CONTEMPORARY METHODOLOGICAL APPROACHES IN HEALTH SCIENCES

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Contemporary Methodological Approaches in Health Sciences

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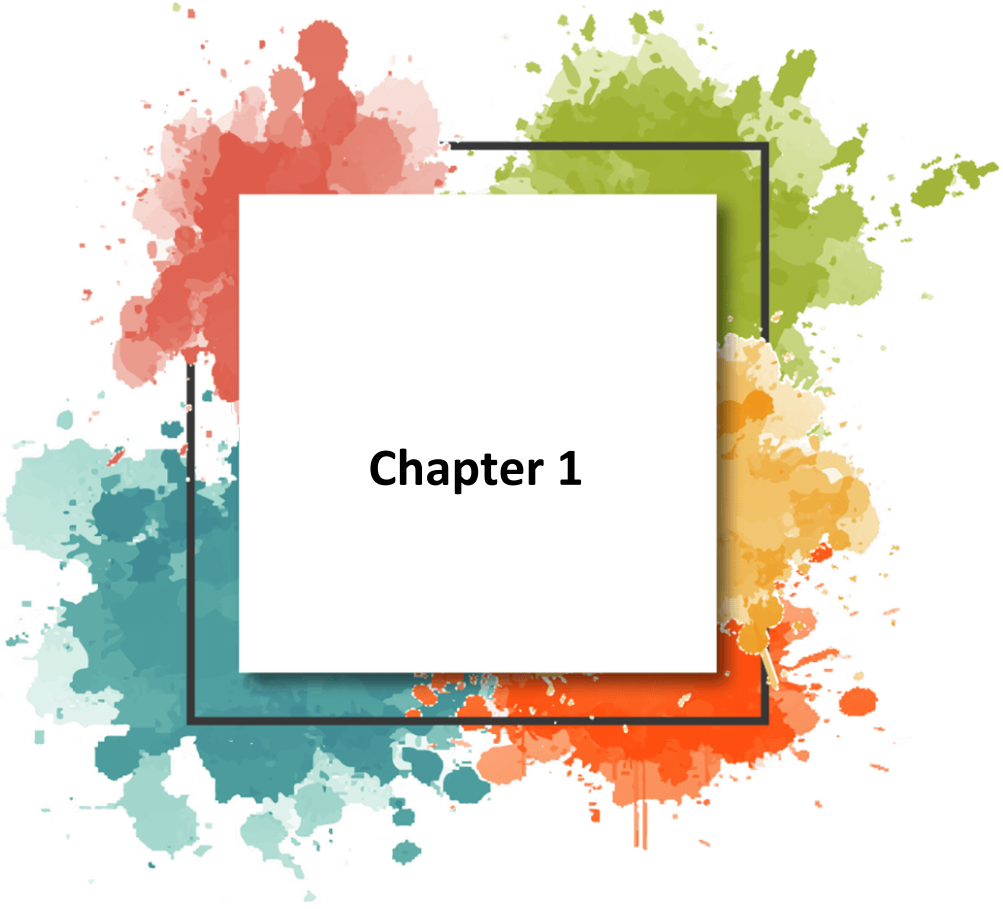
CONTENTS


Chapter 1

Migraine Genetics

***Ferhat Kiliçaslan*[1]**

Migraine is a multifactorial disease in which both genetic and environmental factors play a role in its pathophysiology(1). Family and twin studies reported that heritability of migraine is about %30-60(2–4). Migraine with aura (MA) have higher heritability then migraine without aura (MO) (5) and some motor forms of aura such as familial hemiplegic migraine show Mendelian inheritance(6). Nevertheless, most of migraine which is without aura is polygenetic and environmental(1,6). While the high potency of mutations in monogenetic diseases directly affects clinical manifestations, environmental factors also become important in polygenetic forms. Therefore, migraine research has focused on monogenetic forms due to both methodological and cost considerations(1). Mendelian forms of migraine are heavily considered to be monogenetic, and primarily monogenic migraine type is familial hemiplegic migraine(6).

Familial hemiplegic migraine (FHM)

Hemiplegic migraine is (is considered to be) migraine with motor aura symptoms(7). Hemiplegic migraine can occur sporadically, but familial forms show autosomal-dominant heredity(8). Three genes mutations that is missense reported in FHM are CACNA1A, ATP1A2 and SCN1A (9–11). FMH1 is mutation of CACNA1A, FMH2 is ATP1A2 and FHM3 is SCN1A and in rat models these mutations shows cortical spreading depression (CSD) and behaviours that might be considered migraine like symptoms(12–14). FMH1 gene encodes Cav2-1 protein FMH2 gene encodes ATP1A2 protein and FMH3 gene encodes Nav1-1 protein(9–11). FHM1 and FHM2 genes takes part of glutamatergic signalling and mutations of this genes ultimately increases glutamate in extracellular space(15).FHM 3 gene encodes voltage dependent sodium channels especially in GABAergic inhibitor neurons, this gene previously was known as epilepsy gene but this mutations is complex and there is no animal model available to literature(16–18).

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Gene mutations reported with some FHM patients but mainly associated with other diseases

PRRT2 gene mutations causes a large part of paroxysmal kinesigenic dyskinesia patients (PKD)(19), this mutation also reletad to various conditions such as hemiplegic migraine ataxia and epilepsy(19,20).

PNKD protein: The paroxysmal non-kinesigenic dyskinesia protein interacts with PRRT2 and regulates release inflammatory peptides (19).

ATP1A3, SLC1A3 and SLC2A1mutations are autosomal dominant, reported in FHM patients but associated with alternating hemiplegia of childhood, rapid onset parkinsonism, episodic ataxia type 6, GLUT deficiency syndromes(19,21–24)

Familial non-hemiplegic migraine

Migraine in most cases is polygenetic and multifactorial and yet in some cases it acts like mendelian heredity make researchers suggest that presence of highly penetrant gene variants. KCNK18 encodes potassium channel proposed to monogenetic cause of migraine with aura(25). This mutation leads to increased excitability of neurons(26). CSNK1D encodes casein kinase 1δ (CKIδ) that takes role in circadian cycle. Mutation of this gene caused altered sleep cycle and also co-segregated with migraine with aura(27).

Also, we should note that some cerebrovascular disorders like CADASIL features migraine, is caused by NOTCH3 mutations.

Genome-wide association studies (GWAS)

Both migraines with and without aura shows strong familial aggregation make researchers suggest that Mendelian inheritance. Gene mutations that found in FMH were not linked to MA or MO. Since genetic basis of migraine could not be established with monogenetic mutations researchers turned to GWAS. Since 2010 multiple GWAS studies ~~performed~~ conducted, and ~~altogether~~ approximately 180 low-impact ~~effect~~ genetic variants ~~that have~~ having a role in vascular and neuronal (nerve?) tissues have been identified. Those studies confirm migraine is polygenetic multifactorial neurovascular disorder(28–30).

Treatment response and genetic of migraine

Response to treatment is affected by many conditions. Genetic factors may affect absorption, distribution protein bindings metabolization and excretion of drugs. Most drug studies investigate cytochrome p450. ~~For genetics~~ According to genetic research, ~~that~~ single nucleotide polymorphism (SNP) mutations in C939T cause a negative response to triptans (31), SLC6A4STin2VNTR mutations lead to inconsistent response to triptans in migraine (32) and ~~lastly~~ finally PRDM16rs2651899 mutations ~~effect in~~ affect migraine (33).

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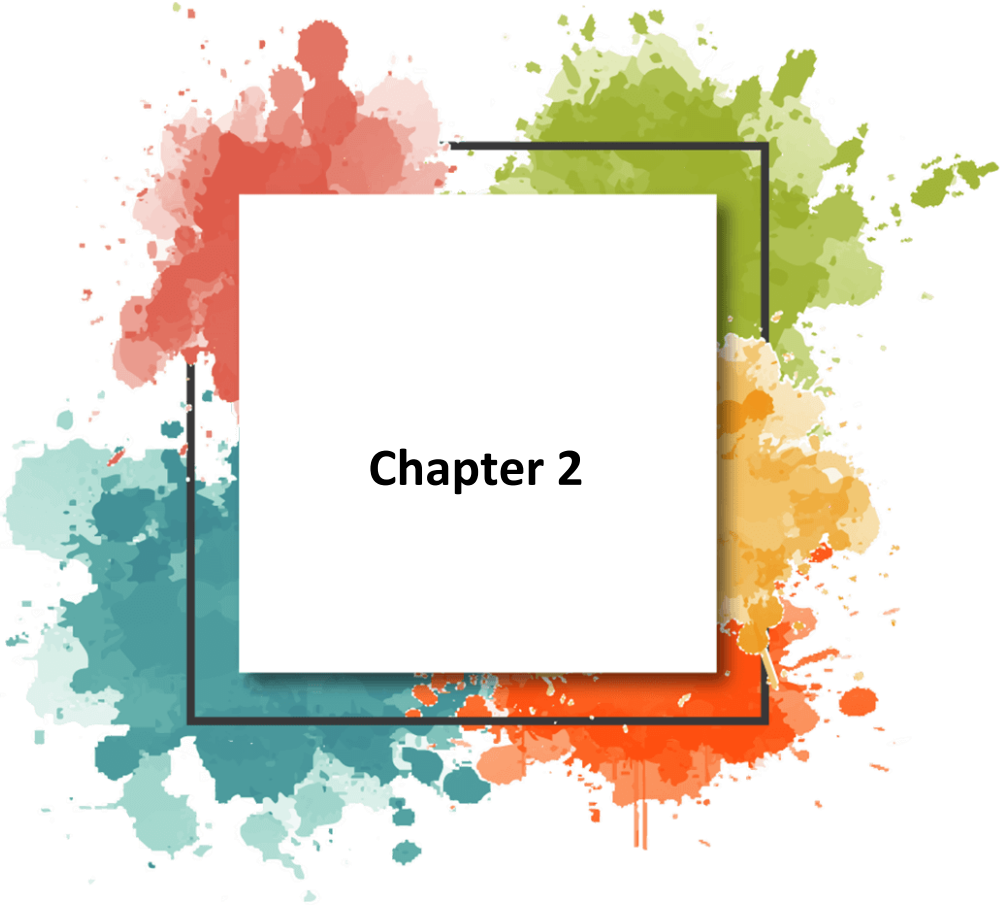
Chapter 2

Pathophysiology of the Tension-Type Headache

***Ferhat Kiliçaslan*[1]**

Tension-type headache (TTH) lasts hours to days, occurs on bilateral location and shows non-pulsatile quality and mild or moderate intensity (1). Historically TTH was considered caused by emotional disturbances and sustained contraction of skeletal muscles of neck and head areas(2). In mid-19. century researchers considered both central and peripheral nervous system contribution of TTH and some trigger areas defined. The hypothesis was hypersensitive peripheral areas overwhelm central nervous system and there should be another mechanism that contributes so pain when peripheric stimuli is absent(3). Later studies observed that first degree relatives of chronic TTH have more than 3-time risk developing chronic TTH compere to population these observational studies open new discussions of genetics for TTH(4).

Pathophysiology

By sustained tooth clenching, increased peri cranial tenderness and lower pain threshold is found in patients develop TTH (5). Additionally, peri cranial tenderness is found in patients with TTH not only during pain but also in pain free periods(6–13). However increased pain sensitivity does not cause TTH but occur as a consequence (14).

Peripheral mechanisms

Local soft tissue injury or inflammation may cause increased or abnormal sensitivity of peripheric neurons that eventually lead to hyperalgesia or even allodynia (11). Myofascial trigger areas are hard muscle areas that are hypersensitive to palpation and pressure(3). Multiple myofascial trigger areas are found around head and neck that cause pain which could mimic TTH. By infusing hypertonic saline in these myofascial trigger areas pain of TTH can be clinically reached(15). Some researchers speculated that muscle ischemia and local vasospasms may induce TTH, but literature does not support this claim(16,17).

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Central mechanisms

Central sensitization is proposed for TTH chronification, Spinal trigeminal nucleus, dorsal horn, somatosensory cortex, thalamus sensitization is considered(18). Functional MRI studies and gray matter density measurements show that decreased neuronal synchronization and altered gray matter density in pain processing areas in patients with TTH. Gray matter density alteration was dynamic and reversible (19,20). Ashina suggested psychological comorbidities may contribute increased pain sensitivity but pathophysiologic mechanism and full role of limbic system has not been discovered.

Molecular mechanisms

Multiple molecules studied in patients with TTH(21). Since nitric oxide has potential to effect both peripheric and central pain regulation, is most investigated molecul(22,23). Nitric oxide has been shown that induce pain in patient with TTH and inhibition of synthase of nitric oxide reduce pain intensity and muscle tenderness(24). Seretonin, substance P, neuropeptide Y and VIP level studies has been designed but no significant differences found.(21)

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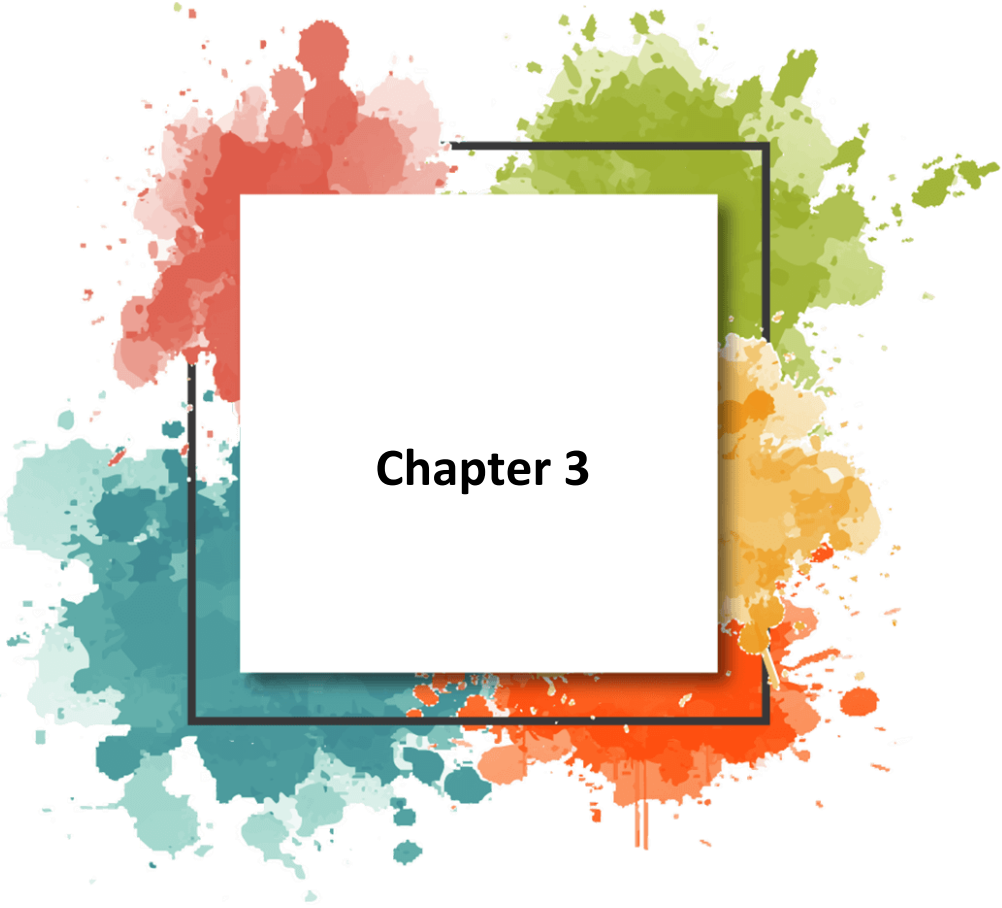
Chapter 3

A Contemporary Perspective on Mitochondria in Wound Healing

***Omer Faruk Taner*[1]**

Introduction

The skin is a protective barrier that covers the outer part of the body and is in constant interaction with the environment. Consequently, it is highly vulnerable to traumas. Furthermore, the skin performs a number of essential functions, including sensation, sweating, regulating body temperature, preventing fluid loss and synthesizing vitamin D (Nuutila et al., 2014). The epidermis, in particular, is a continuously renewed tissue. This is achieved by epidermal progenitor cells, which demonstrate high rates of proliferation and metabolic activity. These highly active cells require a substantial quantity of ATP. The primary source of ATP is oxidative phosphorylation in mitochondria, which serve as the bioenergy center of cells (Casanova et al., 2023).

