cheese	Konya
Kopanisti	Cesme and Karaburun villages
Kozluk Kok cheese	Batman
Köcer (Göcer) cheese	Siirt
Kurci	Erzurum, Rize, Bayburt
Kuru Ezme cheese	Aydın
Kuru Cökelek	İzmir and Aydın in districts,
Küflü cheese	Konya

Traditional Cheese Type	Production Province / Region
Külek (Varella) Cheese	Trabzon, Araklı, Tonya, Caykara, Posof, Of, Rize, Artvin
Künefe cheese	Urfa, Antep, Adana
Küp cheese	Sivas, Sürmene, Yalvac (Isparta)
Küp Cökeleği	Tokat
Küpecik cheese	Ayas, Cankırı
Lor	The East and Marmara Region
Lorlu Kashar Kırığı	Bayburt
Malatya cökeleği	Malatya
Malatya Kelle cheese	Malatya
Maras Parmak cheese	Kahramanmaras
Mengen cheese	Mengen (Bolu)
Mezele cheese	Trabzon (Sürmene)
Mihalic cheese	Bursa, Balıkesir, Canakkale
Minzi cheese	Trabzon, Rize, Artvin,
Minzi Kurut	Trabzon
Motal cheese	Mus, Bulanık
Oğma cheese	Trabzon, Artvin
Olaman	Ordu
Otlu cheese	Diyarbakır-Kars-Siirt-Van, Hakkari, Bitlis
Otlu Cacık cheese	Van
Otlu Lor cheese	Van
Ovma ve Basma	Ankara (Ayas)
Örgü cheese	Diyarbakır
Örme cheese	Bayburt
Pesküten	Sivas
Pestigen	Elazığ, Bingöl, Tunceli, Erzurum
Posa cheese	Bodrum
Sacak cheese	Selim, Sarıkamıs, Hanak, Damal
Sepet cheese	Burhaniye, Karaburun, Foca, Cesme, Ayvalık,
Sepet Loru	Ayvalık
Sırvakta Loru	Bursa, Balıkesir
Siirt Otlu cheese	Siirt
Su (Sulu) cheese	Trabzon
Sürk (Sürke)	Hatay

Traditional Cheese Type	Production Province / Region
Süller Tuluk cheese	Denizli
Sünme	Hatay
Süt Kırması	Trabzon and its surroundings
Sütcüler tortusu	Isparta
Sütlü cheese	Antalya, Mersin
Safak cheese	Erzincan
Savak cheese	Elazığ, Tunceli, Bingöl
Sor cheese	Savsat (Artvin)
Sor Loru	Kars
Urfa Beyaz cheese	Sanlıurfa
Urfa Topak cheese	Sanlıurfa
Tekne cheese	Artvin
Tel cheese	Erzurum and Kars
Telli Peynir	Sürmene, Akcabat (Trabzon), Artvin
Telli Kremalı Peynir	Artvin (Yusufeli)
Testi cheese	Antalya
Tomas (Serto) cheese	Tunceli, Bingöl, Elazığ ve Mus
Tonya Kashar	Tonya (Trabzon)
Tonya cheese	Tonya (Trabzon), Yusufeli (Artvin)
Topak cheese	Sanlıurfa
Trabzon Cami Boğazı	Trabzon
Trakya Kashar	Kırklareli, Tekirdağ, Edirne
Tulum Kashar	Dumanlı (Tokat), Vakfıkebir, Sürmene (Trabzon)
Tulum Kesi	Akseki, Manavgat, Korkuteli
Türkmen Sacak	Ardahan
Varil cheese	Trabzon
Vartu Keci cheese	Mus (Varto)
Yalvac Küp cheese	Yalvac
Yaprak cheese	Hakkâri
Yayla cheese	Trabzon, Artvin
Yer cheese	Trabzon
Yörük cheese	Antalya, Denizli, Isparta, Burdur, Toroslar, Gönen
Yumme cheese	Artvin
Yusufeli küflü köylü cheese	Sarıgöl (Artvin-Yusufeli)
Yüksekova cirek cheese	Hakkari

