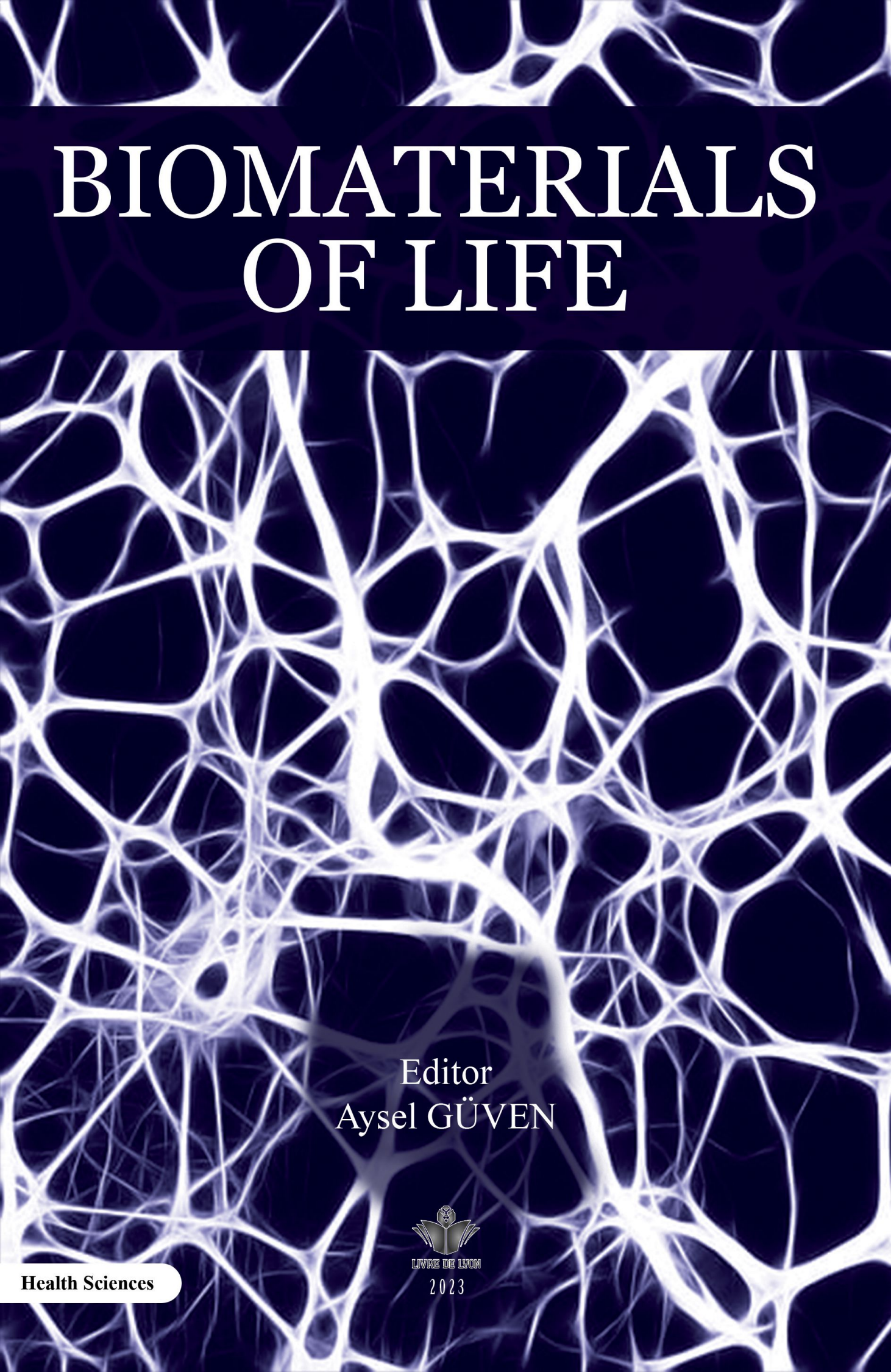
# BIOMATERIALS OF LIFE



Editor
Aysel GÜVEN

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Health Sciences

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**Biomaterials of Life**

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# PREFACE

Depending on the change of technology and natural process, biological molecules, materials and working systems also change. These concepts, which are disease centered, patient-centered, community-centered and human-centered, have been determinative in the development and progress of health services. Innovative studies on disease and health will continue to make this field visible day by day. We hope that our book prepared for this purpose will be useful and contributing. This book is dedicated to commemorating the twelfth anniversary of Prof. Dr. I Abamuslüm Güven. Regards.

**Assoc. Prof. Dr. Aysel GÜVEN**

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# CHAPTER I

# EFFECTS OF NANOPARTICULES ON REACTIVE OXIDATIVE SPECIES FORMATION

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## 1. INTRODUCTION

Nanoparticles (NP) are defined as particles obtained by combining organic and/or inorganic materials with sizes ranging from 1 to 100 nm. The diameter of the nanoparticle is similar to the size of biomolecules in many ways: the size of proteins is 1-20 nm, the diameter of DNA is 2 nm, the virus is 20 nm, the cell surface receptor is 10 nm, hemoglobin is 5 nm, the cell membrane is 6-10 nm in diameter, easily overcoming natural obstacles. They can reach molecules, intracellular organs, tissues, cells and organisms (1, 2). For this reason, their use in the field of health is very important. Nanoparticles have a very large surface area and this surface area has a high affinity for metals such as silver (Ag), iron (Fe), organic chemical combustion products such as polycyclic aromatic carbons (3). NPs are divided into different types according to their structure, size, morphology, physical and chemical properties. Some of these are carbon-based, ceramic, polymeric and metal NPs. Metal nanoparticles are produced from metals. Metal NPs can be produced using gold, silver or platinum. Metal NPs are obtained using physical, chemical or biological methods (4,5). Nanoparticles are particles that can be designed appropriately so that surface modifications suitable for specific applications can be easily carried out. With these features, they are used in the diagnosis and treatment of many diseases (6,7).

NPs are divided into different types according to their structure, size, morphology, physical and chemical properties. Some of these are carbon-

based, ceramic, polymeric and metal NPs. Metal nanoparticles are produced from metals. Metal NPs are methods obtained by using many minerals and using physical, chemical or biological methods and have been used extensively in scientific, biological and medical research recently (5-7). NPs obtained by chemical methods are generally used metals such as Au, Ti, Fe, Zn, Ag. When metal NPs are obtained by chemical methods, many undesirable toxic chemicals can be produced during reduction. The production of metal NPs using bioorganisms is called the biological method (2, 8, 9). Studies are accelerating that these NPs do not impair their biocompatibility, do not cause toxic substance production, and allow production in a short time. Silver NPs (AgNPs) have an important place among metal NPs (10, 11). Due to the abundance of silver in nature, its biocatalytic/photocatalytic properties, ease of synthesis and low cost, the use of AgNPs stands out compared to other metal NPs (12, 13). Silver is also preferred because of its high antimicrobial effect (10, 11). Considering the studies in the field of health, it is seen that there are biodegradable polymers such as NP, silver, iron oxides, gold, zinc, titanium, dendrimers, lipid-based carriers including liposomes and micelles, viruses and even organometal compounds used for this purpose (14). Most of the studies on nanoparticles with multi-effect systems have antimicrobial and antiviral effects, and they also cause anticarcinogenic, oxidative stress, and free radical effects (15,16). Brown et al. also reported that there is a significant correlation between the surface area of nanoparticles and inflammation caused by oxidative stress (17). Papageorgiou et al. showed that cobalt-chromium mixture nanoparticles cause free radicals, DNA damage, aneuploidy and cytotoxicity at a higher rate than micron-sized particles in human fibroblast tissue cultures (18). Research has focused on the development of nanoparticles that can bind to biological molecules such as peptides, proteins and nucleic acids and have optical, electronic and magnetic properties (19).

An important part of the usage areas of nanomaterials is the determination of some microorganisms. Various nanostructures can be developed and sensors that can interact selectively with bacteria, enabling fast and accurate measurement, can be used for bacterial identification. Determination of biomolecules is of great importance in medicine, environment and food analysis. Recently, nanoparticles have been used in various application areas such as soil and groundwater remediation, air pollution control, drinking and wastewater treatment (including garbage leachate treatment) in the field of environmental engineering (20).

While AgNPs show antimicrobial activity against viruses, bacteria, fungi, and protozoa, they also exhibit anti-cancer activity against cancer cells (Asha Mónica and SR, 2020, Yang and Lu, 2005; Sarkar et al., 2005). Recently, NPs in biological systems have been used as disease diagnosis, drug delivery tools for better medical treatment (10,11,21).

## 2. SILVER GROUP ((Ag-NPs)

Silver is used as an antimicrobial agent (22) in water used in food preservation, Russian MIR space station and NASA space shuttle, water disinfection, in the treatment of burns and chronic ulcers, on the grounds that its shelf life is extended for many years. Coli, as well as in the content of important drugs and disinfectants such as eye drops, burn ointments and washing waters (23). Due to its non-toxicity, silver can be modified in order to reduce toxicity and to ensure stability and biocompatibility in the form of silver nanoparticles (AgNP) with its anticarcinogenic and antioxidative effects, which can eliminate bacteria with various action mechanisms. This modification includes coating and functionalization of AgNPs. Unmodified AgNPs are called naked. Bare AgPNs are not stable; a high degree of aggregation and ionization is seen (Figure 1a). Capped AgNPs are coated with polymers, enzymes, amino acids, or proteins. They are stable, showing hydrophobic or hydrophilic properties depending on the type of coating (Fig. 1b). Functionalized AgNPs, on the other hand, are stable, water-soluble, polysaccharide-based biocompatible nanocomposite coated, functionalized nanoparticles equipped with reactive groups (Figure 1c).

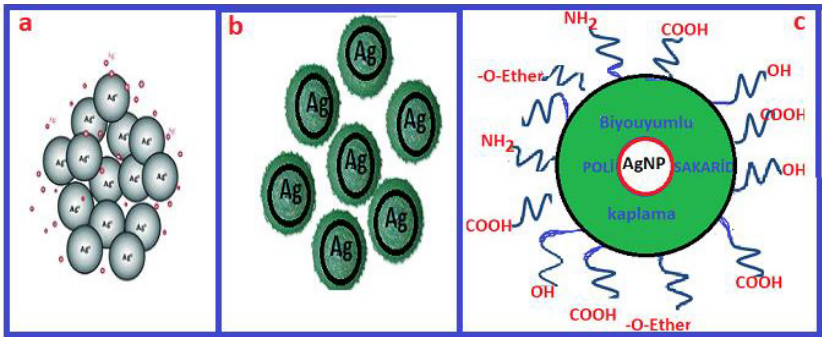


**Figure 1.** (a) Bare AgNPs, (b) Coated AgNPs, (c) Functionalized AgNPs (24)

The distribution of silver nanoparticles in the cell, their cytotoxic, genotoxic and immunological properties, in vivo and in vitro effects depend

on their direction of synthesis, shape, size, surface structure, dose, application time and the type of cell to which they are applied. AgNPs are mostly taken up into cells by mechanisms associated with endocytosis. AgNPs can create toxic effects by undergoing chemical transformation as they move through the cell. During this chemical transformation, they cause dysfunction in mitochondria by creating reactive oxygen species that damage the cell membrane, proteins and DNA. This results in cell cycle arrest and subsequent apoptosis (25-27). AgNPs are known to cause cytokine production and lead to proinflammatory responses. It has also been shown that they cause specific responses, such as the production of interleukin and tumor necrosis factor (TNF) in a dose-dependent manner (28). It has been determined that silver binds to the bacterial cell wall and cell membrane, interacts with thiol groups and inhibits respiratory enzymes, thus causing the death of the microorganism. In silver ion studies, he investigated the effect of silver ions on amino acids containing thiol (-SH) groups and amino acids without thiol (-SH) groups, and it was observed that silver ions were bound to thiol groups in amino acids containing thiol (-SH) group (29). There is also a study that examined the morphological differences of silver ions on Gram-positive Staphylococcus aureus (S. aureus) and Gram-negative E. coli bacteria and showed that they easily and permanently affect the immune system cells on damaged microorganisms (30). With this feature, it has been developed and used in the textile and food industry as well as in the health industry, and positive results have been obtained when it is used as a preservative instead of chemicals in wet wipes (23). These effects of silver can be reduced or even eliminated by functionalized correct coating of AgNPs (31,32).

Biocompatible silver-containing cellulose, chitosan and dextran-like functionalized composites show strong biocompatibility, and their use in the diagnosis and treatment of wounds, cancer and metabolic diseases, as well as their free radical properties that can cause oxidative stress, are presented by bringing together extensive recent research. The abundance of silver in nature, its biocatalytic/photocatalytic properties, ease of synthesis and low cost make AgNPs clinically useful compared to other metal NPs. Silver-containing nanoparticles draw attention with their antibacterial properties, which provide an easy and permanent effect on microorganisms such as bacteria and viruses, which cause problems in human health, and their non-toxic effects on cells. In addition, in vitro and in vivo studies have accelerated whether the new nanoparticles to be developed based on the studies have reached the target, and if they do, the advantageous and disadvantageous experimentally created

in vitro and in vivo (Abitbol, 2016; Almeida et al., 2015; Hamed et al., 2019; Bunyatova et al. , 2021).

**Cellulose:** Among all polysaccharides, cellulose is the most widely used and easily available polysaccharide. Cellulose is a polysaccharide found in plants and is one of the most abundant biomaterials on earth. Cellulose, which is generally synthesized by plants, is also produced by some bacteria. Cellulose, a hard, fibrous and water-insoluble polysaccharide, plays a role in keeping the structure of plant cell walls stable (21). Cellulose, a glucose polymer, is used in biomedical fields, especially in paper production, in the cosmetic industry and in the production of polymer composites (33). Carboxymethyl cellulose is formed by the bonding of carboxy methyl groups to some hydroxyl groups of glycopyranose monomers that form the backbone of cellulose (chemical formula: C8H16NaO8). It is a biocompatible, biodegradable, hydrophilic, non-toxic and inexpensive polysaccharide. It has high water solubility and chemical stability thanks to a series of sodium carboxymethyl (CH2COONa) and hydroxyl (-OH-) groups. It is widely used due to its antitumor and antimicrobial properties (34, 35). Cellulose-based composites; They are widely used for coating metal NPs because they are stable and robust, biodegradable, non-toxic and have low density (36,37).

**Chitosan:** Chitosan is a fascinating substance containing amino and carboxyl groups, biocompatible, biodegradable into harmless substances, non-toxic, stable, with outstanding affinity for proteins, antibacterial, hemostatic, fungistatic, antitumoral and anticholesteremic effects. Carboxymethyl chitosan has anti-cancer, anti-bacterial effects, interacts with cells in events such as tissue regeneration and wound healing, and promotes the proliferation of fibroblasts (38). The source of chitosan is chitin, one of the most abundant organic materials in nature. It is an important component of the exoskeleton in animals, especially crustaceans, mollusks and insects. It is also the main fibrillar polymer in the cell wall of some fungi (Kim, 2008). It is known that nanocomposites formed with chitosan and its functionalized derivatives continue to exhibit these properties (39, 40).

**Dextran (D):** Dextran is a complex, branched bacterial polysaccharide (chemical formula: H(C6H10O5)xOH) composed of linear chains of varying lengths. Dextran, a type of α-glucan, is the first commercialized bacterial polysaccharide that exhibits high biocompatibility and biodegradability, enabling a wide range of applications (36,37). Dextran and its derivatives, which are natural macromolecules, are used as biomaterials and drug

carriers. Dextran is a water-soluble, hygroscopic, biocompatible, non-toxic, and environmentally safe polysaccharide. Therefore, it is of great interest for clinical research. Dextran reduces damage to normal tissues and ensures targeting of anti-cancer drugs (41). For example, dextran has been shown to be the ligand of mannose receptors found in the cell membrane. Recognition of dextran by cells both ensures that agents are taken into the cell by targeting the receptor, and accelerates cell uptake via receptor-mediated endocytosis (42). In this sense, dextran nanocomposite coated AgNPs may be a good alternative to overcome the limitations of conventional drugs. Dextran nanocomposites have a very high biocompatibility and have the ability to reduce the toxicity of metal nanoparticles due to the molecular structure of dextran (having hydroxyl groups andhemiacetal ends).

These are cellulose, chitosan and dextran. AgNPs functionalized with these polysaccharides block the DNA macromolecule by interacting with amine and carboxyl groups and similar groups in cancer cells. It has been stated that the three carboxylic groups (COOH) of this substance react with the amine in the DNA of cancer cells and other reactive groups that are suitable for the growth of cancer cells. It is also stated that functionalized AgNPs have a synergistic effect against β-lactam class antibiotics even at small doses. When uncoated AgNPs enter the mitochondria and nucleus, they can increase reactive oxygen substrates, induce mitochondrial dysfunction, and subsequently lead to DNA damage, chromosomal abnormalities, and cell cycle arrest (15,24). Studies with Functional AgNPs combined with cellulose, chitaso and dextran have also shown that these hydrogels have significant anti-cancer and antimicrobial effects (43,44). It has been shown that functionalized AgNPs significantly reduce cancer cell viability in different types of cancer. The therapeutic and protective effect of functionalized AgNPs in cancer is superior to other chemotherapy treatments, as it has a lethal effect only against cancer cells, not against health cells, and is almost toxic. In recent years, studies on anti-cancer agents that can be applied locally in the tumor cavity and the surgical site, especially after surgery, targeting the residual tumor cells and the immune cell response in the tumor microenvironment, have gained momentum in the treatment of breast cancer. Hydrogels applied inside, around the tumor or in the surgical cavity are used as drug delivery systems that allow targeted effective treatments that can be used in the early post-surgical phase. These AgPNs are three-dimensional materials with high water content prepared from cross-linked polymers (14, 15, 45). They have a soft tissue-like structure and excellent biocompatibility (26).

Functional AgNPs can release drugs directly and for a long time with minimal side effects at once (46, 47).

They can be produced from natural polymers such as polysaccharides and proteins, or they can be produced from synthetic polymers. Chitosan is one of the most commonly used biodegradable polymers in medicine. It is preferred in cell targeting studies and many biomedical applications due to its suitability for chemical modification, lack of toxicity and bioactive properties. AgNPs show antimicrobial activity against viruses, bacteria, fungi and protozoa (11, 48). AgNPs functionalized with cellulose, chitosan and dextran play a role in tissue construction due to their natural antimicrobial effects from polysaccharide, their protective against infectious microorganisms and their protein mineral content. In this case, according to the nanocomposite functional structure of functionalized AgNPs, the interaction mechanism with virus and bacteria goes through stages such as electrostatic attraction, Van der Waals/Hydrogen forces, receptor ligand and hydrophobic interactions (31).

In the light of all these data, the functionalization of nanocomposites with polysaccharides such as cellulose, chitosan and dextran, thanks to their biocompatible, water-soluble and multifunctional structures, can be widely used as treatment and diagnostic agents that can be reached in the field of health with antibacterial, antiviral, anticarcinogenic and regenerative effects in burn-wounds and antioxidative effects at affordable costs. is recommended.

## 3. EFFECTS OF NANOPARTICULES ON ROT FORMATION

Free radicals with one or more unpaired electrons in their outer orbitals are highly radioactive and unstable compounds. In parallel with the increase in the number of studies revealing the relationship between free radicals and diseases in recent years, the interest of the society towards free radicals has also increased. Both endogenous and exogenous free radicals are produced continuously in the cell and in the environment (49-51). Both field and laboratory studies have shown toxicological results such as DNA damage, lipid peroxidation in cell and organelle membranes, inhibition of enzyme systems, disruption of carbohydrate and protein metabolism in living things due to ROS formed under the influence of a wide variety of environmental pollutants. Free radicals, which are formed in the body through natural metabolic pathways, are normally eliminated by radical-degrading antioxidant systems. These antioxidants are generally metal-containing antioxidants. However, a series of pathological events called oxidative stress occur as a result of the increase of reactive oxygen species and

insufficient antioxidant mechanisms for various reasons. The source of free radicals is molecular oxygen. Molecular oxygen has two unpaired electrons in the parallel spin state. Oxidation reactions occur with single electron transfer to molecular oxygen by means of nanoparticles and enzymes containing transition metals (such as Fe, Cu, Ni, Cr) in the organism. Molecular oxygen containing unpaired electrons plays an important role in the formation of ROS (52).

Fenton Reaction

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + .OH + :OH^-$

$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\cdot + H^+$

Fenton-Like Reaction

$Cu+ + H_2O_2 \rightarrow Cu^{2+} + \cdot OH + OH^-$

$Ag + H_2O_2 \rightarrow Ag+ + \cdot OH + OH^-$

Haber-Weiss Reaction

$Fe^{3+} + \cdot O_2^- \rightarrow Fe^{2+} + O_2$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$

Magnetite, which is taken into the cell in different ways (passive diffusion, endocytosis), is most likely broken down into iron ions by the action of hydrolysis enzymes in lysosomes. These free iron ions (Fe2+) can pass through the nuclear or mitochondrial membrane and interact with hydrogen peroxide and oxygen formed in the mitochondria and cause the formation of hydroxyl radicals and ferric ions (Fe3+) through Fenton reactions. That is, the conversion of H2O2 to hydroxyl or superoxide radicals is catalyzed by the Fe2+/Fe3+ ions formed by magnetite in the cell. The resulting radicals also have the potential to damage intracellular structures and cell membranes (53). Transition metals catalyze the decomposition of synthesized lipid hydroperoxides (LOOH) and the chain reactions of lipid peroxidation rather than initiating lipid peroxidation. Thus, they make less harmful radicals more harmful.

Nano silver causes membrane damage, inactivation of proteins such as respiratory enzymes and DNA damage by causing the formation of reactive oxidative species (ROS) even in resistant bacteria that are difficult to treat. Therefore, the formation of ROS and the detoxification mechanism of these products are important. Various enzymes in the normal metabolic pathways of aerobic cells and environmental factors such as trauma, infection, reperfusion

and inflammation create reactive oxygen species (ROS). These radicals are molecules containing one or more unpaired electrons, and they cause damage to cellular macromolecules due to their high chemical activity (54). In recent years, many studies have shown that oxidative stress plays a role in metabolic diseases such as cancer, diabetes, and wound healing (55). There are protective mechanisms defined as antioxidants in the organism against the harmful effects of free radicals, which are the cause of tissue damage. While some of these protective mechanisms prevent the formation of free radicals, some of them act to reduce the harmful effects of the formed free radicals. That is, under normal conditions, oxidant-antioxidant levels are in equilibrium. However, oxidative stress develops as a result of the deterioration of the current balance towards free radicals due to various diseases and stress (52). ROS, which play a role in the pathogenesis of many diseases, cause tissue damage. For example, in burns and wounds, hydroxyl radicals and O- anion cleave hydroxyproline and proline in the collagen structure, changing the adhesion, proliferation and vitality of fibroblasts, and HO, on the one hand, inhibits the migration of keratinocytes and epidermal growth factor (EGF) signal communication and causes serious damage to fibroblasts (56, 57). Therefore, high amounts of ROS cause cytotoxicity and delay wound healing. For this reason, the elimination of ROS emerges as an important condition, especially in the healing of chronic wounds. It has been demonstrated that oxidants cause severe systemic and local damage in thermal wounds. In burns, the influx of neutrophils into the region by intravascular route increases the formation of free radicals. The resulting ROS are responsible for the development of edema permeability.

Nanoparticles (NPs) used in biological systems play an unstable role and can affect all organisms at different levels. It is thought that nanoparticles cause oxidative stress by disrupting the redox balance in the cell, suppressing antioxidant defense systems or in relation to increased intracellular ROS. There are many studies that nanoparticles increase ROS production (58). TiO2–NP has also been shown to cause ROS production (59). Transition metals, especially iron, copper and silver, take part in oxidoreduction reactions that take place in the form of electron exchange under physiological conditions. Due to these properties, transition metals act as catalysts that accelerate free radical reactions. Uncoated. AgNPs tend to aggregate and may not migrate towards the nucleus and mitochondria, but the macromolecule coated AgNPs such as polysaccharide and protein are more likely to enter the mitochondria and nucleus. As it is known, glutathione is a tripeptide and is known as a good reducing agent and

a strong antioxidant against free radicals (60, 61). For this reason, it has been emphasized in various studies that increases or decreases in glutathione levels may be an indicator of the oxidative stress experienced by the organism (61-63). However, the use of AgNPs in medical fields is limited due to their unstable nature and their cytotoxic effects on healthy tissue. However, the use of AgNPs in medical fields is limited due to their unstable nature and their cytotoxic effects on healthy tissue (64). AgNPs are taken into the cell by endocytosis and can cause toxic effects by undergoing chemical transformation as they move through the cell. During this chemical transformation, it causes the formation of reactive oxygen species that damage the cell membrane, proteins and DNA. This results in cell cycle arrest and subsequent apoptosis (26, 27, 47). It is also known that AgNPs cause cytokine production and lead to proinflammatory responses (28). The toxicity of AgNPs is the major limiting factor for their use in vivo (64). The toxicity of AgNPs varies depending on the particle shape and size, as well as the amount of silver used. Studies in zebrafish and guinea pigs have shown inflammatory and dermal complications depending on the silver dose (65). In another study, in which AgNPs were administered to rats orally (5 mg/kg and 10 mg/kg) for 28 days; it has been shown that there are no signs of toxicity in organs such as kidney, brain, lung, heart and testis of rats, but toxic findings in liver tissue of rats at a dose of 10 mg/kg (66). In another study by Tiwari et al., it was determined that AgNP doses lower than 10 mg/kg were safe and had no side effects for biomedical application, but doses higher than 20 mg/kg had toxic effects (67). These undesirable cytotoxic effects are significantly reduced or even eliminated if a correct functionalized coating is applied to AgNPs. AgNPs can be modified to reduce toxicity and ensure that they are stable and biocompatible. This modification includes coating and functionalization of AgNPs. Unmodified AgNPs are called naked. Bare AgPNs are not stable; high degree of aggregation and ionization are seen. Capped AgNPs are coated with polymers, enzymes, amino acids, or proteins. They are stable, show hydrophobic or hydrophilic properties depending on the type of coating.

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properties depending on the type of coating. The number of studies investigating the in vivo effect of AgNPs on cancer cells is very few. Therefore, the molecular mechanisms of intracellular and intercellular effects of AgNP application are largely unknown (68). When we look at the studies examining the effect of AgNPs on breast cancer in the literature, there is not much information available. In another study, AgNPs were found to be highly cytotoxic to triple negative breast cancer cells at doses that were not cytotoxic to healthy breast epithelial cells, regardless of size, shape or stabilizing agent. Exposure to AgNPs has been shown to deplete cellular antioxidants and cause endoplasmic reticulum stress in triple negative breast cancer cells without causing damage to healthy breast epithelial cells. It was also found that systemically administered AgNPs reduced the growth of triple-negative breast cancer in mice and had significantly less AgNP cytotoxicity on normal mammary epithelial cells (69). In another study investigating the effect of soybean agglutinin-conjugated AgNPs on breast cancer cell lines (MDA-MB-231 and MCF-7), AgNPs were found to be cytotoxic specifically to these cell lines but not cytotoxic to healthy breast epithelial cells (MCF-10A). (70).

Studies also show that polysaccharide-coated AgNPs cause stronger DNA damage in target cells compared to uncoated ones. Uncoated AgNPs tend to aggregate, making it difficult to reach the nucleus and mitochondria. However, the coating of AgNPs with macromolecules such as polysaccharides and proteins facilitates their distribution throughout the entire cell and increases their probability of entering mitochondria and nuclei. When AgNPs enter the mitochondria and nucleus, they can increase reactive oxygen substrates, induce mitochondrial dysfunction, and subsequently lead to DNA damage, chromosomal abnormalities, and cell cycle arrest. Cancer cells are more sensitive to AgNPs as they are cells with a high cell cycle, and therefore they become a particular target (21, 71).

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# CHAPTER II

# HEALTH ECONOMICS, COST ANALYSIS METHODS IN HEALTH AND ECONOMIC EVALUATION TECHNIQUES

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## INTRODUCTION

The fact that health services intervene in human life and cause serious consequences such as death in cases where the service is not provided has revealed the characteristics of health services. Health services cannot be postponed. It is not known when the demand for health services will arise and there is no alternative option. When a person is sick, he/she needs to receive health services as it may put his/her and society's health in a dangerous situation. In health services, consumers do not have as much information as providers and accordingly there is a difference in information. Some of the health services are of social nature. Especially preventive health services, the provision of epidemics by the state and the fact that the output of health services cannot be monetized are among these features. These characteristics of health services can be shown among the reasons for the emergence of health economics. (1) Economy in the field of health ensures that people can easily access the health services they need. Off-label examinations, ineffective use of drugs, occupation of beds in hospitals even in full health can both prevent patients from accessing health and are considered to be a burden on the health economy. Rising costs in health services significantly reduce access to health services. For these reasons, health plans, laws and policy makers should consider resource constraints and accessibility while providing health services. As a result, health service providers have to make the best choice and realise the choices that will achieve the highest level of health at the sociallevel. These developments are thought to

have made the effectiveness of medical diagnosis and treatment as well as its cost and its impact on the patient's quality of life important. In order to make an economic evaluation inthe health sector, it is necessary to determine the purpose of the analysis, calculate the opportunity cost and determine the alternatives. (2)

## 1. HEALTH ECONOMICS

Health economics is defined as transferring the rules, methods and analysis techniques of economics to the health sector. It is also defined as one of the sub-branches of the economy that calculates the economic analyses of health services. Health economics reveals alternatives for ensuring efficiency in the health sector and making it more efficient. It contributes to the availability of methods that provide high benefit and low-cost compliance from these alternatives. (3) The aim of health economics is to use the resources allocated for health services efficiently. (4)

Rapid developments in medicine, technology and medical equipment have strengthened the hands of physicians in the fight against diseases. Thanks to the developing pharmaceuticals and technology, alternatives regarding the treatment processes of patients have increased. When these alternatives are evaluated with the changes in the structure of the health services market, diversity in the demand for health services emerges. This diversity is not only in service provision. It is also seen in the payment of service fees and payment methods for this purpose.