One of the most prevalent forms of damage to the skin is trauma (Bernatchez & Bichel, 2023). The skin is susceptible to damage from external factors such as cuts, strokes, burns, and other injuries that are commonly encountered in daily life. Furthermore, surgical procedures, pressure sores in inpatients, diabetes and vascular diseases, etc. It is of significant importance that the skin, which is susceptible to damage from a multitude of sources, is capable of rapid regeneration and healing of the affected area.

Wounds are a common occurrence and can have significant implications for an individual's health, depending on the severity of the wound and the underlying condition. It has been reported that approximately 2 million people in Europe and 6.5 million people in America have chronic wounds at a given time (Lindholm & Searle, 2016; Sen et al., 2009). Europe, it has been reported that 64% of wounds treated at home are chronic, with 16% of these wounds remaining unhealed for a period exceeding one year (Drew et al., 2007). Wound treatments constitute 3% of total healthcare expenditure (Lindholm & Searle, 2016) and £5 billion is spent annually in the UK (Guest et al., 2015). It is estimated that between 27% and 50% of inpatients receive some form of wound treatment (Posnett et al., 2009).

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Wound Healing Process

In light of the vital functions performed by the skin and its significance to the human body, it is evident that severe skin injuries represent a significant life-threatening concern. In an optimal scenario, the skin would return to its original anatomical structure, functionality, and appearance. The regulation of skin integrity and homeostasis is achieved through the activation of complex mechanisms in response to tissue damage (Eming et al., 2014). The aforementioned mechanisms collectively constitute the process of tissue repair, which can be summarized as follows: hemostasis, inflammation, proliferation and maturation. These processes involve cells, cytokines and growth factors (Greaves et al., 2013; Zomer & Trentin, 2018).

In the hemostasis phase of wound healing, platelets interact with collagen in the opened vessel wall, forming a temporary clot that facilitates hemostasis. Thromboxane A2, which is secreted during this process, activates platelets and exerts a vasoconstrictor effect (Scopelliti et al., 2022). Another classification considers the hemostasis phase together with the inflammatory phase. The inflammatory phase follows wound formation and includes the coagulation cascade, the inflammatory pathway and the involvement of the immune system. Hemostasis is achieved by platelet clot formation and the formation of a fibrin matrix, which provides a suitable environment for cell infiltration. As a result of platelet degranulation, release of chemotactic agents from damaged tissue and release of bacterial degradation products, the complement system is activated, and neutrophils arrive at the lesion. Macrophages coordinate the damage response events in this process. These cells are also responsible for fibrin phagocytosis and removal of cellular debris (Tottoli et al., 2020). Molecules such as transforming growth factor-beta (TGF-β), interleukin-1β (IL-1β), tumor necrosis factor α (TNFα) and ROS are among those involved in this process. In addition, preclinical studies have shown that inhibition of molecules such as CCL2, CCL21, CXCL12 and CXCR4 can improve the wound healing process. (Ridiandries et al., 2018). In the subsequent proliferation phase, new tissue formation, granulation, and epithelial tissue formation occur, accompanied by the construction of the vascular network. The formation of the epidermal barrier is the responsibility of keratinocytes, while fibroblasts and endothelial cells are responsible for establishing the vascular network and extracellular matrix. In this process of re-epithelialization, characterized by the proliferation and migration of keratinocytes towards the core of the lesion, the area between the base and the edges of the wound is filled with granulation tissue (Hye Sung Kim et al., 2019). During the maturation phase, the newly formed tissue undergoes a process of reorganization. At this stage, activated processes are deactivated and macrophages, isolated endothelial cells and myofibroblasts undergo apoptosis.

They then migrate out of the wound, leaving behind a region rich in collagen and other extracellular matrix proteins. Thereafter, interactions between the epidermis and dermis ensure the continued regulation of skin integrity (Enoch & Leaper, 2008). The duration of this process can span several years (Monaco & Lawrence, 2003).

The majority of individuals have encountered a wound at some point in their lives. In the majority of cases, the healing process is straightforward and rapid. Even in the event of the formation of scar tissue, there is no permanent loss of function or serious complications. However, in some patients, the healing period is prolonged, resulting in the emergence of symptoms that have a detrimental impact on the quality of life. Such injuries are designated as " hard-to-heal wounds" which fail to demonstrate optimal healing outcomes when treated with conventional methods (Azevedo et al., 2020). The present context allows for the consideration of both acute and chronic wounds, irrespective of the specific type or underlying cause. In a considerable number of cases, particularly in the elderly with comorbidities, the management of wounds becomes challenging (Vowden & Vowden, 2009). Furthermore, additional factors influencing the process of wound healing include wound size, depth, location and biological load (Morton & Phillips, 2016).

Today, optimal wound healing is achieved by a treatment regimen that includes good blood supply, removal of dead tissue, protection from infection and good moisturization of the wound bed. To this end, wound dressings, sharp debridement, wound cleansing, topical antimicrobials, negative pressure wound therapy for deep wounds, rapid closure of acute wounds and various therapies (hyperbaric oxygen, energy wound stimulation, etc.) are used to improve wound healing (Frykberg & Banks, 2015). Despite this, there is no definitive method that can be used for skin regeneration. A significant proportion of the problems caused by wounds, especially chronic wounds, are related to treatment and management practices that limit wound repair rather than restoring tissue integrity (Borena et al., 2015). However, it is believed that new opportunities can be created through interdisciplinary collaboration, particularly in the treatment of difficult wounds.

The Current Approaches to Wound Healing

The presence of permanent scarring has been demonstrated to have a detrimental impact on an individual's quality of life, as well as causing psychological distress. This results in prolonged hospitalization and a notable increase in treatment costs. It is associated with increased morbidity and mortality rates. The impact of wounds on individuals and the healthcare system is significant, yet often overlooked (Lindholm & Searle, 2016). Due to these characteristics, wounds are referred to as "the silent epidemic" (Queen, 2010).

In the present era, the issue of how to meet the growing demand for wound healing with the limited resources available has become a matter of concern. The primary objective is to enhance the efficacy of the therapeutic intervention. Today, a variety of procedures and novel treatment modalities are being delineated with the objective of enhancing the efficacy of wound healing. In order to achieve this, processes such as debridement procedures, infection prevention, moisture balance, exudate management and epithelial edge advancement are given particular emphasis. For instance, advanced wound dressings are being considered as a means of facilitating the healing of complex wounds while reducing costs. The novel types of wound dressings are regarded as a platform that absorbs exudate, safeguards the wound from external influences, regulates the lesion microenvironment and incorporates growth factors, anti-fibrotic, anti-microbial and anti-inflammatory agents (Kamoun et al., 2017; Morton & Phillips, 2016). Furthermore, regenerative medicine constitutes an area of significant emphasis within the domain of wound healing. Regenerative therapy represents a multidisciplinary approach that employs the use of growth factors, stem cells, and biomaterials in conjunction to induce tissue regeneration or stimulate wound healing. This approach is regarded as a potential paradigm shift in treatment (Safferling et al., 2013). Further research is being conducted in a number of other areas, including the development of cellular skin implants that mimic the extracellular matrix, gene therapy, cell programming technologies (such as induced pluripotent stem cells) and tissue engineering (High & Roncarolo, 2019; H. S. Kim et al., 2019; Li et al., 2019; Soriano et al., 2023).

The Role of Mitochondria in Wound Healing

The intricate mechanisms that underpin the healing process following the formation of a wound result in a considerable energy requirement. Furthermore, mitochondria play a pivotal role in the healing process, as evidenced by their involvement in a multitude of essential biological processes, including cell differentiation, inflammation, cytokine production, growth factor production, and neoangiogenesis (Schiffmann et al., 2020). In this context, some studies have reported that the number of mitochondria increases during the process of wound healing (Kotil). Additionally, platelets have been observed to enhance the contribution of mesenchymal stem cells to wound healing through the transfer of mitochondria (Levoux et al., 2021). In this context, it has been demonstrated that mitochondrial respiration plays a regulatory role in neoangiogenesis during the process of wound healing (Schiffmann et al., 2020).

The current research landscape encompasses a multitude of approaches that target mitochondria in the context of wound healing, with a particular emphasis on addressing the heightened cellular energy requirements that accompany this process. In addition to their role in energy production, mitochondria are involved

in a number of other important biological processes, including apoptosis, infiltration and the biosynthesis of cytokines (Marchi et al., 2023; Tait & Green, 2012). Furthermore, numerous diseases resulting from diverse mitochondrial anomalies have been documented. Mitochondrial dysfunction has been linked to a number of dermatological conditions, including skin cancers, hair and hair anomalies, inflammation, pigmentation disorders, acrocyanosis, non-epidermolytic palmoplantar keratoderma (NEPKK), Dupuytren's disease and Rothmund-Thomson syndrome (Bodemer et al., 1999; Maász et al., 2008; Michou et al., 2012). Furthermore, it has been linked to a number of diseases that affect different tissues and organs, including Kearns-Sayre Syndrome, mitochondrial encephalopathy, lactic acidosis, and Stroke-Like Episodes (MELAS), Pearson Syndrome, and MERRF Syndrome (Myoclonic Epilepsy, Ragged-Red Fibres). Additionally, it has been associated with various forms of cancer, cardiovascular disease, neurodegenerative disorders, and metabolic conditions (Picard et al., 2016).

There are many studies investigating the role of mitochondria in wound healing via enhancing intrinsic mitochondrial functions. In one study, it was demonstrated that SkQ1, an antioxidant that targets mitochondria, resulted in a reduction in the production of oxidative agents and a stabilization of mitochondrial functions. Furthermore, evidence indicates that it enhances granulation tissue formation, angiogenesis and epithelialization (Demyanenko et al., 2017).

A considerable number of chronic wounds are the consequence of chronic inflammation. Therefore, research aimed at reducing the inflammatory process in wound healing can facilitate accelerated healing and an improved final scar appearance (Rosique et al., 2015). In addition to the control of inflammation through the use of drugs such as non-steroidal anti-inflammatory drugs and phyto-modulators, novel approaches, including the inhibition of marrow-derived mesenchymal stem cells and microRNAs, are currently under investigation (Shukla et al., 2019). Mitochondria are additionally characterized as an organelle that regulates the process of inflammation (Meyer et al., 2018). Given the pivotal role of mitochondria in the inflammatory response, there is a growing interest in the potential of mitochondrial-targeted therapies to reduce inflammation and enhance the healing process (Hunt et al., 2023).

In a further study of the treatment of diabetic wounds with photobiomodulation, the dynamics of mitochondria were investigated. The results demonstrated that the treatment had a beneficial impact on wound healing by influencing mitochondrial dynamics and maintaining cellular homeostasis. It was posited that mitochondrial dysfunction is associated with diabetes mellitus, which has a deleterious impact on wound healing (Tatmatsu-Rocha et al., 2018).

Another innovative approach is the field of mitochondria transplantation, which has gained significant attention in recent times. Mitochondria possess an autonomous genome. However, it lacks the requisite genetic material to fulfill its functions (Sendra et al., 2021). In this sense, it is a rare organelle with partial connections to the nucleus. Given these characteristics and its double-membrane structure, this organelle is capable of being transferred between cells and transplanted from outside (Caicedo et al., 2017).

Research on Mitochondrial Transplantation Studies in Wound Healing

It is possible for mitochondria to be transferred between cells under a number of different conditions within the human body. In this context, it has been demonstrated that platelets enhance the capacity of mesenchymal stem cells to repair wounds by transferring mitochondria between cells. It has been demonstrated that the process of wound healing can be accelerated by the external transfer of mitochondria isolated from platelets. It has been shown that human dermal fibroblasts are capable of internalizing isolated exogenous mitochondria, which has been shown to result in increased cell proliferation and wound cavity closure (Kim et al., 2023).

Similarly, electron microscopy analysis demonstrated that mitochondria were transferred from activated platelets to target HUVEC cells in both free form and within vesicles. In this instance, it was was found to be effective in reducing ROS levels, enhancing mitochondrial membrane potential and inhibiting apoptosis. In the subsequent phase of the study, mitochondria derived from platelets were introduced into HUVEC cells, with the observation that they entered the cell via dynamin-dependent clathrin-mediated endocytosis. In addition, it was demonstrated that HUVEC oxidative stress was reduced, apoptosis was diminished, and in vivo wound healing was facilitated by mitochondrial transplantation. Platelets were identified as a appropriate source of mitochondria for this process (Jin et al., 2023).

Fibrosis in wound healing represents an irregular wound healing response following tissue damage (Henderson et al., 2020). The pathways involved in the pathogenesis of fibrosis converge in the activation of fibroblasts, which are cells responsible for producing the extracellular matrix (Moore & Herzog, 2013). The primary activator of fibroblasts is elevated transforming growth factor-β (TGF-β) signaling, which culminates in an excessive accumulation of extracellular matrix components (Rockey et al., 2015). It has been demonstrated that TGF-β signaling and fibroblast activation are associated with mitochondrial dysfunction (Gibb et al., 2020; Sun et al., 2020). A study conducted in this context demonstrated that stimulation of fibroblasts with TGF-β was associated with fibrosis-related pathways. Furthermore, it was observed that transplantation of mitochondria to fibroblasts stimulated with TGF-β resulted in the elimination of

the metabolic shift towards glycolysis, a reduction in ROS formation, a reduction in extracellular matrix protein synthesis, and a reduction in fibroblast migration and proliferation. It was therefore concluded that mitochondria-targeted strategies have the potential to be developed into effective therapeutic methods for the treatment of fibrosis (Baudo et al., 2023).