Turkey is a very rich country in terms of traditional foods with its geographical location, rich natural resources, historical and cultural heritage. It is thus one of the few countries in the world with high potential for Geographical Indication (GI) (33-37). Geographical Indications (GI) are signs that indicate products originating from a specific region or identified with the region of origin with a certain quality, reputation or other characteristics (37-38). Geographical Indications are registered as a sign of origin or designation of origin in the Industrial Property Law No. 6797 dated 22.12.2016, which replaced the Decree Law No. 555, in parallel with the European Union practices. Traditional product name protection is not a Cİ, but a tradition that is protected. "Protected Designation of Origin" (PDO): Products originating from a region, territory with defined geographical boundaries or, in exceptional cases, from a country, which derive all or their essential characteristics from the natural and human elements specific to this geographical area, and whose production, processing and other operations all take place within the boundaries of this very geographical area. "Protected Geographical Indication" (PGI): It defines products, at least one of the production, processing and other operations of which is carried out within the boundaries of the designated geographical area (36, 38). The cheeses with geographical indications in Turkey are shown in Table 2. In the world, Parmesan cheese (in the provinces of Parma, Reggio Emilia, Modena and Mantua in Italy and along the banks of the Po and Reno rivers) and Grana Padano cheese (in some regions along the banks of the Po and Reno rivers in Italy) are among the cheeses with geographical indications. In Turkey, the cheeses with geographical indications are shown in Table 2 (36).

Tablo 2. Cheeses with geographical indication in Turkey

Geographical Indication Name	Geographical Boundary
Antakya Carra Cheese	Hatay province; Antakya, Arsuz, Belen, Yayladağ, Altınözü and Reyhanlı districts
Antakya Sürkü (Cökeleği) and Moldy Sürkü	Hatay province
Antep Sıkma Cheese	Gaziantep province
Bolu Kes Cheese	Bolu province Göynük, Mengen and Mudurnu districts, Sakarya province Taraklı districts and Bilecik province Gölpazarı districts
Diyarbakır Örgü (Braided) Cheese	Diyarbakır province and districts
Edirne White Cheese	Turkey borders
Erzincan (Savak)Tulum cheese	Erzincan province and its districts
Erzurum Civil and Moldy Cheese	Erzurum province and its districts
Ezine White Cheese	Ezine, with Bayramic and Ayvacık districts, Serbetli, Etili, Ahlatlıburun, Kücüklü, Alibeyköy, Söğütalan, Karacaören, Kursunlu and Kirazlı villages
Gümüshane Deleme Cheese	Gümüshane province
Hellim Cheese	Kıbrıs island
Karaman Divle Obruk Tulum Cheese	Karaman province Ayrancı district
Kars Kashar	Kars and Ardahan provinces and its districts
Kırklareli White Cheese	Kırklareli province
Malkara Aged Kashar Cheese	Tekirdağ province Malkara district
Manyas Kelle Cheese	Balıkesir province Manyas, Bandırma and Gönen districts
Maras Parmak (Finger)/Sıkma Cheese	Kahramanmaras province
Pınarbası Uzunyayla Cerkes Cheese	Uzunyayla platosu
Sakarya Abhaz (Abaza) Cheese	Sakarya province
Urfa Cheese	Sanlıurfa province
Vakfıkebir Külek Cheese	Trabzon province
Van Otlu (Herby) Cheese	Van and Hakkari provinces
Yozgat Canak	Yozgat

6. Cheeses with geographical indication in Turkey

6.1. Antep Sıkma Cheese

Antep Sıkma is a type of cheese made from the milk of small cattle (sheep, goat or a 1:1 mixture of these) grazing in the pastures of Gaziantep province. This is one of the types of cheese made by boiling the curd, is consumed fresh or kept in brine. Known as “pisken” or “kelle” in the region, this cheese is easily dispersible in the mouth, leaves a squeaking sensation in the mouth when chewed, flexible, non-porous, homogeneous, smooth, firm, does not crumble when cut, gray-white in color and semi-hard. This cheese, which is also rich in flavor components, has a spherical shape with a diameter of about 6-10 cm (23, 39-43).

6.2. Antakya Carra Cheese

Antakya Carra cheese is produced from the milk of cows or goats fed with herbs rich in medicinal and aromatic plants (about 2000 species) and endemic species (280) growing within the designated geographical borders. A layer of cheese, a layer of salt, and a layer of cökelek cheese with zahter or black cumin added to it are placed in a jug called carra (glazed/unglazed). After 3-4 days, the mouth of the jug is plastered with a special mortar and ripened under the ground for about 3 months. The distinctive features of this cheese come from the mountain thyme known as “zahter” in the region, black cumin and the pottery used for packaging (23, 44-45). Depending on the season and animal breed, Antakya Carra cheese is white-cream in color, hard and brittle, salty and rich in aroma components (45-47). At the same time, more than 60 volatile compounds were detected in this cheese and characterized in terms of 3-methyl-2-butanol, ethyloctanoate, 2-isobutyl-3- methoxypyrazine, propanoic, butanoic, 3-methylbutanoic, hexanoic and octanoic acids (45).