Health economics evaluates the alternatives emerging in relation to health service provision, demands and payment methods. This evaluation is of great importance in terms of offering solutions around the characteristics of economics. Saving measures that can be taken due to the increase in health expenditures worldwide are on the agenda of many countries of the world. (5)

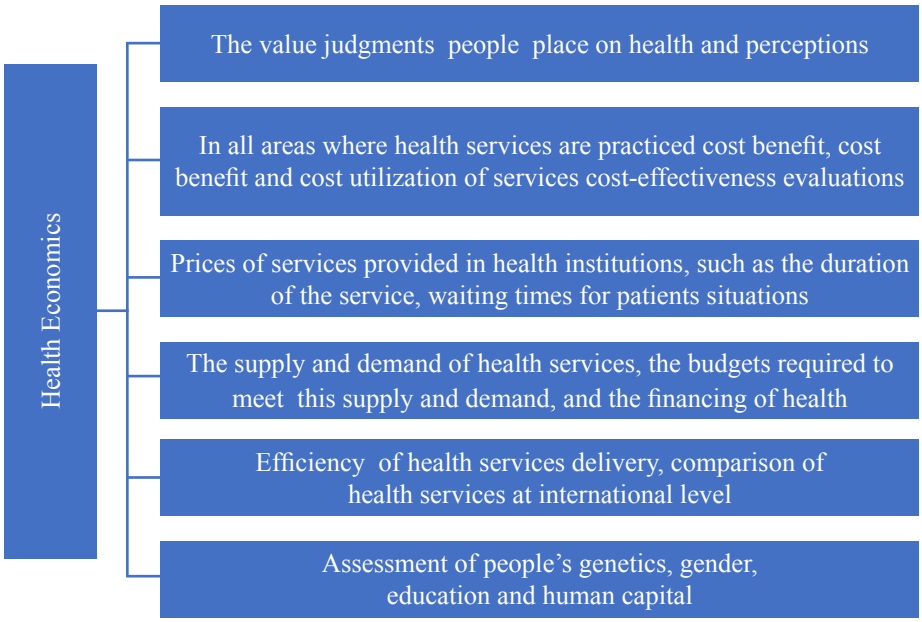
Economics is basically a science that has emerged in order to ensure the efficient and effective use of the resources available in all fields as a resultof resource scarcity. Health economics is the integration of the rules, theoriesand practices defined in the science of economics into the field of health. (6) Health economics examines the effects of services on social welfare and general economy such as production, expenditure, welfare, inputs and outputs affecting the health market in the context of effectiveness and efficiency, the highest level of health service production with the most effective use of resources, the economic consumption of the services produced and their fair distribution to all social groups. In health economics studies, there are basically three problems in the health sector. These are distribution, internal inefficiency and injustice. Distribution is the inability to ensure sufficient cost- effectiveness in

the provision of health services. Internal inefficiency is the expression of waste especially in public service provision. Injustice is expressed as the inability to access health services under equal conditions. Health economics can be defined as a sub-branch of economics that aims to produce solutions to these problems by using the methods of economics. (3) The main objective of health economics is to obtain the highest health output in the health sector by using limited resources in the most efficient way in order to implement the right to live a healthy life, which is one of the fundamental rights of individuals, by providing equality to all individuals constituting the society. (7)

The science of economics is not only concerned with the production of goods or services, but also with how these productions are distributed. Economic theories, concepts and practices aim to explain the production and distribution processes, the reactions of those who produce and consume goods and services, to determine priorities in the light of the information obtained as a result of their evaluation, and to provide information to policy makers about the possible consequences of these priorities. The discipline of health economics differs from general economics in that health has some unique characteristics and providers and service demanders have different characteristics than other sectors. In markets with perfect competition conditions, those who supply goods or services and those who demand them come face to face, and at a point where both parties are satisfied, a compromise is reached and the price is determined. However, there are some conditions for this to happen. In the health sector, these conditions are not always realised. Therefore, it is not possible to use resources effectively and efficiently. The health sector creates serious pressure on resources in all societies. Therefore, it causes a large amount of resource utilization. Especially since the 1980s, the increase in health expenditures has become a problem, and the need to determine priorities in the use of resources and to analyse the results of use is the main reason behind the development of health economics as a separate science. (8)

One of the driving forces in the development of health economics can be said to be that the difficulties encountered in adapting the theories produced about economics to the field of health attracted the attention of scientists. The fact that health is related to people can be considered as one of the most important features of health services. As a result of the services and purchases made in order to protect the society and individuals from diseases and to prevent the progression of diseases, the number of healthy people growing up in a healthier environment increases and the development of the society is ensured. The most important components of the health economy that distinguish it from other

goods and services are that it has no alternative, it cannot be postponed, supply is not equal to demand, asymmetric information is available, the service cannot be stocked, and it is random. (9)




Health services should provide equal and equitable services to all segments of the society. There are two basic conditions that can ensure this. The first of these conditions is that the benefit to be obtained from economic analyses is at the highest point, and the second is that the health of the people in the society is at the highest point. In the researches conducted in the field of health, all results related to the satisfaction of the individuals constituting the society with health services, their accessibility in the time they demand the service, the benefit and satisfaction of patients, doctors and other health professionals in the procurement of goods and services will be examined in every aspect. When all these are evaluated together, the impact of health services on economic recovery and the economic targets to be realised will be largely positive. (3) The issues addressed in health economics should be at a level that can be explained and understood by all individuals in the society. The fact that the public benefiting from health services receive services in a way that they can understand the information provided, that effective policy proposals that can increase efficiency and productivity meet the satisfaction criteria of the public, that health personnel are well-equipped, the production supply of those who produce in the field of health, and the demand of consumers for services are

within the scope of health economics. Supply in health services means that all inputs offered for the service are used in the most appropriate way and the most modern health service is produced and offered to people. Increases in price and quantity are directly proportional. Each unit increase in the price of health services means that those who produce health services increase the amount they want to offer from the service whose price increases. Hospitals, physicians, pharmacies, health institutions, health personnel involved in the provision of health services constitute the supply of health service production. In the service market, it is possible for producers to set, increase or decrease prices. (10) In the provision of health services, this situation is slower. The supply of health services is largely determined by hospitals and physicians. The main problem in health care is that it is difficult to quantify health care services. It is difficult to measure the satisfaction of patients and their relatives who come to the hospital and receive services, and to measure how well the health workers treat the service recipients, which may take time and is difficult to measure completely. (3) Those who produce health services aim to provide the highest level of benefit while providing services in the market and do not aim to make a profit. For governmental organizations, public support and the quality of health service provision are of primary importance. They do not act with the aim of profit. Individuals apply to health services for precautionary purposes when they are sick or when they are suspected of getting sick. Individuals who want to secure themselves against illness consider the share they allocate for health care, which they regard as an investment, as a kind of insurance for the future. (11) In the health market, there are two factors that provide financing and aim to bring together the factors of production. If the probability of financing increases, it expands the market supply in terms of investments. If the probability of financing decreases, the market supply for investments contracts. In the short run, the elasticity of supply for factors of production in the market is rigid, the elasticity of supply measures the sensitivity to changes in the price of the good. Supply in health services cannot be evaluated in the short run. In the short run, supply is inelastic. In the long run, however, there is elasticity. Thereason for this is that increasing the quantity of services in the face of changesin the price of services covers the long term. (3) Although the changes in production quantities are rigid in terms of health services, this situation is theopposite when evaluated in terms of health tourism. Recently, hotels opened in hospitals provide services to health service providers. The demand for hotels by the relatives of patients who are treated in hospitals for a long time and who come

from afar also increases the supply. Production amount and price increase in direct proportion. Demand in health services is defined as thedesire of people to purchase the service. Demand in health services has two basic characteristics. In the market economy, when consumers buy the goodsand services they want to buy, the level of benefit remains the same, while those who benefit from the goods or services in health services cannot remainat the same level of benefit. In the market economy, the demand for substitutable goods is independent of each other. In the health economy, however, substitutable goods are not independent of each other. This is because consumers cannot determine demand. Demands are mostly determined by physicians. Factors affecting demand in the health sector can be listed as follows: (12)

- Price of health services
- Time cost
- Changes in income level
- Prices of substitute, complementary goods and services
- Health insurance
- Quality of goods and services
- The seriousness of his health condition
- Demographic characteristics of the population

In health economics, demand is the situation where individuals make requests as a result of deterioration in their health status. Demanding individuals learn the fee following the service they benefit from at the time of the transaction. (13) Patients who demand health services want to be treated in a service provider that can solve their health problems as soon as possible while benefiting from this service. Adequate number of hospitals, health personnel, physicians and beds are important in terms of providing the service effectively. (11) Demand for health services is a situation that arises according to the needs of individuals. The law of demand in markets is not valid in health services. Individuals who want to restore their health apply to health services. The only place to receive health services is health centers. For this reason, there is no substitution. (3)

## 2. COST ANALYSIS

If we define cost analysis, it combines the information regularly obtained from the cost system with information collected from different sources, and includes studies aimed at evaluating and interpreting it from different aspects.

(14) Cost analyses consist of analyses that guide future financial planning by making use of cost accounting data from the past period. There are various reasons for cost analyses to ensure the highest level of equity and efficiency in health systems. The first thing to be done in cost analysis is to determine whether the service delivery resources in the hospital are distributed efficiently. It allows comparison of cost analysis evaluations of other hospitals. Cost analyses enable cost performance to be monitored over time. Policy makers can benefit from the cost data of various hospitals in their standardization efforts without compromising service quality. In addition, cost analysis studies play a role in determining the reimbursement status of hospitals financed by health insurance. (15) The first task of cost accounting is to record the purchased material, to classify the materials and to reveal the amount used for the service produced. The objectives of your cost analysis are listed as follows: (16)

- Pricing
- Determine unit costs
- Preparing plans and budgets
- Calculate stock values
- Calculating revenues
- To ensure cost control
- Providing information to managers

### *2.1. Disease Cost Analysis*

During the management of the disease state, it is important to choose the cost-effective one among the approaches using the financial information of the disease. Disease Cost Analysis is one of the types of analyses that enable the rational use of the limited resources available. The purpose of this analysis is to guide the decisions to be taken in the future by making use of past experiences. There are many definitions of cost analysis in disease. The most common definition is the analysis that provides cost effectiveness by using cost data in the diagnosis and treatment of diseases in making rational decisions for the patient or the health system with limited resources. (17) Cost analysis in disease is used to calculate the amount of economic cost caused by diseases. It determines the amount of benefit to be provided by the treatment of the disease, changes in the treatment method, early diagnosis or prevention of the disease. Disease-specific cost studies aim to calculate the total cost of the disease by calculating all expense items related to that disease. The aim of general disease

cost studies is to calculate the cost of health services for the year determined by using International Classification of Disease (ICD) codes.

(16) The definition of the economic cost of the disease should be calculated as the total of all costs collected related to the disease. The highest amount ofbenefit to be obtained can be calculated by means of cost analysis studies in the disease. These studies enable the determination of the expenditures made for the opportunity cost of a particular disease in the society. If we detail the study objectives, the prominent headings are as follows: (18)

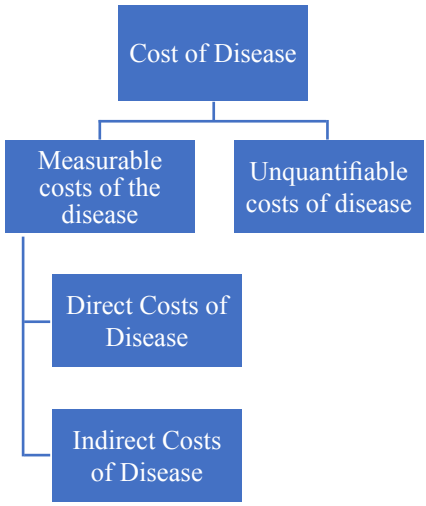
- Identify all costs associated with the disease
- Determining the direct costs of the disease
- Identifying the indirect costs of the disease
- Determine the economic burden of the disease
- Estimating the future economic burden of the disease
- To compare different treatment methods of the disease in terms of cost
- Determining the research costs of the disease
- To assist health managers and decision makers

In disease cost analysis studies, the cost element undertaken by the factors affecting the cost such as the health enterprise, the state, the patient and the reimbursement institution should be determined separately. Calculated costs differ according to the perspective chosen. Costs according to the perspective are as follows: (19)

- Medical costs
- Non-medical costs
- Mortality costs
- Morbidity costs
- Transfer payments

Following the perspective determined in disease cost analysis researches, it is necessary to determine and classify the costs of the disease. Expenses related to the disease are determined according to the expenditures made. (16) Expenditures incurred due to the disease are categorised as medical and non-medical costs. In addition, costs are classified as measurable or unmeasurable. Unquantifiable costs are the physiological and psychological conditions such as pain, stress, anxiety, etc. that the patient endures. Since these situations are

very difficult to calculate financially, they are generally not taken into account in studies. Measurable costs are divided into direct and indirect costs.




### *2.1.1. Direct Costs of Disease*

Direct costs of the disease include the costs incurred during the treatment of the disease. These costs consist of medical costs such as diagnosis, treatment and rehabilitation as well as non-medical costs. Top- down cost management approach and bottom-up cost management approach are the approaches used in the calculation of direct costs. In disease cost analysis studies, the basis of direct cost expenses is the medical care expenses related to the disease. (20)

### *2.1.2. Indirect Costs of Disease*

Indirect costs of the disease include the costs arising from the mortality and morbidity of the disease. Social costs that cause illness, disability or premature death are defined as indirect costs. If we define indirect costs more clearly; loss of labour force, inability to perform routine daily activities, etc. due to the disease can be called morbidity costs, and the loss due to deaths caused by the disease can be called mortality costs. (21)

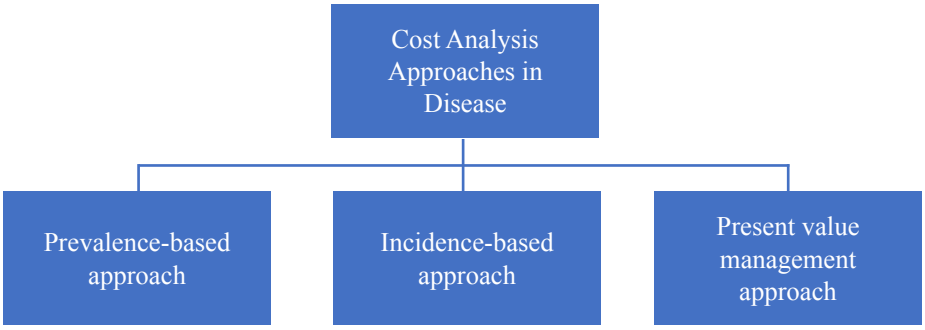
### *2.1.3. Unquantifiable Costs of Disease*

Unmeasurable costs in the disease stand out as costs that occur in the quality of life of patients. Conditions such as stress, anxiety, anxiety can be

given as examples of these costs. They are difficult to measure and are calculated as the value of quality of life. They are generally not considered in disease cost analysis studies. (22)

### *2.2. Cost Analysis Approaches in Disease*

Cost analysis approaches in the disease are as follows: (21)




#### *2.2.1. Prevalence Based Cost Analysis Method*

Prevalence-based cost analysis method estimates the total cost of disease in a given year. It is a method generally used in disease cost analysis studies. (23) In this method, the economic cost of the disease is calculated according to the prevalence of a certain disease in a certain year.

#### *2.2.2. Incidence Based Cost Analysis Method*

The incidence-based cost analysis method includes new cases that have emerged in a certain period of time. Since these are new cases, it enables the calculation of the lifetime costs of these cases. When calculating this cost, direct costs, mortality costs and morbidity costs should be calculated from the date of the first occurrence of the disease. (21)

#### *2.2.3. Present Value Method*

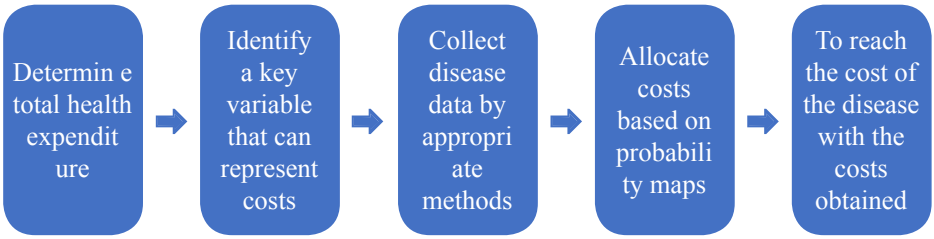
The present value method is the calculation of future costs over a certain discount rate. (24) The approaches used in the calculation of direct and indirect costs in the cost of illness analysis methods are as follows: (16)

- Top-down Cost Analysis
- Bottom-up Cost Analysis

- Retrospective Disease Cost Analysis
- Prospective Disease Cost Analysis
- Electrometric Cost Analysis
- Human Capital Method
- Friction Cost Method
- Willingness to Pay Method

The bottom-up cost analysis method is an approach used to calculate total costs by determining the amount of each resource used to produce a service. In this approach, the total cost is calculated by multiplying the unit costs by the amount of utilization. In this method, health inputs are measured first. Then the unit costs of the inputs are estimated. (25)

The top-down cost analysis method is calculated by allocating total costs to individual services using predetermined criteria as a measure. This method measures the proportion of illness caused by exposure to disease. (21) The stages of the top-down cost analysis method are as follows:




Retrospective disease cost analysis, while conducting disease cost analysis research, data on the disease are collected retrospectively. Since retrospective disease data are used, new cases are not included in the study. Retrospective data in the specified time interval are used. (21)

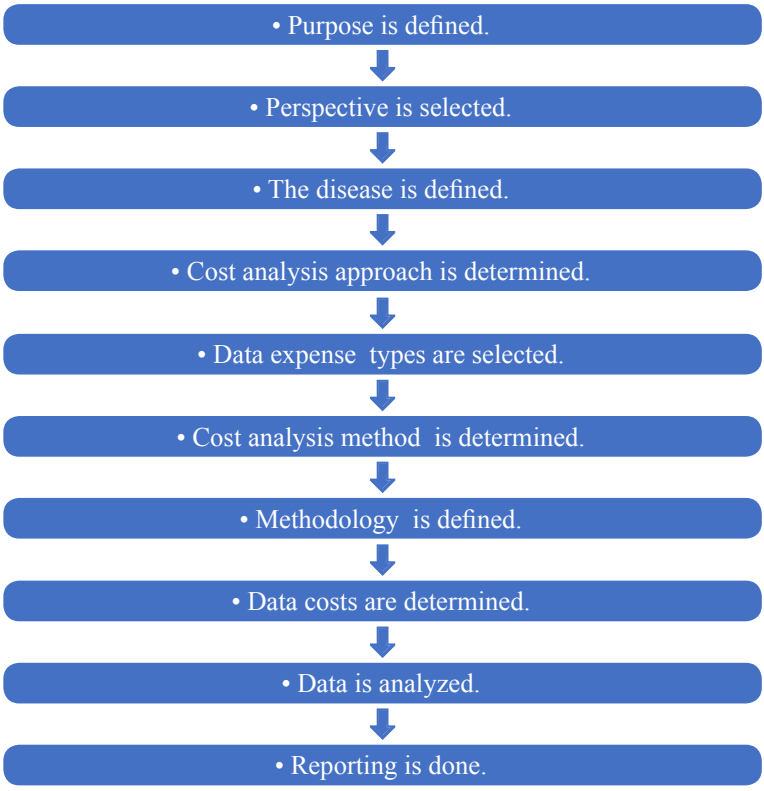
Prospective disease cost analysis is a method of prospective monitoring of disease data over a certain period of time while conducting disease cost analysis research. In this method, new cases should be taken into consideration. More realistic results are obtained when bottom-up cost analysis method and prospective disease cost analysis method are used together. (18)

The econometric cost analysis method is an approach based on the estimation of the cost difference between a cohort with the disease and a cohort without the disease. Since this method measures the incremental difference between those with and without the disease, it is also called the incremental

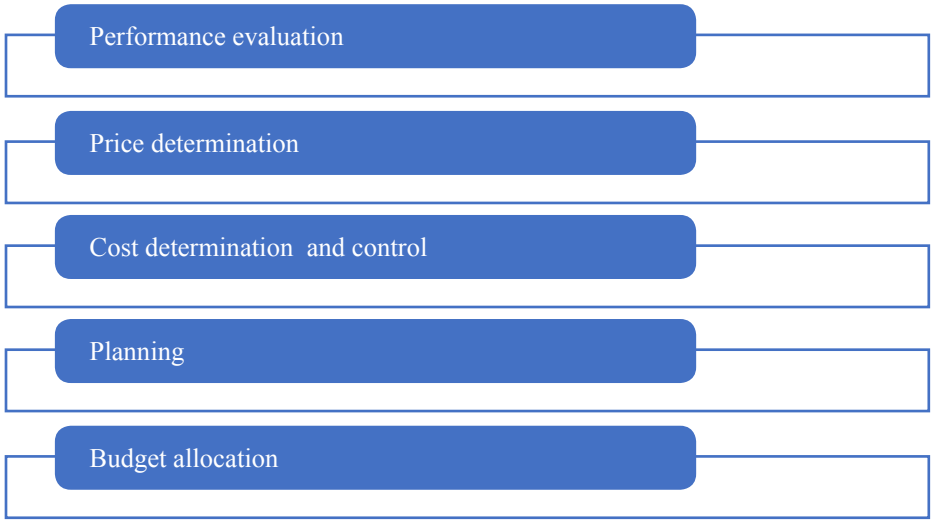
approach. There are two approaches used to estimate costs in this method. These approaches are: (25)

- Multistage regression approach
- Mean differences approach

Cost of Disease Analysis process consists of stages such as objective, perspective, collection of disease data, analysis and reporting. The first stage, the goal setting stage, is very important. Because the shaping of the study depends on this stage. After determining the perspective, which is the next stage, the disease should be defined. ICD codes are used in the disease identification phase. In the next stage, it is decided which cost analysis approach will be used. Subsequently, expense types are determined and the cost analysis method is determined accordingly. After this stage, the methodology is defined. Expenses are determined and the available data are analyzed. Reporting is done at the last stage.



Capacity, technology, infrastructure, steps in the referral chain, population density in the region, epidemiological structure, service quality, image of the hospital, number of health workers on duty, equipment and equipment status and money source can be listed as factors affecting the cost in health institutions. (26) Cost analyses are among the most important financial management tools used by management accounting. It is defined as the process in which the expenses are distributed to the expense locations and the cause and effect relationships of the expenses incurred while the institutions provide services. (27) Determining the unit costs and total costs of their institutions, using cost data while preparing financial statements, determining the cost of units and institutions in institutions, controlling costs, using cost data for methods to be used in pricing, capacity utilization of the enterprise, making choices between machinery and equipment services and determining the cost of diseases are listed as the reasons for performing cost analysis in health institutions. (28) In order to carry out a cost analysis study in a reliable manner, the scope of the study, the sources of information, the period of time the study will cover and the costs of the services to be calculated should be determined correctly. The resources that contribute to service provision should be determined completely and accurately. (29, 30) Cost analysis information is guiding in the following issues in health institutions: (31,32)




Purpose, determination of cost system, expenses and cost centers, harmonization of expenses and cost centers, expense allocations, determination

of cost centers and unit costs, and finally reporting are determined as the stages of cost analysis in health institutions. (16) A six-stage working process is followed in cost analysis studies in health institutions:

Stage 1: In the first stage of cost analysis, outputs are determined. Firstly, the number of outpatient clinics, surgeries, examinations, the number of discharged patients and the number of intensive care patient days are considered as outputs. Using these outputs, outpatient clinic costs, inpatient and patient day costs, examination and surgery costs are calculated. (27)

Stage 2: At this stage, it is necessary to define the cost centers. In health institutions, the service units where the costs arising from service production are incurred are defined as expense locations. In cost analysis studies, it is divided into 4 categories as expense and main production expense locations, auxiliary service and production expense locations and general administrative expense locations. (33)

Stage 3: In the third stage, expense types are determined. Expense types are divided into three groups as labour, material and general production expenses.

Stage 4: In the fourth stage of the cost analysis, the expenses determined should be allocated to the expense locations. Direct costs can be charged directly to the cost centers. However, since indirect costs belong to more than one cost center, they are allocated to cost centers by cost allocators. The first allocation table is formed by allocating the expenses to the expense locations. (27)

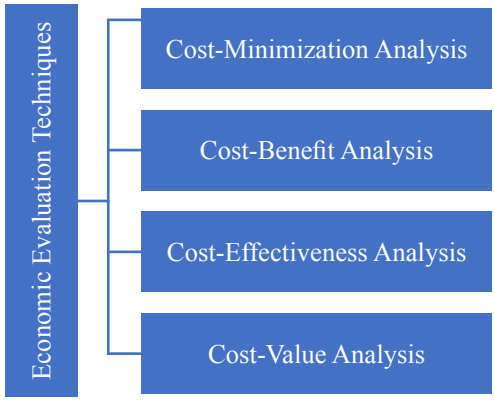
Stage 5: In the fifth stage, the second allocation table is formed by allocating the expenses in the administration and support expense centers to the expense centers. Different allocation methods are used for the second expense allocation process.

Stage 6: The last stage of the cost analysis is the calculation of total and unit costs of auxiliary main cost centers. In Turkey, health institutions receive the reimbursement of the expenses they incur from the Social Security Institution (SSI) according to the outpatient clinic and clinic invoices, which are called the main production expense locations. Therefore, firstly, unit costs of auxiliary production cost centers are calculated, then auxiliary production cost centers are distributed to main production cost centers and polyclinic and clinic unit costs are calculated. (33)

## 3. ECONOMIC EVALUATION TECHNIQUES

The aim of all health systems is to maintain the health level of individuals and society by ensuring social welfare and economic development. Economic

evaluation techniques draw attention in the decision- making process such as which intervention and which drug will be applied to maintain this level. Economic evaluation techniques are as follows: (34)




### *3.1. Cost-Minimization Analysis*

It is the analysis in which the methods give the same result. In this analysis, the costs of the methods are compared. Since the outputs are the same, the efficiency of the method with lower costs is higher. (35)

### *3.2. Cost-Benefit Analysis*

Cost-benefit analysis argues that if a project has benefits that exceed its costs, this project will increase social welfare. In this sense, it criticizes many problems in health economics such as immunization programmes, patient screening and heart transplantation, focusing on cost-benefit thinking. According to cost-benefit analysis, not only the benefits and costs directly contributing to the project should be taken into account, but also the benefits and costs arising through externalities or other third-party effects. For example, factories discharging waste into the air or water create external costs by damaging the environment and adversely affecting third parties (e.g. cancer, respiratory diseases). On the other hand, factories that carry out pollution abatement activities provide external benefits to the environment and third parties without making direct payments. In this analysis technique, policies that generate negative externalities are evaluated lower than those that generate positive externalities, and projects that generate positive externalities are considered to be superior. (36,37)

### *3.3. Cost-Effectiveness Analysis*

Cost effectiveness analysis is a widely used technique that evaluates the expected level of health as a result of interventions together with costs by making comparisons between alternatives. With this analysis technique, the less costly but more effective one is selected among the alternatives. Thus, health interventions are ranked according to their cost-effectiveness ratios and the most cost-effective programmes can be selected as health priorities to be financially supported by governments. (37,38)

### *3.4. Cost-Value Analysis*

Cost-value analysis is an economic analysis method in which two or more alternative strategies are compared in terms of both costs and outcomes. The outcome measure considered in this method is usually quality-adjusted life years. The aim of this analysis is to compare two or more alternative strategies both clinically and in terms of economic parameters, using a cost- effectiveness approach. Cost-value analysis is considered to be the gold standard method for evaluating the cost-effectiveness of alternatives in health services. (39)

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CHAPTER III

# EPIDEMIOLOGY OF FRANCISELLA TULARENSIS, LABORATORY DIAGNOSIS, TRANSMISSION, AND PREVENTION METHODS

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## 1. INTRODUCTION

*Francisella tularensis* is the causative agent of tularemia, a highly serious and sometimes fatal disease in both humans and many animal species. *F. tularensis* was initially isolated from ground squirrels during an outbreak resembling plague in Tulare County, California, USA, and was named Bacterium tularensis (1). *F. tularensis* is also the agent responsible for the disease known as rabbit fever or tularemia (2).

After the isolation of the bacterial agent, it was classified as a new genus by Edward Francis in 1925, and the name tularemia was proposed to describe the various disease syndromes it caused in humans (3). Tularemia, a zoonotic disease caused by *F. tularensis*, is reported to have a wide clinical spectrum, including ulceroglandular, glandular, oculoglandular, oropharyngeal, typhoidal, and pneumonic forms (4).

To date, four subspecies of *F. tularensis* have been identified: *tularensis*, *holarctica*, *mediasiatica*, and *novicida*. Each subspecies is more frequently encountered in certain geographical regions compared to others. While all subspecies share significant similarities in terms of biochemical properties, antigenic characteristics, and phylogenetic proximity, they are known to exhibit different levels of virulence in mammalian hosts (5).

### *1.1. Tularemia Syndromes*

The course of the disease is mostly dependent on the route of entry of *F. tularensis* into the body. The incubation period of the pathogen is observed to be between 3 to 5 days, but it can also range from 1 to 21 days (6). Following the incubation period, the disease manifests itself as early infection syndrome, characterized by sudden fever, myalgia, tremors, weakness, flu-like sore throat, headache, and rhinitis (7). It should be noted that any form of tularemia can result in hematogenous dissemination, leading to the occurrence of other syndromes such as meningitis, peritonitis, pericarditis, ataxia, osteomyelitis, sepsis, and septic shock (8, 9, 10).

### *1.2. Epidemiology of Francisella Tularensis*

Tularemia, a zoonotic disease caused by *F. tularensis*, is also referred to as "Francis disease," "deer-fly fever," "rabbit fever," "water rat disease," or "hunter's disease" in everyday language in many countries. The causative bacterium is known to infect approximately 200 wild and domestic animal species. Although tularemia is rare, it is described as a highly lethal disease with a potential for fatality. Initially defined as a lagomorph disease in the Northern Hemisphere, tularemia has been reported in almost every state in the United States, except for Hawaii (11).

After the identification of the disease and isolation of the pathogen, detailed epidemiological studies were conducted in the former Soviet Union, some Eastern European countries, and certain regions of the United States for decades. It is emphasized that in studies investigating the epidemiology of tularemia, the fundamental differences among *F. tularensis* strains need to be taken into account (12).

Studies conducted in the United States indicated that the number of annual human infections with *F. tularensis* varied between 27 and 698 cases from 1998 to 2007. The annual incidence rate during these years was approximately 0.30, but it ranged from 0.23 to 0.76 in states where tularemia was endemic between 2000 and 2006 (13).

A report by the European Centre for Disease Prevention and Control (ECDC) based on data up to 2019 stated that a total of 1463 confirmed tularemia cases were reported in Europe and European Union countries. The report highlighted that the highest number of cases, accounting for 56%, was reported in Switzerland, while 8 countries had no reported cases, and the overall incidence rate was 0.3 per 100,000 (14).

A review study conducted by Imbimbo et al. in 2020, based on ECDC data, reported a surveillance rate of 0.07 per 100,000 for human tularemia cases in 18 countries. It was emphasized that apart from rare imported cases in Greece, Ireland, Latvia, Luxembourg, Macedonia, Malta, and the United Kingdom, tularemia cases were not encountered (15).

Tularemia cases have also been reported in specific regions of the Asian continent. Cases of tularemia have been documented in China and Japan in the eastern part of Asia. In representative countries from the western part of Asia, such as Iran, Turkey, Azerbaijan, and Armenia, tularemia cases have also been reported (16).

In a study conducted by Njeru et al. between 2014 and 2015, using ELISA and Western blot methods on 730 hospitalized patients in the northern region of Kenya, the presence of *F. tularensis* was detected in 27 patients (3.7%). The study emphasized the existence of *F. tularensis* in Kenya but highlighted the underreporting of cases (17).

Although *F. tularensis* bacteremia has been reported in Sudan, the bacterial isolates have not been fully characterized, suggesting that tularemia in Africa requires further confirmation tests. Similarly, in studies conducted in Egypt, the presence of *F. tularensis* could not be detected in samples collected from camels using PCR or cultural methods, and it is noted that the reported cases from African countries require more verification tests (18).