Mitochondrial damage or dysfunction is a common occurrence in cases of burns, which represent a significant injury type that results in impaired skin integrity. A study investigating the effects of mitochondrial transplantation in burn treatment in vivo and in vitro revealed that cell viability, colony formation, proliferation and migration were enhanced, and apoptosis was reduced in HaCaT cells. In in vivo evaluation, it was observed that the process of wound healing was promoted, the level of inflammation decreased, the rate of proliferation recovery accelerated and the formation of scars was reduced (Li et al., 2024). The study investigating the effects of mitochondrial transplantation on corneal epithelial wound healing demonstrated a two-fold acceleration in the healing process when observed in vivo. It was observed that the wound area underwent re-epithelialization, resulting in a multi-layered appearance and restoration of normal corneal dehydration (Raz et al., 2024).

The favorable outcomes of this preclinical investigation indicate that mitochondrial transplantation may prove to be an efficacious approach to facilitate human wound healing. Indeed, in one study, healthy autologous mitochondria from skeletal muscle were used to promote chronic pressure wound healing. The results demonstrated that mitochondrial transplantation was an effective method for promoting wound healing, as evidenced by a reduction in wound size, an increase in granulation tissue, and an acceleration of epithelialization (Taner et al., 2024).

Conclusion

Mitochondria-based research is currently being conducted in a number of different fields. On the one hand, these studies examine the role of mitochondria in disease formation; on the other, they attempt to develop mitochondria-targeted treatment approaches. Furthermore, research on mitochondria in wound healing is also yielding positive results. It is evident that novel methodologies, such as mitochondrial transplantation, can confer substantial advantages by facilitating wound healing and reducing both patient discomfort and healthcare expenditure. Further research is likely to facilitate accelerated progress in this field.

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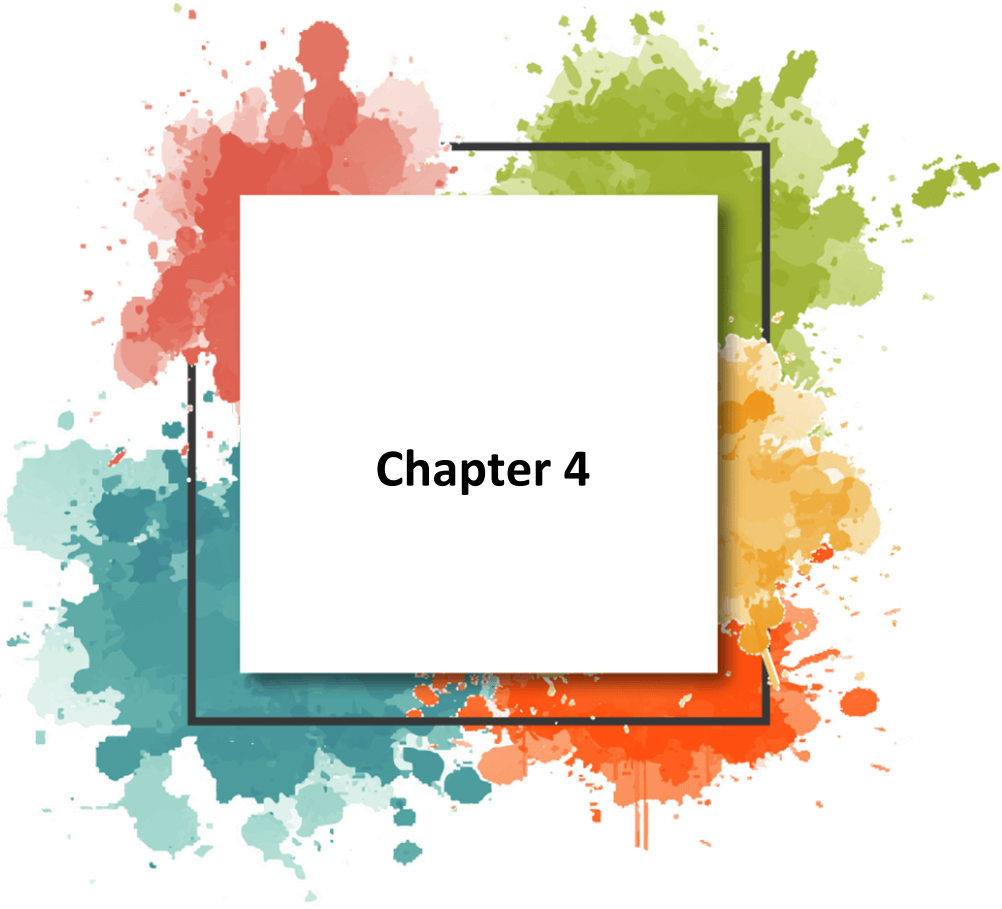
Chapter 4

The Process of Physiological Root Resorption in Primary Teeth

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Root resorption is characterized by the gradual loss of the tooth's mineralized tissues, including cementum and dentin, associated with odontoclastic activity (Patel et al., 2009). While the permanent teeth erupting, resorptive processes occur in the bone overlying the erupting tooth, in the roots of primary teeth, and in supporting tissues such as the periodontal ligament, in order to establish an eruption pathway (Bhaskar, 1991; Ten Cate & Anderson, 1986; Ten Cate, 1998).

Odontoclasts are the primary cells responsible for dental hard tissue resorption, whereas osteoclasts are responsible for bone resorption (Furseth, 1968). Odontoclasts, which are considered osteoclast-like cells, play a key role in the resorption of dental hard tissues during the physiological root resorption of primary teeth (Bhaskar, 1991; Furseth, 1968; Morita et al., 1970; Sasaki et al., 1988; Ten Cate & Anderson, 1986).

During the exfoliation of primary teeth, resorption of bone and dental hard tissues is predominantly mediated by multinucleated resorptive cells, with mononucleated resorptive cells contributing to a lesser extent (Ne, 1999). Parathyroid hormone is involved in the activation and proliferation of osteoclasts, whereas calcitonin plays a significant role in reducing osteoclastic motility and inhibiting resorption (Sasaki, 1988; Ten Cate & Anderson, 1986). Resorptive cells interact closely with macrophages and monocytes, and the biological events underlying the resorption process are regulated by cytokines, enzymes, and hormones (Domon et al., 1997).

Physiological root resorption of primary teeth involves the degradation of both organic and inorganic components of the dental matrix. The breakdown of the organic matrix is mediated by enzymes such as collagenases, proteases, and hyaluronidases, whereas the degradation of the inorganic matrix is carried out by odontoclasts and lysosomal enzymes. These processes may occur concurrently, or organic matrix degradation may precede the demineralization of the inorganic matrix. Physiological root resorption is not a continuous process; rather, it

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progresses with alternating phases of resorption and repair, during which a cementum-like tissue accumulates within resorption lacunae (Bhaskar, 1991; Furseth, 1968; Yawaka et al., 2003; Sasaki, 1988; Sasaki, 1990). It has been reported that the cementoid and/or cementum-like structures deposited on the dentin surface within resorption areas during physiological root resorption are produced by active cementoblasts adjacent to active odontoclasts, and that these cells contribute to the remodeling of the dentin surface in the resorption area (Sasaki, 1990; Yawaka et al., 2003).

In the physiological process of primary tooth root resorption, multiple factors play a role, including the eruptive pressure of the permanent tooth, the activity of endocrine glands, and the degree of vascularization within the resorption area (Obersztyn, 1963; Sasaki, 1989; Sasaki, 1988; Ten Cate & Anderson, 1986; Ten Cate, 1998). It has been suggested that the most significant stimulus for the activity of resorptive cells responsible for root resorption is the development of the permanent tooth located beneath the primary tooth and the internal pressures generated by tooth eruption (Bhaskar, 1991; Furseth, 1968; Obersztyn, 1963; Ten Cate & Anderson, 1986). However, it has also been reported that root resorption may occur, albeit in a delayed manner, in primary teeth lacking an underlying permanent tooth germ (Harokopakis-Hajishengallis, 2007; Obersztyn, 1963; Rune, 1984).

In addition to these factors, the overall growth and development of the organism, the increasing discrepancy between masticatory forces and the morphological structure of primary teeth, excessive occlusal forces affecting primary teeth, and pathological conditions leading to inflammation in primary teeth and periodontal tissues may also contribute to primary tooth root resorption (Bhaskar, 1991; Furseth, 1968; Morita et al., 1970; Obersztyn, 1963; Sasaki, 1988; Ten Cate, 1998).

It is widely accepted that the pressure exerted by the erupting permanent tooth plays a contributory role in determining the pattern and progression of resorption (Sahara, 2001). Nevertheless, the presence of a permanent tooth is not a prerequisite for the occurrence of this process. The resorption process in primary teeth without an underlying permanent tooth germ may occur later than normal (Harokopakis-Hajishengallis, 2007; Obersztyn, 1963).

Biological Mechanisms Influencing Primary Tooth Root Resorption

Root resorption leading to the exfoliation of primary teeth is a physiological phenomenon that results in the eruption of the underlying developing permanent teeth into the oral cavity (Furseth, 1968). The remarkable consistency and symmetry in the timing of exfoliation of primary teeth on the right and left sides of the oral cavity, as well as in the replacement of exfoliated teeth by permanent

successors, are striking and are considered to represent a sequence of programmed biological events (Harokopakis-Hajishengallis, 2007).

Although numerous theories have been proposed regarding the factors influencing this process, none has clearly elucidated the cause–effect relationship. The pressure exerted by the erupting permanent tooth, mechanical occlusal trauma, the overall growth and development of the organism, and inflammatory processes have all been evaluated in terms of their potential roles in this complex biological phenomenon (Obersztyn, 1963).

1. The Pressure Applied by the Permanent Tooth Germ to the Primary Tooth

Two fundamental factors are required for the occurrence of tooth eruption movements. The first is the force that drives the tooth along its eruption pathway, and the second is the removal of bone tissue and primary tooth roots from this pathway. It has been reported that the required force is generated by cellular proliferation at the apex of the erupting tooth. However, it has been observed that severing the apex after the onset of eruption does not halt the eruption process. Cellular activity within the periodontal ligament or variations in blood flow may also contribute to eruption, although no definitive conclusion has yet been reached regarding these mechanisms (Proffit et al., 2009).

It is widely believed that the pressure exerted by the erupting permanent tooth plays a contributory role in determining the pattern of resorption. In general, the pressure generated by the permanent tooth is considered an important factor influencing odontoclast differentiation and activation. Nevertheless, no clear cause–effect relationship has been established to date (Sahara, 2001). Moreover, the presence of a permanent tooth is not a prerequisite for the occurrence of this process. Although delayed compared to normal conditions, root resorption ultimately occurs in primary teeth lacking an underlying permanent tooth germ. This finding suggests that permanent teeth do not exert a direct effect on the initiation of the resorption process; however, the eruptive movement of the permanent tooth plays a crucial role in determining the direction and spatial pattern of primary tooth resorption (Obersztyn, 1963; Sahara, 2001).

Studies conducted by Marks and Cahill demonstrated that the dental follicle, rather than the permanent tooth itself, plays a controlling role in the tooth eruption process. In animal experiments, developing tooth crowns were removed and replaced with inert materials such as silicone and metal replicas, which were placed within the dental follicle. These materials were shown to erupt successfully into the oral cavity. These findings indicate that the dental follicle coordinates and regulates resorptive events, including the resorption of the bone overlying the follicle and possibly the roots of primary teeth (Marks, 1984).

In a histological and radiological study conducted in puppy dogs, root resorption of primary teeth and resorption of the bone overlying the erupting tooth occurred uneventfully, even when the eruptive movement of the permanent premolar tooth was mechanically inhibited using stainless steel wires. This observation suggests that the formation of the eruption pathway is not dependent on the direct and continuous pressure exerted by the erupting tooth on the surrounding bone (Cahill et al., 1969).

2. Role of Endocrine Functions and Nutrition

The eruption process of permanent teeth is influenced by various factors, including the function of endocrine glands (pituitary, thymus, and thyroid glands), malnutrition (calcium and magnesium deficiency), and vitamin deficiencies (vitamins A, C, and D). Therefore, these factors exert indirect effects on the root resorption process of primary teeth (Bastos et al., 2007; Obersztyn, 1963).

Conditions such as hypothyroidism, pituitary dwarfism, and chronic malnutrition have been reported to delay the exfoliation of primary teeth, presumably due to their inhibitory effects on the eruption of permanent teeth (Harokopakis-Hajishengallis, 2007; Kjellberg et al., 2000).

Some authors have attributed the development of primary tooth resorption to the activity of endocrine glands, the pressure exerted by the erupting permanent tooth, regulation by the nervous system, and the degree of vascularization in the resorption area (Obersztyn, 1963).

Mechanisms of Primary Tooth Root Resorption

Histological studies investigating root resorption have reported that it occurs through the activity of odontoclasts located along the apical region of the root or in the interradicular areas in primary molars (Furseth, 1968; Morita, 1970; Sahara et al., 1994; Sahara et al., 1996; Sasaki et al., 1990).

Root resorption in primary teeth begins in the region closest to the permanent successor. For example, in anterior teeth, the permanent teeth are positioned lingually to the apical and middle thirds of the roots of the primary teeth. With the eruptive force of the permanent teeth, resorption starts in the area of the primary tooth root that is closest to the permanent tooth. After the labial surface is resorbed, the permanent tooth becomes positioned beneath the root of the primary tooth. From this stage onward, resorption continues horizontally in a coronal direction. Once the process is completed, the primary tooth exfoliates and the permanent tooth takes its place in the oral cavity.

In some cases, permanent mandibular incisors may fail to move sufficiently in the labial direction during eruption. In such situations, incomplete or delayed

resorption of the roots of primary incisors may be observed, and the permanent teeth may erupt into the oral cavity from the lingual side of the primary teeth. In the primary molar region, developing permanent teeth are initially located lingually to the overlying primary teeth. As growth continues, the developing permanent tooth migrates beneath the divergent roots of the primary teeth. The position and dimensions of the dental follicle influence the pattern of root resorption.

At any given time, approximately 36% of primary teeth exhibit an uneven resorption pattern affecting one or more roots. The roots of mandibular second primary molars are highly curved and divergent, and the interradicular distance is greater than the size of the underlying follicle. Depending on the position of the permanent tooth follicle, unequal effects may be exerted on the roots. More than one-third of all mandibular second primary molars demonstrate an uneven pattern of root resorption.