6.3. Antakya Sürkü (Cökeleği) and Moldy Sürkü

Antakya Sürkü (Cökeleği) is obtained from cow›s milk with increased acidity or by boiling buttermilk. It is a type of orange colored and conical shaped cökelek cheese that contains bitter, sour and salty flavors, obtained by adding salt, pepper paste and optionally various spices (such as black pepper, red pepper, cumin, coriander, mint, mahlep and ginger) together with wild thyme collected from the mountains, known as «zahter» in the region (41, 43, 47-49).

6.4. Antakya Moldy Sürkü (Cökeleği)

Antakya Moldy Sürkü is a separate type of moldy cheese obtained as a result of the natural molding and ripening of Antakya Sürkü. During the ripening process, the cheese undergoes changes in appearance, taste and smell. However, unlike other moldy cheeses, Antakya Moldy Sürkü is consumed after the molds are removed from the surface (50). While a hard crust is observed on the outside, industrial production is not yet in question for this type of cökelek cheese, whose color changes from dark red to brown (36).

6.5. Bolu Kes Cheese

Bolu Kes cheese is a traditional Turkish cheese that has been produced and consumed in Central Asia and Anatolia for centuries. This type of cheese, which has many varieties with different characteristics (such as leather kes, gök (moldy) kes, dry kes, yellow kes and fresh kes) varying according to the region where it is produced (such as Bitlis, Bolu, Hatay, Giresun, Kars and Van), the producer and consumer preference, is a salty and hard-structured cökelek cheese with a distinctive taste, smell, aroma and color (matte-white), low fat content, usually produced from cow's milk in May and June. The most important distinguishing feature of Bolu kes cheese is the use of strained yogurt made from partially skimmed cow's milk in its production and its longer shelf life due to its production technology (salting and drying) (51-52).

6.6. Diyarbakır Örgü (Braided) Cheese

Diyarbakır örgü cheese is a type of brined cheese with high fat content, homogeneous, plastic curd, elastic structure, semi-hard, distinctive taste and aroma, high nutritional value and shaped like a braid. This cheese, which can be separated into threads by hand, has a shiny appearance and its color is cream white or slightly yellowish (39, 53-54). This cheese, which is made from sheep's milk in the spring time, can also be produced from a mixture of goat and cow's milk. The distinguishing features of Diyarbakır örgü cheese stem from the use of milk obtained from special sheep breeds (Karakas, Karacadağ, Zom and Ivesi sheeps of the Akkaraman variety) that are fed with various grasses in pastures with rich vegetation and the production method that requires a great deal of skill. The most important distinguishing feature is that it is shaped like a pigtail, its taste and smell are between white cheese and kashar cheese, and there are no physical defects such as crusting and pore formation (36, 43). Diyarbakır örgü

cheese is similar to white cheese in terms of composition and to kashar cheese in terms of production technology. Although this cheese is usually made from raw sheep's milk in spring, goat and cow's milk and their mixtures can also be used in its production (41, 54).

6.7. Edirne White Cheese

Edirne white cheese is a yellowish-white colored and rectangular shaped brined type of white cheese made from milk (cow, sheep and goat) obtained from dairy animals (cow, sheep and goat) fed by the vegetation under the influence of the deltas formed by the Tunca, Meric, Arda and Ergene rivers of Edirne Province and the climate specific to the region. Edirne white cheese is produced by brine method or tinning method. One of the most important points in the production of white cheese, which is salted and ripened in brine, is that the cheese has a flat and non-porous structure. The distinctive feature of this cheese, which has its own unique structure, taste and smell, is the geographical region where it is produced as well as the type of milk in the region, the use of different yeast and salt. It is the most widely consumed geographically indicated cheese in our country (23, 26, 43, 55).

6.8. Erzincan Tulum Cheese

Erzincan tulum cheese is the most well-known and consumed type among tulum cheeses. It is Turkey's first cheese with a certificate of registration. Erzincan Tulum cheese is "made from the milk of Karaman sheep fed in the highlands of Erzincan (Munzur, Cimen, Cayırlı, Tercan and Kemah Oluk), which has 90-100 different kinds of plant richness" (56-58). It is also known as "Savak" cheese in Tunceli, Erzincan and Elazığ provinces. It is pressed into jerry cans or skin bags with Kemah salt and ripened in caves for about 1 year (23). The sheep's milk, rennet, Kemah salt, production method and packaging in goat skin bags are the distinguishing features of this tulum cheese. Thus, it appears as a white-cream colored, high fat content, fragile, homogeneous structured and delicious cheese with a distinctive acidic, buttery and rancid taste, easily melting in the mouth (26, 59).