*F. tularensis* infection is mostly identified through case reporting in humans. Research has indicated that *F. tularensis* infection is rarely found in free-living animals because infected animals are usually found dead or dying. Therefore, reports of *F. tularensis* infection in wildlife are limited (19).

Currently, the presence of *F. tularensis* or tularemia in animals has been reported by 11 European countries to the World Organization for Animal Health. Among the affected countries, tularemia cases were reported in farm rabbits in 8 countries, while only Bulgaria reported cases in other animal species, particularly dogs, cattle, and sheep. Existing research shows limited case reports or seropositivity studies in pet animals (20).

### *1.3. Sources of F. Tularensis Infection and Modes of Transmission*

The causative agent of tularemia, *F. tularensis*, can affect various vertebrate and invertebrate animals. The natural reservoirs of the infectious agent include lice, mosquitoes, horseflies, and fleas found in lagomorphs and rodents. It is noted that domestic animals such as sheep and cats could accidentally serve as

hosts for *F. tularensis* and potentially act as a source of infection for humans as well (21).

Some studies have shown that specific strains of *F. tularensis* are associated with certain mammals. In the United States, it has been reported that human case prevalences coincide with cottontail rabbits, with Type A strains being most frequently isolated from these animals. It has been clarified that Type A.I strains are isolated from cottontail rabbits living in the eastern United States, while Type A.II strains are found in desert cottontail rabbits. However, no vertebrate animal has been associated with Type B strain (22).

Tularemia, the disease caused by F. tularensis, is commonly associated with the environment in Eurasia and North America. Agricultural activities, rodents contaminating water sources, trapping of these animals, as well as outbreaks from feces or carcasses are among the causes of the disease. In the Castilla y Leon region of Spain, typhoidal tularemia has been linked not only to contact with rodents but also to crop harvesting (23). It is reported that the understanding of the role of animals that survive *F. tularensis* infection in spreading the agent into the environment is still insufficient. It is known that the increase in the number of herbivores living in the wild, such as field mice and rabbits, is associated with the human cases reported in European countries for a long time (24).

In the past 20 years, tularemia cases have been associated with drinking water in Turkey, Kosovo, Georgia, Bulgaria, Macedonia, Norway, Sweden, Italy, and Germany (25). In 1988, waterborne cases of oropharyngeal tularemia were reported in Turkey. Between 1988 and 2018, 28 tularemia cases in the same country were linked to contaminated water (26). Experimental studies have shown that *F. tularensis* can remain viable in water and aquatic environments for 1 to 70 days. The viability of F. tularensis is affected by both water temperature and salinity. In a study by Gilbert and Rose, it was observed that *F. tularensis* sub.spp. holarctica could be cultured for 1 day at 5°C or 25°C, and for 28 days at 8°C (27).

The ability to form biofilms has been proven to be an important mechanism for bacteria to survive in water environments. Biofilms are naturally occurring communities of bacteria that are attached to each other within an extracellular polymeric matrix (28). Recent studies have indicated that *F. tularensis* has the capability to form biofilms. However, there is currently no established link between biofilm formation and the virulence of *F. tularensis*. It has been emphasized that this biofilm formation may enable the bacteria to survive in the surrounding environment (29).

It is known that free-living amoebae feed on bacteria, fungi, and algae. However, certain microorganisms have evolved resistance against these protists. Amoebae-resistant microorganisms include pathogens such as *Cryptococcus neoformans*, *Legionella spp*., *Chlamydophila pneumoniae*, *Mycobacterium avium*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *F. tularensis*, as well as some emerging pathogens (30). In a study conducted by Abd et al. in 2003, they observed that Acanthamoeba castellanii cells were killed at a rate of 25% when co-cultured with *F. tularensis*. Based on this study, they concluded that *F. tularensis* infects *Acanthamoeba castellanii*, leading to its death (31).

Arthropods have long been associated with the transmission of *F. tularensis*, and early studies have primarily focused on deer flies (32). While *F. tularensis* has been shown to be vector-borne, involving arthropods and blood-feeding insects in many studies, it is noted that the presence of these vectors is not necessary for *F. tularensis* to survive in the environment (33). *F. tularensis* has been demonstrated to be present in various arthropods, including ticks, fleas, flies, mosquitoes, and lice. Despite the existence of many arthropod species infected with *F. tularensis* in nature, only a small fraction of these species are considered important in transmitting the pathogen to humans (34). Tularemia outbreaks have occurred multiple times, mostly in the 1900s, and were reported to have occurred most recently in 2007 in the state of Utah, USA (35).

Studies have shown that flies do not harbor *F. tularensis* for more than 5 days, and it has been concluded that F. tularensis does not multiply in flies. Artificial infection experiments with deer flies have not been conducted thus far, but it is assumed that transmission occurs because *F. tularensis* is present in the mouthparts of deer flies. Naturally infected tabanid flies and horse flies, including *C. discalis*, *C. fulvaster*, *C. eastuans*, and *C. relictus*, have been frequently associated with epidemiology and outbreaks (35, 36).

Laboratory studies with mosquitoes have shown that mosquitoes feeding on infected animals can transmit *F. tularensis* for up to 27 days. However, despite these studies, no evidence has been found indicating the multiplication of *F. tularensis* in mosquitoes or their eggs in the wild. Based on the findings, it has been concluded that mosquitoes do not play a significant role in the long-term persistence of *F. tularensis* outbreaks in nature (35).

It has been proven that *F. tularensis* can infect not only humans but also over 100 animal species, including domestic animals, wild animals, amphibians, reptiles, and birds (37). In the United States, it is particularly reported that lagomorphs are commonly infected with *F. tularensis* and play an important role in transmitting the pathogen to humans. Emphasis is placed on the role of

ticks as both vectors and reservoirs of *F. tularensis* throughout their life cycle, especially in lagomorphs (38). In addition to contact with lagomorphs, various sporadic cases have been reported in humans as a result of contact with squirrels, pheasants, sheep, and other non-human primates (38, 39).

In a case presentation following the deaths of three tamarins (Sanguinus nigricollis) and one talapoin monkey (Cercopithecus talapoin) at a zoo in Canada, it was stated that *F. tularensis* was isolated and identified from the liver, lungs, and spleen of each deceased monkey. The same study highlighted the presence of abscesses in the tongue and submandibular region of another infected talapoin monkey that was successfully treated. It was disclosed that the attending veterinarian also contracted the infection as a result of being bitten by the sick tamarin monkey. The study further emphasized that ground squirrels were the source of the disease, and the causative organism *F. tularensis* was also identified in the liver, spleen, and fleas living on the ground squirrel (40).

In a case presentation published by Meinkoth et al. in 2004, it was noted that a 4-year-old female Irish Setter dog exhibited symptoms of anorexia, acute lethargy, and weakness upon examination. The same study recorded the presence of depression, 40.5°C fever, mildly enlarged mandibular lymph nodes, bilateral mucoid discharge, and mild conjunctival hyperemia in the dog. It was noted that the dog had consumed an adult rabbit 36 hours prior, and laboratory investigations confirmed the dog›s infection with *F. tularensis* based on culture, agglutination test, and PCR test using specific primers from lymph node samples (41). Gustafson and De Bowes reported a case of lymphadenopathy, anorexia, and tonsillitis in a German Shepherd dog, which tested positive for *F. tularensis* using an antibody test in 1996 (42).

It was noted that domestic cats infected with *F. tularensis* exhibited a clinical picture similar to that of dogs. Woods et al. reported in 1998 that two domestic cats exposed to rabbits showed symptoms of anorexia, vomiting, dehydration, and lymphadenopathy. These cats were confirmed to be infected with *F. tularensis* through culture and antibody testing (43). Fatal and non-fatal tularemia cases in domestic cats continue to be reported from various regions. Serological studies in some geographical areas have indicated that 12% of domestic cats have antibodies against F. tularensis. It is emphasized that there is still a lack of information regarding the rate at which domestic cats transmit *F. tularensis* to humans (44).

| Animals with the presence of *F. tularensis* Representative figure | Represantive figures | Number of species |
|---|---|---|
| Mammals |  | 190 |
| Invertebrates |  | 88 |
| Birds |  | 23 |
| Amhibians |  | 3 |
| Reptiles |  | Very few |
| Fishes |  | Very few |

## 2. LABORATORY DIAGNOSIS

*F. tularensis*, the causative agent of tularemia, is a highly virulent gram-negative bacterium that is non-spore forming, non-capsulated, non-motile, coccobacillus in appearance, and facultatively intracellular (45). It has been reported that the direct clinical diagnosis of tularemia is challenging due to mild symptoms or a similar presentation to other diseases. Therefore, a series of methods are used for the etiological diagnosis (46).

### *2.1. Culture*

It is stated that the initial difficulty in diagnosing *F. tularensis* in individuals infected with tularemia is culturing this organism (47). F. tularensis is a bacterium that is difficult to culture and requires cultivation on enriched media containing cysteine or other compounds containing thiol groups. For *F. tularensis* culture, 9% sheep blood Cysteine Heart Agar, chocolate agar, Thayer-Martin Agar, and Mueller Hinton Agar can be used. The optimal temperature for growth in the culture medium is reported to be 37°C, but it has been shown that it can grow weakly at 28°C as well. *F. tularensis* forms gray-white, slightly mucoid colonies of 2 to 4 mm in size on sheep blood-enriched chocolate agar after incubation. The bacterium can be weakly stained in Gram staining and appears as individual coccobacilli under light microscopy. It is noted that even with the addition of cysteine to the medium, *F. tularensis* does not grow well in liquid culture and requires a large inoculum in liquid medium (48). The direct isolation of the bacterium can be done from nodule scrapings, lymph node biopsies, or sputum samples. Although blood isolation of *F. tularensis* is mostly unsuccessful despite the use of enriched media, it is mentioned that the isolation of the organism from stool or urine cultures is very rare. Despite optimizing all conditions for culturing

the organism from samples obtained from patients, it has been documented that it often fails (49).

### *2.2. PCR*

It is stated that due to the high virulence of *F. tularensis*, the need for a biosafety level 3 laboratory for culture, and its difficult growth in enriched media, culture of this bacterium is rarely attempted. Recent studies have emphasized that in the outbreak of tularemia among meadow dogs, 46 meadow dogs succumbed to the disease, and culture was found to be approximately 87% in tissue biopsy samples, while PCR research showed a 100% detection rate (50). Real-time PCR is stated to be more sensitive and faster than conventional PCR for the detection of *F. tularensis*, and it can be used in routine investigations. Some sources mention the disadvantages of PCR, stating that it can yield false negative and positive results. It is particularly emphasized that PCR may not be very useful in clinical samples due to the presence of inhibitory substances, and the fact that antibiotic susceptibility tests can only be performed on live bacteria is also highlighted as a limiting factor for PCR methods (49).

### *2.3. Serological Tests*

It is mentioned that serological tests such as tube agglutination, slide agglutination, microagglutination, and ELISA have been used for the diagnosis of *F. tularensis* for many years. The simplicity of serological tests, which rarely give incorrect results, is considered an advantage. However, the disadvantages of serological tests include their tendency to yield negative results at the early stage of the disease, their low specificity, and the fact that they can yield positive results even years after the individual has recovered from the disease (51).

## 3. PREVENTION AND CONTROL

*F. tularensis* is a highly virulent bacterium that can infect many animal species. It is emphasized that in areas where the infection is endemic, people can become infected while removing ticks from animals or working with hay, and they need to be cautious. It is advised not to mow areas where sick or dead animals are present and to avoid touching these animals (51, 52). *F. tularensis* can also be transmitted through inhalation or by consuming the meat of infected animals. In endemic regions where the disease is observed, the Centers for

Disease Control and Prevention (CDC) recommends wearing long-sleeved clothing and socks to protect against arthropods such as ticks and deer flies (52, 53).

It is known that *F. tularensis* can also infect small rodents and rabbits, and humans can contract the disease through contact with these animals, skinning them during hunting, or touching their carcasses. It is recommended for hunters to use gloves when hunting wild animals or trapping them, and to thoroughly cook the meat of the captured animals before consumption. Although *F. tularensis* has not caused pandemics to date, veterinarians in endemic areas are advised to consider this pathogen. Laboratory personnel working with clinical materials and other healthcare workers at risk are advised to work under minimum BSL2 or preferably BSL3 conditions and to be vaccinated, although it is noted that obtaining an *F. tularensis* vaccine is not always possible.

## 4. CONCLUSION

Although *F. tularensis* was initially observed in the United States, it can now be found in many countries around the world. While it has not caused pandemics throughout history, it can lead to sporadic endemic outbreaks. F. tularensis is described as a highly virulent bacterium with the potential to be used as a biological weapon. It is noteworthy for its ability to infect various animal species and its modes of transmission such as inhalation and contact with infected animals. Due to the similarity of tularemia, the disease caused by *F. tularensis*, to other syndromes, it is often mistaken for other pathogens. We recommend considering this pathogen, especially in areas where the disease is reported. Culture, ELISA, and PCR are used as methods for *F. tularensis* detection. It has been reported that each method has advantages and disadvantages, and all three methods are necessary for the definitive identification of the pathogen. In terms of biosafety, laboratory personnel working with this pathogen should adhere to the necessary rules, receive vaccinations, and veterinarians and other individuals working in human or animal health should take necessary precautions, considering this pathogen in areas where the disease is reported.

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CHAPTER IV

# LEISHMANIA, LEISHMANIASIS AND IMMUNOPATHOLOGIA OF THE DISEASE (LEISHMANIASIS: A TROPICAL DISEASE)

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## 1. INTRODUCTION

### *1.1. Leishmania*

*Leishmania* is a parasitic protozoa that causes leishmaniasis, a disease that affects humans and animals. The parasite is transmitted through the bites of infected female sandflies, which feeds on the blood of humans and animals and they are commonly found in tropical and subtropical regions of the World (1). There are several species of *Leishmania* that can cause different types of leishmaniasis. Cutaneous leishmaniasis is the most common form of the disease, accounting for around 80% of all cases. This form of leishmaniasis is characterized by skin sores that can appear anywhere on the body, usually weeks or months after being bitten by an infected sandfly. These sores can be painless or painful and can take months to heal, leaving behind scars. Cutaneous leishmaniasis is typically not life-threatening but can cause disfigurement and disability if left untreated (1, 2). Visceral leishmaniasis is a more severe form of the disease, which affects internal organs such as the spleen, liver, and bone marrow, and can be fatal if left untreated. Symptoms include fever, weight loss, enlargement of the spleen and liver, and anemia. It can take several months or years for symptoms to appear after infection (4, 5). Mucocutaneous leishmaniasis is a rare but severe form of the disease that affects the mucous membranes of the nose, mouth, and throat, causing ulcers and significant tissue destruction,

leading to disfigurement. Also, this form of leishmaniasis leads to disfigurement and disability if left untreated. (1-3).

*Leishmania* can infect a wide range of animals, including dogs, rodents, and livestock, and can be transmitted between animals and humans. In humans, the disease can affect people of all ages, but children and people with weakened immune systems are more susceptible. Treatment for leishmaniasis typically involves a combination of drugs, which can vary depending on the type of *Leishmania* causing the infection and the patient's immune system. Treatment can take several weeks or months and may have side effects. In some cases, the disease may recur after treatment (6-8). Prevention measures can help reduce the risk of infection, such as using insecticide-treated bed nets, wearing protective clothing, and using insect repellents. Control of sandfly populations is also important in areas where leishmaniasis is endemic (8).

### *1.2. Taxonomy of Leishmania*

*Leishmania* is a genus of parasitic protozoa belonging to the family Trypanosomatidae. Within the genus, there are several species that are known to cause disease in humans, including: *L. donovani*, *L. infantum* (also known as *L. chagasi*), *L. tropica*, *L. major*, *L. mexicana*, *L. braziliensis*. Each species of *Leishmania* is associated with specific types of leishmaniasis and has unique morphological and genetic characteristics. For example, *L. donovani* and *L. infantum* are associated with visceral leishmaniasis, while *L. tropica* and *L. major* are associated with cutaneous leishmaniasis. The taxonomy of *Leishmania* is constantly evolving as new species are discovered and genetic analysis techniques improve. Additionally, there is ongoing debate about the classification of certain *Leishmania* species, particularly those that cause atypical forms of the disease (9-11).

### *1.3. Morphology of Leishmania*

*Leishmania* is a genus of protozoan parasites that has a complex morphology and life cycle. The morphology of *Leishmania* varies depending on the stage of its life cycle. In its flagellated stage, known as the promastigote form, *Leishmania* has a long, whip-like structure called a flagellum that it uses for movement. The amastigote forms are immobile forms without flagellum and oval shape (Figure 1). The promastigote form of *Leishmania* is found in the midgut of sandflies, which serve as the vector for transmitting the parasite

to vertebrate hosts. When *Leishmania* enters a mammalian host, it transforms into its oval, non-flagellated stage called the amastigote form. In this stage, the parasite loses its flagellum and becomes round or oval in shape. The amastigotes of *Leishmania* reside inside host cells, especially macrophages. *Leishmania* amastigotes have a single nucleus, a kinetoplast (a specialized mitochondrial structure), and a paraxial rod (a unique structure that extends along the length of the cell). They also possess a cell membrane and a cell wall. The surface of the cell membrane is covered with glycoproteins that help the parasite evade the host immune system. Overall, the morphology of *Leishmania* is essential to its complex life cycle and ability to infect and survive inside mammalian hosts (12-14).

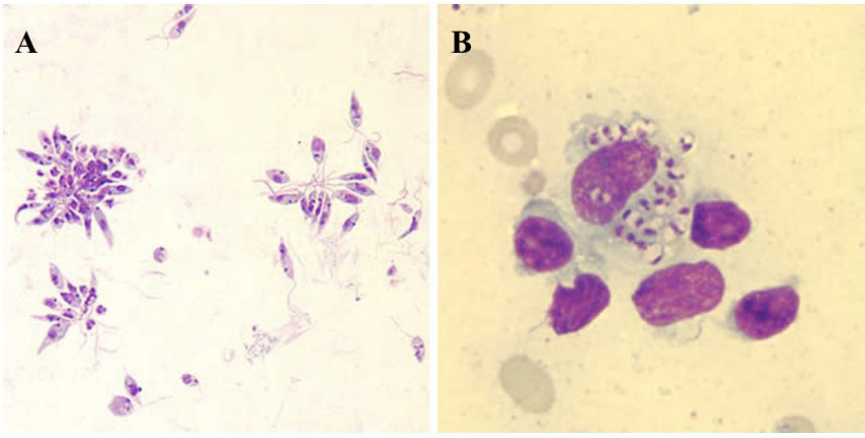


**Figure 1.** (A) Promastigote and (B) amastigote forms of *Leishmania* (2)

### *1.4. Lifecycle of Leishmania*

The life cycle of *Leishmania* involves two stages: a promastigote stage that develops in the gut of the sand fly vector and an amastigote stage that infects the macrophages of the vertebrate host. Here is a more detailed explanation of the life cycle of *Leishmania*: Sand fly bites an infected mammal, which introduces the promastigotes of *Leishmania* into the blood of the mammal. The promastigotes are then phagocytosed by macrophages, where they differentiate into amastigotes and multiply inside the macrophages. The sand fly then feeds on the infected mammal, ingesting the amastigotes along with the blood. Inside the gut of the sand fly, the amastigotes differentiate into promastigotes, which multiply and migrate to the proboscis of the sand fly. When the sand fly feeds

on another mammal, it injects the promastigotes into the skin of the mammal, where they are phagocytosed by macrophages and the cycle begins again. This life cycle of *Leishmania* involving sand flies as vectors and vertebrate hosts as reservoirs allows the parasite to maintain its life cycle and spread the disease (Figure 2) (15).

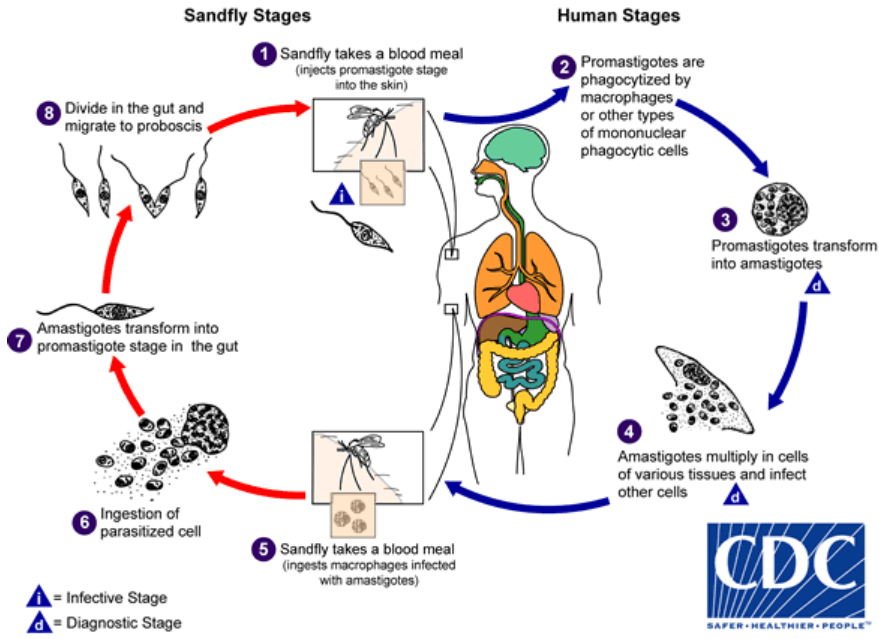


**Figure 2.** Lifecycle of *Leishmania* (2)

### *1.5. Genomic Organization of Leishmania*

*Leishmania* is a genus of protozoan parasites that have a complex genome structure. They possess a diploid genome with 36 chromosomes, including 34 chromosomes that are uniparentally inherited and two that are biparentally inherited. The size of the *Leishmania* genome varies between species, ranging from 28-35 Mb, and is organized into several chromosomes with variable sizes. Trypanosomatid organisms, including *Leishmania* species, have genomic DNA in the nucleus and a mitochondrial genome called kinetoplast DNA (kDNA) located in the basal part of the flagellum. kDNA contains a DNA network called “maxicircle” and “minicircle”. It is known that there are approximately 10,000 minicircles and 50 maxicircles in the genome. Maxicircle has a homogeneous

structure with a size of 20-35 kilo bases (kb), while minicircle has a heterogeneous structure with a size of 0.5-1.5 kb (16). The minicircle include about 800 bp, and all *Leishmania* species have a conserved similar nucleotide sequence of 120 bp in length. The maxicircle region in the kDNA genome differs between species and contains variable and non-coding regions. Therefore, kDNA is widely used for the molecular diagnosis of *Leishmania* and gene regions such as internal transcript spacer I (ITS I), splice leader mini-exon (SLME), SSU rRNA gene, tubulin and gp63 (17-19). *Leishmania* genomes also exhibit significant synteny with other trypanosomatids, such as *Trypanosoma brucei* and *Trypanosoma cruzi*, despite significant differences in their life cycles and pathogenicity. Comparative genomics studies have revealed important differences in gene expression patterns and metabolic pathways between *Leishmania* and other trypanosomatids, providing insights into the molecular basis of their distinct biology and pathogenicity. Advances in genomics and genetic manipulation techniques have enabled researchers to identify and study genes involved in virulence, drug resistance, and host-parasite interactions in *Leishmania*. These studies have revealed important insights into the molecular mechanisms underlying *Leishmania* pathogenesis and have facilitated the development of new diagnostic and therapeutic strategies (20, 21).

## 2. LEISHMANIASIS

Leishmaniasis is a vector-borne disease caused by the protozoan parasite of the *Leishmania* genus. It is transmitted to humans through the bite of infected female phlebotomine sandflies, which are found in tropical and subtropical regions of the world. Leishmaniasis is a neglected tropical disease that mainly affects people living in poverty, with limited access to healthcare and proper sanitation. The disease is endemic in 98 countries, with an estimated 1 million new cases and 20,000-30,000 deaths per year. Control and prevention measures include early diagnosis and treatment, vector control, and development of vaccines and new drugs. Three main forms of the disease, which are generally accepted as cutaneous leishmaniasis, visceral leishmaniasis and mucocutaneous leishmaniasis, are reported. In addition to these, diffuse cutaneous leishmaniasis, post-kala-azar dermal leishmaniasis and canin leishmaniasis forms are also known (1).

Cutaneous leishmaniasis is the most common form of the disease, characterized by skin lesions that can be ulcerated, nodular, or papular.

Mucocutaneous leishmaniasis affects the nasal and oral mucosa, causing destruction of the tissues and disfiguration of the face. Visceral leishmaniasis, also known as kala-azar, affects the internal organs such as the liver, spleen, and bone marrow, and can be fatal if left untreated (1, 22).

Visceral leishmaniasis, also known as kala-azar, is the most severe form of the disease, affecting the internal organs such as the spleen, liver, and bone marrow. The disease is caused by species such as *L. donovani* and *L. infantum*, and can be fatal if left untreated. Visceral leishmaniasis is endemic in parts of Asia, Africa, and Latin America, and accounts for an estimated 200,000 to 400,000 cases and 20,000 to 40,000 deaths each year (1, 23, 24).

Mucocutaneous leishmaniasis is a more severe form of the disease, affecting the mucous membranes of the nose, mouth, and throat. The disease is caused by certain species of the parasite, such as *L. braziliensis*, and can lead to severe tissue destruction and disfigurement. Mucocutaneous leishmaniasis is more common in South America, particularly in Brazil, where it accounts for up to 10% of all cases of leishmaniasis (1, 25).

Diffuse cutaneous leishmaniasis is a rare form of the disease, characterized by widespread lesions that can affect large areas of the body. The disease is caused by certain species of the parasite, such as *L. aethiopica*, and is typically found in East Africa. Diffuse cutaneous leishmaniasis is often difficult to diagnose and treat, and can lead to severe disability and social stigma (1, 26).

Post-kala-azar dermal leishmaniasis is a complication that can occur following treatment for visceral leishmaniasis, particularly in the Indian subcontinent. The disease is characterized by skin lesions that can persist for months or years, and can act as a reservoir for the parasite, contributing to the continued transmission of the disease (1, 27).

Canine leishmaniasis is a form of infection that occurs in dogs, which is the most important reservoir of the parasite in nature. The causative species of KanL include *L. infantum*, *L. tropica*, *L. chagasi*, *L. major* and *L. braziliensis*. The causative agent of canine leishmaniasis is known as *L. infantum* in the Old World and *L. chagasi* in the New World (1, 28-30).

Overall, the clinical manifestations of leishmaniasis can vary widely depending on the species of the parasite, the immune status of the host, and other factors. The diagnosis and treatment of leishmaniasis require careful consideration of these factors, as well as the geographic and epidemiological context of the disease.

## 3. EPIDEMIOLOGY OF LEISHMANIASIS

Leishmaniasis, an intracellular protozoa, is one of the most important public health problems in the world. About 70 animal species, including humans, are known as natural reservoir sources of *Leishmania* parasites. Leishmaniasis is at the top of the World Health Organization (WHO)'s list of the most important tropical diseases. The disease occurs in both tropical and subtropical regions of the world, including parts of Asia, Africa, South America, Central America, and southern Europe. According to the WHO, an estimated 700,000 to 1 million new cases of leishmaniasis occur each year worldwide. However, due to under-reporting and lack of accurate data, the actual number of cases may be much higher. Leishmaniasis is found in 98 countries, with the majority of cases occurring in developing countries. The disease is most common in areas where poverty, malnutrition, and poor sanitation are prevalent, as these factors increase the risk of infection. In terms of distribution, leishmaniasis is more common in rural areas than in urban areas. The disease is also more prevalent in certain regions, such as the Indian subcontinent, East Africa, and Brazil (1-3, 31, 32).

The risk of leishmaniasis is highest for people who live or travel in areas where the disease is endemic, as well as for those who have weakened immune systems, such as HIV/AIDS patients or people undergoing chemotherapy (32).

## 4. TREATMENT OF LEISHMANIASIS

There is currently no definitive treatment method developed for leishmaniasis. Various antibiotics, trivalent and pentavalent antimony compounds are tried in the treatment of the disease, but trivalent antimony compounds are not preferred because of their high toxicity. Although the mechanism of action in treatment is not completely clear, pentavalent antimony compounds, which are thought to affect the molecular structure of the parasite and the macrophage-host relationship, are known as the most used compounds today because of their low toxicity and clearer response to treatment (33-35).

Treatment of leishmaniasis depends also on the type and severity of the disease, as well as the patient's age, health, and other factors. Treatment for cutaneous leishmaniasis typically involves the use of antimonials, miltefosine, or paromomycin, which are medications that can be administered orally or topically. Treatment may last for several weeks or months, depending on the extent of the infection and the response to treatment. Visceral leishmaniasis, which is a more serious form of the disease that affects the internal organs,

requires prompt and aggressive treatment. The recommended treatment for visceral leishmaniasis is a course of medication, such as liposomal amphotericin B, miltefosine, or paromomycin. In some cases, a combination of medications may be used. Treatment may need to be continued for several weeks or months to ensure complete clearance of the parasite from the body. Mucocutaneous leishmaniasis, which affects the mucous membranes of the nose and mouth, can be more challenging to treat and may require a combination of medications and surgery. Treatment options may include antimonials, amphotericin B, or miltefosine, in combination with surgery to remove the affected tissue. In addition to medication, supportive care may be necessary to manage symptoms and prevent complications. This may include pain management, wound care, and nutritional support. Patients may need to be hospitalized for treatment, especially in cases of severe disease. In conclusion, to summarize, It is important to note that treatment of leishmaniasis can be challenging, and the effectiveness of treatment may vary depending on the species of the *Leishmania* parasite, the location and severity of the infection, and the overall health of the patient. In some cases, the disease may recur after treatment, and long-term follow-up may be necessary. If you suspect you may have leishmaniasis, it is important to seek medical attention promptly. A healthcare professional can help determine the appropriate treatment for your individual case (1, 2, 36).