In the maxilla, resorption is observed to begin behind the highly divergent palatal root of primary molars. In approximately 56% of maxillary second primary molars, the palatal root exhibits less resorption compared with the other roots. The incidence of uneven root resorption is lower in mandibular first primary molars, which has been attributed to the relatively small discrepancy between the interradicular distance and the crown size of the underlying permanent tooth (Avery & Chiego, 2006; Harokopakis-Hajishengallis, 2007).

Resorption occurring on the inner surface of the bony roof surrounding the permanent tooth follicle is accompanied by apposition on the outer surface of this roof. The bone surrounding the permanent tooth undergoes continuous remodeling by osteoclasts and osteoblasts in order to adapt to the eruptive movement of the tooth. When the rate of resorption exceeds that of apposition, the bony septum between the primary and permanent teeth progressively thins and eventually disappears. As the bone is lost, it is replaced by granulation tissue, allowing the crown of the permanent tooth to come into close contact with the root of the primary tooth. It has been reported that the physiological root resorption of the primary tooth begins precisely at this stage (Avery & Chiego, 2006; Patel et al., 2009).

Root Resorption in Primary Teeth

The root resorption process in primary teeth is generally described in three stages.

1. Active Root Resorption

The most distinctive feature of this stage is the presence of numerous giant odontoclasts with various configurations within the resorption area (Sasaki et al.,

1990). During the active root resorption phase, resorbed dentin surfaces exhibit resorption lacunae of varying depths and configurations. These lacunae are predominantly formed by odontoclasts, and some are partially covered by cementoblast-like cells.

In addition to odontoclasts, mesenchymal cells, neutrophils, fibroblasts, cementoblasts, and macrophages have been identified as playing significant roles within root resorption cavities (Sasaki et al., 1990). Demineralized zones are present around these lacunae. Demineralization of the extracellular matrix mediated by odontoclasts is followed by the release of apatite crystals. In this region, complete degradation of the organic matrix has been observed. This finding has been interpreted as evidence that the organic and inorganic components of dentin are degraded simultaneously (Avery & Chiego, 2006; Sasaki et al., 1988; Sasaki et al., 1990).

2. Resting Phase

During the resting phase of root resorption, a large portion of the relatively softened dentin surface is covered by cementoblast-like cells resembling bone-lining cells. These cementoblasts are not actively functioning cells. Macrophages may be present to some extent during this phase; however, active odontoclasts are absent. Collagen fibers and fibroblasts are also abundantly present.

Following the disappearance of odontoclasts, newly formed odontoclasts do not immediately appear. Occasionally, these cementoblastic lining cells form a structure resembling a small clear zone on the surfaces facing the resorbed dentin. During this intermediate period, in which resorption has ceased and odontoclasts are absent, this structure facilitates the absorption of small dentin particles released during resorption into deeper regions through deep membrane invaginations. The absorbed dentin particles occlude dentinal tubules, thereby protecting the pulp tissue from external stimuli (Sasaki et al., 1990).

3. Repair Phase

The repair phase is characterized by root repair associated with cellular cementum formation, during which cementoblasts produce precement and/or cementum matrix on the resorbed dentin surface (Sasaki et al., 1990). The deposited cementum appears as a band-like structure and consists of a single layer. This layer exhibits a relatively homogeneous structure and contains abundant microfibers, fine granular materials, granular substances, and collagen fibrils. The accumulated cementum structure differs markedly from the original cementum of primary teeth and from the cementum of permanent teeth (Yawaka et al., 2003).

These findings indicate that primary tooth root resorption is not a continuous process but rather one that includes resting periods. During these resting periods, cementum deposition may occasionally be observed on the resorbed root surface. As resorption progresses, repair processes occur simultaneously. These two processes do not occur sequentially but concurrently. Although both processes continue together, the resorption process predominates over repair, thereby driving the overall progression of the process in the direction of resorption (Domon, 2000; Yawaka et al., 2003).

According to some authors, resorption processes also occur in the pulp chamber and coronal dentin of primary teeth approaching exfoliation, and this phenomenon has been considered a fourth stage. Based on histological criteria, four stages can be observed in the pulp chamber of a primary tooth close to exfoliation. In the pre-resorptive stage, the walls of the pulp chamber are covered by an odontoblastic layer, and multinucleated odontoclasts are absent within the pulp chamber. In the early resorptive stage, multinucleated odontoclasts are present on the walls of the pulp chamber; however, the remaining surfaces of the pulp chamber are still covered by the odontoblastic layer. In the late resorptive stage, the odontoblastic layer disappears, and the entire surface of the pulp chamber is surrounded by multinucleated odontoclasts (Sahara et al., 1992). In the final resorptive stage, it has been reported that the resorbed dentin surface of the pulp chamber can be partially or completely repaired by cementum-like tissue (Sahara et al., 2004).

After root resorption is completed, the primary tooth begins to exhibit increased mobility due to the eruptive force of the underlying permanent tooth and masticatory forces, and exfoliates shortly thereafter (Avery & Chiego, 2006; Sahara et al., 1993; Patel et al., 2009). At the site of the exfoliated primary tooth, a physiological wound forms spontaneously. When a primary tooth exfoliates naturally in humans, minimal gingival bleeding is observed, and healing in this area has been reported to occur more rapidly than healing following tooth extraction (Sahara et al., 1993).

Factors Affecting the Timing and Sequence of Permanent Tooth Eruption

It is widely accepted that various biological and environmental factors influence the timing and sequence of permanent tooth eruption. Biological factors are related to innate and genetically determined biological variability. These factors include sex-related differences among individuals, ethnic variation, craniofacial growth patterns, and craniofacial morphology. Environmental factors, on the other hand, are acquired and not genetically determined. Examples of environmental factors that may affect the timing and sequence of tooth eruption include fluoride intake, dental caries, premature extraction of primary teeth, body constitution, and nutritional status (Shaweesh, 2012).

Factors influencing tooth eruption are generally classified as general and local factors. General factors include certain systemic diseases and syndromes, such as cleidocranial dysplasia, osteoporosis, Hutchinson–Gilford progeria syndrome, Gardner syndrome, and endocrinopathies. Examples of local factors include retention of primary teeth, ankylosis of primary and permanent teeth, space deficiency in the dental arches, mucosal barriers, radiation-induced damage, dense alveolar bone, odontomas or supernumerary teeth located between the crown of the permanent tooth and the alveolar crest, and eruption cysts (Proffit et al., 2009; Rhoads et al., 2013; Sivakumar et al., 2007; Wise, 2009; Wise & King, 2008).

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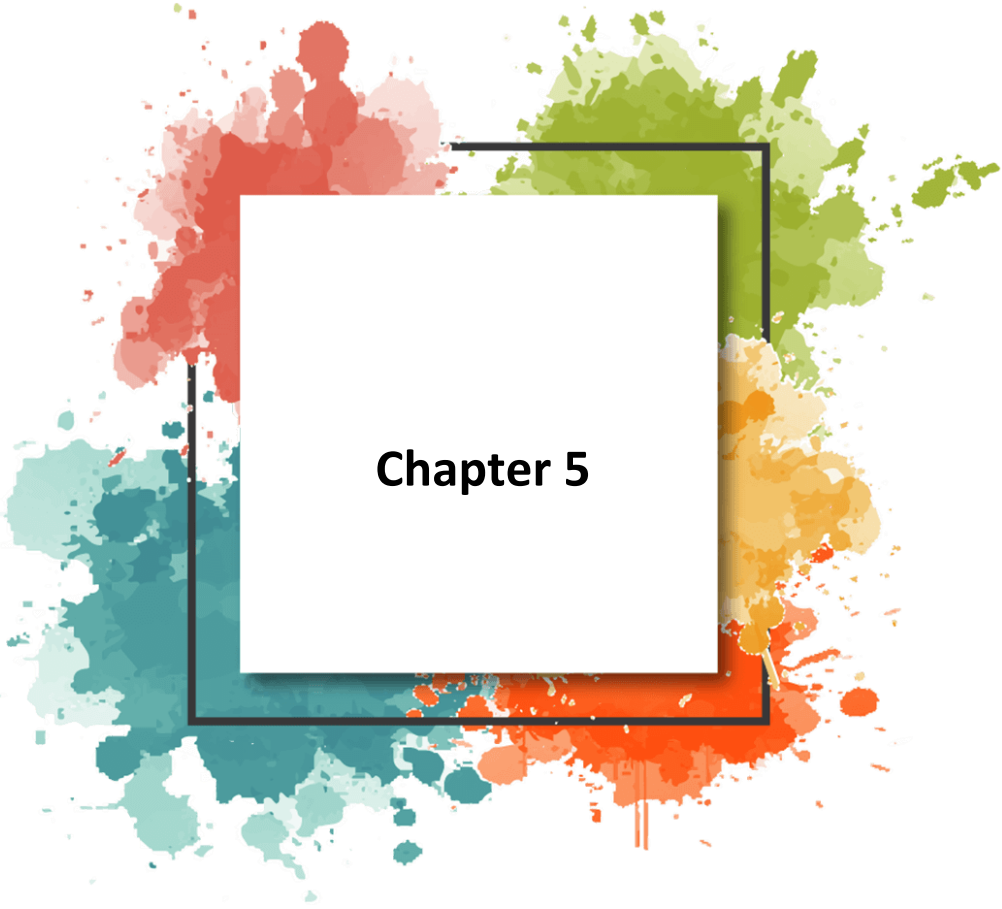
Chapter 5

Comprehensive Perspectives on Burnout and Chronic Fatigue Syndrome: Diagnosis, Neurobiological Bases, and Psychosocial Approaches

***Samet Kaya*[1]**

1. Introduction

In contemporary society, the phenomenon of being "completely burned out" has transitioned from a colloquial expression to a critical public health concern (CESUR, ÜNAL, BÜKER, ÇAĞLAYAN, & OKYAY, 2023; Demerouti, 2024). Although burnout and ME/CFS are distinct entities, they share a common thread of persistent, non-physiological tiredness that is not alleviated by rest (Grach et al., 2023; Schaufeli et al., 2009). Burnout predominantly targets the workforce, particularly those in "helping" professions like medicine and teaching, while ME/CFS presents as a complex biological disorder affecting millions worldwide (Arron et al., 2024; Jing et al., 2025; Ungur et al., 2024). Understanding the interplay between genetic vulnerability, environmental triggers, and neurobiological responses is essential for accurate diagnosis and effective recovery (Arron et al., 2024; Chmiel & Kurpas, 2025; König et al., 2022).

2. Conceptual Definitions

2.1. Burnout Syndrome

The concept of burnout was first introduced in 1974 by Herbert Freudenberger and later refined by Christina Maslach (Freudenberger, 1974; Maslach & Jackson, 1981). It is defined by three specific dimensions: emotional exhaustion, depersonalization (cynicism), and a reduced sense of personal accomplishment (Maslach, Schaufeli, & Leiter, 2001). The ICD-11 classifies it not as a medical condition but as an occupational phenomenon resulting from chronic workplace

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stress that has not been successfully managed (Bianchi et al., 2021; Schaufeli et al., 2009).

2.2. Chronic Fatigue Syndrome (ME/CFS)

ME/CFS is a debilitating chronic multisystem illness (Arron et al., 2024; König et al., 2022). Its primary hallmark is Post-Exertional Malaise (PEM)—a worsening of symptoms following even minor physical, cognitive, or emotional exertion (Grach et al., 2023). Fatigue in ME/CFS is profound, newly onset, and leads to a significant decrease in pre-illness activity levels (Arron et al., 2024; Grach et al., 2023).

3. Prevalence and Epidemiology

3.1. Global Burden of Burnout

Burnout is endemic in high-stress occupations (Bridgeman, Bridgeman, & Barone, 2018). Among physicians, median point-estimates suggest approximately 67% meet burnout criteria (Chmiel & Kurpas, 2025). In nursing, one-third experience high emotional exhaustion (Cañadas-De la Fuente et al., 2015). Prevalence rates in teachers are reported as high as 30% (Weber & Jaekel-Reinhard, 2000).

3.2. Global Burden of ME/CFS

ME/CFS has a global prevalence estimated between 0.1% and 0.8% (Arron et al., 2024). It affects all ages and races but is reported 2-3 times more frequently in women than men. It carries a massive economic burden, costing the U.S. economy billions annually due to lost productivity and medical expenses (Arron et al., 2024)

4. Etiology and Causes

4.1. Burnout: The Person-Environment Misfit

The etiology of burnout is multifactorial, centering on a mismatch between the worker and the job environment across six areas: workload, control, reward, community, fairness, and values (Schaufeli et al., 2009). Personality also plays a role; individuals high in neuroticism or those with perfectionist traits are more susceptible (Cañadas-De la Fuente et al., 2015; Schaufeli et al., 2009).

4.2. ME/CFS: Infectious and Biological Triggers

Up to 80% of ME/CFS cases are associated with an antecedent infection (9). Common triggers include viruses like Epstein-Barr Virus (EBV) and SARS-CoV-2 (Arron et al., 2024; Grach et al., 2023). Twin studies suggest a heritable

predisposition, potentially involving genes related to the immune response and hormone action (Arron et al., 2024; König et al., 2022).

5. Neurobiological Foundations

5.1. Brain Structure and Connectivity in Burnout

Neuroimaging reveals that chronic occupational stress leaves "unmistakable fingerprints" on the brain. Key findings include amygdala hypertrophy, which leads to heightened emotional reactivity, and atrophy of the prefrontal cortex, which weakens executive control over emotions. Furthermore, the disintegration of "rich-club" networks causes the brain to work harder but less efficiently, leading to "compensatory executive overdrive" (Chmiel & Kurpas, 2025).

5.2. Pathophysiology of ME/CFS

ME/CFS involves complex pathological responses, including neuroinflammation and mitochondrial dysfunction (König et al., 2022). PET scans show increased activation of astrocytes and microglia in the brain (Arron et al., 2024). Dysbiosis in the gut microbiome increases intestinal permeability ("leaky gut"), allowing bacterial toxins to enter the blood and trigger systemic inflammation (König et al., 2022).

5.3. HPA-Axis Alterations

Both conditions involve Hypothalamic-Pituitary-Adrenal (HPA) axis dysfunction. Burnout is often associated with hypocortisolism (adrenal burnout), marked by a lower cortisol awakening response (CAR) (Kakiashvili et al., 2013). In ME/CFS, HPA axis hypofunction leaves the individual more vulnerable to minor stressors (Arron et al., 2024).

6. Psychosocial Perspectives

The psychosocial impact of these conditions is devastating, with ME/CFS patients often reporting a lower quality of life than those with multiple sclerosis(Arron et al., 2024; Kingdon, Bowman, Curran, Nacul, & Lacerda, 2018).