6.9. Erzurum Civil Cheese

Erzurum civil cheese is produced under names such as Cecil cheese, string cheese, yarn cheese and pull cheese in the Eastern and Northeastern

parts of Turkey, especially in Erzurum, where acid, rennet and heat treatment are used together (60-61). Although containing high levels of protein, calcium and phosphorus, it has a low fat content (62). According to the Geographical Indication (GI) registration certificate, Erzurum Civil cheese is defined as "a type of local cheese classified as fat-free or low-fat cheese produced by obtaining the milk from animals fed with various nutritious grasses in the pastures between the high mountains of Erzurum province, passing it through separators and removing the fat, and then acidifying the remaining skim milk at a certain level, fermenting it with liquid sirden rennet and heating it, mixing and kneading the clot and then hanging it on hangers to form a string in the mass". It is consumed both fresh and brined (63- 64).

6.10. Erzurum Moldy Civil Cheese (Göğermis Cheese)

Erzurum Moldy Civil Cheese (Göğermis Cheese); Erzurum Civil cheese is a ripened cheese with a unique flavor obtained by shredding Erzurum Civil cheese and mixing it with lor cheese or by putting Civil cheese in plastic drums suitable for food packaging, removing the water and letting it naturally mold. It is also known as göğermis (turned green) cheese or moldy lor cheese. This cheese, produced by being put on materials such as wood and leather as well as plastic materials, is molded in blue-green color (60, 64-67). It is an aromatic cheese type that has an important place in the breakfast culture of Erzurum province and its surroundings, is known all over Turkey, has a flavor similar to the Roquefort cheese of France, is perceived as a fatty variety due to the effect of biochemical reactions during ripening although it is produced from skim milk, has been investigated in all aspects with projects supported by TÜBİTAK and all its properties revealed, whose strains of Penicillium roqueforti have been identified as starter molds. (60, 68-69).

6.11. Ezine Cheese

It is the most produced and consumed white cheese type in Turkey, with the highest economic value. It is a geographically indicated cheese among the white cheese varieties that are widely produced in almost every region of our country, especially in the Aegean, Marmara, Thrace and Central Anatolia regions (70-71). This full-fat brined type white cheese is produced from a mixture of cow's milk (maximum 25%), sheep's milk (35-45%) and goat's milk (minimum 40%) obtained from animals fed with natural endemic vegetation (such as sage, oregano, thyme, marjoram and hairy mint) and water resources in

the geographically demarcated regions located in the northern and western parts of the Kaz Mountains; with a white to light yellow color, medium hardness, non-fragile structure, few and small diameter pores in its mass (26, 43, 72-74). Ezine cheese has a "creamy" taste and aroma due to milk fat and "baked milk" taste and aroma due to the heat treatment applied. The milk or milk mixture used, the yeast prepared from calf sirden in whey, the sea salt used and different production methods constitute the distinctive features of Ezine cheese. The milk obtained from special animal breeds (Tahirova, Sakız, Dağlıc breed sheep, Holstein breed cows and Karakeci and Turkish Saanen goat breed) between March and July is used in the production of this cheese (72). Starter culture and calcium chloride ($CaCI_2$) are not used in its production (74).

6.12. Gümüshane Deleme Cheese

Gümüshane Deleme Cheese is a homogeneous, non-porous, slippery, semi-hard cheese with a slightly sour taste and high milk flavor, which can melt when heated, which is obtained by boiling the cökelek cheese produced by curdling/coagulation of cow›s milk with naturally increased acidity with fat milk and kneading and shaping it after pre-treatments (75).

6.13. Hellim Cheese

Hellim (Halloumi) cheese is a semi-hard traditional cheese type specific to Cyprus and is also widely produced in Eastern Mediterranean countries (76). The cheese is available in the market in two forms: fresh and mature halloumi. Fresh halloumi is a double-layered, yellowish-white colored, easy-to-slice, semi-hard and elastic cheese obtained by the production method specific to the region with the addition of rennet to milk. Mature Halloumi, on the other hand, is a double-layered, yellowish-white, easy-to-slice, semi-hard and firm cheese obtained by maturing fresh Halloumi cheese in salty whey brine. Halloumi cheese's distinctive characteristics stem from the use of raw or pasteurized sheep, goat and cow's milk and mixtures thereof, obtained from dairy animals fed on the rich vegetation of Cyprus, and its characteristic production method (77).