## 5. IMMUNOPATHOLOGY OF LEISHMANIASIS

The immunopathology of leishmaniasis refers to the way in which the body's immune system responds to the presence of the *Leishmania* parasite. The interaction between the parasite and the immune system can lead to a range of clinical manifestations, from asymptomatic infection to severe disease. It is known that *Leishmania* infections depend on factors such as the type and pathogenicity of the parasite, the immunity developed by the host against the infection, and the genetic background of the host. One of the most important characteristics of *Leishmania*, which is a eukaryotic pathogen, is that it can evolve in the host organism and cause long-term chronic infections (37, 38). The organism produces a complex set of immune responses against Leishmaniasis. Resistance or susceptibility to *Leishmania* infections depends on the T helper (Th)1/Th2 balance, and T cells are thought to play an important role in protective immunity. Th1/Th2 balance varies depending on many factors such as pathogen type, host immune system and genetic background, virulence factors, interactions of antigen presenting cells (APC) with T cells, and amount

of secreted cytokines. Upon infection, the parasites are phagocyted by immune cells such as macrophages, dendritic cells and neutrophils. These cells recognize the presence of the parasite and trigger an immune response that involves both innate and adaptive immunity. Innate immunity is the first line of defense and involves non-specific mechanisms that provide immediate protection, whereas adaptive immunity is a more specific response that is tailored to the specific pathogen (39). In cutaneous leishmaniasis, which affects the skin, the immune response is typically dominated by a T-helper (Th) 1 response, characterized by the activation of $CD4^+$ T cells that produce interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α). These cytokines are involved in the activation of macrophages, which in turn phagocytose and kill the parasites (40). In contrast, in visceral leishmaniasis, which affects the internal organs, the immune response is typically characterized by a Th2 response, which is associated with the production of interleukin-4 (IL-4), IL-10, and other cytokines that suppress the activation of macrophages and promote parasite survival. Mucocutaneous leishmaniasis, which affects the mucous membranes of the nose and mouth, is associated with a mixed Th1/Th2 response, and the immune response can also be influenced by host genetic factors. In addition to the Th1/Th2 balance, other factors such as regulatory T cells, B cells, and natural killer (NK) cells have been implicated in the immunopathogenesis of leishmaniasis (41-43). Regulatory T cells (Tregs) are another type of T cell that plays a role in the immune response to *Leishmania* infection. Tregs suppress the activity of other immune cells and can help to limit excessive inflammation and tissue damage. B cells, which produce antibodies, have also been implicated in the immune response to *Leishmania* infection. However, the role of antibodies in protection against *Leishmania* is not well understood. Overall, the immunopathology of leishmaniasis is complex and influenced by a range of factors, including the species of *Leishmania* parasite, the immune status of the host, and the site and severity of the infection. A better understanding of the immunopathology of leishmaniasis is essential for the development of effective therapies and vaccines for this important neglected tropical disease (44).

## 6. CONCLUSION

Leishmaniasis is a complex and challenging disease to control and eliminate. The lack of effective vaccines and limited treatment options for some forms of the disease, coupled with the high burden of poverty and lack of resources in endemic areas, present significant obstacles to controlling the

spread of the disease. In recent years, however, there have been important advances in the development of new diagnostic tools, vaccines, and therapies for leishmaniasis. Rapid diagnostic tests based on immunochromatographic and molecular techniques have been developed, enabling early detection and treatment of the disease. Several vaccines against leishmaniasis are currently under development, with some showing promising results in pre-clinical and clinical trials. These vaccines aim to stimulate the immune system to recognize and eliminate the parasite, providing long-lasting protection against the disease. In addition, new drugs and drug combinations are being developed for the treatment of leishmaniasis, including oral and injectable formulations with improved efficacy and safety profiles. The use of combination therapies is also being explored as a means of reducing the risk of drug resistance. Vector control measures, such as the use of insecticide-treated bed nets and indoor residual spraying, have been effective in reducing the transmission of leishmaniasis in some areas. However, the complexity of the transmission cycle and the presence of multiple sandfly species that can act as vectors make it challenging to implement effective vector control measures in all endemic areas. Overall, a multi-faceted approach is needed to control and eliminate leishmaniasis, involving improved access to healthcare, sanitation, and education, as well as increased funding for research and development of new tools and interventions.

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CHAPTER V

# INTESTINAL PROTOZOAN INFECTIONS TRANSMITTED BY WATER

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## 1. INTRODUCTION

Water, which is an indispensable part of the human body, is also known as the source of life. Today, the need for access to safe water is increasing. It is known that 1.3 billion people in the world suffer from access to healthy and safe water and this number will double for people who cannot obtain water resources. Increasing industrialization, household waste, pesticides used in agriculture, fertilizers and frequent contamination of groundwater have the potential to pollute the seas, oceans, rivers and stagnant waters (1). Insufficient water consumption causes a public health problem related to the increase of many microorganisms that can cause diseases. Parasitic infections are among the microorganisms that can be transmitted by water. Most of these parasitoses occur due to insufficient implementation of hygiene rules and settle in the gastrointestinal tract (GIS) (2).

Intestinal parasitoses are among the first diseases to be controlled by the World Health Organization (3). These parasites can be identified by serological, immunological, pathological, molecular and epidemiological methods.

Besides being an important problem of hygiene and sanitation, their supply through water increases the importance of water in our lives. Parasitic diseases affect all segments of society, especially children and adolescents. Parasites can mix with environmental waters and drinking waters through oocysts or cysts released into the environment with human and animal feces and can infect undesirably. These infective agents dispersed to the environment with infected human and animal outputs have the potential to cause disease even at very low doses.

Although the diseases caused by intestinal parasites can generally progress with asymptomatic findings, they can sometimes cause general symptoms such as mental and physical development retardation, loss of labor and gastroenteritis, diarrhea, abdominal pain and vomiting (4).

## 2. INTESTINAL PAROTOZOAN INFECTIONS

### *2.1. Entamoeba histolytica/dispar*

It is the third leading cause of death from parasitic infections (5). The infestation by this parasite occurs after the ingestion of cysts in contaminated food and water or due to a lack of handhygiene (6). these cysts can remain in the intestinal lumen and invade the intestinal wall to form new cysts after bipartition, which are eliminated to the outside by fecal matter and re-contaminate water, soil and food. In the invasion process of the intestinal mucosa and submucosa, they produce ulcerations responsible for some symptoms of amebiasis and distant dissemination and involvement of other organs. It has two forms as *E. histolytica* or *E. dispar*. Although *E. histolytica* is a pathogenic form that can cause amoebic colitis and extraintestinal amoebiasis, *E. dispar* is considered non-pathogenic (7). The clinical manifestations can vary and be asymptomatic, representing 90% of the total. In the case of invasive intestinal amebiasis or dysenteric amoebic colitis,the prevalence is 10%. Ingested amoebae pass into the large intestine, where they develop. In some cases, amebiasis can cause discomfort and either diarrhea or constipation. It can also cause dysentery and painful diarrhea with abundant blood and mucus (8). *E. histolytica* is a protozoan that needs to be treated because of its potential to develop extraintestinal infections (9).

### *2.2. Blastocystis hominis*

*B. hominis* is a universally distributed anaerobic protozoan affecting both man and many animals. Although it is the most common parasite in stool samples, its clinical significance is still unclear. The infection consists of water-resistant cysts and is divided into two groups. These are thin-walled ones that can auto-infect, and thick-walled ones that can be transmitted through water and food (10). It is a microorganism with marked genetic heterogeneity (genotypes), and presents multiple morphologies (vacuolar, granular, multivacuolar, avacuolar, amoeboid and cystic) with different replication strategies. It was initially considered as commensal. However current epidemiological studies suggest that *Blastocystis* spp. is pathogenic and is associated with a wide range of

gastrointestinal and extra-intestinal disorders (11,12). *B. hominis* is transmitted by fecal contamination. It is one of the most common intestinal parasites in tropical areas. It is generally non-pathogenic as it does not invade the intestinal tissue. Some studies have affirmed its pathogenicity by causing diarrhea and other digestive symptoms. Human infection is acquired by fecal contamination from other people or reservoirs. Its infective form is not clearly defined, but it is the most widely accepted that it is made up of hard-walled cysts. This parasite is found in the colon (10).

### *3.2. Giardia duodenalis (syn.G. lamblia, G. intestinalis)*

*Giardia duodenalis* is the most common intestinal parasite in the World and is a flagellated protozoan parasite. It is estimated that giardiasis causes 280 million diarrheas per year (13). It causes disease through feco-oral infectable cysts. Cysts are contagious in water for months in cool, moist areas. 10 cyst infection can cause the disease (14). *Giardia* has been recognized as one of the neglected diseases by WHO (15). Giardia has also been widely reported in wild and farm animals (16). *G. lamblia i*nfection is cosmopolitan because it can develop both endemically (basically affecting the child population, with frequent reinfections) or epidemic (outbreaks affecting closed communities or ravellers visiting endemic areas). *Giardia* is responsible for 2-3% of all ravellers' diarrhea. The infection is acquired by ingesting cysts or, more rarely, by trophozoites originating from fecal matter. Another way to become contaminated is by ingesting drinks or food contaminated by the infectious forms of *Giardia* (17*)*. It can be asymptomatic or cause infections ranging from chronic diarrhea to malabsorption (18). Giardiasis can cause diarrhea, nausea, vomiting and weight loss (19). It has been proven that children with diarrheal cases in developing countries are associated with this infection. This disease is also more likely to occur in waterborne outbreaks in developed countries or in acute diarrhea cases travelers to endemic areas (20).

### *3.3. Cryptosporidium* spp

The genus *Cryptosporidium* has several species that affect human beings and many animals. Many species of *Cryptosporidium* spp. cause infection in animals. The parasite's outer shell is highly sheltered and this prevents chlorine disinfection as well. This coccidia reproduce in the small intestine, where it causes an inflammatory reaction. Oocysts infect orally; by asexual reproduction,

they release sporozoites that invade intestinal cells. They reproduce and form merozoites (meronts) that gives rise to oocysts, and are eliminated in the fecal matter (21). The parasite can be transmitted especially with drinking and utility water. *Cryptosporidium* is leading cause of waterborne disease among humans. The disease begins 2-10 days after being infected with the parasite and watery diarrhea is the most important symptom (22). Although these symptoms can cause disease in healthy hosts, they can cause serious illness in immunocompromised hosts (such as people with HIV/AIDS, cancer and transplant patients, immunosuppressive medication and immunocompromised patients) (23). *Cryptosporidium* accounts for 60.3% of waterborne protozoan parasite outbreaks reported worldwide between 2004 and 2010 (24). Most cases detected in humans are caused by *C, parvum* and *C. hominis*. This is also common in HIV-positive patients. In addition, there are infections with subspecies such as *C. meleagridis, C. felis,* and *C. canis. C. wrairi* from guinea pigs, *C. felis* from cats, *C. andersoni* from cattle, *C. canis* from dogs, *C. hominis* from humans and *C. molnari* from fish were detected. Numerous new species have been described, including *C. agni* in sheep*, C. anserinum* in geese, *C. bovis* in calves, *C. cuniculus* in rabbits*, C. garnhami* in humans, and *C. rhesi* in monkeys (25).

### *3.4. Cyclospora cayetanensis*

*Cyclospora* spp. is a coccidian parasite in the phylum Apicomplexa where people are at risk for infection by consuming food or water contaminated with the parasite, or by travelers living in endemic countries (26). Water contaminated with faeces can transmit *C. cayetanensis*. In endemic areas, it can develop as a source of infection in drinking water. In endemic areas, it can develop as a source of infection in drinking water (27) *C. cayetanensis* is the only species known to infect humans (28). *Cyclospora* needs at least 1-2 weeks to become contagious in humans (29). It then infects the small intestine and results in explosive diarrhea. There may also be symptoms of loss of appetite, weight loss, stomach cramps/pains, bloating, increased gas, nausea and fatigue. If *C. cayetanensis* is left untreated, it can last for a long time and has the potential to recur (26). Cyclosporiasis is seen in many countries, especially in tropical and subtropical regions, and it can be seen seasonally in some regions (30) Considering that the infection is caused by sporulated oocysts, direct person-to-person transmission is unlikely. It is not life-threatening without disease

complications (malabsorption, cholecystitis and reactive arthritis, etc.). Studies show that it is not possible to kill *Cyclospora* with routine chemical disinfection or sanitation methods (31). *Cyclospora* can easily be overlooked due to the very low number of shed oocysts (32). For this, the stool sample should be examined after it has been concentrated by the formalin-ethyl acetate technique (33). In developed countries (34), Cyclosporiasis has been observed regardless of age. young children are the most affected in endemic areas (35). Poverty and low socio-economic status are accepted as risk factors for increased transmission and infection due to poor water and food sanitation. *Cyclospora* has been detected in the feces of many animals, including cattle, dogs, mice, rats, monkeys, ducks, chickens and other bird species (36). Studies have shown that this increases the risk of infection in humans. *C. cayetanensis* is an important risk factor for children, foreigners and immunocompromised patients in endemic and developing countries. The presence of schizonts in intraepithelial enterocytes has been reported in HIV-positive patients (37). Therefore, it should be carefully studied without overlooked.

## 3. INTESTINAL PROTOZOAN INFECTIONS TRANSMITTED BY WATER IN TURKEY

Studies on waterborne protozoan infections in Turkey have been reviewed and documented. When we look at these studies, *E histolitica, G. duedonalis, Cryptosporidium, B. hominis* are mostly seen. The high number of these shows that water health is sometimes unsuitable for our country. The fact that infections can be seen with these infectious agents even in developed countries shows us the importance of water sanitation and hygiene. Table 1 shows the studies conducted on waterborne protozoa in Turkey.

**Table 1:** Studies on waterborne protozoa in Turkey

| Time | Place | Parasitosis | Reference |
|---|---|---|---|
| 2009-2019 | Van | Giardia intestinalis %9,3, Entamoeba histolytica/Entamoeba dispar %0,8, Cystoisospora belli %0,004 | 38 |
| 2009-2010 | Bursa | Giardia intestinalis 3.23%, Entamoeba coli 2.34%, Entamoeba histolytica 0.59% | 39 |
| 2003-2007 | Eskişehir | Entamoeba histolytica/dispar 31%, Giardia intestinalis 19%, Blastocystis hominis 7%, Cryptosporidium parvum %4.5 | 40 |
| 1999-2009 | İstanbul | Blastocystis hominis %2,1, Giardia intestinalis %1,4, Entamoeba histolytica/ dispar %0.05 | 41 |
| 2005-2008 | Kayseri | Blastocystis hominis, %19.72, Entamoeba coli % 3.15, Giardia intestinalis %1.96, Entamoeba histolytica/dispar %0.87 | 42 |
| 1997-2001 | Malatya | Giardia intestinalis %25.1, Entamoeba histolytica in %4.0 | 43 |
| 2004-2005 | Elazığ | Entamoeba histolytica/dispar %31, Giardia intestinalis %19, Blastocystis hominis %7, Cryptosporidium parvum %4.5 | 44 |
| 2006-2018 | Sivas | Entamoeba histolytica/dispar %1,5, Cryptosporidium parvum %0,3 | 45 |
| 2011-2015 | Bursa | Giardia intestinalis %0.9 | 46 |
| 2004-2009 | İstanbul | Entamoeba histolytica %5.2, Giardia %11.1 | 47 |
| 1993-2006 | Sivas | Entamoeba coli, %3,7, Entamoeba histolytica/ dispar %12,4, Giardia intestinalis %4,1 | 48 |
| 2009-2010 | Erciyes | Blastocystis hominis %15.8, Entamoeba coli %2.1, Giardia intestinalis %1.9 | 49 |
| 2012-2013 | Rize | G. intestinalis %12,1, Entamoeba histolytica/ dispar %9,6 | 50 |
| 2005 | Hatay | Giardia intestinalis %31,5, Blastocystis hominis %25,3, Entamoeba coli %18,30, Entamoeba hystolytica/dispar %13,4 | 51 |
| 2002-2004 | Sivas | Giardia intestinalis %3,7, E. histolytica/dispar %2,4, Entamoeba coli %2,5, Iodamoeba butschlii %0,6, Blastocystis hominis %0,4 | 52 |

| | | | |
|---|---|---|---|
| 2006-2010 | Manisa | Blastocystis spp. %7.64, Giardia intestinalis %1 | 53 |
| 2011-2012 | Gaziantep | Entamoeba histolytica/dispar %12.45, Giardia intestinalis %2.15 | 54 |
| 2003-2005 | Kocaeli | G. intestinalis %24,95, E. histolytica/dispar %3,43, %17,54) E .coli %17,54, B. hominis %20,97 | 55 |
| 2006-2004 | Şanlıurfa | E.histolytica/E.dispar %26,4 | 56 |
| 2005-2008 | İzmir | Blastocystis hominis %4,83, Giardia intestinalis %1,24, Entamoeba histolytica/ dispar %0,24 | 57 |

It is necessary for these parasitoses not to be overlooked and to increase the identification methods for a successful treatment. This is only possible with appropriate sample acceptance, development of highly sensitive examination methods and training of experienced parasitologists. Increasing research on water-dependent parasitosis in Turkey is important in terms of determining the distribution of parasitic diseases in our country. For this reason, new studies should be carried out to identify parasitoses and ensure water safety.

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CHAPTER VI

# A COMPREHENSIVE STUDY ON CHALCONES AND THEIR BIOLOGICAL ACTIVITIES

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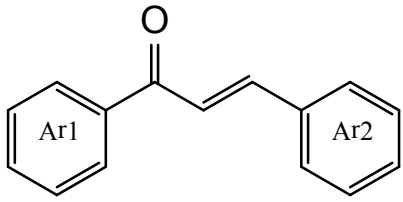
## 1. INTRODUCTION

Chalcones are a class of organic compounds characterized by the presence of connecting two aromatic rings linked by a three-carbon α,β-unsaturated carbonyl system. Chalcones, known for their diverse biological activities, are gain a huge attention in the realm of biological chemistry. These compounds, which are considered as precursors in the biosynthesis of flavonoids, exhibit promising antibacterial(1), antiinflammatory,(2) antileishmanial,(3) antimalarial,(4) antioxidant,(5-8) and antitumor (1) properties. Due to the cromophor and the auxochrome groups in their structure, chalcones are often coloured.(10) The name of chalcones are given these structure by Kostanecki and Tambor.(11)

Due to this interesting structure, chalcones can undergo various chemical reactions, making them versatile scaffolds for the design of new drugs with desirable pharmacological properties(12) and demonstrate a broad spectrum of biological activities.(13)The general structure of chalcones can be represented as: Ar1 – CH = CH – CO – Ar2 Where Ar1 and Ar2 represent two different aromatic rings.(14)

**Fig.1.General Chemical Structures of Chalcones**



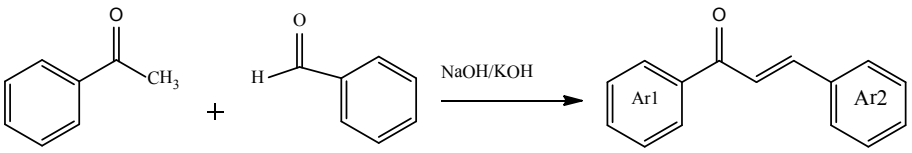
## 2. SYNTHESİS OF CHALCONES

Chalcones can be synthesized in many different ways; catalytic reactions such as coupling of Suzuki-Miyaura (15), Heck-Mizoroki(16) and Sonogoshira (17); electrophilic aromatic substitution such as Claisen Schmidt condensation (18) using solvents protic polar, acid, and basic catalysts or different reaction conditions. But among these synthesis methods the most functional one is Claisen Schmidt condensation method.

### *2.1.Claisen Schmidt Reaction*

The other name of this reaction is crossed-aldol reaction. This reaction involves the condensation of an aromatic aldehyde with an activated methylene compound (usually a ketone) in the presence of a base leading to β-hydroxycarbonyl compounds that has a vital importance in synthetic organic chemistry. (19-24) The reaction occurs at room temperature. Occured chalcone compounds can be characterized using various analytical techniques, such as infrared (IR) spectroscopy, proton nuclear magnetic resonance (1H-NMR) spectroscopy, and elemental analysis.

**Fig.2. Sythesis Of Chalcone**




Besides these synthesis methods, chalcones also can be synthesized by microwave assisted synthesis methods. This method is superior to other methods in terms of time and yield. The experiment can be done at very high temperatures. (25)

## 3. BİOLOGICAL APPLICATIONS OF CHALCONES

Chalcones are widely distributed in nature and can be found in various plant sources, such as fruits, vegetables, and medicinal plants.(26)

Chalcones are known for their diverse biological activities due to this feature have gained significant attention due to potential therapeutic applications. Here are some of the biological activities associated with chalcones:

### *3.1. Antioxidant Properties of Chalcones*

Chalcones have been found to possess potent antioxidant properties, which can be attributed to their ability to scavenge free radicals and reactive oxygen species (ROS). Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the ability of cells to detoxify these harmful compounds or repair the resulting damage. (27-28) ROS are highly reactive molecules containing oxygen, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals.(29) Forming of Reactive oxygen species (ROS) in normal cell occurs during aerobic respiration and can cause oxidative stress even to cell death. (30)

Antioxidants reduce free radical toxicity (31) and play crucial a role in reduction of many diseases stemming from free radicals in the living organisms. (32-33) Hence, in recent years scientist have been intensevely tried to synthesis and caracterization of new effective antioxidants. Scientist also have succeeded to synthesis of bioactive molecules to treatment of various diseases. For this purpose a large number of chalcone species have been synthesized and have taken their place in the literature.

Especially heteroaryl-chalcones shows antioxidant properties and hydroxyl groups attached the phenyl ring can act a critical role for these antioxidant agents. (34) The number and position of hydroxyl groups as well as methoxy groups in the antioxidant molecule are important for scavenging of different reactive oxygen and nitrogen species. Numerous of articles reported antioxidant activity of polyphenolic chalcones involving single proton or electron. Some chalcone compounds that have -OH and -$OCH_3$ groups in vicinal position, can be utilized to scavenge double or triple free radical mechanisms. Also, the chalcones which have -$OCH_3$ group can involve in the scavenging process by breaking Ph-$OCH_3$, $PhOCH_2$-H and PhO-$CH_3$ bonds, that's why these compounds deserve more attention. (35) Synthetic β-chalcone derivatives was sythesized and studied for its antioxidant activity using the DPPH and hydrogen-peroxide ($H_2O_2$) assays; these chalcones showed significant antioxidant radical-scavenging activity in the DPPH and $H_2O_2$ assays. (36)

Both synthetic and natural chalcones can exhibit antioxidant properties depending on the variation in their structure. The results of experimental studies have shown that chalcones are more effective antioxidants than the corresponding flavones. (37)

### *3.2. Anticancer Activity of Chalcones*

Some of the natural chalcone derivates has been discovered to exhibit potent anticancer effects. So many new chalcone derivates have been synthesized and investigated their antitumor effects. Especially the scaffold of chalcone consist of two aryl groups and an α,β-unsaturated alkenone. During the recent years scientist have studied on modification of this α,β-unsaturated alkenone structure. The investigations showed that 4-hydroxyl and methoxy substituents of chalcones exhibit the excellent antitumor activity. Nowadays the researchers try to replace the to aromatic rings of chalcones with heterocycles.

Microtubules are cytoskeletal structures that hold or release receptors have been essential for cell division. Tubulin is a protein in dimer structure and polimerization of this protein cause to uncontrolled cell division. It has been found that chalcones containing qualine, benzoxazole, benzotriazole, indole, benzopyran, benzofuran, heterocyclic naphthalene in A and B rings show excellent inhibitory and cytotoxic effects against tubulin polymerization. Furthermore, It has been also observed that the methoxy group in the heterocyclic ring improves the anticancer activity of chalcone derivatives. (38)

**Breast Cancer:** In recent years, breast cancer has become a global health problem. A wide range of drugs have been developed to destroy the root cause of breast cancer. In order to develop drugs of natural origin against cancer, scientists have focused on this field.(39-40)

The chalcones bearing -OH and -$OCH_3$ moeity groups were isolated from Syzygium samarangense and exhibited that these compounds show antiproliferative activity in MFC-7 and SKBR-3. (41)

When the hydroxy group of A-ring replaced by methoxy group, none of the synthesized compounds show relevant cytotoxic activity.

The results exhibit that chalcones bearing -OH and -$OCH_3$ moeity groups are isolated form Syzygium samarangense showed significant inhibitory effects against the growth of human cancer cell lines in vitro. These molecules based on the natural compounds scaffold represent good lead compounds in the search for new pharmaceuticals to fight breast cancer.(42)

Luo et al. synthesized a series of novel ligustrazine-chalcone hybrids and evaluated for their in vitro and in vivo antitumor activities. The results showed that most of these compounds exhibited significant in vitro cytotoxicity against MDA-MB-231, MCF-7, A549 and HepG2 cell lines with IC50 values as low as sub-micromole. (43)

Apart from chalcone, the indole family represents an important class of natural small molecules that are useful in cancer chemotherapy. Jumaah et al. Twenty-seven new ortho-hydroxy chalcone and a series of indole-chalcone derivatives. (44) The researchers evaluated these molecules for cytotoxicity activity against human breast cancer (MCF-7) cell lines using a reported MTT assay.(45)

Extensive studies regarding anticancer activity proved that chalcone derivates can present antiproliferative activity on cancer cells.

**Brain Cancer:** Brain cancer refers to the abnormal growth of cells in the brain. There are different types of brain tumors, some of which are benign (non-cancerous) and some that are malignant (cancerous). Malignant brain tumors can be primary, originating in the brain, or secondary, spreading to the brain from another part of the body.

Some studies have investigated the potential anti-cancer effects of chalcones. Particularly in relation to various types of cancer cells, including brain cancer cells, these studies have mostly been conducted in laboratory settings or on animal models. The results have shown promising anti-cancer activities, such as inhibiting the growth of cancer cells, inducing apoptosis (programmed cell death), and interfering with cancer cell signaling pathways.

Today so many pharmacologic antitumor therapies have been investigating or using in clinic.

The most important features to achive novel, effective and safe pharmacological modulation of therapies are the selectivity and the ability to induce controlled cell death of carcinogenic cells.

Glioblastoma multiforme (GBM) is a highly aggressive and malignant type of brain tumor that typically occurs in the brain's supportive tissue. It is the most common and deadliest form of primary brain cancer in adults. Patients suffering from this cancer type face significant morbidity and mortality. It grows rapidly and has a high turnover rate.

*Mendanha* et al. Synhesized and characterized three new chalcone derivates bearing methoxy groups in the structure and tested their antitumor activities aganist two different GBM cultures namely human glioblastoma astrocytoma (U87) and murine glioma (GL261) cells, as well as a non-tumor cell line (bEnd.3), at different time points. This study has exhibited that the chalcone derivates has a potential to enhance new treatment alternative for brain cancer.(46)

It's important to note that while these findings are promising, much of the research on chalcones and brain cancer is still in the preclinical stage. More studies, including clinical trials, are needed to determine the full potential of chalcones as therapeutic agents for brain cancer treatment.

**Skin Cancer:** Skin cancer is a type of cancer that develops in the cells of the skin. It occurs when the DNA of skin cells is damaged, typically by ultraviolet (UV) radiation from the sun or tanning beds. The damaged cells then begin to grow and divide uncontrollably, forming a tumor.

Chalcones has been reported to improve vision, memory, joint and muscle discomfort, liver and kidney function, sleep, prevents cancer, strengthens the immune system, and beautifies skin and hair. Nardoaristolone A is a natural chalcon. It can be extracted from Nardostachys chinensis and classified as terpenoid chalcones. Due to Nardoaristolone A both red blood cell count increase and it aids in small bowel movement.(47)

It has been designed and prepared a series of furan-substituted chalcone derivatives, in which the bis-furan units are at the A- or B-rings (Figure 2), utilizing the appropriate bis-aldehydes or bisketones as precursors. And to demonstrated the anticancer activity, the these new chalcones were tested against some human cancer cell lines and the results exhibit that they are active aganist A431 (skin cancer) and BJ1 (normal skin fibroblast), the other cancer derivates for example A549 (Lung carcinoma), HCT116 (Colon cancer), HePG2 (hepatocellular carcinoma cell line), PC3 (Prostate cancer), A43.(48)

These furan-substituted chalcone derivatives also showed a significant anticancer activity against lung and skin cancer cell lines compared with known chemotherapeutic drug (doxorubicin).

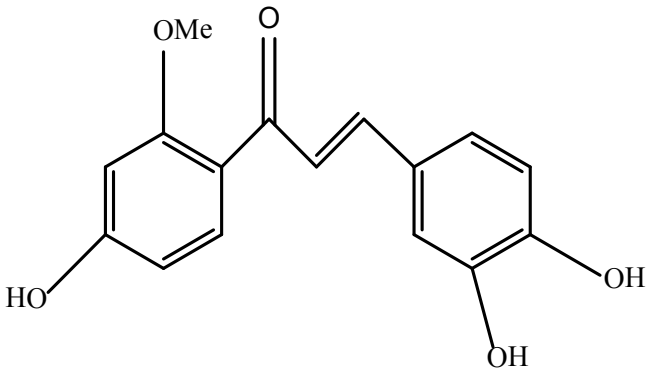
Isobavachalcone (IBC) is a natural compound that belongs to the class of chalcones and one of the most useful active compounds among numerous chalcones, it is especially obtained from plants of the Fabaceae and Moraceae families (49) Studies reported that IBC inhibits tumor formation in mouse skin cancer.(50)

**Colon Cancer:** Colon cancer, also known as colorectal cancer, is a type of cancer that affects the colon or rectum, which are parts of the large intestine. It typically starts as small, noncancerous clumps of cells called polyps that form on the inner lining of the colon or rectum. Colon cancer is one of the most common types of cancer worldwide, and its development is often influenced by various risk factors. For example age, genetic syndromes, unhealthy lifestyle, etc...

The studies of researchers have shown that bis chalcone derivatives play a major role in inhibiting pair of isogenic HCT116 colon cancer cell lines. (51)

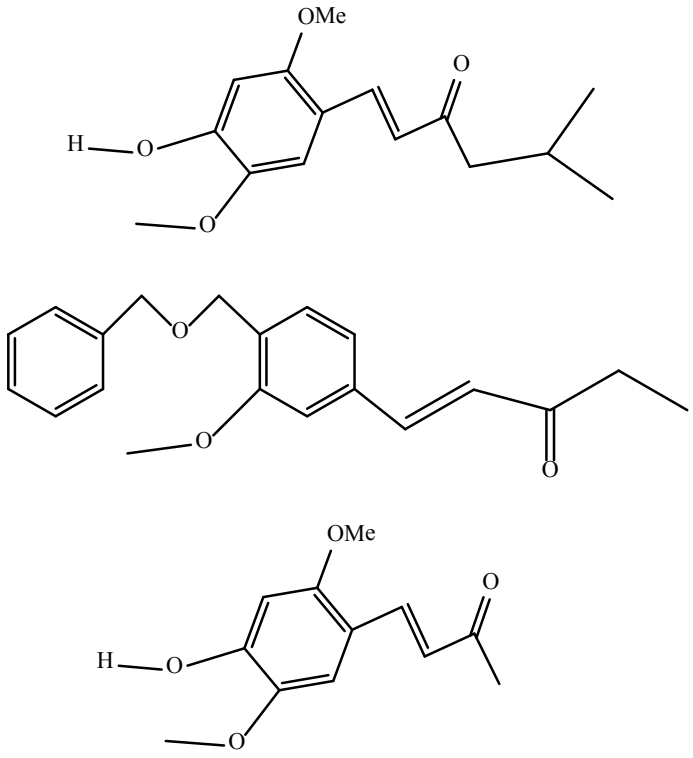
Sappanchalcone is a natural molecule isolated from Caesalpinia sappan L. It can be used on human colon cancer treatment P53 and HCT116. Colon cancer cells with mutated p53 have been found to be treatable with sappanchalcone. A study by Shin et al. showed a high level of p53 and apoptosis induction in chalcone-treated HCT116 cells. Interestingly, they also found apoptosis induction in p53-null HCT116 cells, suggesting that both p53-dependent and p53-independent mechanisms can play roles in apoptosis that is induced by this chalcone.(52) In addition, several other publications have revealed an association between p53 activation and apoptosis induction in chalcone-treated colon cancer cells (53-56).