- **Stigmatization:** Both burnout and ME/CFS sufferers face "disbelief" from medical professionals who may dismiss symptoms as purely psychosomatic (Arron et al., 2024; Chmiel & Kurpas, 2025).
- **Relationship Strain:** The spillover of work-related exhaustion often damages family life, leading to isolation (CESUR et al., 2023).
- **Economic Vulnerability:** Inability to work leads to financial instability and a loss of career identity (Arron et al., 2024).

7. Diagnosis and Differential Diagnosis

7.1. Burnout Assessment

Diagnosis typically relies on self-assessment tools, primarily the Maslach Burnout Inventory (Schaufeli et al., 2009; Ungur et al., 2024). Clinicians must distinguish burnout from Major Depressive Disorder; while they overlap, burnout is primarily work-related (Bianchi et al., 2021).

7.2. ME/CFS Diagnosis

Diagnosis is based on clinical criteria such as the 2015 NAM guidelines, requiring persistent fatigue, PEM, unrefreshing sleep, and cognitive impairment or orthostatic intolerance (Grach et al., 2023).

8. Management and Therapeutic Strategies

8.1. Psychosocial and Behavioral Interventions

- **Pacing:** One of the most critical strategies for preventing PEM is pacing, which is a strategy in which individuals consciously balance activity and rest according to their available energy (Casson et al., 2023). This approach helps individuals regulate their energy use to minimize exertion that might trigger symptom exacerbations (Sanal-Hayes et al., 2023). Avoiding overexertion and the subsequent worsening of symptoms offers significant benefits for clinical trajectory and functional outcomes (Schaufeli et al., 2009).
- **Mindfulness:** It has been demonstrated that mindfulness practice reduces amygdala volume and restructures connectivity among regulatory networks(Fox et al., 2014). Moreover, because it enhances psychological resilience, it may be beneficial both in preventing and ameliorating PEM (Ueno & Amemiya, 2024; Yuan & Hu, 2025).
- **CBT and ACT:** Cognitive Behavioral Therapy reduces perceived stress and the risk of work-related burnout by facilitating the management of secondary anxiety and the development of functional coping strategies (Kuut et al., 2024; White et al., 2023). Acceptance and Commitment Therapy, through value-based processes, enhances psychological flexibility and provides a complementary approach that supports adherence to energy management and pacing strategies (Jonsjö, Wicksell, Holmström, Andreasson, & Olsson, 2019).

8.2. Neurobiological and Medical Approaches

- **Physical Activity:** Moderate aerobic exercise has been shown to contribute to the partial reversal of stress-related prefrontal cortical

changes and to increases in brain-derived neurotrophic factor (BDNF) levels, supporting neuroplasticity and cognitive regulation (Chmiel & Kurpas, 2025; Erickson et al., 2019).

- **Pharmacotherapy:** In ME/CFS, pharmacological agents such as low-dose naltrexone or aripiprazole may be helpful in the management of fatigue symptoms (Grach et al., 2023). In burnout, anxiolytic and antidepressant treatments should be used cautiously as supportive and symptomatic approaches for the management of subthreshold anxiety and depressive symptoms (Bianchi et al., 2021; Kakiashvili et al., 2013).
- **Nutraceuticals:** Supplementation with coenzyme Q10 and NADH has been suggested to support mitochondrial function and cellular energy metabolism, potentially contributing to a reduction in overall symptom burden (Castro-Marrero et al., 2016; Chmiel & Kurpas, 2025) (9, 13).

8.3. Organizational Solutions

The most effective approach to addressing burnout lies in organizational and institutional interventions rather than solely individual-level strategies. Evidence consistently indicates that modifying workload, improving job control, and increasing worker autonomy are central to reducing burnout risk and sustaining long-term occupational well-being (CESUR et al., 2023; West, Dyrbye, Erwin, & Shanafelt, 2016)

9. Conclusion

Burnout and ME/CFS represent significant and increasingly prevalent challenges for modern medicine. Although these conditions share common neurobiological substrates, including hypothalamic–pituitary–adrenal (HPA) axis dysfunction and structural brain alterations, they differ substantially in their clinical trajectories and therefore require distinct management approaches (Arron et al., 2024; Chmiel & Kurpas, 2025). Recognizing these conditions as legitimate medical entities constitutes a fundamental step toward reducing stigma and supporting effective recovery processes (Chmiel & Kurpas, 2025). In particular, the implementation of preventive strategies in high-stress occupational settings is of critical importance in burnout(Ersoy, Yıldırım, & Edirne, 2001). For identified cases, psychosocial and behavioral interventions should form the foundation of management, while nutraceuticals and psychopharmacological agents may be considered as supportive options in selected cases (Schaufeli et al., 2009).

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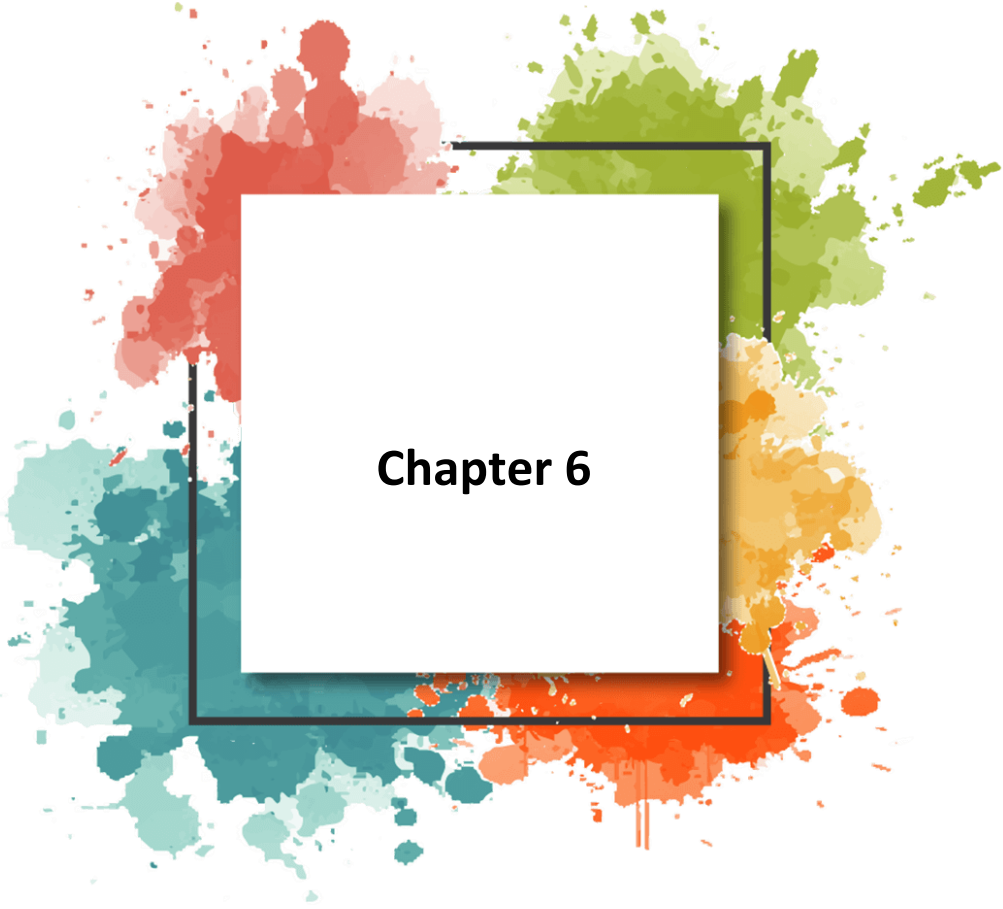
Chapter 6

Nocturnal Enuresis Prevalence in School-Age Children (2000-2025): A Comprehensive Review for Meta-Analysis

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Introduction

Nocturnal Enuresis (NE) is defined as the involuntary urination during sleep beyond the expected age of nighttime bladder control in children, usually after age five (Ozden et al., 2007). It is a common pediatric condition affecting a significant proportion of school-aged children worldwide (Adisu et al., 2025).

Although NE is generally considered a developmental delay rather than a serious medical condition, it can have profound emotional, psychological, and social impacts on affected children and their families (Lauters Rebecca A. et al., 2022). This can negatively impact quality of life and family dynamics. NE-related peer bullying and social exclusion can lead to limitations on participation in social activities like sleepovers or camping trips (Adisu et al., 2025).

The prevalence of NE tends to naturally decrease with age due to spontaneous resolution. A spontaneous remission rate of approximately 14–15% is reported annually (Lauters Rebecca A. et al., 2022). However, this condition remains a significant pediatric public health problem.

This article covers nocturnal outcomes in school-aged children published between 2000 and 2025. This study aims to systematically review and synthesize the current literature on the prevalence of enuresis. The aim is to provide a

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comprehensive overview of reported prevalence rates, associated factors, methodological issues, and identified gaps.

The wide variability in prevalence rates (ranging from 2% to 75%) (Adisu et al., 2025) is largely attributed to differences in study designs, definitions of EN (e.g., minimum wetting frequency, age cutoff points), and cultural perceptions (Adisu et al., 2025). This represents not only a numerical difference but also a methodological one. This heterogeneity poses a critical challenge for meta-analysis. Simply aggregating numbers without careful consideration of these definitional and methodological differences can lead to inaccurate or misleading conclusions. This means that the 'true' prevalence is obscured by these discrepancies. Therefore, meta-analysis needs to account for this heterogeneity, perhaps through subgroup analyses based on definitions (e.g., ICCS vs. DSM-IV-TR), age ranges, or study designs (e.g., cross-sectional vs. longitudinal studies). This report highlights this challenge and suggests strategies for addressing it.

1. Nocturnal Definitions and Classification of Enuresis

a. Standardized Definitions (e.g., ICCS, DSM Criteria)

NE is generally defined as intermittent sleep wetting after organic causes have been excluded and generally requires a minimum frequency of wetting, typically at least once a month or twice a week in children older than five years (Lauters Rebecca A. et al., 2022). The International Children's Continence Society (ICCS) offers widely accepted definitions (Sarici et al., 2016). Differences in diagnostic criteria (e.g., ICD-10, DSM-IV-TR/DSM-5) can significantly impact reported prevalence rates (Scheffel Elizabeth C. et al., 2017). For example, some studies define enuresis as once a month, while others define it as once a week or twice a week, significantly impacting reported rates (Lauters Rebecca A. et al., 2022).

b. Enuresis (Primary, Secondary, Monosymptomatic , Non-monosymptomatic)

Enuresis is divided into different types based on its etiology and associated symptoms:

- **Primary Nocturnal Enuresis (PNE):** This is a condition in which the child has never experienced prolonged periods of dryness at night (e.g., six months or more) (Scheffel Elizabeth C. et al., 2017). It accounts for the vast majority of cases (80-90%) and is generally associated with genetic predisposition, biological, and developmental factors (Ozden et al., 2007).
- **Secondary Nocturnal Enuresis (EN):** This is the recurrence of

bedwetting after a period of dryness of at least six months (Adisu et al., 2025). This type is often triggered by psychological factors or stressful life events (Scheffel Elizabeth C. et al., 2017).

- **Monosymptomatic Nocturnal Enuresis (MNE):** Characterized by bedwetting only at night and not associated with daytime urinary incontinence or other lower urinary tract symptoms (LUTS) (Lauters Rebecca A. et al., 2022).

- **Non-monosymptomatic Nocturnal Enuresis (NMNE): Includes** bedwetting plus daytime urinary symptoms (e.g., frequent urination, urgency) or other comorbid conditions (Lauters Rebecca A. et al., 2022) NMNE is less likely to resolve spontaneously and usually requires treating the underlying pathology first (Lauters Rebecca A. et al., 2022).

Primary/secondary and monosymptomatic / non-monosymptomatic: The distinction between enuresis and enuresis is crucial for understanding etiology and guiding treatment (Ozden et al., 2007). Inconsistent reporting of these classifications can confound prevalence studies. These classifications are not only of academic importance but also have clinical significance for diagnosis and management. If prevalence studies do not clearly distinguish and report these subtypes, overall prevalence figures may mask important epidemiological patterns. For example, a high prevalence of NMNE may indicate a higher burden of underlying bladder dysfunction or psychological problems in a population, which would require different public health interventions than a high prevalence of PNE. Meta-analysis should aim to extract and compare the prevalence rates of these subtypes across included studies, if possible. The importance of consistent reporting of NNE subtypes in future research should be emphasized to improve comparability and the clinical utility of prevalence data.

2. Global and Regional Prevalence of Nocturnal Enuresis (2000-2025)

a. General Prevalence Rates

The prevalence of NE varies significantly across geographic and sociocultural contexts (Adisu et al., 2025). A global systematic review and meta-analysis (2024) encompassing 128 studies across 39 countries reported an overall prevalence of NE among children and adolescents of 7.2% (95% CI: 6.2%–8.1%) (Adisu et al., 2025). In contrast, another meta-analysis focusing on Saudi Arabia (2024/2025) found a significantly higher overall prevalence of 24.8% (95% CI: 17%–34%) across 16 cross-sectional studies (Almutairi Nehal Ghannam et al., 2024).

This marked difference between the global prevalence (7.2%) and the prevalence in Saudi Arabia (24.8%) indicates significant regional variability, requiring further investigation into contributing factors beyond general demographics. This difference is not random. The Saudi Arabia meta-analysis notes regional variation within Saudi Arabia (highest in the southern region, lowest in the central region) and rates its prevalence higher than in Iran (8.25%-8.8%), Turkey, and Burkina Faso (Alqahtani et al., 2024). This suggests that specific environmental, sociocultural, genetic, or healthcare access factors may be influential within regions/countries. For example, the Saudi Arabia study notes "no association with male gender or age" in its meta-regressions for overall prevalence (Almutairi Nehal Ghannam et al., 2024), contradicting some individual studies and suggesting that other factors are more dominant in this context. Therefore, meta-analysis needs to consider geographic subgroup analyses and investigate potential region-specific factors (e.g., climate, diet, access to healthcare, cultural attitudes toward bedwetting, and treatment seeking) that may explain these important differences.

b. Prevalence Data by Continent/Region and Country

The prevalence of NE varies considerably worldwide and even within the same country:

- **Asia:**

  - **China (2007):** The overall prevalence was 5.66% in children aged 5-12 years (Qing et al., 2007) .Regional variations within China (4.07% to 10.3%) were also noted (Cher et al., 2002; Qing et al., 2007). In Hong Kong (2005), the prevalence was reported as 3.5% in children aged 4-12 years (Cher et al., 2002).

  - **India (2023):** In selected villages in Aurangabad district, the prevalence was found to be 10.91% in children aged 5-10 years. National estimates range from 7% to 15% in children, decreasing to 3% to 5% by age 10 (Khadke et al., 2023).

  - **Iranian:** In Urmia, it was reported as 18.7% in children aged 7-11 (2013) (Mahmoodzadeh Hashem et al., 2013). Another Iranian study from 2008 reported a rate of 7.7% in children aged 7-12 (Pashapour N. et al., 2008).