6.14. Karaman Divle Obruk Tulum Cheese

Tulum is a type of cheese that is widely produced and consumed after white cheese in our country and has many varieties such as Cimi, Divle, Erzincan and

İzmir tulum cheeses. Tulum cheese is mostly produced in small family-type enterprises (78). Karaman Divle Obruk tulum is a type of cheese that is offered for consumption after the teleme cheese made with a mixture of cow (10%), sheep (80%) and goat (10%) milk obtained from animals fed in the highlands and pastures within the borders of Ayrancı district of Karaman province (fed with roughage such as dried alfalfa, hay, etc. in seasons when they cannot go out to pasture) is pressed into goat or lamb skin bags and ripened in Divle sinkhole for 5-6 months. Its distinguishing feature from other cheeses is that the cheeses pressed into the skin bags have a unique mold flora, blue, then white and then brick red colored mold fungi develop after ripening, and it is ripened at 4ºC and 80% relative humidity in a cave called "obruk" (sinkhole), which is a natural cold storage (39, 79-80).

6.15. Kars Kashar

Kashar is the most produced and consumed semi-hard or hard cheese type in our country after white cheese. The production methods of this cheese vary according to regions. In our country, it is produced in many provinces such as Kars, Tekirdağ, Edirne, Kırklareli, Kocaeli, Mus, Erzurum and Trabzon (81). Kars Kashar, the most well-known of these, is a type of cheese with a distinctive taste, smell, aroma and color, which can be consumed fresh or ripened, and which can be produced by mixing sheep and goat milk with cow's milk, although it is generally made from cow's milk grazing on pastures with rich vegetation between May and August in Kars and Ardahan provinces. Fresh Kars kashar (in the pre-ripening stage) is whitish in color and tastes slightly salty, bland and reminiscent of milk when it comes first out of the mold, but within a week, it turns yellowish with a crust. Aged kashar (after 3 months of ripening), on the other hand, is harder, slightly salty, more aromatic and easily dispersed in the mouth (41, 82). The most important feature distinguishing Kars kashar from other kashars is that its color, which is close to white when fresh, turns yellow after a week. Another distinguishing feature is the milk used in its production. Kashar cheese is produced from the milks obtained from animals that feed on endemic plants and grasses in the region (23, 83).

6.16. Kırklareli White Cheese

Kırklareli white cheese is a type of cheese produced from a mixture of milks obtained from cows (15-30%), sheeps (30-45%) and goats (25-45%) which are grown in the pastures and meadows of Kırklareli and fed with grasses, including endemic species, and consumed by ripening in oil and brine (84-85).

6.17. Malkara Aged Kashar Cheese

Malkara Aged Kashar cheese is a hard, cylindrical, fully ripened cheese with a distinctive taste, smell, aroma (terpene-derived components such as alpha pinene and calarene from green forages are dominant) and color (straw yellow), made from the milk of cows, sheeps and goats grazing on pastures with rich vegetation (especially Lathyrus L., clover and thyme, which are native to the region) in April and July in Malkara district. The most important distinguishing characteristic of this cheese is that it is produced from the milk of animals fed with high levels of grasses with various flavors originating from the vegetation of the region. Sensory-wise, it has a salty, umami and sour taste; cooked, creamy-milk fat, sulfur, PAS, rancid, animal-like, yeast/mold, fruity and nutty aroma (10, 41, 86).

6.18. Manyas Kelle Cheese

Manyas Kelle cheese is a type of full-fat or half-fat hard cheese made with cow's and sheep's milk or a mixture of these milks obtained from animals fed with the natural vegetation and water resources of Manyas District of Balıkesir province, with a hard rind 2-3 mm thick, yellow-white color, without cracks, porous structure, unique taste and aroma (slightly acidic and salty), ripened in brine (87). Manyas Kelle cheese, which has been produced from raw sheep milk in Balıkesir and Bursa provinces for 250 years, also goes by different names such as Mihalic, Maglıc and Mahlıc (88-89).