Fig.3. The Structure of Sappanchalcone




Vanillin-based chalcone analogues and their antitumor activity were studied by Lukovic et.al. (57) The results showed that IC50 values observed in the HCT-116 cell models were promising. Furthermore, vanillin-based chalcone analogues caused over expression and activation of mitochondrial Bax protein and caspase-3 in HCT-116 cells, indicating that their antitumor mechanism of action was mediated by activation of the internal apoptotic pathway. Here are the chalcone compounds studied.(Fig 3)

Fig.3. The Structures of Studied Chalcone Analogs




Mohammed M. Amin et al. Synhesized novel 8-hydroxyquinoline/chalcone hybrids and investigated their cancer activity. 8-Hydroxyquinoline (8HQ) is one of the important heterocyclic scaffold that was implied in several clinically used chemotherapeutics. the anticancer, antimalarial, antifungal, and antiprotozoal. The study group obtined promising results.(58)

Several studies have been achived in the field of anticancer properties of chalcone. Apart from the cancer types mentioned above; chalcones are demonstrate hopeful result for; lung cancer;mouth cancer, etc...

It's important to note that while chalcones show promise as anticancer agents in preclinical studies and in vitro experiments, further research, including clinical trials, is needed to fully understand their efficacy, safety, and optimal dosage for human use. Additionally, chalcones are just one of many compounds being studied for their potential anticancer properties, and a comprehensive approach to cancer prevention and treatment involves various strategies and interventions.

### *3.3. Antimicrobial Properties Of Chalcones:*

Several studies have investigated the antimicrobial activities of chalcones against a wide range of microorganisms, including bacteria, fungi.. Many types of chalcones have been synthesized up to now. Some of these molecules are served as antimicrobial agent. For example pyrazolyl–chalcone derivatives have exhibited antimicrobial activity aganist Gram-negative and Gram-positive bacteria, as well as non-flamentous fungus, thiazole based chalcone derivates have been exhibit promising antimicrobial properties.(59-60)

Scientists focus on natural antimicrobials since there is increasing resistantance to antimicrobials having become a ring of the human food chain.(61) Nowadays, the consumers prefer less refined, natural products are in demand.(62)The chalcones Antimicrobial chalcones can be obtained from plant metabolites or they can be pure compounds or extracts to obtain new antibiotics.(63)

#### *3.3.1. Antibacterial Properties of Chalcones:*

Chalcones have been recognized for their diverse biological activities, including antibacterial properties. Many diseases have been caused by bacteria result in morbidity and mortality worldwide since low cost, less toxic, and powerful antibiotics have not been developed. Chalcone-based drugs have been tought promising natural agent aganist to bacterias. (64)

A series of chalcone derivates were sythesized and investigated their antibactrial activity . against both Gram-positive and Gram-negative bacterias. This includes common pathogens such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella spp. This study showed that (E)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one ($C_{15}H_{11}ClO$) compound was the most active against E. Coli; (E)-1-phenoxy-3-(3-phenylprop-1-en-1-yl)benzene ($C_{21}H_{16}O_2$) compound was the most potent against S. typhi; (E)-3-(3-nitrophenyl)-1-phenylprop-2-en-1-one ($C_{15}H_{11}NO_3$), (E)-3-(3,4-dimethoxyphenyl)-1-phenylprop-2-en-1-one ($C_{17}H_{16}O_3$) and (E)-1-phenoxy-3-(3-phenylprop-1-en-1-yl)benzene ($C_{21}H_{16}O_2$) compounds were against S. aureus; (E)-3-(3,4-dimethoxyphenyl)-1-phenylprop-2-en-1-one ($C_{17}H_{16}O_3$) compounds and 3'-hydroxy-4-methoxy chalcone ($C_{16}H_{14}O_3$)compounds were the most active (MIC 0.4 mg/mL) against B. subtilis only compound 4'-phenoxy chalcone ($C_{21}H_{16}O_{2)}$ showed activity (MIC 0.8 mg/mL) against C. albicans, only 3'-hydroxy-4-methoxy chalcone ($C_{16}H_{14}O_3$) showed activity against P. aeruginosa while all the compounds were active against A. niger.(65)

Especially fluorine based chalcones have been demonstrated superior biological activity. The fluorine in these compounds improves their pharmacological properties.(66) A series of (E)-1-(4 fluorophenyl)chalcones were evaluated and these compounds demonstrated antibacterial activities against E. coli and P. aeruginosa and, antifungal activity against A. niger. (67)

(E)-3-(4-fluorophenyl) chalcones have been reported to show antitubercular and antibacterial activities against S. aureus and antifungal activity against C. albicans. The research further revealed that fuorinated chalcones with fuoro-substitution in position 2 and or position 5 of its B ring showed higher antibacterial potency against S. aureus, S. pyogenes, E. feacalis, E. coli, P. aeruginosa and antifungal activity against C. albicans, C. glabrata and C. parapsilosis.(68) Also, fuorinated chalcones-1,2,3-triazoles conjugates have been reported to show signifcant activity against some bacterial and fungal strains (69)For instance, (E)-1-(2-hydroxyphenyl)-3-(4-fuorophenyl)chalcones was shown to possess antibacterial activity against E. coli, B. pumilis and B subtilis and, antifungal activity against A. Niger (70)

A series of 4-fluoro-2-hydroxychalcone have been reported that they have shown antibacterial activities against MRSA, VRE, Staphylococcus aureus, Streptococcus pyrogenes, Streptococcus feacalis (Gram-positive) and Escherichia coli, Salmonella typhi, Corynebacterium ulcerans, Proteus mirabilis, Pseudomonas aeruginosa (Gram-negative). (71)

Chalcones can be obtained in naturally or syntheticly. There are a wide range of chalcones syhthesized by naturally. (72) In 2016 Three chalcones, 2'-hydroxy-4-4',6'-trimethoxychalcone were syhthesized. These chalcone compounds were isolated from leaves of Piper hispidum. P. hispidum leaves was determined against bacteria Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus and yeasts Candida albicans, C. parapsilosis and C. tropicalis. The result of study showed that e. P. hispidum had good antibacterial activity against S. aureus and B. subtilis, with MICs of 62.5 and 7.8 mg/mL, respectively.(73)

Glycyrrhiza, one of the oldest medicinal plants, is often referred to as Chinese licorice, which belongs to the family of Leguminosae(74-75)

Isoliquiritigenin (ISL) is a chalcone, obtained naturally from Glycyrrhiza plant showed a more essential antibacterial activity against three major periodonto pathogens, that is, Porphyromonas gingivalis, Fusobacterium nucleatum, and Prevotella intermedia, and a stronger inhibitory activity to P. gingivalis collagenase and human MMP-9 (76).

Recently, Zhao et al. found that ISL showed a strong inhibitory activity against Ralstonia solanacearum with an inhibition zone diameter of 14.15 mm. (77)

### *3.3.2. Antifungal Activities Chalcones:*

Fungal diseases, also known as mycoses, are caused by various types of fungi. These infections can affect different parts of the body, including the skin, nails, respiratory system, and internal organs. Fungal diseases can range from mild, superficial infections to severe and life-threatening conditions, depending on the type of fungus and the individual's immune response.

In 2013, Kakati et.al sythesized a series of new steroidal chalcones and investigated their antibacterial activities against B. subtilis and E. coli bacterias. And antifungal activities aganist Aspergillus niger and Candida albicans. Some of the compounds showed excellent activity against the tested fungies. (78)

The novel chalcone derivatives were synthesized and screened biologically for antifungal activity 2-benzylidene-3,4-dihydronaphthalen- 1(2H)-one and 2-(4-chlorobenzylidene)-3,4-dihydronaphthalen-1 (2H)-one among the compounds exhibited antifungal activity against Microsporum gypseum. But the results for antifungal activity of these compounds weren't positive aganist Candida albicans and Aspergillus niger (79)

3,4,6trimethoxyacetophenone isolated from Croton anisodontus and eight chalcones were synthesized by the reaction of benzaldehyde and 3,4,6 trimethoxyacetophenone. Then investigated the in vitro antifungal activity of these eight chalcone derivates aganist C.ablicans fungi. The study showed that the chalcone have good fungal acivity. The in vitro antifungal potential of the chalcones showed that chalcones (E)-3-(furan2-yl)-1-(2-hydroxy-3,4,6-trimethoxyphenyl) prop-2-en-1-one (E)-1-(2-hydroxy-3,4,6- trimethoxyphenyl) -3-(4-methoxyphenyl)prop2-en-1-one were considered as chalcones of better activity aganist C. albicans. (80)

Tri-fluorinated chalcones from 1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl) ethan-1-one. The (E)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en1-one (DBTFP-1) and (E)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3-(4-(trifluoromethoxy)phenyl)prop-2-en-1-one (DBTFP-2) were synthesized and tested for their antifungal activity aganist A. niger and C. albicans fungal species. And it is proved that the compouds has shown a potent antifungal action against A. Niger and C. albicans. (81)

Substituted chalcone Schiff bases were studied to investigate their fungal activity aganist yeasts Saccharomyces cerevisiae and Candida albicans yeasts. Antifungal activities were determined via minimum inhibitory concentration method.(MIC), antifungal activity of these molecules depend on their capability of distirbution the cell wall. Sorbitol was used as osmeoprotectant to help cell wall in protection against osmotic stress.(82) The study proved that nitro group substituted chalcones demonstrate antifungal activity against yeast cells.

In a recent study (E)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one chalcone compound was sythesized and investigated antifungal properties aganist Mucor sps and Candida albicans fungies. The results of this study showed that the antifungal effect of the mentioned chalcone compound was higher on mucor sps than on candida albina fungus. It is observed that the zone of inhibitions against Mucor sps was dramatically higher to the standard drug.(83)

### *3.4. Antiparasidal Activity of Chalcones*

Parasites are organisms that live in or on another organism, known as the host, and obtain nourishment from the host at the host's expense. Besides their signification for health and finance, parasites are an inseparable part of the ecosystem. They have a huge effect on host health that they infect all living organisms. Parasites also can influence public health, food economy, structure of population.(84) They can be found in various forms, including viruses, bacteria, fungi, and multicellular organisms such as worms and insects. Several studies have investigated the potential antiparasitic activity of chalcones against various parasites, including protozoa and helminths.

21 new chalcone derivatives bearing different substituents were synthesized and investigated antiparasitic activity aganist Leishmania braziliensis, Trypanosoma cruzi, and Plasmodium falciparum parasites. Depending on their moieties the molecules exhibited different antiparasidic activities which have the substituents H, OH, or $NH_2$ in C-2′, whereas, in ring B, they have groups with different electron-donating and electron-withdrawing properties, such as $OCH_3$, $NO_2$, and halogens (Cl, F). In vitro effects of these substances on L. braziliensis, T. cruzi, and P. falciparum were evaluated. The results showed that electron-donating groups in ring, the hydrogen bonds and carbonyl group affect the antiparasitic activity. The compounds exhibited high antiparasidal activity aganist L.braziliensis depending on their structural features. This is the main

reason why only one compound very active aganist T.cruzi, but none of them can affect the other one.(85)

In another study; (E)-3-(phenyl)1-(phenyl)prop-2-en-1-one chalcone compound sythesized by classical Claisen Schmidt method. The antiparasitic activity of sythesized chalcone compound evaluated aganist Toxoplasmosis is a very widespread infection caused by Toxoplasma

Gondii parasites. And to comprehense activity of this chalcone, it was compare with a synthetic drug spiramycin. Vero culture used for progation of Toxoplasma Gondii. The result of this study exhibited that newly sythesized chalcone can be utilitied to inhibate Toxoplasma Gondii parasites instead of spiramycin.(86)

2,4'- dimethoxy-6'-hidroxychalcone and 2,5'- dimethoxy-4,6'-hidroxychalcone and two flavanone synthesized and antiparasitic activity of these compounds were evaluated aganist Polygonum salicifolium, Trypanosoma congolense ,Leishmania mexicana; Trypanosoma brucei brucei parasites. The result showed that chalcones exhibited interesting activity against T. b. brucei; congolense; and, to a lesser extent, L. mexicana, and provide further evidence for the potential uses of natural plant extracts for combating these global parasitic diseases.(87)

Leishmaniasis, a parasitic disease transmitted by the bite of infected female sandflies (phlebotomine sandfly), can be treated with a wide range of drugs. But the drugs have some disadvantages for example serious side-effects, not accesible to everyone and limited efficiency.

A group of scientist synthesized and characterized a new series of prenylated chalcones with attracting or electron-donating functional groups attached to the A and B ring were. And tested antileishmanial activity of these prenylated chalcones aganist L. mexicana promastigotes. These new prenylated chalcones exhibited selectivity against the parasite and low cytotoxic activity on mammalian macrophages.(88)

## 4. CONCLUSIONS:

Chalcones are a group of chemical compounds that have been intensively studied by scientists in recent years due to their biologically active properties. Especially the anticancer activity of fluorine-substituted chalcones (66) and the widespread biological activity of some naturally occurring chalcone compounds can be counted among the superior properties of chalcones.

In this study, these superior biological properties of chalcones were investigated and it was observed that chalcones showed promising biological activities that can be used in the treatment of different diseases. Chalcones can be occured naturally or sythetically. So they are still being intensively studied, predicted that have the potential to be used in the treatment of lethal diseases such as cancer in the next years. In addition, antimicrobial and stotoxic properties of chalcones enable them to be included in the class of compounds that can be used for the development of drugs with fewer side effects in the coming period.

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CHAPTER VII

# HYPERMOBILITY AND HYPERMOBILITY-RELATED CONDITIONS

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## 1. INTRODUCTION

Joint hypermobility is the range of motion of the joints above the normal range of motion, independent of any rheumatic disease. Hypermobility was first described by Finkelstein in 1916. In the 1900s, this condition received various names such as "joint hypotonia", "arthrochalasis multiplex congenita", and Graham, who has been researching hypermobility for many years, first defined it as "hereditary hypermobility". The term "hypermobility syndrome" was first defined by Kirk et al. in 1967 as joint laxity accompanied by musculoskeletal symptoms. In those years, Kirk emphasized that hypermobility syndrome is associated with many musculoskeletal symptoms, that this syndrome is predisposing to degenerative joint disease and the importance of early diagnosis. (1) Since then, interest and awareness of hypermobility syndromes has increased. Numerous studies have been conducted on hypermobility syndrome in the last 60 years since then, and versatile information has been obtained on this subject. In those studies hypermobility syndrome has been named as "Generalized joint hypermobility (GJH)", "Joint Hypermobility Syndrome (JHS)", "Symptomatic Joint Hypermoblity", "Benign Joint Hypermobility Syndrome (BJHS)", "Ehler Danlos Syndrome Hypermobility Type (EDS-HT or EDS-Type III)" and "Hypermobile EDS (HEDS)".(2–4) When we look at the studies in the literature before 2017, individuals who only meet the Beighton score but do not have any complaints are called "Generalized joint hypermobility (GJH)"; Those who have complaints in other systems and meet Brighton criteria are defined as joint hypermobility syndrome (JHS).(2,5–7) In 2017, The International Classification

System of Ehler Danlos Syndrome was revised and 13 subgroups of EDS were defined. This new classification system has defined the term Hypermobility Spectrum Disorder (HSD) for individuals with GJH who do not meet the criteria for HEDS.(8) This definition, which is still relatively new, has been used frequently in the literature and strategies for the management of these patients have been revised.(9–11) The clinical findings and conservative treatment strategies of these individuals will be discussed later in this section.

### *2. EPIDEMIOLOGY*

In order to determine the prevalence of hypermobility, the countries where the studies are applied, ethnic characteristics, age ranges of the participants, the tests used as diagnostic criteria and even the cut-off values taken for diagnosis in these tests vary. For these reasons, it is very difficult to determine the precise prevalence value for hypermobility. Normal values of the joint range of motion (ROM) are under a wide physiological variation. It is well known that race, age, and gender affect ROM.

When various races were examined, it was noticed that Asian, Caucasian and Indian origins had a wider ROM than European and American people. For example, the prevalence of hypermobility in the adult population was 5% in the USA, 25-38% in Iraq, and 43% in a tribe named Noruba in Nigeria. Among 267 healthy students at a university, 17.1% of North American origin and 33.3% of Asian origin were observed to be hypermobile(5).

The age factor can also change the value of ROM that should be considered normal. The joint range of motion of a healthy newborn is above normal and decreases over time. There is a much wider spinal and peripheral joint range of motion (ROM) in childhood compared to adults, and it is seen that these ROMs decrease as age progresses. For many joints, this is considered normal (except for the knee and elbow joints: Genu recurvatum of the knee joint and hyperextension of the elbow joint are not considered normal, even in children). (12) In a systematic review of studies on the prevalence of hypermobility in children, the prevalence of hypermobility, adapted to the world, was determined as 34.1%, and it was stated that this rate was higher in girls and younger ages. (13) In a study in which 861 healthy high school children from Turkey were evaluated, the prevalence of joint hypermobility was found to be 11.7%.(14)

Considering the genders, it has been supported by many studies that joint hypermobility is more common (approximately 3 times more) in females than

males.(6,12,14–16) Hormonal factors are not associated with the development of hypermobility, but with worsening of its symptoms (especially during the estrogen peak before menstrual bleeding).

Despite all these given epidemiological figures, the general belief is that individuals with hypermobility are actually more frequent in clinics than is realized.

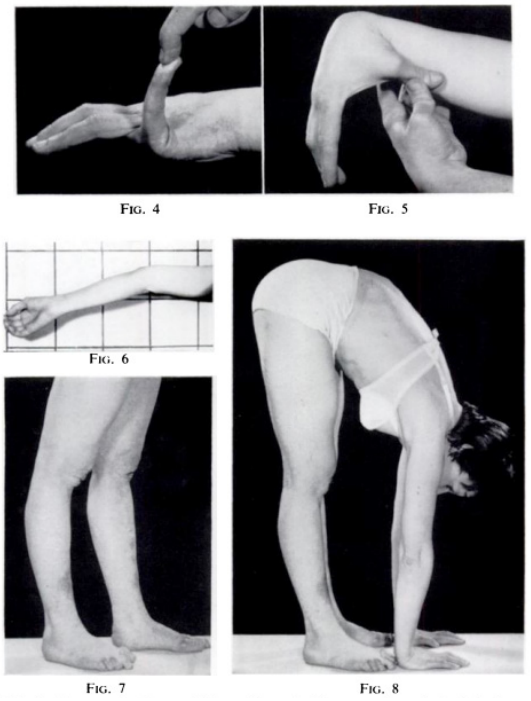
## 3. DIAGNOSTIC CRITERIA

Carter and Wilkinson criteria were first presented in 1964 for the diagnosis of hypermobility, and the Beighton Score was created by revising these criteria shortly after.(17) In 1973, the Beighton Score was revised and the 9-point Beighton Scale was created, and it is still valid from the past to detect hypermobility.

### *3.1. 9-point Beighton Scoring System:*

The 9-point Beighton scoring system evaluates joints symmetrically. In this scale, passively touching the thumb to the inner surface of the forearm, dorsiflexion of the 5th metacarpophalangeal joint exceeding 90 degrees, hyperextension of the elbow exceeding 10 degrees, and hyperextension of the knee exceeding 10 degrees are evaluated separately on the right and left sides (8 points). Finally, while standing, it is requested to bend and touch the palm of the hand to the ground (1 point) while the knees are fully extended. As a result, the individual is evaluated over a total of 9 points. Those who score 4 and above on the Beighton Scale are diagnosed as generalized joint hypermobility (GJH) (Figure 1).

**Figure 1.** The Beighton Scale




*Beighton & Horan, 1969*

The Beighton score detects the presence of hypermobility in a person, but does not give information about its severity. It also assesses the mobility of some joints predominantly in the upper extremities, but not the shoulders, hips, ankles, and feet. For these reasons, some authors argue that the diagnosis of GJH should not be made with an isolated Beighton Score, but a clinical judgment should be made by combining it with expert opinion.(18) Despite some criticisms, Beighton scoring is a well-accepted scale in daily practice and literature because it is comprehensive and applicable.(2,19–21)

### *3.2. The Brighton Criteria*

The Brighton Criteria, which were revised from the Beighton score, were developed in 2000, as the Beighton scoring and Carter criteria pointed to GJH but were insufficient to make the definitive diagnosis of joint hypermobility syndrome (JHS). The Brighton Criteria are used today as a comprehensive and useful diagnostic tool for JHS (Table 1). (22)

**Table 1.** The Brighton Criteria For Diagnosing JHS*

| |
|---|
| **Major criteria:**<br>Generalised Joint Hypermobility (Beighton Score ≥ 4/9)<br>Pain in ≥ 4 joints for ≥ 3 months<br><br>**Minor criteria:**<br>Beighton score of 1-3/9<br>History of recurrent joint dislocation<br>Pain in 1-3 joints or back pain for ≥ 3 months<br>≥ soft tissue injuries ie tenosynovitis<br>Marfanoid habitus<br>Skin involvement for example thin, strechy<br>Eye involvement for example droopy eyelids, shortsightedness<br>Varicose veins, hernia or organ prolapse |
| **Diagnosis requires:**<br>**"2 major"** or **"1 major and 2 minor"** or "**4 minor criteria"**<br>and<br>**"other heritable disorders of connective tissue have been excluded"** |
| *JHS: Joint Hypermobility Syndrome |
| *Graham et al., 2000* |

### *3.3 5-Point Hypermobility Questionnaire*

Another questionnaire that can be used very easily in daily practice to detect the presence of hypermobility is the 5-point Hypermobility Questionnaire (Table 2). (23) In this questionnaire, the individual tested is asked 5 questions; receiving 2 or more "yes" responses supports the presence of hypermobility:

**Table 2.** 5-Point Hypermobility Questionnaire

| |
|---|
| 1. Can you now (or could you ever) place your hands flat on the floor without bending your knees?<br>2. Can you now (or could you ever) bend your thumb to touch your forearm?<br>3. As a child, did you amuse your friends by contorting your body into strange shapes or could you do the splits?<br>4. As a child or teenager, did your kneecap or shoulder dislocate on more than one occasion?<br>5. Do you consider yourself "double-jointed"?<br><br>Answering **yes to ≥2 of these questions** suggests hypermobility with sensitivity of 85% and specificity of 90%. |
| *Hakim & Graham, 2003* |

### *3.4. Possible Quantitative Measurements in Diagnosis*

Studies in which quantitative measurements of tissue mechanics are performed in the diagnosis of hypermobile patients are rare, and a gold standard quantitative measurement method has not yet been determined for diagnosis. Quantitative measurements of skin, muscle (brachioradialis and biceps brachii), tendons (patellar and achilles), and ligaments (anterior talofibular ligament) have been observed in these rare studies. Although "soft tissue stiffness meter" and "strain elastography with diagnostic ultrasound" methods seem to be useful in measurements, it has been emphasized that they need validation and association with clinical scenarios. (24,25)

## 4. SYSTEMIC PROBLEMS ASSOCIATED TO HYPERMOBILITY SYNDROME

Individuals with hypermobile joints present to healthcare providers with a wide range of symptoms (Table 3). It is noteworthy that individuals with hypermobility are associated with some clinical conditions such as anxiety, chronic pain, temporomandibular joint problems, autonomic problems, mitral valve prolapse, gastrointestinal diseases, gynecological problems, degenerative joint diseases, and osteoporosis in older ages. Although these relationships have been frequently examined in the literature and found to be significant by some authors, they have been reported as "variant of normal" or " too weak to cause disease" by some other authors(26).

### *4.1 Musculoskeletal System:*

The most common complaints in hypermobile individuals are musculoskeletal complaints. Pain is often the main complaint. Causes of pain include injuries due to overuse of joints with increased range of motion, recurrent dislocations, muscle imbalance and spasms secondary to postural dysfunction or scoliosis, muscular trigger points, sprains, tendinitis, bursitis, and tendon ruptures. Even if there is no pain, patients can also apply for complaints such as trauma, injury, joint dislocation, posture asymmetry, muscle weakness. All these symptoms are actually the most common patient group that individuals dealing with the musculoskeletal system encounter in daily practice. The important thing here is that the healthcare professional can distinguish whether the patient has hypermobility and/or hypermobility syndrome. Making this distinction will be the main guide in conservative or invasive treatment interventions to be given, recurrence, prognosis and patient education.(17,27–31)

### *4.2 Autonomic System:*

One of the systems most affected in hypermobile individuals is the autonomic nervous system, especially cardiac dysautonomia is common. Cardiac dysautonomia presents with orthostatic hypotension and/or Postural Orthostatic Tachycardia Syndrome (POTS). In these patients, complaints such as tachycardia, low blood pressure while standing, edema, exercise intolerance, presyncope/syncope, thermoregulation disorder, bruises on the skin, anxiety, brain fog, concentration problems, chronic fatigue and sleep disorders can be seen. (32) Postural orthostatic tachycardia syndrome (POTS) is also known to be associated with hypermobility. There are many publications in the literature that support this association. In a study examining large groups of patients diagnosed with POTS, researchers found that more than one-fifth of individuals with POTS had EDS and more than one-third had HSD.(33)

### *4.3 Cardiovascular and Respiratory System:*

Cardiovascular system complaints are common in hypermobility syndrome. Mitral valve prolapse (MVP) and aortic root dilatation were included in the hEDS International Classification Criteria in 2017.(8) Dizziness, palpitation and chest pain are the most common complaints in these patients. These individuals may have less cardiovascular fitness. Cardiac dysautonomies (POTS and orthostatic hypotension) are common. Although these individuals also have additional

abnormalities, such as traces of valve insufficiency, low-grade diastolic dysfunction, and minor pleural effusions, they often do not lead to a significant cardiac clinical condition. Many studies examining the cardiac status of these individuals reported that ECG findings did not change significantly compared to non-hypermobile individuals.(34) In a study conducted with patients with fibromyalgia, the frequency of MVP was 9 times higher in those with benign joint hypermobility syndrome.(35)

### *4.4 Gastrointestinal System:*

Gastrointestinal system problems are one of the most common reasons for hypermobile individuals to apply to health services. Chronic gastritis, heartburn, rectal prolapse and herniations can be given as examples of gastrointestinal system findings that are common in hypermobility. Irritable bowel syndrome is a syndrome with constipation and diarrhea attacks, nausea, gastroparesis, food sensitivity, bloating and abdominal pain, and its frequency is increased in hypermobility. In a study evaluating a large group of patients who applied to the gastroenterology clinic with the complaint of burning in the upper gastrointestinal tract, it was found that one third of these individuals had joint hypermobility.(7) In addition to all these, somatic symptoms that can affect functional gastrointestinal symptoms are also common in these individuals, which complicates the management of patients.(36)

### *4.5 Immune System:*

The relationship between immune system functions and hypermobility and EDS is not fully known. The relationship between mast cell-related conditions and hypermobility syndromes is studied in the literature. Reviews evaluating large patient groups on this subject have found that mast cell related disorder or mast cell activation Disorder (MCAD) is associated with many systemic and local findings of EDS and HSD patients. This supports the idea that mast cells may play a critical role in the development of the EDS clinic.(37)

### *4.6 Skin Involvements:*

The dermis layer, which is 70% of its dry weight is collagen, is often held responsible for skin findings in hypermobile individuals. Hyperextensible skin, poor wound healing, easy bruising, scars are common cutaneous and subcutaneous findings in individuals with hypermobile EDS. In ages around

puberty, cracks may occur on the skin. Paradoxically, striae gravidarum is often absent during pregnancy.

**Table 3.** Systemic Signs of JHS, HSD and hEDS

| | |
|---|---|
| Musculoskeletal | Bones & Joints<br>• Frequent or recurraint subluxations, dislocations<br>• Scoliosis<br>• Decreased BMD*<br>• Persistent joint pain<br>Soft Tissues<br>• Sprains<br>• Tendinitis, tendinosis, tendon ruptures<br>• Bursitis<br>• Fasciitis<br>• Muscles: spasms, trigger points, reduced muscle strength |
| Autonomic | Cardiac Dysautonomia<br>• Orthostatic Intolerance<br>• POTS**<br>Raynaud Syndrome |
| Cardiovascular and Respiratory | Aortic dilatation<br>Mitral valve prolapse<br>Varicose veins<br>Dysfunctional breathing<br>Asthma like symptoms<br>Reduced cardiorespiratory fitness |
| Dermatological | Hyperextensible skin<br>Scars<br>Poor wound healing<br>Easy bruising |
| Gastrointestinal | Gastroesophageal reflux, chronic gastritis, heartburn<br>Irritable bowel syndrome – constipation/diarrhea, bloating, gastroparesis, food sesitivities, nausea, abdominal pain<br>Rectal prolapse<br>Hernias |

| | |
|---|---|
| Neurological | Central sensitization-Fibromyalgia, hyperalgesia<br>Headache, migraine, dizziness<br>Paresthesias, nerve entrapments<br>Restless leg syndrome<br>Poor proprioception - frequent falls<br>Motor delay in children |
| Mental or Cognitive | Poor motor planning / coordination<br>Anxiety<br>Panic disorder<br>Depression<br>Memory or concentration problems |
| Urogenital | Urinary incontinence<br>Prolapsed bladder, cervix or uterus<br>Urinary tract infections<br>Dysmenorrhea, endometriosis, vulvodynia, pelvic pain, painful intercourse |
| Immune | Mast cell activation syndrome (thought to be related many systemic symptoms of hypermobility syndromes) |
| Nonsystem | Insomnia<br>Sleep Disturbance<br>Chronic fatigue |
| | *BMD: Bone Mineral Density |

## 5. DIFFERENTIAL DIAGNOSIS OF JOINT HYPERMOBILITY SYNDROME

### *5.1 Marfan Syndrome*

Marfan syndrome is an autosomal dominant multisystem connective tissue disorder. It is characterised by ectopia lentis, aortic dilatation, moderate joint hypermobility and marfanoid habitus. Marfan Syndrome is usually associated with mutation in fibrillin-1 gene, and sometimes in TGF-ßR-1 or 2.(38) People with Marfan syndrome are often quite tall and thin. Arms, legs, fingers and toes are disproportionate and may appear too long compared to the rest of the body. Their joints may be weak and easily dislocated. People with Marfan syndrome often have a long, narrow face and their palate may be higher than normal. Features of the Marfanoid habitus are;

- Arachnodactyly
- Scoliosis

- Pectus excavatum/carinatum
- Span/height ratio ≥1,03
- Crown/pubis:pubis/floor ratio <0.89
- Hand: height ratio >11%
- Foot: height ratio >15%

### *5.2 Osteogenesis Imperfecta*

Osteogenesis imperfecta (OI) is another autosomal dominant genetic disease. Patients have varying degrees of bone fragility, recurrent fractures, blue sclera, short stature, and moderate hypermobility.

### *5.3 Other Types of Ehler's Danlos Disease*

Ehler Danlos Syndrome consists of a genetically and clinically heterogeneous group of syndromes. Its main clinical features are joint hypermobility, skin hyperextensibility (easy bruising, delayed wound healing resulting with atrophic scars) and generalized connective tissue fragilitiy. Many authors consider JHS synonymous with EDS-Hypermobility Type (Type III). When making the differential diagnosis of hypermobile individuals, differential diagnosis should be made with the classical type, vascular type, type I, II or IV of EDS.