  - **Saudi Arabia:** A meta-analysis reports an overall prevalence of 24.8% , while individual studies (Almutairi Nehal Ghannam et al., 2024) show rates of 31.1% (2024) (Alqahtani et al., 2024) and 48% in the eastern region (2021) (Almutairi Nehal Ghannam et al., 2024).

- **Turkey:** Various studies provide varying rates: 9.52% in children aged 6-13 (2016) (Sarici et al., 2016), 17.5% in children aged 6-12 (2007) (Ozden et al., 2007), and 14.9% in schoolchildren (2008) (Gunes et al., 2009). The overall prevalence in Turkey ranges from 9.5% to 12.9% in children aged 6-12 (Aygun et al., 2025).

- **Europe:**
  - **Spain:** One study reported a prevalence of 7.8% in school-aged children (Taborga Díaz et al., 2021).
  - **Finland:** The reported prevalence is 8.2% (Taborga Díaz et al., 2021).
  - **United Kingdom:** 2.6% at age 7 (defined as 2 or more episodes per week) (Taborga Díaz et al., 2021).
- **North America:**
  - **United States:** Affects 5–7 million children. Prevalence decreases with age: 15% at age 5, 6% at age 10, and 1% at age 15. (Texas Children's Hospital, 2025)General estimates range from 15% to 25% at age 5 (Lauters Rebecca A. et al., 2022).
  - **Canada (Quebec, 2005):** Approximately 10% in children aged 53 months (approximately 4.5 years) (Touchette et al., 2005).
- **Africa:**
  - **Dominican Republic:** more than 25% (Taborga Díaz et al., 2021).
  - **Jamaica:** up to 50% (Taborga Díaz et al., 2021).
  - **Nigeria (Southwest):** 17.5% (Mahmoodzadeh Hashem et al., 2013).
  - **Burkina Faso:** It has been reported to have a higher prevalence than Iran (Alqahtani et al., 2024).

The wide range in prevalence rates, even within the same country (e.g., Iran: 7.7% vs. 18.7%; Turkey: 9.52% vs. 17.5%), suggests that, beyond definitional differences, local factors (specific socioeconomic conditions, regional healthcare practices, or even sample selection biases) play an important role. This is not only a global variation but also a significant within-country variability. While definitional differences are the primary cause, specific study populations (e.g., urban vs. rural, different socioeconomic strata, different school types such as day

and boarding schools (Gunes et al., 2009) and local cultural factors (e.g., stigma, willingness to report) may contribute to these differences. The Iranian study (Mahmoodzadeh Hashem et al., 2013) explicitly cites 'sociocultural differences between countries and regions' as a cause. Therefore, meta-analysis should consider these subnational or local contextual factors whenever possible. Researchers should be cautious in generalizing prevalence from a single study in a country and acknowledge the influence of specific study settings.

3. Factors Affecting the Prevalence of Nocturnal Enuresis

The prevalence of nocturnal enuresis is influenced by a complex array of demographic, familial, socioeconomic, clinical, physiological, psychological and behavioral factors.

a. Demographic Factors

- **Age:** A consistent and strong trend across studies suggests that the prevalence of NE decreases significantly with age. For example, in a study from Turkey, 30.8% of 6-year-old children wet the bed, while none of the 12-year-old children wet the bed (Ozden et al., 2007). Similarly, in Iran, 31.3% of 7-year-old children experienced NE, decreasing to 13.3% by age 11 (Mahmoodzadeh Hashem et al., 2013). In the United States, the prevalence decreases from 15-25% at age 5 to 1-2% by age 17 (Texas Children's Hospital, 2025). This spontaneous recovery rate is approximately 14-15% per year (Lauters Rebecca A. et al., 2022).

- **Gender:** Many studies report a higher prevalence of NE in boys than in girls, with the ratio generally being approximately 2:1 or 3:1 in younger age groups (Ozden et al., 2007). For example, a Turkish study found a prevalence of 12.4% in boys and 6.5% in girls (Sarici et al., 2016). However, some studies, particularly those from Iran and Turkey, have found no statistically significant gender difference (e.g., 20.9% in boys vs. 16.5% in girls in Iran (Mahmoodzadeh Hashem et al., 2013); 14.3% in boys vs. 16.8% in girls in Southeastern Turkey (Gunes et al., 2009)). A meta-analysis from Saudi Arabia found no association with male gender (Almutairi Nehal Ghannam et al., 2024). The inconsistent findings regarding gender prevalence suggest that the role of gender may be modulated by other factors, possibly cultural or age-specific factors, and is not a universal, independent determinant. If sex differences disappear with age (Taborga Díaz et al., 2021) or are not significant in certain populations , this (Almutairi Nehal Ghannam et al., 2024) suggests that (Almutairi Nehal Ghannam et al., 2024) biological predisposition (e.g., faster sphincter control maturation in girls) may be influenced by sociocultural factors (e.g., parental attitudes, treatment-seeking patterns, or even reporting bias) in some contexts .

The Saudi Arabia meta-analysis explicitly states in its meta-regressions that 'no association with male sex or age was found', suggesting that other factors (such as family history) are more dominant for that particular population. It is important that the meta-analysis conduct subgroup analyses by sex and age groups to investigate where and when male predominance is observed and to assess whether cultural or regional factors are associated with these differences.

- **Birth Order:** Some studies suggest that being the second or subsequent child may be associated with a higher risk of (Cher et al., 2002) enuresis. A global meta-analysis found that first birth order was a protective factor (AOR 0.5) (Adisu et al., 2025).

- **Rural/Urban Setting:** Living in rural areas has been identified as a predictor of bedwetting in Chinese children and was associated with more severe bedwetting (Qing et al., 2007). Similarly, a Turkish study found a higher prevalence in children living in villages (Gunes et al., 2009).

b. Familial and Socioeconomic Factors

- **Family History:** A positive family history of enuresis (in parents or siblings) is consistently identified as one of the strongest predictive factors. Children with one enuretic parent have a 44% and children with both enuretic parents have a 77% probability of developing (Ozden et al., 2007) enuresis , highlighting a significant genetic component.

- **Parental Education/Employment:** Lower parental education (both father and mother) is often associated with (Ozden et al., 2007) higher rates of enuresis . Working mothers and unemployed fathers have also been found to be associated factors in some studies, potentially due to their stress on children (Mahmoodzadeh Hashem et al., 2013).

- **Family Structure:** Large families, increased number of siblings, and single-parent households (divorced or deceased) are generally associated with a higher prevalence of (Ozden et al., 2007) enuresis . Parental death was found to be a significant associated factor in a global meta-analysis (Adisu et al., 2025).

- **Low Income:** An increased prevalence of enuresis has been observed in children from low-income families or living in villages (Gunes et al., 2009).

The combination of socioeconomic and familial factors (low parental education, unemployed fathers, working mothers, large families, single parents, low income) points to a broader "stress-vulnerability" model for NE. In this model, environmental stressors increase a child's susceptibility. Seemingly

disparate factors such as parental education, employment, family size, and income are consistently associated with NE (Ozden et al., 2007). While these factors are not direct physiological causes, they may represent a socioeconomic environment, such as increased stress, decreased parental involvement/support, or less access to healthcare and hygiene, which may exacerbate or prolong enuresis. For example, 'unemployed fathers and working mothers' have been thought to create stress in their children. This suggests a complex interaction in which environmental stress or lack of resources may impede the child's development of bladder control or exacerbate an underlying vulnerability. Meta-analysis should evaluate these (Mahmoodzadeh Hashem et al., 2013) socioenvironmental factors as potential modulators or mediators of NE prevalence, particularly when comparing studies across different socioeconomic contexts.

c. Clinical and Physiological Factors

- **Urinary tract infection (UTI):** A history of recurrent UTI is an important associated factor for NE (Ozden et al., 2007).

- **Constipation:** While some studies have found no significant association, others (Sarici et al., 2016) have identified constipation as (Gunes et al., 2009) a risk factor for NE , highlighting a potential link between bowel and bladder dysfunction (Daley et al., 2024).

- **Daytime Incontinence (GI)/ Lower Urinary Tract Symptoms (LUTS):** The presence of daytime incontinence or other LUTS (e.g., urgency , frequency) is a common comorbidity, occurring in 15-30% of enuretic children (Ozden et al., 2007). These cases are non-monosymptomatic. It is classified as enuresis and usually requires treating daytime symptoms first (Lauters Rebecca A. et al., 2022).

- **Sleep Arousal Disorder:** Poor sleep arousal is hypothesized to be a primary cause (Texas Children's Hospital, 2025)of NE . Children are unable to awaken when their bladders are full. Deep sleep has also been identified as a contributing factor (Ozden et al., 2007).

- **Bladder Capacity/Urine Production:** Inadequate production of the hormone vasopressin (ADH) leading to excessive urine volume at night and small bladder storage capacity are physiological factors contributing (Texas Children's Hospital, 2025) to NE .

- **Vitamin D Status:** Vitamin D deficiency plays a role as it can increase uninhibited bladder contractions (Adisu et al., 2025).

The interaction between physiological factors (e.g., bladder function, hormone levels, sleep arousal) and clinical comorbidities (UTI, constipation, LUTS) suggests a multifactorial etiology for NE and highlights the need for a holistic assessment. This multifactorial nature means that prevalence studies may capture different "types" of enuresis depending on the underlying causes prevalent in a given population. For example, a population with high rates of UTI may exhibit a higher prevalence of NMNE. This complexity also explains why treatment approaches must be individualized. When interpreting prevalence data, it is crucial to consider the prevalence of these associated clinical conditions in the studied population, as they can significantly influence overall NE rates and guide targeted public health interventions.

d. Psychological and Behavioral Factors

- **Poor School Performance:** It is frequently observed in children with NE , indicating a potential influence on academic outcomes or shared underlying factors (Ozden et al., 2007).

- **Stress, Anxiety, and Emotional Problems:** Secondary enuresis often stems from psychological factors. Environmental stressors, family problems, and exposure to bullying have been reported as risk factors (Adisu et al., 2025).

- **Developmental Delays:** Associated with bedwetting, suggesting a possible delay in central nervous system development, particularly in motor skills and language (Lauters Rebecca A. et al., 2022).

- **Attention Deficit/Hyperactivity Disorder (ADHD) and other psychological disorders:** Children with NMNE have a higher incidence of (Lauters Rebecca A. et al., 2022) comorbid psychological disorders such as ADHD, conduct disorder, oppositional defiant disorder, and separation anxiety .

- **Potty Training:** Improper or delayed potty training is a controversial but stated cause (Wikipedia, n.d.).

The strong associations between NE and psychological and developmental factors suggest that NE may be a marker of broader developmental or mental health difficulties and highlight the need for a comprehensive pediatric assessment. NE is not simply a bladder problem but also a condition with significant psychosocial dimensions. The co-occurrence of NE with these factors suggests a bidirectional relationship: NE can cause psychological distress, while preexisting psychological or developmental issues can also contribute to or exacerbate NE. This means that effectively addressing NE may require a multidisciplinary approach that includes not only urological interventions but

also psychological support and developmental screening. It is important for the meta-analysis to assess whether studies report these psychological comorbidities and whether these influence the reported NE rates. This also highlights the importance of including quality of life measures in future research.

4. Effects of Nocturnal Enuresis

1. Psychosocial Burden on Children (Self-Esteem, Social Shame)

NE can lead to significant emotional and psychological distress in children, including low self-esteem, lack of self-confidence, and feelings of shame and social embarrassment (Lauters Rebecca A. et al., 2022). The stigma associated with bedwetting can lead to peer bullying and social exclusion, restricting social activities such as sleepovers and camping trips (Adisu et al., 2025). Treatment has been shown to improve health-related quality of life scores, including children's self-esteem and emotional well-being (Lauters Rebecca A. et al., 2022).

2. Family Dynamics and Parental Concerns

Parents often experience feelings of frustration and helplessness, which can strain family dynamics. It has been noted that (Adisu et al., 2025) many parents lack sufficient interest or knowledge in managing (Sarici et al., 2016) enuresis , and many children go untreated, which can lead to future problems and the development of lower urinary tract symptoms in adulthood. A significant percentage of parents and children with enuresis are concerned about the impact of enuresis (for example, 46.4% of parents and 57.1% of children (Gunes et al., 2009) with enuresis in one study). In Saudi Arabia, 63% of children with NE experienced shame and social embarrassment (Almutairi Nehal Ghannam et al., 2024).

The significant psychosocial burden and low rates of treatment seeking point to a systemic problem related to awareness, stigma, and access to appropriate healthcare. It has been observed that NE causes significant emotional distress and social limitations, yet many parents do not seek treatment (Lauters Rebecca A. et al., 2022; Ozden et al., 2007). This suggests a disconnect between the impact of the problem and the perceived need for intervention. This disconnect may stem from cultural perceptions (e.g., some cultures view bedwetting as a normal part of childhood (Adisu et al., 2025)), a lack of knowledge among parents and healthcare providers about effective treatments, or the stigma associated with the condition that inhibits open discussion and help-seeking. One source explicitly states, the majority of parents do not pay sufficient attention to enuretic children,

and many children go untreated. Physicians should inform parents of (Sarici et al., 2016) enuretic children to prevent future problems and the development of lower urinary tract symptoms in adulthood." This points to a public health and education challenge. Meta-analysis should acknowledge this gap in treatment seeking and its consequences for the long-term well-being of affected children. Future research could examine barriers to care and the effectiveness of awareness campaigns.

5. Treatment-Seeking Behavior and Management Approaches

1. Professional Consulting Rates

Despite its prevalence and impact, the percentage of children with enuresis seen by a physician for treatment is generally low. For example, one Iranian study reported 19.8% and a Turkish study reported 17.2% (Mahmoodzadeh Hashem et al., 2013; Ozden et al., 2007). Another Iranian study indicated that only about one-third of families sought help (Pashapour N. et al., 2008). In Saudi Arabia, 54.4% of mothers received medical counseling (Almutairi Nehal Ghannam et al., 2024).

2. Common Treatment Modalities

- Medication is often the most preferred treatment option (e.g., 64.5% in Iran (Mahmoodzadeh Hashem et al., 2013); 59.5% in Turkey (Ozden et al., 2007)). Desmopressin is a commonly used medication (Aygun et al., 2025).

- Although alarm therapy is highly effective, with initial and long-term success rates of 70–90% and 50–70%, it is often the least preferred or underutilized method (e.g., only 2.4% in one Turkish study (Ozden et al., 2007); 2.6% in another Iranian study (Pashapour N. et al., 2008)).

- Other methods include waking up to urinate, fluid restriction, and waiting for maturity (Ozden et al., 2007).