6.19. Maras Parmak/Sıkma Cheese

It is a local type of cheese whose curd is boiled, homogeneous, non-porous, not easily disintegrating, low-salted, white colored, called "parmak" (finger) or sıkma cheese because of its finger-shape, produced in Kahramanmaras and its surroundings (90). Although it can be considered in the same group with many other cheese varieties because it is shaped during the boiling of the cheese curd and preserved in brine, it is in fact a typical local cheese variety thanks to its origin, milk quality, external and internal qualities and shaping method (91).

6.20. Pınarbası Uzunyayla Cerkes Cheese

Pınarbası Uzunyayla Cerkes cheese is a traditional type of cheese produced by coagulating the milk obtained from cows or sheeps grazing in the plateaus and pastures at 1600-2000 altitude in Uzunyayla plateau with fermented whey at the boiling point and dried in the wind. This semi-hard cheese has a smooth texture,

white-straw yellow color depending on the drying conditions, is oily, hard rind, slightly salty, with a slightly sour and cooked taste and basket appearance (92).

6.21. Sakarya Abhaz (Abaza) Cheese

Sakarya Abkhaz (Abaza) cheese is a type of ripened cheese that has been shaped by the culture of the Abkhaz people settled in Sakarya province 700 years ago, has the characteristics between Kashar cheese and dil (string) cheese, has a fibrous structure, amber yellow color, and its appearance is usually braided. The distinctive features of the cheese are due to the rennet (sirden) used in its production, different seasonings (optionally black cumin and thyme) and the traditional production method (93).

6.22. Urfa Cheese

Urfa cheese is produced in the Southeastern Anatolia Region, especially in and around Sanlıurfa, using mostly sheep and goat milk (94-95). This is a type of local cheese that has become increasingly popular in recent years. Urfa cheese is a semi-hard cheese produced from sheep milk and ripened in brine (96).

6.23. Van Otlu (Herby) Cheese

Otlu cheese is a type of cheese that has been produced and consumed in Eastern and Southeastern Anatolia regions of our country (Van, Diyarbakır, Siirt and Ağrı) for many years and is mostly produced in Van province (39, 97-98). Van Otlu cheese is made from sheep, cow or goat's milk or a mixture of these, with the addition of about 20-25 herbs called Sirmo, Mendi, Thyme, Siyabo, Wild mint and Heliz, which grow in the region and surrounding provinces (47, 97, 99), with a color ranging from white to yellowish due to the differences in the milk and herbs used, delicious (dominating garlic and thyme aroma), consumed fresh after salting or ripened in brine, semi-hard and flat, shiny looking cheese (39, 100). The most important feature of the herbs used in production is that they have antimicrobial effect against coliform group bacteria. In addition, it is reported that the unique color, taste, smell and appearance of the cheese are due to the herbs in question (101). This traditional cheese is similar to white cheese in terms of production method and structure, but has different characteristics due to the presence of endemic herbs in its structure. Van Otlu cheese, which is made in the spring time, is produced in two different ways in terms of salting method: "brined" or "dry salted". There are differences between the two types regarding the structure and appearance (39, 98).

6.24. Vakfıkebir Külek Cheese

Vakfıkebir Külek cheese is a traditional type of cheese obtained by pressing the cheese and cökelek cheese produced from cow's or sheep's milk tightly in layers in wooden containers named külek and ripening them under the ground or in hazelnut shells. The distinctive characteristics of this cheese stem from the traditional sirden rennet used in its production and the pots made of spruce trees used for packaging (102).

6.25. Yozgat Canak Cheese

Yozgat Canak cheese is a low-ripened and half-fat cheese with a unique taste and aroma, made from cow, sheep and goat milk fed with the natural vegetation and water resources of Yozgat center and its districts. As the name suggests *[canak meaning bowl],* this cheese is ripened by pressing it into an earthen bowl and then burying it in sand. It is important that the buried sand is moist and humid during the ripening phase (43, 103, 104).

7. Conclusion

The Turks have changed their homeland frequently throughout the world and spread over a wide area and have been under the influence of many cultures. The different civilizations they have lived in have created a cheese culture that has changed from Central Asia to the present day. Cheese, which is consumed with pleasure in our country as well as all over the world, is a durable dairy product with a wide variety and it is as well an indicator of the cultural richness of our country. Although there are many cheeses recorded in Turkey, many of them are in danger of extinction. While some cheeses are produced locally, others are recognized throughout the country. In our country, it is of great importance to obtain Geographical Indication (GI) in order to classify and standardize cheeses according to the region or area where they are produced. This will ensure that our traditional cheeses are produced at a certain standard in the food sector, encourage other local cheeses to obtain the GI mark and thus our traditional cheese varieties will be introduced to the world.

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