## 6. GENETICS AND PATHOGENESIS

When twin studies on hypermobility are examined, genetic variance of up to 70% is observed. Basically, the opinion in the literature is that JHS is caused by a defect in the genes encoding connective tissue collagen and proteins and has an autosomal dominant inheritance. (38)

Type I collagen has a high tensile strength and is densely located in the tendon joint capsule, bone and skin. Type III collagen is more flexible and disorganized and is concentrated in the intestines, skin, and blood vessels. Collagen content appears to be increased in favor of Type III collagen in JHS patients. As a result of this, clinical symptoms may be encountered in individuals with JHS as a result of relatively lower tensile strength and more fragile tendons and ligaments, skin and vascular structures.(5,39)

Today, there appears to be research on the genetic heterogeneity of these patients and/or the interactions of multiple gene loci. Examining immunofluorescence analyzes and gene expression profiles from fibroblast cell cultures taken from the skins of 5 women diagnosed with JHS/EDS-HT, Chiarelli

et al. detected irregularities in several matrix structural components such as fibrils, tenascins, elastin, collagen, fibronectin and their integrin receptors. Their transcriptome analysis indicated confusion in a number of genes that affect many signaling cascades required for extracellular matrix architecture, homeostasis regulation, cell adhesion, immune/inflammatory pain responses, and redox balance. Ultimately, they profiled the dysregulated pathways and gene expression of skin fibroblasts in JHS/EDS-HT patients that correlated well with systemic phenotype.(40)

With a detailed algorithm proposed by Malfait, Hakim and Graham for the differentiation of hereditary connective tissue diseases, the diagnosis of the patient presenting with hypermobility can be made. According to this algorithm, when the patient's clinical findings and family history are evaluated, if other Ehlers-Danlos Syndrome (non-Type III) subgroups or Osteogenesis imperfecta is suspected, the definitive distinction is to take a skin biopsy and look at the type I-II-V collagen structure, and molecular analysis. Molecular analysis will be possible by investigating the presence of COL1A1, COL1A2, COL3A1, COL5A1 and COL5A2 mutations. If Marfan Syndrome is suspected, the diagnosis can be made by performing FBN1 molecular analysis in the blood sample. However, except for the FBN1 analysis, the detailed laboratory-based diagnostic studies mentioned are almost impossible to implement in daily practice. Figure 2 shows the algorithm created by Marfait et al. This algorithm may guide health professionals in the clinical and laboratory-based distinction of individuals with joint hypermobility.(38)

## 7. EFFECTS OF NUTRITION ON HYPERMOBILITY

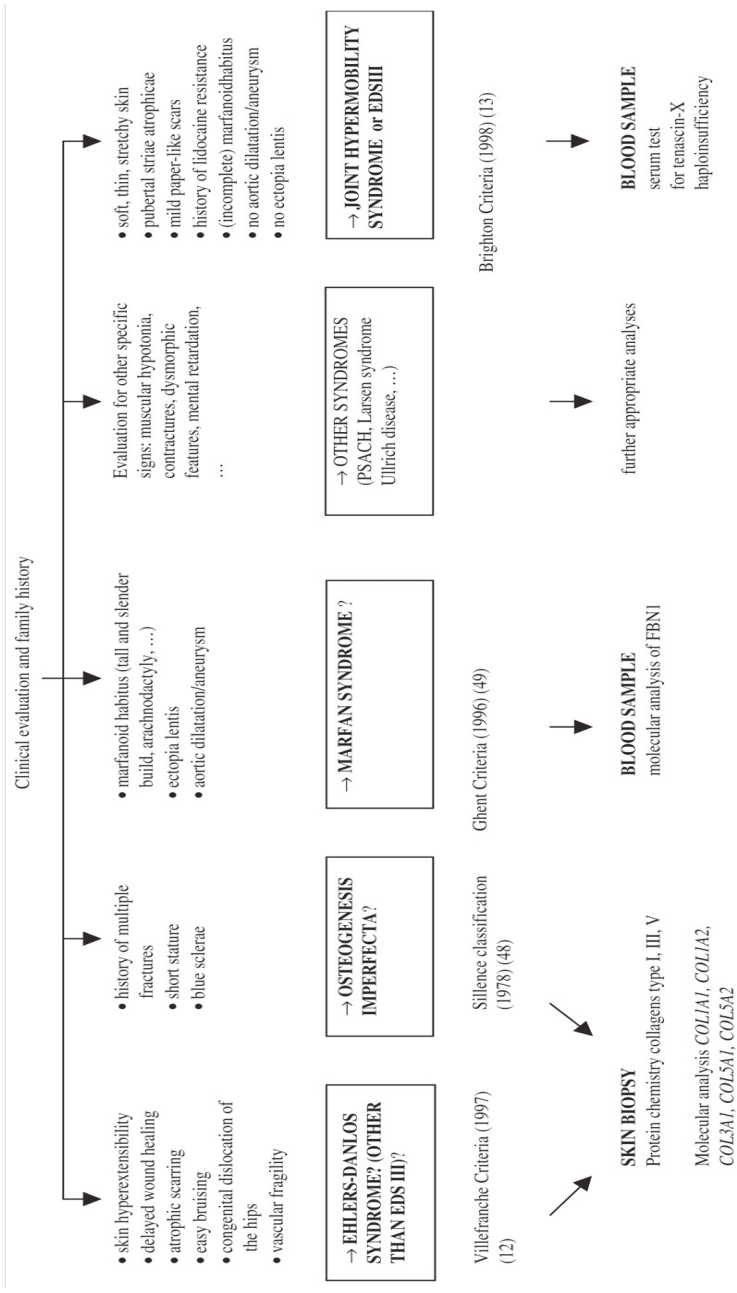
Investigating the possible contribution of nutrition to joint laxity, Indian authors found that the prevalence of hypermobility in children with high-grade malnutrition exceeded 60%, as a result of the evaluation of 869 children aged 3-19 years.(41) However, our knowledge about the effects of nutrition or nutritional support preparations on joint laxity is not sufficient in the literature.

## 8. NON-PHARMACOLOGICAL TREATMENT, EXERCISE AND REHABILITATION

Although joint hypermobility syndrome is a multisystemic condition, joint laxity and pain are prominent in patients.(29) While regulating the exercise and lifestyle of these individuals, it is basically aimed to protect and strengthen the control and stability of the joint. For this purpose, posture, core stabilization,

muscle strengthening and proprioception are important. Because of the increased susceptibility to oint injuries, injury prevention strategies are important.

**Figure 2.** Diagnostic Algorithm For Patients With Joint Hypermobility (38)




*Malfait et al. 2006*

As the frequency of injuries has increased in daily life, injuries may occur during physiotherapy or home exercises. Approximately one in three individuals with joint hypermobility reported physiotherapy-related injury. Although these injuries cannot be directly associated with physiotherapy, it has been observed that the perception of being injured by physiotherapy can occur in the individuals mind. Therefore, physiotherapy approaches and management can be challenging in these individuals.(42) Although periarticular muscle strengthening is recommended rather than stretching exercises for hypermobile individuals, patients tend to like stretching exercises. These patients should be informed that short-term stretching may be beneficial in pain and spasms, but that stretching may increase joint laxity and injuries in the long term.

Weak posture components such as pes planus, genu recurvatum, pelvic anterotation, lumbar hyperlordosis, dorsal hyperkyphosis, and shoulder internal rotation are common in hypermobile individuals. Poor posture, stance and swing phase abnormalities, and lower extremity proprioception disorders can lead to increased energy consumed in walking, resulting in fatigue. Pain, fatigue and fear of injury are also common in these individuals, which may prevent them from exercising, and kinesiophobia may develop. Pain, fatigue, and kinesiophobia may cause a decrease in the quality of life of these individuals. (28,29,43)

The effectiveness and evidence levels of each of physiotherapy and occupational therapies in individuals with hypermobility syndrome have not been fully determined, but researches are continuing on this subject.(27,29,44–46) The information obtained in recent studies is that exercise programs aimed at improving motor functions, improving posture and increasing core stabilization are beneficial. The use of adaptive devices (insoles, corsets, etc.), manual therapies, complementary medicine practices (yoga, pilates, etc.) can contribute to functional well-being, but the level of evidence is still insufficient. Respiratory exercises can be beneficial for dyspnea. There is a need for rigorous, multicenter, randomized controlled studies on the clinical results and cost analyzes of physiotherapy applications.(27,44)

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CHAPTER VIII

# HEAT SHOCK PROTEINS (HSP) EXPRESSED IN THE LUNG AND THEIR FUNCTIONS

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## 1. INTRODUCTION

Heat shock proteins (HSP) are synthesized in body cells, cytosol and cell organelles. These proteins, which are shaped due to increased high temperature, also occur in organ or tissue damage. These are small proteins with different molecular weights. (1) These proteins were discovered in the early 1960s. This was recognized as a protein synthesized by the larvae of the fruit fly Drosophila by applying a temperature above body temperature. (2) These heat shock proteins were later found to exist in homologous form in prokaryotic and eukaryotic cells. They observed that the synthesis of these proteins can be triggered not only by heat shock but also by various other lesions. The common point of all mentioned cases is that the formation of HSP is due to the increase in the number of unsynthesized or mis-synthesized proteins in a cell. (3)

One of the main functions of this protein is to prevent undesirable interactions between proteins that cause aggregation. For this reason, they are also called molecular chaperones. These molecular chaperones belong to the family of HSPs. Because these proteins increase significantly in cells when exposed to high temperatures (approximately 42°C for at least 30 minutes). (4) Normal protein formation is accompanied by chaperones. For example, they can prevent polypeptide chain mis-synthesis and thus the formation of insoluble inclusion bodies when proteins are expressed. Other functions of HSP are also discussed, for example, they can perform many functions such as suppressing

apoptosis under physiological conditions by preventing activation of procaspases and preventing the formation of apoptosomes and inducing immunosuppression by inhibiting proinflammatory non-stress proteins. (5)

HSPs get their name from their molecular weight. HSPs are found in the cytosol, mitochondria, ER and nucleus. They typically have a relatively long half-life. HSP78, 75, 60, 10 are mainly found in cell organelles, while HSP110, 90, 73, 72, 20 are found in the cytosol and nucleus. (6)

## 2. HSP EXPRESSED IN THE LUNG

### *2.1. HSP32*

HSP32, also known as hemoxygenase (HO), belongs to the monooxygenase family. HSP32 catalyzes the oxidative cleavage of protoporphyrin heme with the help of NADPH and oxygen. So far, three isoforms of HO have been identified, which are products of different genes. Hemoxygenase type 2, HO-2 or HSP is constitutively expressed at 36 kDa and is mainly found in the central nervous system. Hemoxygenase type 3 (HO-3, 33 kDa) has low catalytic activity. Its physiological functions are not yet fully elucidated, but its involvement in heme binding is suspected. Inducible hemoxygenase type 1 (HO-1, 32 kDa) is ubiquitous in the organism and is one of the heat shock proteins. The functional significance of hemoxygenase-1 induction has not yet been clarified. Despite all this, results from several laboratories support the hypothesis that HO 1 induction has a significant effect on cellular protection against both heme and non-induced oxidative damage. (7)

Increased expression of HO-1 mRNA is also observed in cultured cell exposed to hyperoxia in vitro. They are also found in lung fibroblasts, pulmonary epithelial cells, peritoneal and alveolar macrophage cells. Induction of HO-1 mRNA in cultured cells in the absence of the in vivo environment and inflammatory response can transmit intracellular signals to induce hyperoxia directly or indirectly to induce HO-1 gene expression. (8) HO-1 is not only induced by the natural substrate heme molecule, but also by a wide variety of structurally different agents. In addition to heat shock and oxidative stress, UV light, heavy metals, endotoxins, prostaglandins and inflammatory cytokines are among the most important inducers. Therefore, HO-1 appears to be an enzyme that protects cells from oxidative stress. Due to the differential functionality of the inducers, HO-1 is also predicted to have an important effect in maintaining cellular homeostasis. Therefore, it is a highly conserved enzyme. These findings highlight the importance of hemoxygenase in regulating critical cell processes. (9, 7)

Inducible hemoxygenase is one of the most important protective proteins in the organism due to its anti-inflammatory, anti-apoptotic, antioxidant and anti-proliferative properties. Protection against pro-oxidative agents due to induction or overexpression of HO-1 has been demonstrated in various cell types. (9) This protein has an important role in the cell-protective and modulating effect, especially on inflammatory processes. (10) Some studies have also shown that the enzyme has an effect on the development and progression of malignant tumor diseases. (11, 12) It is also increased in a number of different tumors such as bronchial carcinoma, renal cell carcinoma, prostate carcinoma and sarcoma. They also observed that there are different localizations of hemoxygenase between tumor and non-tumor tissue. Although hemoxygenase is located in the endoplasmic reticulum, it reaches the cell nucleus via proteolytic cleavage, where it promotes tumor progression and invasion regardless of enzymatic activities. In addition, hemoxygenase induces angiogenesis by causing expression of angiogenic factors. (13)

### *2.2. HSP47*

HSP47 has a small molecule structure. It is among the stress proteins. It is localized in the endoplasmic reticulum. It is a specific chaperone for procollagen and its levels increase during hyperthermia and cellular stress. (14, 15) Various experiments combined with cross-matching and immunoprecipitation analysis found that HSP47 acts as a collagen-specific molecular chaperone during the formation and secretion of procollagen in the endoplasmic reticulum. HSP47 has an important role in the formation of collagen fibrils during the development of pulmonary fibrosis. (16) They stated that due to the selective inhibition of HSP47 expression, it also decreased in collagen synthesis. (17) High levels of heat shock protein, particularly cytoplasmic overexpression of HSP47, are found in cancerous lung tissue compared to normal lung tissue. In addition to overexpression, they also detected autoantibodies against HSP40 in serum. (18) Higher levels of HSP47 have also been found in metastasizing malignant diseases. (19)

### *2.3. HSP60*

HSP60 is in the mitochondrial enzyme complex. It is a mitochondrial matrix protein that ensures the synthesis and proper attachment of polypeptides. It is especially involved in the delivery of mitochondrial enzymes out of the cell. One possible cause of deficiencies in many enzymes is a defect in

mitochondrial biogenesis. (20) It is known that partial HSP60 deficiency causes neonatal mitochondrial myopathies and enzyme accumulation, while complete HSP60 deficiency is lethal. (21) Increased expression of this family of proteins is observed in normal bronchial epithelium and alveolar macrophages, as well as in bronchial carcinomas of various histological types. It is also stated that it occurs in a direct immune response against tumor cells. HSP60 appears to increase macrophage and neutrophil cell activation in chronic lung disease. (22)

### *2.4. HSP70*

HSP70 is found in almost all organisms, in all cellular compartments and even in extracellular spaces. (23) The main function of HSP70 is to be a molecular companion. In cells with normal metabolic activity, HSP70 takes part in two different functions. Its first task is to take part in protein synthesis. Its second task is to establish the translocation of proteins into different cell compartments. HSP70 is also involved in the regulation of the cell cycle. Binding to monocytes leads to the release of proinflammatory mediators, TNF alpha, IL-6 and IL-1 beta. (24) That is, it serves as a signal not only for the protective function, but also for stimulating the immune system.

HSP70 expression is also significantly increased in the airway epithelium and alveolar macrophages of asthmatic patients. The expression level is related to the severity of the disease and the level of eosinophils in the bronchial fluid. (25) Different studies have shown that HSP70 has a role in various aspects of the immune system. Relatively large amounts of HSP72 and HSP90 have been found in the columnar epithelium of the upper respiratory tract in healthy non-smokers. (26) The involvement of HSP70 in apoptotic processes is diverse. It affects both AIF (apoptosis inducing factor) independent and dependent processes and different time points in apoptotic signaling pathways. (27) In addition to physiological functions, it supports processes that serve to protect the cell or repair damage caused by stress. HSP70 is expressed not only in physiological conditions but also in various tumor diseases. Increased concentration of HSP70 is associated with tumor proliferation, metastasis and poor prognosis. (28) The result of increased HSP70 expression in tumor cells is the ability of this molecule to protect against apoptosis. The expression of HSP70 on the cell surface of tumor cells is dependent on the cytoplasmic amount. In vitro studies report that HSP70 protects monocytes and other cells from hydrogen peroxidase, apoptosis and denaturation temperature. (29)

### *2.5. HSP90*

This family of proteins has important roles in stress tolerance and protein synthesis. These proteins belong to ubiquitous molecular chaperones. HSP90 synthesis is increased in response to cellular stress and in tumor cells of various histological types of bronchial carcinoma. HSP90, together with some accessory proteins, binds to newly synthesized receptors of the steroid hormone family in a ATP-dependent manner and keeps them in a conformation that prevents their transport into the cell nucleus. Heat shock proteins are removed after binding to the respective hormone. After the hormone-receptor complex is formed, it can enter the nucleus and be activated. Probably the most important function of HSP90 is to form a superstructure on which chaperones of the HSP90, HSP70, HSP60 group and other proteins newly synthesized or conformationally disrupted by various damages are attached. This allows them to convert to their natural, biologically active form. (30) HSP90 can prevent protein aggregation and promote expression in vitro. (31) However, in vivo, it works in conjunction with a multiprotein complex containing a number of related proteins. (32)

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CHAPTER IX

# A PHENOMENON THAT HAS BECOME A THREAT TO ANESTHETISTS: CHILDHOOD OBESITY

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## INTRODUCTION

In today's world, the high prevalence of childhood obesity is recognized as a global public health priority. Based on statistical studies, considering that by 2020, 38.9 million children under the age of 5 are moderately or severely overweight; it can be said that cases of severe pediatric obesity have continued to increase in the last few years, especially in high-risk groups. (1)

In the text published by the World Health Organization on World Health Day in this year, more than 1 billion people worldwide stated that they are obese. According to the World Health Organization, 650 million of this number are adults, 340 million are adolescents and 39 million are children, and the number is increasing day by day. WHO estimates that by 2025, approximately 167 million people, including adults and children, will become less healthy because they are overweight or obese (2). It is estimated that obesity with the prevalence of pediatric obesity exceeding 20% in high-income countries. (3,4)

Therefore, considering its relationship with increased morbidity and mortality and the risk of its continuation throughout adulthood, more diligence should be paid to the early diagnosis and treatment of pediatric obesity.

Obesity-related comorbidities are generally thought to affect children before they reach adulthood. (5) In evaluations related to pediatric obesity; as well as serious emotional problems; psychosocial complications such as worsening quality of life, unhealthy tendencies for weight control, depression and poor self-image appear to be associated with obesity.

From the point of view of anesthesia; considering the increasing prevalence of overweight in children, anesthesiologists are likely to see a corresponding increase in the proportion of these patients who present for surgical procedures and anesthesia.

However, it remains unclear whether obese children are prone to the same comorbidities and anesthetic risks as adults. Therefore, pediatric obesity is also special in terms of anesthesia due to accompanying comorbidities.

## 1. DEFINITION OF PEDIATRIC OBESITY

The International Classification of Diseases 11 (ICD-11) defines obesity as "a chronic complex disease defined by excessive adiposity that can impair health (6)

Body mass index is the most commonly used indicator to evaluate overweight and obesity in children and varies according to age, gender and maturity in children and young adolescents. (7) Efficacy was measured as change in BMI, because it is the most commonly used indicator to assess overweight and obesity in children and defining overweight vary by age and gender in infants, children and adolescents (7,8).

Table 1: Classification of the BMI in children and adolescents according to the American Center for Disease Control and Prevention

(CDC) and the World Health Organisation (WHO)

| BMI classification | WHO perc. scores for children and adolescents | CDC perc. scores for children and adolescents |
|---|---|---|
| **Underweight** | < 15 | < 5 |
| **Normal weight** | ≥ 15 to < 85 | ≥ 5 to < 85 |
| **Overweight** | ≥ 85 to < 97 | ≥ 85 to < 95 |
| **Obesity** | ≥ 97 | ≥ 95 |

**Table 1** is referenced from the publication of Kolimechkov et al [9].

Abbreviations: perc: percentile

## 2. ETIOLOGY AND RISK FACTORS

Obesity in most cases; it is a multifactorial disease due to obesogenic environments, psycho-social factors and genetic variants. These factors, including genetic ones, account for less than 10% of pediatric obesity (10).

### *2.1 Genetic Risk Factors*

Studies have shown that childhood obesity is associated with parental obesity. Apart from their family's genetic heritage, children share their habitat and especially their eating habits. Therefore, outside of certain genetic diseases, it can be difficult to determine genetic risk.

Parents are role models for children's food choices and eating behaviors; Children learn eating habits from their parents at a very young age. Therefore, increasing obesity levels in parents result in increased obesity levels in children. In most cases of chilhood obesity, the cause is exogenous. Endogenous causes constitute a limited part of these patients. Nevertheless, the existence of monogenic and polygenic causes in the development of obesity should not be ignored.

Syndromic, hormonal and monogenic disorders listed below may predispose to childhood obesity. (11)

- Monogenic disorders

– Leptin deficiency, melanocortin 4 receptor mutation, Proopiomelanocortin deficiency

- Syndromic disease

– Prader-Willi syndrome, Alstrom syndrome, Albright's hereditary osteodydrophy, Trizomy 21, Cohen syndrom (Frontometaphyseal dysplasia), Bardet –Biedl, WAGR (Wilms' tumor, aniridia, genitourinary anomalies, retardation

- Hormonal disorders

– Cushing's syndrome, precocious puperty, dyslipidemia, polycystic ovary, hypothyroidism

### *2.2 Behavioral Variability*

In general, it would not be wrong to say that behavioral risk factors such as the child's physical activity, nutritional habits and diet, sleep patterns and stress also play an important role in childhood obesity. In recent years, there has been increase in studies showing that behaviors that make children accustomed to inactivity, such as playing computer games and watching television, are associated with development of obesity.

### *2.3 Nutrition*

Nutritional habits are one of the factors that play an important role in childhood obesity. Certain dietary habits in childhood trigger childhood obesity and can cause children to become obese child, adolescents and adults.

Although increased total energy intake is seen as a risk factor in the development of childhood obesity, it is not supported by sufficient evidence. Beverage selection is an important issue.In particular, excessive consumption of sugar-sweetened beverages, fruit juice, and sodas has been associated with childhood obesity (12).

### *2.4 Physical Activity*

In general, it would not be wrong to say that decreased physical activity among children is associated with obesity. (13)

In recent years, there has been an increase in studies showing that being more engaged in sedantary behaviors, particularly watching TV or playing video games, is associated with an increased risk of future obesity.

Therefore, efforts to lower the sedentary activity time and increase the physical activity will play a key role to preventing obesity development. (14)

### *2.5 Stress and Sleep Factors*

Several types of stress which we can call personal, parent and family related, can be independent sources of stress. Each of these factors may be positively associated with the risk of childhood obesity, especially if they cause chronic stress. This can occur during childhood and continue into adulthood.

While we do not have much evidence; it has been observed that shorter sleep duration is associated with chilhood obesity. (15) It is stated that increasing the sleep time by shortening the time spent in front of the screen in positive home routines is inversely related to childhood obesity. (16)

## 3. PEDIATRIC OBESITY RELATED CO-MORBIDITIES

In recent years, considering the studies carried out obesity-related comorbidities seem to be described in more detail for adults. However, with the increase in studies on pediatric obesity, it is seen that comorbidities become more common in children and the risk of side effects due to anesthesia and surgical procedures increases.

Diseases such as hypertension, asthma, obstructive sleep apnea (OSA), hyperlipidemia, insulin resistance and adult heart disease are among obesity-related multi-organ pathophysiological changes and co-morbidities (17) These diseases have been associated with obesity not only in adults but also in children, and the risk of developing bronchial hyperreactivity, asthma and obstructive sleep apnea in obese children is significantly increased. (18,19) These pathophysiological changes on organs and systems and co-morbidities significantly increase the risk of anesthesia, especially in obese children who will undergo surgical procedures and require anesthesia.

Desaturation attacks up to 70% during sleep in children with obstructive sleep apnea pose a serious risk in anesthesia applications due to left ventricular dysfunction and pulmonary hypertension; In addition, obese children are more prone to respiratory tract infections than normal weight children, which increases airway problems in the perioperative period (20).

In addition to these comorbidities, recent epidemiological studies have shown that with childhood obesity; showed an association between leukemia, Hodgkin lymphoma and increased risk of cancer types, some of which are more common in adulthood (such as colorectal, breast cancer). (21) Pathophysiologic changes and co-morbities in multiorgan system associated with childhood obesity are shown in Table 2. (22)

**Table 2.** Pathophysiologic changes and co-morbities in multiorgan system associated with childhood obesity

| System and effects of obesity |
|---|
| **Cardiovascular** |
| - Increased left ventricular mass |
| - Pulmonary hypertension |
| - Hypertension |
| - Dislipidemia |
| - Increased risk of coronary artery disease |
| **Respiratory** |
| Increased |
| - Work of breathing |
| - Risk of upper airway |
| - Incidence of Astma |
| - Risk of OSA (obstructive sleep apnea) |
| Decreased |
| - Lung and chest wall compliance |
| - Critical airway closing pressure |
| **Hepatik and Renal System** |
| - Cellüler hepatocytes damage |
| - Non-alcoholic fatty liver disease |
| - Non -alcoholic steatohepatitis (NASH) |
| - Increase in kidney size |
| **Metabolic syndrome*** |
| - Central obesity |
| - Elevated triglycerites, low high density lipoprotein |
| - Elevated fasting glucose |
| *presence of 3 of the 5 criteria above is among the definitions used in the diagnosis of metabolic syndrome |
| **Endocrine system** |
| - Increasing Type 2 diabetes mellitus |
| - Growth and thyroid hormone deficiency |
| - Impaired glucose tolerance |

(Table is remodified from V. Chidambaran et al (22).

## 4. COMMON SURGICAL PROCEDURES IN OBESE CHILDREN

Treatment of severe obesity requires a lifelong multidisciplinary approach (lifestyle changes, nutrition, medications). In patients who do not respond with these approaches, it may become a necessity in surgical applications. Children presenting for surgery are a heterogeneous group. Obese children often require surgery due to problems related to otolaryngology, orthopedic and general surgery. Bariatric surgical interventions, which have increased especially in recent years (especially in diabetic obese children), should be added to these surgical procedures (23)

## 5. PREANESTETIC EVALUATION

There is a motto especially emphasized in the anesthetic approach of pediatric patients: "Children are not miniature adults".

Children's differences from adults in terms of anatomical, physiological and pharmacological features are among the factors to be considered in the anesthetic approach. These differences and some surgical procedures performed more frequently in childhood (ear, nose and throat surgery, endoscopic bronchoscopic procedures, etc.) are associated with an increased risk of anesthetic complications, especially in obese children.

As in all other patients undergoing surgery, the anesthetic approach begins with preoperative evaluation in obese children. The opinion that obese children are not affected by comorbidities associated with obesity in adults has changed with recent studies. Conversely, in a study by Tait et al, (24) it was shown that obese children are affected by co-morbidities such as hypertension,asthma, type II diabetes obstructive sleep apnea and gastroesophageal reflux as adults.

Therefore, for safe anesthesia applications, a comprehensive preoperative evaluation is required in pediatric patients because of obesity-related multi-organ pathophysiological changes.

### *5.1 Obstructive Sleep Apnea*

All obese children should be evaluated for signs of sleep-disordered breathing. The prevalance of OSA in obese children is 13-59 % compared with 2-3% normal weight children. (25)

The ASA practice guidelines for the management of patients with OSA recommend that all adult and pediatric patients over 1 year of age, be screened for OSA prior to undergoing surgery. (25) If the child has OSA, it is important

to determine its severity and perioperative anesthesia management. The use of bi-level positive airway pressure, home monitoring modalities, supplemental oxygen administration, and the need for positioning during sleep should be questioned. Overnight, polysomnography (PSG) is currently considered the gold standard for diagnosing pediatric OSA. (26) But, as it is not feasible to perform PSG in all obese children, blood gas analysis, normal sleep studies and other pulmonary function tests may be needed.

### *5.2 Organ and System Evaluation*

Obese children may have significant desaturation episodes, reaching 70% during sleep (27).

In these patients group and in patients with systemic hypertension or signs of right ventricular dysfunction 12 lead EKG and ECO stress test evaluation is required. Cardiac angiography and cardiac imaging may be required in addition to these tests, especially in patients with dilated cardiomyopathy and pulmonary hypertension.

Preoperative evaluation in obese children should include a careful physical examination of organs and systems, as well as necessary laboratory and imaging techniques.

Physical examination of the upper airway in these children should include head and neck mobility, jaw movement and mouth opening, and oropharyngeal examination. Fatty tissue accumulation in the upper respiratory tract, an increase in the tendency to collapse of the pharynx, a short and thick neck, and limited neck extension are seen in obese children.

Especially in major surgical procedures such as bariatric surgery, laboratory tests should include complete blood count, liver, kidney, and thyroid function tests, as well as lipid profile and hormonal evaluations (such as parathyroid hormone, adrenocorticotropic hormone (ACTH), cortisol).

The blood group and coagulation profile should be studied, and the adequacy of glycosylated hemoglobin and fasting blood sugar and glucose control should be evaluated. Diabetic complications, particularly heart disease, kidney disease, and autonomic dysfunction should also be evaluated.

Preoperative fasting guidelines and preoperative instructions for obese pediatric patients are similar to non-obese children. Because the gastric fluid volume corrected for ideal body weight (IBW) in children is 1 ml kg-1 on average, independent of BMI and fasting range. In their study, Cook et al. stated

that obese pediatric patients can also be allowed to drink clear liquids 2 hours before the surgical procedure. (28)

## 6. PERIOPERATIVE ANESTHETIC MANAGEMENT

A careful preoperative evaluation is one of the factors that play an important role for anesthetists in the perioperative management of obese children. However, the period starting with the induction of anesthesia and extending to the safe postoperative follow-up of the patient can be challenging for anesthesiologists.

### *6.1 Anestetic Drugs and Anesthesia Induction*

Obesity is associated with physiological and pharmacological changes affecting drug absorption, metabolism and elimination. (29)

The volume of distribution, which determines loading dose and onset of action, may be altered in severely obese children. Drug clearance, which is one of the factors that determine the maintenance dose of the drug, may alter in obese children, although it is not certain. (30) It is important to identify the most appropriate scalar (weight measure scaled to which the drug is dosed) to use in obese children.

In a review published by Harskamp-van Ginkel MW et al. it has been reported that approximately two-thirds of drugs prescribed to children with obesity remain at sub-therapeutic or supra-therapeutic concentrations, leading to drug toxicity and risk of treatment failure. (31)

In pediatrics, it is typically used to determine the drug dose by calculating the patient's total body weight (TBW), ideal body weight (IBW), and lean body weight (LBW). Dosage recommendations are derived from pharmacokinetic data in children. (32) However, these weight scales may differ from actual body weight because dosing based on total body weight (TBW) can lead to overdose.

For most obese children, iv induction is preferred to perform rapid sequential induction due to the increased risk of acid aspiration, whereas venous access may be more difficult secondary to increased subcutaneous fat deposition in obese children. Nevertheless, if anesthesia induction will be done intravenously; it should be noted that drug dosing in obese children poses a particular challenge.