- Motivational therapy that includes rewards for dry nights or certain behaviors is recommended for children ages 5 to 7 (Daley et al., 2024).

The underutilization of highly effective treatments, such as alarm therapy, despite their proven effectiveness, represents a significant missed opportunity in managing NE and improving children's quality of life. Alarm therapy has been observed to have high success rates but very low utilization. (Daley et al., 2024; Ozden et al., 2007) This suggests a gap between evidence-based best practice and actual clinical practice. This gap may be due to barriers such as lack of physician knowledge/education, parents' preference for "easier" solutions (such as

medication), and lack of awareness about the cost or effectiveness of alarms. This contributes to the long-term distress and psychosocial burden of NE. Meta-analysis should highlight this discrepancy between treatment effectiveness and utilization. Future research or public health initiatives should focus on understanding and overcoming these barriers to ensure more children receive optimal care.

6. Considerations for Meta-Analysis

a. Heterogeneity in Study Designs and Definitions

As noted earlier, the wide variability in reported prevalence rates (2% to 75%) is largely attributable to differences in study designs, inclusion criteria, definitions of enuresis (e.g., age cutoffs, frequency of wetting), and cultural perceptions (Adisu et al., 2025). Some studies define enuresis as once a month, others as once a week or twice a week, significantly influencing reported rates. The age ranges (Lauters Rebecca A. et al., 2022) for 'school-aged children' also vary (e.g., 5-10, 6-12, 7-11, 6-13, 5-18 years), requiring careful consideration for data aggregation.

Varying quality and reporting standards in primary studies will directly impact the robustness and generalizability of meta-analysis findings. While a variety of methodologies (surveys, online surveys) and sample sizes are observed, some meta-analyses also include quality assessments (Almutairi Nehal Ghannam et al., 2024). Not all studies are equally reliable or comparable. Poorly designed or reported primary studies can increase bias and heterogeneity, making it difficult to draw definitive conclusions. Users should be aware of these limitations during data extraction and synthesis. Therefore, rigorous quality assessment of included studies and transparent reporting of their limitations in the meta-analysis are crucial. Sensitivity analyses can also be conducted by excluding lower-quality studies.

b. Recommendations for Data Synthesis

Given the significant heterogeneity, a simple pooled prevalence would be misleading without subgroup analysis. Therefore, subgroup analyses should be based on the following factors:

- **Geographic Region/Continent:** To explore regional differences (e.g., Asia, Europe, North America, Middle East, Africa).
- **Age Groups:** To account for the age-related decline in prevalence (e.g., 5-7 years, 8-10 years, 11-13 years).

- **Enuresis Definition:** If adequate studies are available, enuresis may be grouped according to specific diagnostic criteria (e.g., ICCS, DSM) or minimum frequency of wetting.

- **Study Design:** Focus should primarily be on cross-sectional prevalence studies, but systematic reviews/meta-analyses should be considered as higher level evidence.

- **Meta-Regression:** Meta-regression should be performed to investigate the effect of continuous variables or study-level characteristics (e.g., mean age of participants, year of publication, certain consistently reported associated factors) on prevalence rates.
- **Quality Assessment: A robust quality assessment of individual studies** should be included to inform weighting in the meta-analysis and assess potential bias (Almutairi Nehal Ghannam et al., 2024).
- **Reporting Associated Factors:** Whenever possible, data on associated factors (gender, family history, socioeconomic status, comorbidities) should be extracted and synthesized to provide a richer understanding beyond prevalence figures.

7. Conclusion and Future Research Directions

a. Conclusion

Nocturnal Enuresis remains a common and impactful condition among school-aged children globally. Prevalence rates vary significantly across regions and populations and are influenced by the complex interplay of age, sex, and familial, socioeconomic, clinical, and psychological factors. Despite its high prevalence and psychosocial burden, treatment-seeking rates are often low, and effective interventions such as alarm therapy are underutilized. Heterogeneity in definitions and methodologies poses a significant challenge to synthesizing prevalence data and highlights the need for careful consideration in meta-analytic approaches.

Despite the known psychosocial impact, persistent inadequate treatment and lack of parental involvement present a critical gap in the translation of medical knowledge into public health practice. Low rates of professional counseling and treatment, and parental disinterest or ignorance, are consistently reported (Ozden et al., 2007). This contrasts with the negative impact of NE on quality of life (Lauters Rebecca A. et al., 2022). This is not only a research problem but also a public health problem. This means that current medical knowledge about NE and its management is not effectively reaching or being adopted by affected

populations. This may be due to cultural stigma, lack of accessible health services, economic barriers, or inadequate training of primary care providers. Addressing this problem requires more than simply determining prevalence; it requires implementation science and public health interventions.

b. Future Research Directions

- **Standardization of Definitions:** In future prevalence studies, consistent application of internationally standardized definitions (e.g., ICCS) should be encouraged to increase comparability across populations.

- **Longitudinal Studies:** Further longitudinal studies are needed to better understand the natural history, remission rates, and long-term outcomes of NE, as well as the temporal relationships among factors associated with NE.

- **Interventionist Research:** Studies are needed that focus on the effectiveness of public health campaigns to raise awareness, reduce stigma, and improve treatment-seeking behavior for NE.

- **Regional Specificity:** Further research is needed to determine the exact causes or influences contributing to the varying prevalence (Almutairi Nehal Ghannam et al., 2024) of NE, particularly in areas with high or low rates.

- **Multidisciplinary Approaches: Future research** should investigate the effectiveness of multidisciplinary interventions that address not only urological aspects but also the psychological, social, and developmental needs of children with NE and their families.

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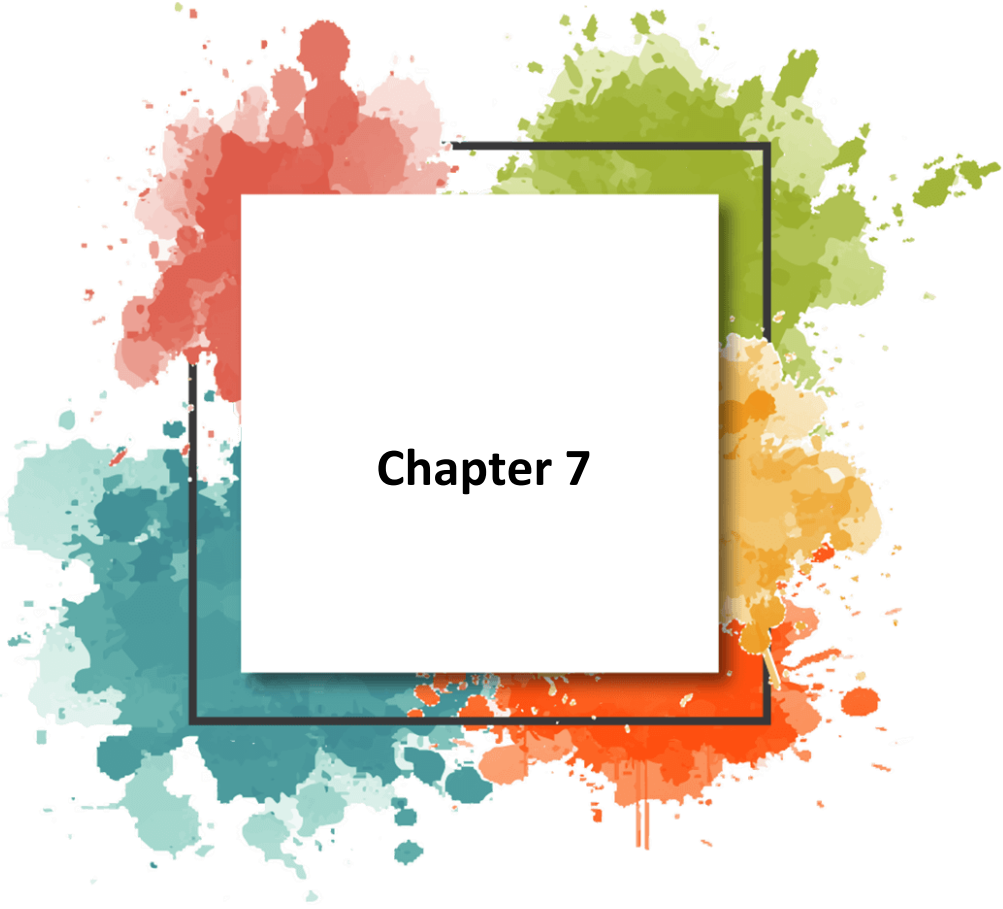
Chapter 7

Bibliometric Studies in Medicine: Global Trends, Collaborations and Scientific Impact

Naime Meriç Konar[1]

Introduction

Bibliometric analysis is an approach that provides a statistical analysis of scientific communication or academic literature (1). It is the process of investigating publications, ranging from articles in scholarly journals, books, and conference proceedings, to see how often and where they have been cited within the literature on a particular topic or field. Indicator factors are references, publication number, author numbers, institutions and journal impact factor. This method helps researchers understand the development of a field, influential authors or works, and emerging research areas (2). Besides, it is a relevant method to unveil the research fields that need further attention (3). This method is commonly applied in research assessments, strategic planning, and informing policy decisions in academia and science (4-6).

In recent years, bibliometric analyses have gained increasing recognition due to their broad applicability across scientific disciplines. The expansion of digital databases such as Scopus, Web of Science, and Google Scholar has facilitated access to large volumes of structured publication and citation data, enabling researchers to conduct more comprehensive and precise analyses (7). Bibliometric indicators, including citation counts and the h-index, provide practical measures of research productivity and impact, which are widely used by institutions, funding bodies, and governmental organizations for academic evaluation, promotion, and funding decisions. In addition, bibliometric approaches allow researchers to track the evolution of scientific fields, identify emerging topics, and address existing knowledge gaps. Their visualization-based techniques—such as co-citation networks, keyword mapping, and collaboration analyses—help clarify complex relationships within the literature and make them more accessible to researchers from diverse disciplines. Moreover, the ability of bibliometric tools to manage large datasets offers a scalable and relatively objective means of assessing scientific output beyond traditional peer-review-

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based evaluations. Collectively, these strengths have positioned bibliometric analysis as a key component of contemporary research assessment.

Over the past decades, bibliometric studies have become increasingly common in medical research, largely due to the rapid growth of scientific output that has made it challenging for clinicians and researchers to remain up to date. By condensing extensive bodies of literature into quantitative indicators, bibliometric analysis can help identify influential studies, leading authors, and prominent journals. Given the highly competitive and well-funded nature of medical research, these tools are particularly valuable for funding agencies and academic institutions seeking to evaluate research productivity and inform resource allocation in a data-driven manner. Furthermore, bibliometric analysis supports the exploration of interdisciplinary connections between medicine and related fields such as public health, bioinformatics, and genetics, thereby promoting collaborative and integrative approaches to research development (8,9).

Bibliometric approaches are widely used to examine research trends related to major global health issues, such as pandemics, cancer, and mental health disorders (10–12). These analyses help identify areas where research activity remains limited, reveal gaps in specific fields, and provide insight into how different countries address shared health challenges. During the COVID-19 pandemic, bibliometric studies played an important role in tracking global research output, identifying leading institutions, and highlighting dominant research themes, including vaccine development, viral variants, and public health responses. Such analyses supported governments and public health organizations by helping them follow rapidly evolving scientific knowledge, prioritize research funding, and strengthen international collaboration. Healthcare organizations and policymakers also use bibliometric evidence to guide decisions on research priorities, ensuring that investments translate into meaningful improvements in healthcare. Similarly, bibliometric analyses in oncology have been used to assess research trends across cancer types, treatment strategies—such as immunotherapy—and highly cited publications, thereby helping to identify underfunded areas and inform more targeted allocation of resources and research efforts.

Recent years have witnessed the massive growth in bibliometric analysis publications in nearly every scientific discipline (13-17). Due in part to the COVID-19 pandemic, the production of medicine-related bibliometric analyses has shown an extreme rise in the last few years. Therefore, there is a need to investigate the progress of these bibliometric researches regarding which country, region, affiliation, publisher, etc., contributes the most to these studies, as well as

to identify the collaboration patterns between countries and sources, to display the general framework of the medical bibliometric analyses.

Materials and Methods

The catalogue of the Web of Science Core Collection (WoSCC) database (www.webofknowlege.com) was accessed to retrieve the data. The search was begun and ended on October 22, 2025, to cover daily updates. The keywords ("bibliometric analysis") OR ("bibliometrics") OR (“bibliometric study”) were entered in "topic" search mode for the retrieval. No time limit was selected. "Article" and "English" were selected as the research type and language, respectively. WoS Index except Science Citation Index (SCI-E) and Social Science Citation Index (SSCI) were eliminated.

WoS categories, “Medicine, General & Internal”, “Medicine Research Experimental”, “Orthopedics”, “Oncology”, “Surgery”, “Public Environmental Occupational Health”, “Immunology”, “Psychiatry”, “Pharmacology Pharmacy”, “Clinical Neurology”, “Cardiac Cardiovascular Systems”, “Radiology Nuclear Medicine Medical Imaging”, “Endocrinology Metabolism”, “Ophthalmology”, “Microbiology”, “Pediatrics”, “Dermatology”, “Gastroenterology Hepatology”, “Infectious Diseases”, “Integrative Complementary Medicine”, “Urology Nephrology”, “Tropical Medicine”, “Parasitology”, “Rheumatology”, “Otorhinolaryngology”, “Respiratory System”, “Anesthesiology”, “Obstetrics Gynecology” , “Emergency Medicine”, “Peripheral Vascular Disease”, “Genetics Heredity”, “Critical Care Medicine”, “Physiology”, “Transplantation”, “Medical Informatics”, “Hematology”, “Medicine Legal”, “Anatomy Morphology”, “Geriatrics Gerontology”, “Pathology”, “Medical Ethics”, “Virology”, “Medical Laboratory Technology”, “Andrology”, “Allergy” were selected to evaluate the performances of bibliometric outputs produced in these aforementioned areas.

The total value of goods and services produced in a nation over the course of a year is known as the gross domestic product, or GDP. GDP divided by the population at the halfway point of the year is known as GDP per capita (18). The association with GDP-PPP values and Number of Publications (NP) and Citation Counts (CC) statistics, both in total and in terms of income classifications, was identified in this present research. Countries’ WHO-based regional classifications were utilized in group comparison analyses to investigate each region’s contribution to medical bibliometric studies. Lists of countries in each region were retrieved from the WHO website (https://www.who.int/).