Additionally,in most children, prolonged and repeated cannulation attempts (even when topical techniques are used) are difficult and distressing for most children. In these cases, the route of induction with inhalation agents may

need to be considered. However, it should be kept in mind that gas induction may cause an increased risk of airway complications. For this reasons, dose recommendations are made for anesthetic drugs, taking into account these characteristics.

Considerations for specific anesthetics in obese pediatric patients can be seen in Table 3

**Table 3** Recommendations for dosing of commonly used anesthetic drugs

| Drug | Recommended scalar for dosing | Comments |
|---|---|---|
| Propofol | | |
| Induction dose | ?LBM | Titrate to clinical effect for induction |
| Maintenance infusion | TBW (allometric) | Allometric weight = 70 × (TBW/70)$^x$ where exponents (x) of 0.72 to 0.8 have been proposed |
| Fentanyl | LBM/PK | Lipophilic, elevated $V_d$ in obese; clearance linearly related to PK mass |
| Remifentanil | LBM/IBW | PK unaffected in obese, but more side effects if dosed by TBW |
| Morphine | IBW | Hydrophilic, $V_d$ does not change with obesity, does not accumulate in body fat |
| Sufentanil | TBW | Lipophilic; increased $V_d$ in body fat, but risk for accumulation. Loading dose based on TBW, decrease maintenance doses |
| Alfentanil | LBM/TBW | |
| Succinylcholine | TBW | May max out dose at 150 mg |
| Non depolarizing muscle relaxants (vecuronium, rocuronium, cisatracurium) | IBW | No differences in PK parameters between lean and obese patients. However, prolonged duration of action when dosed by TBW |
| Benzodiazepines | Loading: ABW/LBM<br>Maintenance: IBW | Lipophilic; *CYP3A4* metabolism decreased in obese; Higher than IBW, less than TBW – no particular scalar has been studied. Daily clinical re-titration recommended |
| Lidocaine | | |
| Initial dose | TBW | Intermittent doses may be preferable to infusions. |
| Maintenance infusion | IBW | Monitor clinically |
| Ketamine | - | Lipophilic; Limited PK studies in obese |
| Acetaminophen (oral) | - | Similar plasma levels with normal doses as non-obese |
| Acetaminophen (intravenous) | - | Lower levels achieved with usual doses in obese, but higher *CYP2E1* mediated metabolite production may preclude dose adjustment. Check liver enzymes |
| Ibuprofen | - | Vd increased in obese; Increase dose without changing dosing intervals |
| Neostigmine | TBW | Maximum dose 5 mg |

Abreviations: TBW: Total body weight; IBW: Ideal body weight; LBM:Lean body mass; PK Mass: Pharmacokinetic mass; - no recommended scaler; Vd: volume of distrubition; CYP3A4-CYP2E1: Hepatic enzymes

Table 3 (reproduced with Chidambaran V et al reference [22])

### *6.2 Intraoperative Period*

#### *6.2.1 Airway Management and Ventilation Strategies*

Fatty tissue accumulation in the upper respiratory tract in obese children and the increased tendency of the pharynx to collapse after induction of general anesthesia (especially in the presence of obstructive sleep apnea) may cause difficult mask ventilation. (33)

In the study of Nafiu et al (33); it has been reported that the frequency of difficult mask ventilation (7.4% vs. 2.2%, respectively), difficult laryngoscopy (1.3% vs 0.4%) and postoperative airway obstruction (1,6 % vs. 0.07 5) in obese children are higher than in normal-weight children.

Additionally, increased oxygen consumption, functional residual capacity (FRC) and decreased lung compliance in obese children may lead to rapid oxygen desaturation by impairing the tolerance capacity of the apneic period, especially in the supine position. (22)

Although children may not find face masks comfortable and not comply with this; adequate preoxygenation is recommended before induction of anesthesia in obese children as well as in obese adults. Effective pre-oxygenation in children; it can be defined as tidal breathing with 100% O2 for 3 minutes. (34)

In the follow-up of adequate ventilation in obese children who are planned to be followed up with spontaneous breathing in the intraoperative period, especially minute ventilation and end-tidal CO2 parameters should be closely monitored. In addition, CPAP is recommended for adequate oxygenation.

In obese children requiring controlled breathing may require higher ventilation pressures due to reduced lung compliance and more frequent positive pressure ventilation with the addition of PEEP to prevent alveolar collapse.

Protective ventilation strategy is a recommended technique to provide oxygenation, maintain normocapnia and prevent lung damage when controlled ventilation is required in obese pediatric patients as in adult obese patients. In this strategy, 6-8 ml/kilogram/(IBW) tidal volume, 0.5-0.8 FiO2 value, PEEP and application of intermittent recruitment maneuvers are important for absorption atelectasis and oxygen toxicity. (35)

Due to the possibility of difficult airway, necessary preparations should be made before induction of anesthesia. Although the use of a laryngeal mask airway (LMA) is controversial in obese children due to the risk of aspiration and the need for higher ventilation pressures, it can be life-saving in case of difficult intubation.

The efficacy of laryngeal mask airway (LMA)-Supreme in 100 obese boys was examined in a study by Tian Y et al., and concluded that LMA supreme can be easily deployed and effectively used for airway management in obese children undergoing minor surgery. (36)

### *6.2.2 Postoperative Period and Pain Management*

Obese children may experience airway problems such as laryngospasm, bronchospasm, and oxygen desaturation at a higher frequency during anesthesia induction and intraoperative period than their non-obese peers; which makes them a high-risk group for surgery. These airway problems we mentioned are also frequently encountered in the postoperative period and can prolong the stay in the post-anesthesia care unit (PACU) after the procedures. (33, 37)

Since airway problems are common, extubation should be performed while the child is awake after airway reflexes have returned completely. It should be kept in mind that oxygen may be needed in the PACU despite following these instructions.

In order to prevent airway obstruction and to minimize diaphragmatic splint, the patient should be placed in the appropriate position with the head slightly elevated using several pillows and constantly monitored with oxygen saturation monitoring.

In particular, children with a history of obstructive sleep apnea may need to be followed up in a highly dependent unit on the first day after surgery rather than outpatient follow-up. (38)

Although there is not enough evidence that it increases the risk of aspiration in awake children recovering after surgery; in these patients, it is important to prevent nausea and vomiting in order to provide a comfortable recovery and reduce the possibility of aspiration. The use of multimodal antiemetics will need to be considered.

One of the challenges for anesthesiologists is effective pain control in the postoperative period in obese pediatric patients. Although opioids are effective agents for pain management, they should be carefully titrated and used in obese children because of the risk of delayed airway obstruction. (39) Therefore, a multimodal approach to analgesia with the co-administration of nonopioid adjuvant analgesic agents and regional anesthesia is recommended. (40)

Although regional anesthesia techniques have reducing effects on postoperative atelectasis, venous thrombosis, blood loss and ileus development; It should be kept in mind that with the same volume of local anesthetic, more neuraxial spread and higher block levels can be seen and subclinical neuropathy may occur in diabetic patients. (41)

## 7. Conclusion

Childhood obesity is a complex and serious medical problem that represents the interaction of genetic, physical and environmental factors. And it continues to increase all over the world.

Early intervention in childhood is important as obesity associated with increased morbidity and mortality. Comorbidities often affect children before they reach adulthood. This situation brings a predictable increase in health expenditures, along with the need to pay more attention to the evaluation and treatment of obesity.

From the point of view of anesthesiologists, these children are at an increased risk of problems occurring at any stage of the anaesthetic process, some of which may be potentially life threatening.

Therefore, it is very important to understand the pathophysiological changes associated with severe obesity in obese children and to know the relevant pharmacological variability for commonly used anesthetic drugs.

Weight scales and dose recommendations are at the forefront of

maximizing the safe anesthetic management of these challenging patients.

In these patient population are needed improved guidelines for risk stratification, perioperative anesthetic management, and further studies evaluating the pharmacology of drugs for anesthetic administration.

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CHAPTER X

# THE EFFECT OF GREEN SPACES ON ELDERLY HEALTH

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## 1. INTRODUCTION

As the global population undergoes a demographic shift towards an increase in aging, several studies have begun to consider the living environment as a crucial determinant of health, impacting various aspects of well-being. There is a growing scientific interest in exploring the potential benefits of green areas, a significant component of the living environment, on the physical and mental health of the elderly. Research in this area is gaining momentum, revealing that green spaces offer numerous advantages for the elderly population.

Firstly, green areas promote and facilitate regular physical activity, particularly among the elderly. Additionally, they help reduce the risk of cardiovascular diseases, respiratory problems, high blood pressure, diabetes, and other chronic diseases and mental disorders. Moreover, green spaces contribute to mental health and well-being by providing interaction environments for the elderly, whose social interactions tend to diminish over time via aging.

As individuals enter retirement as they age, they not only distance themselves from their workplaces but also from the interpersonal encounters provided by working life and social networks. Since the elderly face a higher

risk of losing their partners and friends compared to younger individuals, this significantly impacts their mental health, limits their social relationships, and contributes to increased loneliness. Loneliness, combined with reduced mobility and physical problems associated with aging, is increasingly recognized as a significant public health concern (1). Consequently, efforts to enhance the physical and mental well-being of the elderly are gaining momentum, with a focus on identifying beneficial activities and opportunities.

Studies conducted in this field have revealed the multifaceted benefits of urban green spaces. These spaces provide social interaction for the elderly, encourage physical activity, offer a relaxing and peaceful environment, and help reduce noise and air pollution. Contact with green areas has been found to be essential and beneficial for the well-being of the elderly.

The aim of this study is to discuss recent research highlighting the benefits of contact with urban parks and green spaces for the elderly, who are susceptible to various physical ailments and mental health problems such as depression and stress. Furthermore, the study aims to provide practical recommendations for welfare policies aimed at improving the well-being of the growing urban elderly population.

Overall, the research supports the positive impact of green areas on the health and well-being of the elderly, emphasizing the need for incorporating and prioritizing green spaces in urban planning and welfare policies.

## 2. DEFINITION OF GREEN SPACE AND THEIR BENEFITS

Green space is generally defined as any vegetation or water found within an urban area, including parks, gardens, playgrounds, lawns, and greenways such as paths, disused rail lines, rivers, and canals. It also encompasses natural areas like woodlands, forests, and wilderness areas (2). There are two commonly used methods to define green space. The first method considers green space as an inclusive concept of nature, which stands in contrast to urbanization. The second method refers specifically to vegetated open spaces and focuses on the presence of vegetation within the city (3,4).

Studies investigating the impact of green spaces have revealed that the positive outcomes associated with green spaces, defined and classified in various ways, also differ. For instance, woodlands are believed to have a more restorative effect compared to general urban green spaces, farmland, or wetlands (5,6). Within this context, it has been suggested that exposure to natural environments

influences health through biopsychosocial pathways. These pathways can be broadly categorized into three functions: reducing harm (e.g., minimizing exposure to air pollution, noise, and heat), restoring capacities (e.g., enhancing attention restoration and recovering from physiological stress), and building capacity (e.g., promoting physical activity and fostering social cohesion) (7). Moreover, nature also aids in providing social support and facilitating adaptation, while reducing deaths caused by physical inactivity through increased physical activity.

Air pollution stands out as one of the significant environmental factors contributing to the global burden of disease. Green space is believed to possess the potential to mitigate or reduce the negative impacts of environmental hazards, particularly those experienced by the urban population (3). Green space contributes to cleaner air by removing air pollutants through dry deposition and by lowering temperatures, thereby reducing smoke generation (8). The ability of green areas to lower temperatures is particularly valuable in cities due to the urban heat island effect. Green space can reduce heat in urban areas by providing shade, evapotranspiration, and indirectly sequestering carbon (9,10). It has been suggested that nature-based climate change mitigation and adaptation strategies have the potential to mitigate these effects and improve people's quality of life (11).

On the other hand, environmental noise has emerged as a public health concern due to its various detrimental effects on human health and well-being. It has been emphasized that green areas can serve as physical buffers against noise by refracting, absorbing, or interfering with sound waves, thereby reducing noise exposure (7). A study aimed at evaluating the scientific evidence supporting the effectiveness of urban green spaces as psychological buffers against the negative impacts of noise pollution on human health found moderate evidence suggesting that the presence of vegetation can mitigate the overall negative perception of noise (12).

## 3. GENERAL AND MENTAL HEALTH BENEFITS OF GREEN SPACES

Green space has been confirmed to have a positive impact on general health and mental well-being (13). Interdisciplinary evidence from the health and social sciences emphasizes the importance of nature experiences for mental well-being (14). A study reviewing epidemiological evidence from longitudinal studies on

green spaces and their association with all-cause mortality suggested that green spaces improve health and well-being through various mechanisms. The results showed a significant association between increased proximity to green space and reduced all-cause mortality (15). Many studies suggest that living near or having regular contact with nature has beneficial effects on various health and well-being outcomes (14,16).

While many studies have confirmed the public health benefits of green space for different social groups in North American and European cities (17-19), there is limited research on how social groups in China differ in terms of these benefits. It has been suggested that exposure to nature may enhance prosocial behaviors through increased positive emotions (20). Furthermore, higher frequency of visits to urban green spaces has been associated with lower mortality rates in older adults (21).

In a study investigating the relationship between greenery, walkability, and self-reported chronic conditions and self-assessed health in older Canadians over a three-year follow-up period, the analysis showed that living in greener neighborhoods positively influenced self-assessed measures of healthy aging, general health, and mental health (22). Another study examined the effects of behavioral, environmental, and intermediate attributes of greenways on the well-being of older adults, particularly focusing on the role of place attachment as an intermediary factor for aging in place. Data from environmental assessments and user surveys of 769 participants aged 55 and over were analyzed. The regression models revealed that greenway quality, perceived pollution, activities on greenways, neighborhood social capital, and place attachment significantly influenced well-being. Additionally, the greenness of greenways had a more positive effect on the well-being of older individuals with higher levels of place attachment (23).

## 4. THE EFFECT OF GREEN SPACES ON THE PHYSICAL HEALTH OF THE ELDERLY

The elderly population faces numerous challenges and difficulties, making them one of the most vulnerable groups today. Inactivity among elderly individuals living in urban areas not only leads to physical ailments that diminish their quality of life but also imposes social and economic costs on society, highlighting it as a significant public health issue. Aging is a natural part of the human life cycle and is associated with a higher risk of cardiovascular

disease, depression, and/or mental illness (24). Physiological changes due to aging, combined with potential underlying health conditions, increase the elderly's susceptibility to serious illnesses (25). Moreover, aging also raises the likelihood of developing neurodegenerative diseases that result in memory problems and contribute to an unsafe environment (26).

In a study that recruited 33 elderly men and women, the effects of short-term exposure to different urban green spaces and street environments on physiological and psychological parameters related to cardiovascular health were investigated. Participants were exposed to a well-established city park with mature trees, a newly developed park offering various amenities, and a busy street environment in the city center. Continuous monitoring of electrocardiogram (ECG), blood pressure, mood, and heart rate variability was conducted using psychological questionnaires and subjective assessments of restoration. The results indicated significant reductions in systolic blood pressure, pulse pressure, and improved cardiovascular health, indicating the protective effects of green space exposure (27).

In another study conducted in Toronto, Canada, the relationship between comprehensive measurements of greenness and health was examined. High-resolution satellite images and individual tree data were combined with questionnaire-based self-reports to assess general health perception, cardio-metabolic states, and mental illness. The findings demonstrated that individuals residing in neighborhoods with a higher abundance of trees on their streets reported significantly better health perceptions and fewer cardio-metabolic problems (28).

## 5. IMPACTS OF GREEN SPACES ON THE PHYSICAL ACTIVITY OF THE ELDERLY

Overall, physical inactivity is one of the major risk factors for death worldwide (29). On the other hand, the environmental conditions in which older people live play an important role in promoting or inhibiting physical activity. In this context, especially green space can be seen as an important behavioral environment for physical activity. Many studies have investigated this relationship, but the reported findings are inconsistent (3).

Engaging in physical activity offers numerous lifelong health benefits and has the potential to prevent or improve various age-related health issues. For older individuals, outdoor environments can serve as valuable settings to

participate in physical activity (30). Factors such as walkability, accessible green spaces, equipment used for exercise, and amenities in the outdoor environment contribute to promoting physical activity, particularly among vulnerable populations (31,32). However, some older adults may experience reduced mobility and increased physical limitations, leading to a shrinking effective range of their neighborhoods and local public spaces, limiting their access to areas beyond their immediate vicinity. Therefore, when designing and planning outdoor environments in residential areas, special consideration must be given to the unique physical needs of older individuals (32).

A study highlighting the positive influence of urban green spaces on public health discovered that individuals residing in greener neighborhoods exhibited higher levels of physical activity compared to those in less green neighborhoods (33). The physical and social attributes of the neighborhood environment are widely acknowledged to have a significant impact on the various types and levels of physical activity participation and psychological well-being among older adults (34-36).

## 6. THE EFFECT OF GREEN SPACES ON SOCIAL SUPPORT AND SOCIAL COHESION OF THE ELDERLY

Loneliness is recognized as a significant problem during the aging process. As individuals grow older, their interpersonal relationships tend to diminish, leading to feelings of social isolation and loneliness. Insufficient fulfillment of the elderly's need for interaction and social support can contribute to various mental health issues. However, the presence of green spaces in a neighborhood can foster social interactions and cultivate a sense of community (17). Research has shown that an increased quantity and/or quality of green space in the neighborhood is associated with reduced feelings of loneliness, a lower risk of lacking social support, and improved social cohesion (37,38). Conversely, strong social relationships and successful social adjustment have been linked to improved health and well-being (39). Studies have also observed that greater social support is linked to a lower risk of death and cardiovascular diseases, as well as protection against physical disability and mental health problems such as depression (40) and dementia (41), among others. Apart from providing an appealing environment for neighborly communication, green spaces can also encourage neighbors to spend more time outdoors, facilitating visual contact and opportunities for interaction.

## 7. THE EFFECT OF GREEN SPACES ON MENTAL HEALTH OF THE ELDERLY

In recent times, there has been a growing focus on finding effective strategies to cope with stress and enhance the mental well-being of the elderly. One such method that has been identified is contact with nature. Exposure to nature is regarded as a beneficial approach to improving both the psychological and physiological conditions of individuals. Numerous studies have demonstrated that contact with nature contributes to better mental health and psychological well-being (42-44). Similarly, studies conducted at the urban level have affirmed the positive impact of green spaces on the psychological health of individuals.

Research has demonstrated a positive association between street trees (45), per capita green space (46), street green images (47), natural green spaces (48), and the mental health of people in Guangzhou. It has been suggested that the susceptibility to diseases and the effects of aging on bodily functions create stress among the elderly (49). Recent studies have revealed that urban areas have a relatively higher risk of serious mental illnesses compared to rural areas (50). Consequently, it is crucial to consider the well-being of the elderly, encompassing both physiological and psychological health, in order to maintain their emotional stability, prevent anxiety and stress, and ensure their comfort.

In contemporary societies, nature and green spaces have been shown to improve both the physical and mental health of individuals, enhance social cohesion, enhance the appeal of cities, and reduce psychological distress (51). Korea Centers for Disease Control and Prevention conducted Community Health Survey in 2015. The findings from the survey indicated that the prevalence of mental health issues in urban areas generally decreases with a higher proportion of green space within an area (52).

Data from 5,014 middle-aged adults were analyzed in the HABITAT study conducted at two different time points (2009 and 2011) to explore the associations between perceptions of urban green space and psychological well-being. It was found that perceptions of the amount of urban green space were significantly and positively related to psychological well-being at both time points (53).

Another study investigated the health and well-being benefits of spending time in natural environments and examined the exposure-response relationships. The study examined the association between recreational nature exposure and self-reported health and well-being in the past week among 19,806 participants. The participants' weekly contact with nature was categorized in 60-minute

intervals using the Natural Environment Questionnaire. The results demonstrated that older adults and individuals with long-term health problems who spent at least 120 minutes in nature per week experienced significant benefits compared to those who had no contact with nature the previous week (54).

In a separate study focusing on the relationship between parks and mental health, the presence, quantity, and quality of public green spaces in newly developed neighborhoods were investigated in relation to the mental health of local residents (n = 492), particularly in terms of the presence or absence of mental illness. The findings indicated that both the total number and area of public green spaces were significantly associated with better mental health. Positive mental health outcomes were associated not only with nature-oriented parks but also with green spaces that offered recreational and sporting activities (55). It has been suggested that perceiving a garden environment can reduce negative psychological states and enhance positive emotions, as green gardens have been found to possess emotional and therapeutic values (42,56). Furthermore, gardening activities have been shown to promote physiological and mental relaxation among older adults (57).

A study conducted in Japan aimed to explore the relationship between depression in older adults and three types of neighborhood green spaces: trees, meadows, and fields. The study included 126,878 older adults residing in 881 neighborhoods. The analysis revealed that areas with a higher amount of green space had a lower probability of depression. In urban areas, a greater presence of trees was associated with a lower probability of depression. In rural areas, moderate grassland was linked to a lower probability of depression compared to areas with less grassland. Both urban areas with higher tree density and rural areas with moderate grassland showed a lower probability of depression (58). The study also found that elderly individuals with poor health and low income tended to score higher on tests for depressive symptoms, indicating that physical health and economic factors are associated with depression in older people. Depressive symptoms were more prevalent among the elderly (59). Conversely, higher green coverage in established urban areas was associated with lower levels of depressive symptoms among older residents, highlighting the importance of having ample urban greenery, larger green spaces, improved quality, and enhanced safety.

Another study conducted in Beijing, China, surveyed 701 residents from 16 neighborhoods to explore the complex relationship between neighborhood green space and residents' mental health in an urban setting. The findings

provided strong evidence supporting the positive relationship between green space and mental health in a densely populated urban environment. The study suggested that physical activity and social interaction and cohesion might play a mediating role in this relationship (60).

A systematic literature review focused on urban green space and the subjective well-being of the elderly. The review analyzed 65 articles. The results further supported the strong connection between the subjective well-being of older adults and various characteristics of urban green spaces. However, the relationship between urban green spaces and the subjective well-being of older adults was found to depend not only on the features of the green spaces themselves but also on the characteristics of the older adult population who utilized them (61).

A study conducted over a 10-year period (2004-2014) examined the impact of changes in green areas on changes in mental health. The study utilized data from 3,175 Dutch adults and correlated it with measures of accessibility and usability of green spaces at three different time points (2004, 2011, and 2014), using the Mental Health Inventory-5. The study revealed significant associations between Euclidean distances to the nearest green area and mental health.

Another study investigated whether the relationship between green space and mental health was influenced by age and gender. The study involved 4,924 individuals from the general Dutch population. It was found that green space was associated with good mental health only in specific age and gender groups, specifically within a radius of 3 km. The presence of abundant greenery in open spaces seemed to promote more frequent visits and better health outcomes in older individuals (62).

## 8. PROBLEMS OF ACCESS OF THE ELDERLY TO GREEN SPACES

Research has demonstrated that engaging with nature can have a positive impact on various aspects of well-being, including happiness, positive emotions, connectedness, and a sense of purpose in life (14,63). However, despite the significant benefits that green spaces offer for the healthy aging of older adults, as well as their physical, mental, and social well-being, these spaces are often underutilized by older adults. This can be attributed to the increasing scarcity of natural environments and limited accessibility, particularly for older adults with mobility challenges or those residing in urban areas.

A qualitative investigation was conducted to explore the physical environmental factors of parks that influence the use of parks by older adults. Fifteen out of twenty older adults in Belfast participated in walk-in interviews, which involved capturing visual and audio data, while audio-only interviews were conducted with the remaining participants in their homes. Eight themes emerged as important factors, including park accessibility, natural features, park facilities, sports facilities, maintenance and aesthetics, walking and cycling facilities, safety, and slope. It is crucial to ensure that parks are well-maintained and visually appealing, incorporating natural or semi-natural elements such as landscaping and wooded areas. Additionally, specific park facilities like restrooms and benches, as well as sports facilities such as children's playgrounds, were found to play a significant role in supporting and encouraging park use among older adults (64).

A systematic review focused on the needs and preferences of older adults included 44 articles. The findings highlighted that older adults who engage in recreational activities in green spaces highly value naturalness, aesthetics, and diversity. However, simply creating green spaces may not be sufficient to encourage individuals, including older adults, to engage in walking and cycling activities throughout their lives (66). Safety concerns also act as a barrier, particularly for older adults and young people, limiting their use of these areas (67). Therefore, ensuring accessibility to green spaces and incorporating well-maintained pathways and roads are essential for older adults to fully enjoy the benefits of nature (65).

## 9. THE EFFECT OF INDIRECT CONTACT WITH NATURE ON ELDERLY HEALTH

Older individuals may not always have the ability to access natural environments directly, but they can still experience the restorative effects of nature through indirect means. Going outside may not always be feasible for elderly and unhealthy individuals. In such cases, indirect experiences of nature can be tailored to their specific needs. This allows the elderly, who may be unable to venture outdoors, to benefit from the positive effects of observing nature. Research conducted in this area has demonstrated that visual stimulation through nature images can help individuals in stressful situations or with health problems to connect with nature, relax, feel at ease, and avoid negative emotions. The visual stimulation of nature has been recognized as an effective means of enhancing human health and well-being. Furthermore, indirect experiences

with nature have been found to foster children's attachment to nature, without disregarding the importance of direct experiences (68).

In a specific study conducted in this context, thirty-four participants with an average age of 82.9 ± 0.78 were asked to view bamboo and urban images for 2 minutes. During this visual stimulation, the researchers measured alpha relative waves using electroencephalography as an indicator of brain activity. They also evaluated indicators of arousal such as heart rate variability and skin conductivity, as well as psychological responses and mood states. The results indicated that indirect contact with nature improved the physiological and psychological well-being of the elderly. These findings have implications for the design, renovation, and modification of living environments for the elderly and individuals who are unable to go outside (43). Another similar study demonstrated that observing a money plant for 5 minutes improved both psychological and physiological relaxation in older adults (57). Additionally, research by (69) showed that visual stimulation with forest images significantly increased perceptions of feeling "comfortable" and "relaxed."

## 10. DISCUSSION

Although the global importance of green spaces in terms of public health remains uncertain, studies reveal that green spaces provide better health outcomes. A study of 34 cities around the world on the topic suggested that green spaces were associated with better health outcomes in wealthier cities, but these results did not hold for relatively poorer cities, thus suggesting that the positive effect of green spaces may be context dependent (70). Undoubtedly, there are many factors that affect mental health, and green spaces are only one of them, and in the absence of the positive effects of other factors, it may not be possible for green space alone to reveal the positive effect at a significant level.

While there has been increasing academic interest in the relationship between green space and mental health in the last few decades, most of the existing studies have been conducted in developed countries and less scientific attention has been paid to rapidly urbanizing countries, which has led the academic community to reach unequivocal agreement on the cause and effect relationship of green space to mental health. Another reason is the difficulty of defining the relationship between green space and mental health as causation. This challenge is due to the lack of a complete understanding of the mechanisms involved in the dynamics of green space's mental health impact, as well as the

lack of consistent evidence. It is stated that it is related to the fact that the issue is not revealed and the subject is examined meticulously with longitudinal studies and cannot be adequately supported (71,72).

A study conducted in China aimed to assess the impact of urban green spaces on depressive symptoms among urban residents aged 45 and older. The study included 7,397 participants from urban areas, and it was found that nearly one-third (31.20%) of the sample population experienced depressive symptoms. The findings revealed a negative association between the green coverage rate of neighborhoods and the prevalence of depressive symptoms. Furthermore, public recreational green spaces were found to be beneficial in reducing depressive symptoms in older adults (73).

This review focuses specifically on the findings from experimental and observational studies conducted within the last decade, with a specific emphasis on the elderly population. The results consistently indicate positive relationships between exposure to nature and various aspects of well-being, including improved cognitive function, enhanced brain activity, better regulation of blood pressure, positive mental health outcomes, increased physical activity levels, and improved sleep quality. Moreover, experimental studies provide evidence for the protective effects of being in natural environments on mental health and cognitive function. Many studies further demonstrate the positive correlation between nature exposure and higher levels of physical activity, along with a reduced risk of cardiovascular disease. Some longitudinal observational studies in the field underscore the long-term effects of nature exposure on mitigating depression, anxiety, cognitive decline, and chronic diseases such as cardiovascular conditions.

## 11. CONCLUSION

The findings from this compilation study highlight the vulnerability of the elderly population to mental health problems, which can be influenced by their demographic and social characteristics. Additionally, the study emphasizes the significance of the urban green space ratio in improving the mental health outcomes of elderly individuals residing in cities. It is recommended to identify the specific mental health issues faced by elderly individuals based on their demographic and social characteristics and ensure their access to urban green spaces within the community. These findings can guide the development of a green welfare policy targeting the elderly population.

Gaining an understanding of the fundamental elements required in neighborhoods and outdoor spaces is vital to establish inclusive and secure public areas that cater to the physical and mental well-being of elderly and disabled individuals. By improving the capabilities of neighborhoods and outdoor spaces to facilitate walking, resting, and engaging in leisure activities, as well as enhancing access to nearby amenities and social attractions, we can encourage physical activity among the elderly and disabled population. Additionally, the creation of physical environments that foster good health can contribute to mitigating socioeconomic health disparities. It is important to implement strategies and policies that sustain the progress made in improving outdoor public spaces, particularly by investing in green space initiatives that focus on areas with a high concentration of vulnerable populations, including the elderly and individuals with disabilities.

Access to walkable areas can be challenging for many older adults due to obstacles such as stairs, busy roads, long distances, and other mobility limitations. Ensuring the safety of roads leading to urban green spaces and parks is crucial for people of all ages. It is essential to prioritize the creation of sufficient urban green spaces for all citizens in densely populated cities and develop plans that consider the potential health limitations of the elderly population, such as providing adequate facilities and equipment like benches and toilets.

Loneliness and isolation are particularly challenging issues for older adults, and green spaces can play a significant role in alleviating these feelings. Easily accessible natural outdoor spaces can provide valuable opportunities for social interactions, even for elderly individuals with limited mobility. Simply looking through a window at a garden, forest, or nature pictures can contribute to stress reduction and improve cognitive health. These mental benefits are especially important for older individuals experiencing chronic stress or facing challenging life events such as the loss of a loved one. Moreover, proximity to nature can enhance social connections at the neighborhood level through various means, including the planning and creation of neighborhood green spaces and positive social encounters during walks or while enjoying nature, ultimately enhancing the quality of life for the aging population.

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CHAPTER XI

# INTERSTITIAL PNEUMONIA IN SHEEP

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## 1. INTRODUCTION

Sheep farming utilizes sheep as an important economic resource worldwide, including our country, not only for their meat and milk but also for their wool and skins, which are necessary raw materials for textiles and various industries. The fundamental principle of animal husbandry is to generate economic profit, and combating diseases plays a significant role in achieving this profit. Respiratory system infections account for 5.6% of all diseases observed in sheep, regardless of their etiology (1). Sheep pneumonias, along with condition loss, decreased productivity, and secondary infections, cause significant economic losses through deaths (2).

Pneumonias, although referred to as inflammation of lung tissue, occur due to pathogenic agents such as bacteria, parasites, viruses, fungi, and chlamydiae, as well as predisposing factors like overcrowding, management and feeding errors, sudden climate changes, inadequate ventilation, starvation, transportation,

secondary diseases, and stress (3-5). The classification of pneumonias in veterinary pathology exhibits diversity. Pneumonias are classified based on the type of inflammation (exudative and proliferative), duration (acute, subacute, chronic), and site of involvement (lobar pneumonia, interstitial pneumonia, and bronchopneumonia) (6,7). According to another classification, pneumonias in domestic animals are divided into four groups: interstitial pneumonia, bronchopneumonia (catarrhal purulent, fibrinous), granulomatous pneumonia, and embolic pneumonia (2, 3, 8, 9).

Interstitial pneumonias (IP) are a disease characterized by specific clinical, radiological, and histopathological features. They affect the lung parenchyma and are characterized by fibrosis and inflammation. IP can have an acute or chronic course. [10-12] In interstitial pneumonias, the target structure is the interstitial tissue spaces. In the advanced stages of the disease, bronchial and bronchiolar lumens, alveolar spaces, and alveoli can be affected, leading to involvement of the entire lung parenchyma. For this reason, the term Diffuse Parenchymal Lung Diseases (DPLD) is also used instead of IP (12, 13).

In veterinary medicine, the term IP has been used synonymously with several different clinical conditions such as acute lung injury/acute respiratory distress syndrome (ALI/ARDS), clinical syndrome of interstitial pneumonia, pathogenic/pathological interstitial lung disease (ILD), diffuse alveolar damage (DAD), and pulmonary fibrosis. This has resulted in significant confusion when defining IP (14, 15) Generally, the term IP can be used to describe both infectious (e.g., viral) and non-infectious (e.g., toxic) diseases of the interstitium when there is damage to the alveolar epithelium and endothelium or an increase in cell proliferation and leukocyte count in the alveolar septa (14).

## 2. ETIOLOGY AND PATHOGENESIS

Interstitial pneumonias can occur due to various etiological factors. Aerogenic viral infections caused by viruses such as Adenovirus, Herpes virus, Parainfluenza 3 (PI3) virus, Lentivirus, Respiratory Syncytial Virus (RSV), and Reovirus are common causes. In addition, parasitic, mycoplasma, and chlamydial agents, major lung traumas, drowning, aspiration, inhalation of smoke during fires, surfactant deficiency due to premature births, inhalation of toxic gases like sulfur dioxide, chlorine, ammonia, nitrogen dioxide, chemical toxins, intravascular clotting, heart failure, hypersensitivity reactions, and septicemia can also lead to interstitial pneumonias. (2, 14). Pathogens acquired

through hematogenous, aerosol, and inhalation routes can produce different clinical manifestations depending on their mode of acquisition (16).

When viruses are acquired through aerosol transmission, they initially cause destruction of the respiratory epithelium and then reach the bronchi, bronchioles, and alveoli. They primarily settle in the cranioventral regions of the lungs, leading to sublobular or lobular lesions. In advanced stages, these lesions can spread to almost the entire lobe, resulting in lobar pneumonia. If the pathogen enters through the hematogenous route, pneumonia lesions predominantly develop in the alveoli and alveolar walls. Viral pneumonias are generally observed as interstitial pneumonia, but when accompanied by secondary agents, interstitial pneumonias can transform into purulent, fibrinous, and catarrhal bronchopneumonias (17,18). After infecting the upper respiratory tract and lungs, viruses with selective tropism for monocytes spread to macrophages throughout the body via viremia (19, 20).

Interstitial pneumonias are characterized by septal thickening consisting of macrophages and alveolar necrotic debris. Additionally, features such as hyperplasia of alveolar macrophages, rarely syncytial cells and type II pneumocytes, perivascular infiltrates of lymphocytes and plasma cells, and hypertrophy of peribronchial lymphoid tissue can be observed (14, 19). The etiology of certain subtypes of interstitial pneumonias is still not fully understood. However, these subtypes can be predicted based on their clinical, physiological, pathological, and radiological characteristics (12, 21). Interstitial pneumonias can be categorized into three stages: the acute or exudative phase, the proliferative (subacute) phase, and the fibrotic or chronic phase (2, 14).

The initial stage of IP is the exudative (a Toxic cute) phase, characterized by damage to type I or II pneumocytes or endothelial cells and accompanied by an exudative inflammation. This phase is also referred to as atypical interstitial pneumoni (3,5,22). Substances and infectious agents, whether endogenous or exogenous, cause diffuse destruction, particularly in type 1 cells of the alveoli, leading to alveolar edema (9). As a result, the functions oz and their ability to deal with foreign particles are compromised (23,24). Additionally, alveolar macrophages and epithelial cells release cytokines such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL-6, IL-8), chemokines such as CCL2, CXCL8, CXCL1, CCL20 (25), numerous proteases, reactive oxygen species (ROS), and neutrophil extracellular traps (NETs) (26,27). Inflammatory macrophages and neutrophils also induce factors that cause apoptosis of epithelial cells (such as FasL and TRAIL) (28,29) leading to the loss of connections

between epithelial cells, inflammatory cell infiltration, and several vascular changes. This process results in the formation of hyaline membranes composed of cellular debris, plasma proteins (albumin, fibrinogen, immunoglobulins), and surfactant components on the alveolar surfaces (9, 14).

Patients who have survived the exudative phase enter the proliferative or organizing (subacute) phase, which occurs 3 to 6 days after the onset of alveolar damage (14). As a result of the proliferation of lymphocytes and macrophages, thickening of the interstitium and an increase in alveolar type II cells are observed. There is lymphoid hyperplasia around the blood vessels, bronchi, and bronchioles (6-8). In interstitial pneumonia, the proliferation of alveolar type II pneumocytes indicates the transition from the acute exudative phase to the beginning of the proliferative phase, typically occurring around days (6-8). Epithelialization refers to the lining of alveoli with cuboidal epithelial cells during the subacute and chronic stages of interstitial pneumonia. In the proliferation phase, there is no inflammatory exudate in the bronchial and bronchiolar lumens. Type II pneumocytes replace type I pneumocytes, and interstitial fibrosis may occur (5). The development of fibrosis is an important finding in chronic interstitial pneumonia. Lung fibrosis progresses over time, leading to decreased respiratory parameters, lung function, and quality of life for patients (15, 30-32). Fibrosis is the final stage of interstitial pneumonia, where the lungs are irreversibly damaged, and gas exchange becomes inefficient. The key differentiating factor in the fibrosis stage is TGF-β. TGF-β promotes the activation and phenotypic differentiation of fibroblasts and sustains the necessary cycles for ongoing TGF-β production (33, 34).

### *2.1. Epidemiology*

It is estimated that interstitial pneumonias account for approximately 20% of all lung diseases encountered in humans worldwide (35). The diagnosis of interstitial pneumonias (IPs), which is a common problem in animals, has not been fully elucidated (15).

Interstitial lung diseases are commonly observed in sheep worldwide. In the Shimla and Himachal Pradesh states of India, a study of 127 lung tissues revealed interstitial pneumonia in 38 sheep and 89 goats, accounting for 16.54% of cases (36). In the Rajasthan state, the prevalence of interstitial pneumonia in sheep was reported as 26.50% (37). Furthermore, recent literature searches on the incidence of interstitial pneumonia yielded the following results: 20.93%

in Iran (38), 40.4% in Libya (39), and 41.9% in Ethiopia (40). In another study conducted in Ghana and Nigeria, out of 805 collected lung samples, pneumonia was detected in 70 samples, of which 9 were diagnosed as interstitial and 9 as bronchointerstitial pneumonia (41). Dünyadaki yaygınlığının yanı sıra ülkemizde de önemli bir sorun olarak görülmekte olan koyun İP insidensi farklı bölgelerde yapılan çalışmalarda %13.1 (42), %18 (43), %18.32 (44), %36.24 (45), %58.5 (46), %77.21 (47) olarak farklı oranlarda bildirmiştir.

In addition to that, Eser (2019) found a prevalence of 6% for pulmonary adenomatous pneumonia, 7% for granulomatous pneumonia, 14% for catarrhal-purulent bronchopneumonia, 19% for verminous pneumonia, 23% for fibrinous bronchopneumonia, and 69% for interstitial pneumonia (2). Similarly, in another study by Usta (2019) in Balıkesir province, Turkey, histopathological examination of sheep lungs classified pneumonias as 34% interstitial pneumonia, 32% bronchointerstitial pneumonia, 26% fibrinous bronchopneumonia, and 8% granulomatous pneumonia (9). When examining these studies conducted globally and in our country in recent years, it has been determined that the incidence of interstitial pneumonia is higher compared to other pneumonias in most studies.

### *2.2. Clinical Findings*

In animals with clinically observed ARDS, rapid respiratory distress is initially observed, followed by an increase in respiratory and heart rate due to hypoxia, as well as decreased chest movements. Paradoxical respiration, characterized by chest expansion and abdominal distension, and increased respiratory effort, are prominent (14,48). Diffuse alveolar damage (DAD) is a distinct histopathological finding of ARDS with specific histological features. Acute lung diseases are defined as a milder form of ARDS (49).

In interstitial pneumonias in animals, a decrease in rumen movements and coughing, along with bilateral dull sounds on pulmonary auscultation, are observed. In interstitial emphysema, bronchial and friction sounds are heard on the right side, dorsally to the chest, while purulent bronchopneumonia is characterized by harsh sounds (50, 51). In advanced cases, diarrhea and tympany can be observed. Body temperature generally ranges from 38.3°C to 41.1°C. In some cases, death can occur without any cliPathologica (51). Radiographic evaluation reveals alveolar opacity in the cranioventral pulmonary region (50).

### 3. PATHOLOGICAL FINDINGS

Lungs affected by interstitial pneumonia do not have a specific macroscopic appearance, but as a general consensus, they do not collapse and are usually wet, heavy, pale, swollen, spongy, and have an elastic consistency [Figure 1] (2, 7, 52). Rib impressions are often observed on the surface of the lungs. Upon sectioning, there is no presence of exudate or fluid leakage. Although macroscopic lesions can be observed throughout the lungs, they are more commonly found in the caudodorsal lobes. In chronic cases, thickening of the interstitium due to increased connective tissue, peribronchial nodules, and widespread emphysematous foci in the lungs can be seen (5, 6, 52).

Microscopically, in interstitial pneumonia, during the exudative phase, the alveolar lumens show a serous-fibrinous exudate, and there is edema and congestion in the alveolar walls. Epithelialization and hyaline membranes form in the alveolar epithelium during this phase (2,9). In the proliferative phase, there are mononuclear cell infiltrations, proliferation of type II pneumocytes, and thickening of the interstitium due to increased connective tissue composed of fibrocytes and fibroblasts. Lymphoid hyperplasia occurs around blood vessels, bronchi, and bronchioles [Figure 2] (52,53). In the fibrotic stage, fibrosis develops in the alveoli and interalveolar spaces, and type II pneumocytes become pefibrotic (14).

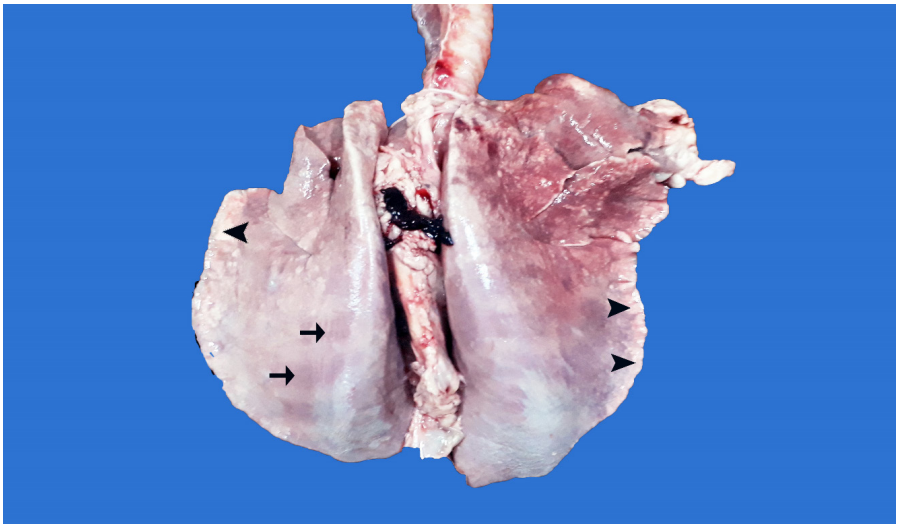

**Figure 1.** Interstitial pneumonia. The lungs exhibit a spongy and elastic texture with rib impressions (arrows) and areas of emphysema (arrowheads) on their surface.

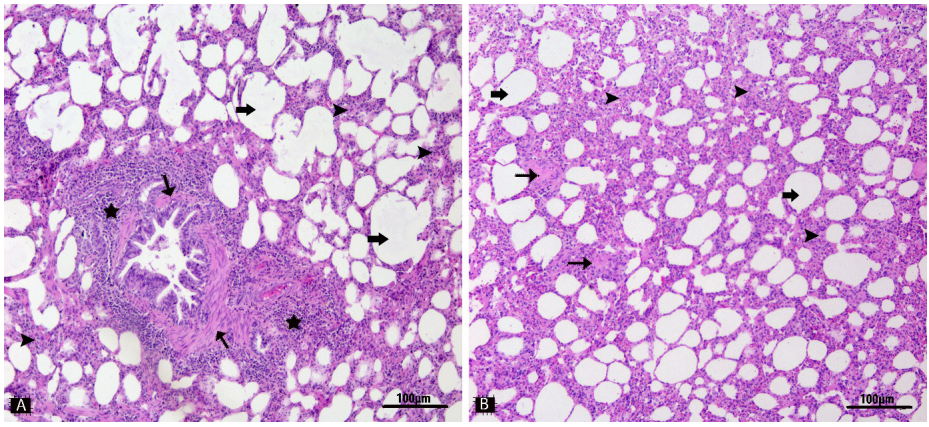


**Figure 2.** Thickening of the interalveolar septum due to mononuclear cell proliferation (arrowheads), alveolar emphysema (thick arrow), fibromuscular hyperplasia (arrows) (A, B), peribronchiolar and perivascular lymphoid hyperplasia (star) (A), HE

**Diagnosis**

The clinical diagnosis of pneumonia is challenging and typically involves physical examination, imaging, serology, nasal swabs, bronchoalveolar lavage, and even fecal samples in cases of atypical pneumonia, along with necessary laboratory investigations (40, 54)

Diagnosing interstitial pneumonia postmortem can often be difficult as it lacks a typical macroscopic appearance. Microscopic, bacteriological, and virological confirmation is required to differentiate it from other conditions presenting with edema or emphysema. In live animals, the disease is diagnosed based on the presence of symptoms consistent with interstitial pneumonia. However, a definitive diagnosis cannot be made without a biopsy sample. Additionally, the bacteriological and virological examination of nasal swabs plays a diagnostic role in this disease (55). Radiographic evaluation showing alveolar opacities in the cranioventral pulmonary region is a general finding for detecting pneumonia (50).

### *3.1. Treatment*

In cases where the disease has a mild course, patients should be placed in a warm, well-ventilated, and humidified hygienic environment. Improving the environmental conditions in the area where the patients are located, such

as ensuring a dust-free, clean, and well-ventilated space with dry bedding, can lead to spontaneous recovery over time. In cases where pneumonia symptoms are severe, treatment may involve the administration of antibiotics such as procaine penicillin G, ampicillin trihydrate, benzathine penicillin G, tetracycline hydrochloride, oxytetracycline hydrochloride, erythromycin, amoxicillin trihydrate, dihydrostreptomycin sulfate, ceftiofur sodium, florfenicol, tylosin, and tilmicosin. Oxygen therapy, bronchodilators, mucolytic agents, fluid therapy, correction of nutritional status, and physiotherapy may also be employed. Antibiotic resistance, delayed initiation of treatment, and the occurrence of irreversible pathological changes can render antibiotics ineffective in the treatment of pneumonia. In cases with severe symptoms, respiratory stimulants containing 5-10% $CO_2$ in oxygen, nikethamide, picrotoxin, leptazol, amphetamine meleate, and caffeine can be used for short periods. Additionally, the use of corticosteroids during the acute phase of pneumonia can reduce fever and increase appetite. However, corticosteroids can lead to disease recurrence and prolonged duration. Tracheostomy may be performed to alleviate respiratory distress. NSAIDs such as flunixin meglumine and ketoprofen can also be used in patients. Diuretics may be administered in the presence of pulmonary edema, while fluid therapy can be employed in cases of dehydration without pulmonary edema (56).

### *3.2. Prevention and Control*

Animals should be housed in clean, dust-free, and well-ventilated environments. Dry bedding should be provided, and clean water should be available. Environmental conditions where the animals are kept should be improved. During outbreaks, infected animals should be isolated to prevent transmission. In farms where pneumonia occurs annually, chlortetracycline can be added to the feed before the onset of the disease to reduce morbidity and mortality. The use of vaccines prepared against viral diseases demonstrates a protective effect against pneumonia (56).

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# CHAPTER XII

# COPPER TOXICOSİS IN SMALL RUMINANTS

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## 1. INTRODUCTION

Copper toxicosis is a significant health problem commonly observed in sheep, often resulting in death. This disease is characterized by the excessive intake or accumulation of copper in the body of sheep. Copper plays an important role in the normal metabolic functions of sheep, but toxic effects occur when it accumulates in excessive amounts. Copper toxicosis typically occurs due to the consumption of feeds containing excessive copper, which can be caused by incorrect formulations or erroneous animal feeding practices. Symptoms of copper toxicosis include loss of appetite, weakness, weight loss, watery diarrhea, anemia, jaundice, neurological symptoms, and death. The safest diagnosis of the disease is usually made through the evaluation of history, clinical signs, and the assessment of copper levels in blood, liver, and kidney. Treatment may involve transitioning to feeds with lower copper content, using medications that reduce copper absorption, and providing symptomatic support. Additionally, proper feeding programs, appropriate feed formulations,

and regular monitoring of water sources are important for preventing copper toxicosis. Copper toxicosis is a serious condition that adversely affects the health and productivity of sheep. Therefore, along with correct feeding and care practices, regular monitoring and diagnosis by veterinarians are crucial.

Copper is a metal widely used in many industrial applications and agriculture. However, excessive exposure to copper or its improper use can lead to copper poisoning in both humans and animals. Copper poisoning usually occurs when there is excessive copper intake or impaired copper excretion, resulting in its accumulation in the body. In this condition, the body absorbs and processes more copper than it should, leading to its buildup. The accumulated copper exhibits toxic effects and causes damage to tissues and organs. (1-3).

Small ruminants, despite being commonly raised animals in the agricultural sector, are particularly susceptible to copper toxicity (4, 5). Copper is an essential micronutrient for the health of small ruminants. It plays a role in protein metabolism, iron absorption, immune system functions, and the formation of red blood cells. Maintaining a proper balance of copper is necessary for the healthy growth, development, and production of small ruminants. Inadequate copper intake can lead to problems such as osteoporosis (bone loss) and growth disorders. However, high doses of copper intake and accumulation can cause liver damage and even death in animals. This condition is referred to as copper toxicosis. Copper toxicosis poses a significant health problem for breeders and veterinarians, necessitating the implementation of proper diagnosis, treatment, and prevention strategies (9-12).

## 2. EPIDEMIOLOGY

High Copper-Containing Feeds: High concentrations of copper in the feeds given to animals can lead to copper toxicosis. This can be associated with the improper preparation of feeds or the excessive consumption of naturally occurring plants with high copper content. The risk of copper toxicosis increases when copper-fortified feeds are given carelessly or when minerals with excessive copper content are mixed into the feeds(1, 2, 9, 11, 12).

Mineral Imbalances: Mineral imbalances in feeds, especially when other mineral supplements are added at high concentrations, can result in copper toxicosis. For example, feeds with high levels of molybdenum negatively affect copper absorption and metabolism in small ruminants, leading to copper accumulation.(2- 4, 13, 14).

Water Sources: Some water sources naturally contain high levels of copper. Excessive intake of copper from such water sources can lead to toxicity(1, 2)

Genetic Factors: Certain breeds of small ruminants have genetic predispositions related to copper absorption and metabolism. This can result in better copper absorption by the animals and weaker mechanisms for copper excretion from the body. These genetic predispositions increase the risk of copper toxicosis(1, 5, 6).

Environmental Factors: Factors such as consuming grains treated with copper sulfate, grazing in environments contaminated with copper residues, or being fed with feeds stored in copper containers for a long time are among the causes of copper toxicosis(1,2,5,7,10).

## 3. MECHANISM OF EFFECT

Copper is primarily absorbed in the small intestine and transported to the blood by transcuprein and albumin, reducing the oxidative effects of divalent copper ($Cu^{+2}$). Unused copper ions are excreted via the bile pathway (13,14). The mechanism underlying copper toxicosis in sheep is based on the inability of sheep to increase copper excretion through the bile pathway in response to increased copper intake. Copper toxicity is generally associated with two main mechanisms:

Excessive copper intake: Feeding animals with high copper content feeds or using copper-containing water sources can result in excessive copper intake. In this situation, the amount of absorbed copper exceeds normal levels and accumulates in the body (3,15).

Impaired copper excretion: Copper is normally excreted through the bile and feces. However, in some cases, copper excretion through the bile is compromised, leading to copper accumulation in the body. This is typically associated with a weakening or impairment of the copper excretion mechanism in the liver (8, 14-17).

Copper toxicosis results from the toxic effects of accumulated copper on tissues and organs. Copper ions bind to organic molecules and enzymes in the blood and protoplasm, rendering them unable to perform their physiological functions (9, 14, 18, 19). Additionally, copper increases the formation of free radicals and causes cellular damage due to oxidative stress (11, 16). The liver plays a crucial role in copper metabolism. Copper toxicosis primarily damages hepatocytes by causing excessive copper accumulation in the liver, leading to impaired liver functions and tissue destruction (21-23).

In small ruminants, there is an antagonistic relationship between copper and molybdenum and sulfur in the diet. When these elements are present in excessive amounts in the diet, they inhibit copper absorption and utilization. Many sources report that feeds with a copper-to-molybdenum ratio (Cu/Mo) higher than 6: 1 result in copper accumulation (9, 11, 24).

## 4. TOXICITY AND CLINICAL SYMPTOMS

Araştırmalra büyük ve küçükbaş hayvanlarda bazı minerallerin fazlalığı ve eksikliğine bağlı olarak birçok kronik hastalık oluşurken toksik etkilerinden dolayı böbreklere ve diğer organlarda harbiyata neden olmaktadır (25-27).

The symptoms of copper toxicity can be observed in a wide range, affecting various systems. Initial signs include loss of appetite, weight loss, weakness, and fatigue. Additionally, yellow-green colored feces and pale mucous membranes can be observed in affected animals (12, 13, 17, 23). The mortality rate in affected animals can reach up to 75%. In cattle and sheep, a copper intake of 3.5 ppm of body weight in the diet leads to chronic toxicity. Similarly, for sheep, forage or feed containing 15-20 ppm copper on a dry weight basis can cause chronic toxicity. In acute poisoning, clinical symptoms rapidly manifest. It is characterized by severe gastroenteritis with symptoms such as anorexia, salivation, colic, diarrhea, dehydration, and shock. The feces are mucousy and appear dark gray-green due to the combination of copper and chlorophyll (23-31).

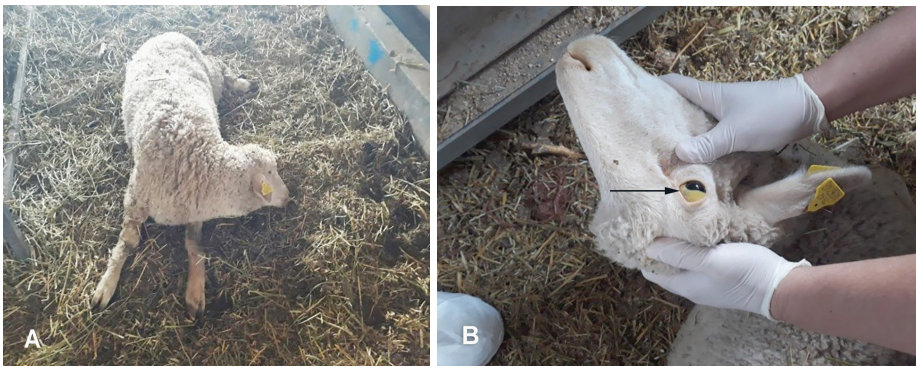


**Figure 1.** A. Lying face down with the neck bent to the side. B. Icteric appearance in the mucosa (arrow)(30).

Prolonged exposure to low doses of copper for various reasons leads to toxic copper accumulation in the liver. When the copper level in the liver reaches a critical level, it is suddenly released into the bloodstream, and signs of toxicity start to appear. The symptoms are mostly associated with hemolytic crisis. Affected animals may show depression, lethargy, weakness, distension of the abdomen, anorexia, thirst, dyspnea, pale mucous membranes, hemoglobinuria, and jaundice (10-12, 24-29).

## 5. DIAGNOSIS

The diagnosis of copper toxicity is made through clinical examination, symptom assessment, necropsy findings, and laboratory tests, all of which should be consistent for an accurate diagnosis. Veterinarians attempt to make a correct diagnosis by evaluating the clinical signs and laboratory results of the animals. Tests such as blood and fecal analysis, copper level measurements, and even liver biopsy can assist in the diagnostic process (19, 27, 31). However, it is possible for copper toxicity to be confused with other toxicosis cases that present with a hemolytic picture, such as zinc and onion poisoning, as well as diseases like hemoglobinuria, leptospirosis, and babesiosis(1, 4, 15).

Copper toxicity often leads to death. During necropsy, important indicators of copper toxicity include extensive liver damage, jaundice, enlarged copper-colored kidneys, ulcerations and hemorrhages in the gastrointestinal system, a tense and dark bile bladder, and an enlarged spleen with dark brown-black parenchyma (3, 7, 19, 22, 24, 29, 231).

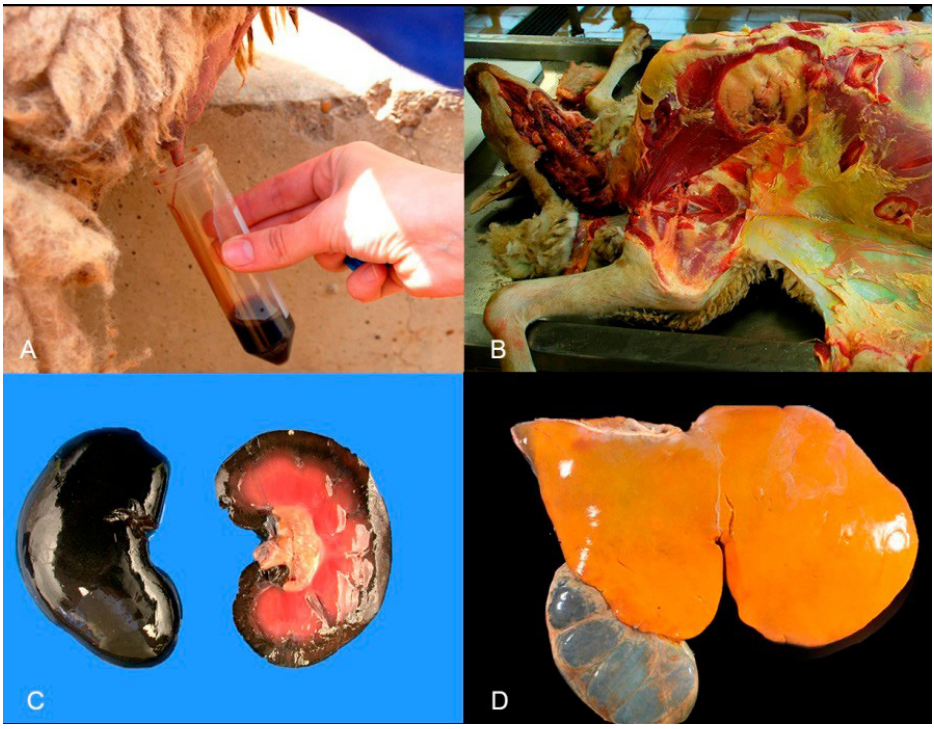


**Figure 2**. Sheep. Clinical signs and gross pathology of chronic copper poisoning. (A) Reddish brown urine. (B) The subcutaneous connective tissue showing an intense yellow colour interpreted as marked icterus. (C) Kidney. Dark "gunmetal" colour of the kidney surface and the renal cortex. Note the yellowish colour of the fat in the renal pelvis. (D) Liver. The organ shows a slight increase in size with rounded margins and orange-yellow discoloration (18).

In cases of acute copper poisoning, the diagnosis can be made based on the history, clinical signs of toxicity, and the presence of dark green-colored feces. Elevated blood or serum copper levels and fecal copper levels of 8,000-10,000 ppm also indicate acute toxicity. However, high blood copper levels are not persistent (9,19).

Chronic copper toxicity results from the excessive accumulation of copper in the liver due to low doses of ingestion, leading to a hemolytic crisis characterized by jaundice and hemoglobinuria. This condition can be mistaken for various parasitic or other disease conditions. Therefore, in order to reach a definitive diagnosis in chronic copper toxicity, measurement of copper levels

in liver and kidney tissue is necessary. In sheep, liver copper levels above 150 ppm and kidney copper levels above 15 ppm are considered toxic (9, 11, 19, 30, 31). Additionally, in chronic toxicity, liver enzyme concentrations, especially ALT and AST, are usually elevated a few days or weeks before the hemolytic crisis. During the hemolytic crisis, the blood profile shows methemoglobinemia, hemoglobinemia, decreases in PCV (packed cell volume), and blood glutathione concentration (12, 15, 16, 20).

## 6. TREATMENT AND PREVENTION

When hemolytic crisis and liver damage occur, the success of treatment is low. The treatment of copper toxicity aims to control the symptoms and restore the health of the animals. Prevention strategies play a crucial role in controlling copper toxicity. The primary goal should be the identification and elimination of the source of high levels of copper.

Copper poisoning is a serious condition, especially in sheep, and it is important for the veterinarian to make an accurate diagnosis and treatment plan. In acute cases, treatment mainly consists of supportive therapy targeting shock, dehydration, and gastrointestinal system damage(5, 6, 15, 16, 29). Similar to other toxicities, emetic drugs may be administered to rapidly empty the digestive system. Additionally, chemical antidotes such as magnesium oxide, sulfur, and potassium ferrocyanide are applied against unabsorbed copper in the digestive system. Furthermore, administration of penicillamine (50 mg/kg/day, orally, for 6 days) or vitamin C (500 mg/day per sheep, subcutaneously) in the early stages of the disease can be beneficial in increasing copper excretion (11, 23-28,32).

To prevent or control chronic toxicity, sodium thiosulfate or ammonium molybdate can be added to high-risk sheep rations. In addition, oral administration of 50-500 mg of ammonium molybdate and 300-1000 mg of sodium thiosulfate significantly reduces liver copper load after 6 weeks (19, 22-24).

Copper toxicity in sheep is a significant clinical condition that adversely affects animal health and production. Therefore, sheep farmers and veterinarians should be aware and take appropriate measures for the early diagnosis and effective treatment of copper toxicity. This way, the effects of copper toxicity in sheep can be minimized, and high levels of productivity can be achieved.

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