Statistical Analysis

The median, minimum, and maximum of the numerical variables were reported and frequency (n) and percentage (%) were recorded for categorical

variables. All income classifications, population sizes and GDP-PPP of countries were obtained from the https://databank.worldbank.org/ site. The assumption of normality was analyzed using Kolmogorov-Smirnov test and the assumption of homogeneity of variance was also tested by Levene Test. Group comparisons were performed with the Kruskal-Wallis (K-W) test. Pairwise comparisons were made using Dunn test. The associations between NP and CC values were investigated by Spearman Correlation Coefficients. Further, WoS category-specific predictions of NP were visualized with the help of the Linear Extrapolation Method. A bar graph was provided to summarize both the top ten WoS categories where the most of bibliometric papers were published and the future production capabilities for certain categories. The Biblioshiny web application (19), VOSviewer software (v.1.6.16), and the R statistical programming language were utilized for bibliometric analyses, graphics, and the remaining analyses, respectively. A two-sided p-value ≤ 0.05 was taken as statistically significant.

Results

A total of 4,132 bibliometric analyses were produced in medical disciplines between 1991 and October 22, 2025. The annual growth rate was 21.61%, indicating an increasing trend in the medicine-related bibliometric papers. The distributions of papers and citations were depicted in Figure 1 (Figure 1). A sharp increase in NP was observed, and the peak was reached in 2024 with 848 outputs. For the citations; significant rise was also seen, even though in recent years a decrease was observed, a linear pattern was noticed for average CC per year (Figure 1).

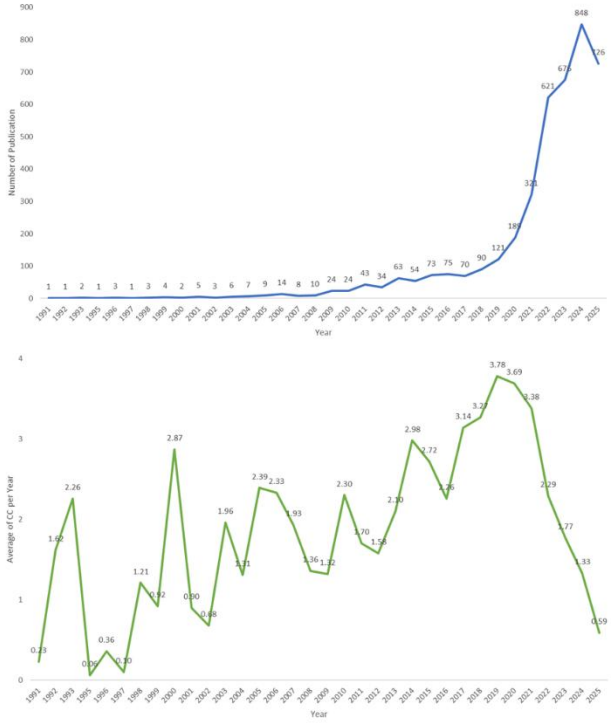

Figure 1. Annual publication and citation frequencies

Bibliometric analyses in various medical disciplines found their places in 871 different sources, while 3.46 years was calculated as the documents' average age. From a collaboration perspective, 5.81 co-authors per document were listed.

The major part of the research (4062 publications; 98.31%) was published in SCI-indexed journals, whereas much of the work was documented in both Q_1 (n=289, 33.2%), and Q_2-class (n=296, 34%) journals, followed by Q_3 (n=194, 22.3%) and Q_4-class (n=92, 10.6%) journals. Sustainable Development Goals for the bibliometric analyses ranked first, second, and third as 03-Good Health and Well-Being (2924 publications), 05- Gender Equality (162 publications), and 04-Quality Education (143 publications), respectively.

Median, minimum, and maximum statistics for Single Country Publications (SCP), Multi-Country Publications (MCP), and total NP were found to be 3 [0 - 2185], 3 [0 - 180], and 6 [1 - 2365], respectively. Further, findings suggested NP (p=0.023) counts significantly differed across income classes, whereas SCP (p=0.612), MCP (p=0.272), and CC (p=0.055) counts were similar across low, lower-middle, upper-middle and high-income countries. High-income, upper-middle-income, lower-middle-income, and low-income countries' descriptive statistics for NP were calculated as, 7.5 [1 - 398], 9 [1 - 2365], 2 [1 - 45] and 1 [1 - 2], respectively (p=0.023). On the other hand, the distribution of bibliometric

studies across WHO regional classes was found to be similar for each bibliometric indicator (p>0.05, for all), indicating similar contributions of each region to medicine-related bibliometric research. (Table 1).

Table 1. Group comparison results of bibliometric indicators across economic level and WHO regional class*

Variable	Economic Level				p-value	p-value (post-hoc)
	Low[a] (n=3)	Lower-Middle[b] (n=17)	Upper-Middle[c] (n=19)	High[d] (n=40)		
NP	1 [1 - 2]	2 [1 - 45]	9 [1 - 2365]	7.5 [1 - 398]	0.023	0.023[a,d] 0.024[a,c] 0.035[b,d] 0.049[b,c]
SCP	2 [0 - 32]	3 [0 - 2185]	5 [0 - 59]	2 [0 - 314]	0.612	-
MCP	1 [0 - 26]	3 [0 - 180]	7 [0 - 28]	2 [0 - 84]	0.333	-
TC	9 [2 - 59]	21 [0 - 249]	43 [0 - 15788]	102.5 [0 - 7471]	0.055	-
Variable	WHO Regional Classification					p-value
	EURO (n=33)	EMRO (n=12)	RAM (n=13)	WPRO (n=14)	AFRO (n=7)	
NP	6 [1 - 91]	3.5 [1 - 39]	10 [1 - 398]	8.5 [1 - 2365]	2 [1 - 15]	0.435
SCP	3 [0 - 314]	7 [0 - 2185]	3 [0 - 50]	3 [0 - 59]	2 [0 - 5]	0.476
MCP	2 [0 - 84]	6 [0 - 180]	2 [0 - 28]	3 [0 - 24]	1 [0 - 5]	0.325
CC	69 [0 - 2031]	30.5 [4 - 301]	67 [0 - 7471]	65 [4 - 15788]	21 [1 - 341]	0.694

*Abbrevations: , EURO: European Region, EMRO: Eastern Mediterranean Region, RAM: Region of Americas, WPRO: Western Pacific Region, AFRO: African Region.

Further, a strong association was noted by correlation analysis between NP and CC values for all countries (r=0.926; p<0.001), while they were r=0.866; p=0.333 for low, r=0.822 p<0.001 for lower-middle, r=0.946; p<0.001 for upper-middle, and r=0.947; p<0.001 for high economic-level countries.

Most productive countries (in terms of number of outputs published and citation counts), journals, and publishers were outlined in Table 2 (Table 2). China (2365 publications), the USA (398 publications), Spain (91 publications), the United Kingdom (88 publications), and Turkey (86 outputs) were in the top-five list regarding the number of papers. On the other hand, China (15788 citations), the USA (7471 citations), the United Kingdom (2031 citations), Canada (1402 citations), and Turkey (1261 citations) were placed top five in terms of citation counts obtained. "Medicine" was listed as the most productive journal (340 publications), and "Scientometrics" was reported as the most cited source (3348 citations) (Table 2).

Table 2. Most productive countries, journals, and most cited countries and journals

Country	NP	SCP	MCP	Country	CC	Source	CC	Journal	NP	Best Quartile
CHINA	2365	2185	180	CHINA	15788	SCIENTOMETRICS	3348	MEDICINE	340	Q_2
USA	398	314	84	USA	7471	LANCET	2773	FRONTIERS IN ONCOLOGY	171	Q_2
SPAIN	91	66	25	UK	2031	NEW ENGL J MED	2640	FRONTIERS IN MEDICINE	141	Q_1
UK	88	58	30	CANADA	1402	PLOS ONE	2194	FRONTIERS IN PHARMACOLOGY	139	Q_1
TURKEY	86	82	4	TURKEY	1261	NATURE	1925	DISCOVER ONCOLOGY	127	Q_2
GERMANY	83	59	24	SPAIN	1190	JAMA-J AM MED ASSOC	1663	WORLD NEUROSURGERY	112	Q_2
CANADA	70	36	34	GERMANY	1177	P NATL ACAD SCI USA	1654	FRONTIERS IN PUBLIC HEALTH	104	Q_1
ITALY	64	36	28	ITALY	927	FRONT IMMUNOL	1467	JOURNAL OF PAIN RESEARCH	65	Q_2
BRAZIL	62	50	12	AUSTRALIA	847	SCIENCE	1143	FRONTIERS IN SURGERY	59	Q_2
AUSTRALIA	58	32	26	BRAZIL	659	INT J MOL SCI	1089	FRONTIERS IN CARDIOVASCULAR MEDICINE	57	Q_2

Countries' productivities were standardized to simplify the evaluation and to avoid the uncertainty caused by distinctions emerging from population size and GDP-PPP values. Influenced by Konar et al.'s work (20), several indexes were calculated for the standardization. Results revealed that Ireland (4.832), Israel

(2.206), and Australia (2.132) were placed in the top three in terms of NP after adjusting for population size, while Lebanon (15.151), Jordan (7.199), and China (6.193) were observed to be the first, second, and third countries, respectively, after adjusting for GDP-PPP. Furthermore, Ireland (11.802) ranked first, followed by Greece (5.775) and Canada (3.396) regarding total CC, after adjusting for population size; on the other hand, Lebanon (27.135), Greece (13.104), and Ireland (8.997) were listed as first, second, and third after adjusting for GDP-PPP. Standardized lists were provided in Table 3 (Table 3).

Table 3. Publication/GDP index, Publication/population index, , CC/GDP index, and CC/population index for top countries on bibliometric research**

ID	Country	NP / GDP-PPP Index*	Country	NP / Population Index*	Country	CC/ GDP-PPP Index*	Country	CC / Population Index*
1	Lebanon	15.151	Ireland	4.832	Lebanon	27.135	Ireland	11.802
2	Jordan	7.199	Israel	2.206	Greece	13.104	Greece	5.775
3	China	6.193	Australia	2.132	Ireland	8.997	Canada	3.396
4	Israel	3.961	Switzerland	2.103	Israel	5.797	Lebanon	3.393
5	Greece	3.931	Lebanon	1.895	Canada	5.187	Israel	3.228
6	Ireland	3.684	Spain	1.864	UK	4.840	Australia	.3.113
7	Spain	3.275	Greece	1.733	Australia	4.373	UK	2.934
8	Australia	2.995	Canada	1.695	Spain	4.283	Switzerland	2.679
9	Peru	2.791	China	1.679	Jordan	4.239	Spain	2.438
10	New Zealand	2.720	New Zealand	1.499	China	4.134	USA	2.197

* NP / GDP-PPP Index =(NP/ GDP-PPP)*100000; NP/ Population Index = (NP/Population)*1000; CC/ GDP-PPP Index= (CC/GDP-PPP)*10000; CC/Population Index= (CC/Population)*100

** Countries with NP ≥ 5 were analyzed. Abbreviations. GDP, Gross Domestic Product per capita; CC, Citation Counts, NP, Number of Publications.

Bibliometric analyses that were produced in various medical disciplines were recorded and analyzed within the context of the current manuscript. Medicine General & Internal (n=715), Surgery (n=612), Oncology (n=508), Public Environmental Occupational Health (n=371) and Clinical Neurology (n=321) were listed as the most active areas in terms of bibliometric analysis. The top ten categories were visualized in Figure 2 (Figure 2).

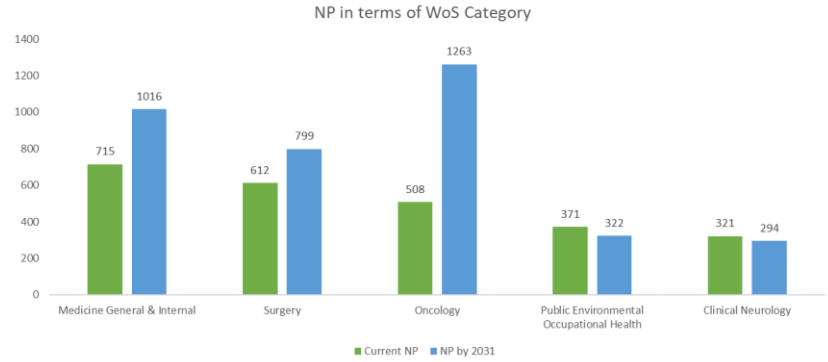

Figure 2. Current and future frequencies of medical bibliometric analysis counts in terms of WoS categories.

Medical literature has been published by 2039 different institutions over the years. Central South University (131 publications), Capital Medical University (103 publications), China Academy of Chinese Medical Sciences Peking Union Medical College (102 publications), Beijing University of Chinese Medicine (87 publications), and Shanghai Jiao Tong University (86 publications) were recorded as top contributors. The National Natural Science Foundation of China (NSFC) (650 publications) was listed as the top funding agency, and "Frontiers" (843 publications) was noted as the most active publisher (Table 4).

Table 4. Most active affiliations, funding agencies and publishers in *medical* bibliometric analyses.

Affiliation	**NP**	**Funding Agencies**	**NP**	**Publisher**	**NP**
CENTRAL SOUTH UNIVERSITY	131	NATIONAL NATURAL SCIENCE FOUNDATION OF CHINA NSFC	650	FRONTIERS MEDIA SA	843
CAPITAL MEDICAL UNIVERSITY	103	NATIONAL KEY RESEARCH DEVELOPMENT PROGRAM OF CHINA	58	SPRINGER NATURE	712
CHINESE ACADEMY OF MEDICAL SCIENCES PEKING UNION MEDICAL COLLEGE	102	UNITED STATES DEPARTMENT OF HEALTH HUMAN SERVICES	58	Lippincott Williams & Wilkins	520
BEIJING UNIVERSITY OF CHINESE MEDICINE	87	NATURAL SCIENCE FOUNDATION OF HUNAN PROVINCE	56	ELSEVIER	495
SHANGHAI JIAO TONG UNIVERSITY	86	NATIONAL INSTITUTES OF HEALTH NIH USA	55	WILEY	204

SICHUAN UNIVERSITY	84	CHINA POSTDOCTORAL SCIENCE FOUNDATION	41	DOVE MEDICAL PRESS	128
CHINA MEDICAL UNIVERSITY	75	NATIONAL KEY R D PROGRAM OF CHINA	29	TAYLOR & FRANCIS	115
NAVAL MEDICAL UNIVERSITY	75	BEIJING NATURAL SCIENCE FOUNDATION	25	AME PUBLISHING COMPANY	79
CHINA ACADEMY OF CHINESE MEDICAL SCIENCES	74	NATURAL SCIENCE FOUNDATION OF SHANDONG PROVINCE	22	MDPI	78
PEKING UNIVERSITY	73	NATURAL SCIENCE FOUNDATION OF ZHEJIANG PROVINCE	21	SAGE	76

WoS categories' publication counts were predicted via the Linear Extrapolation Method. Unlike the current rank, Oncology will be the lead WoS category by 2031, and Medicine General & Internal, and Surgery-based bibliometric research will follow oncology-related bibliometric studies, as projections revealed (Figure 3).

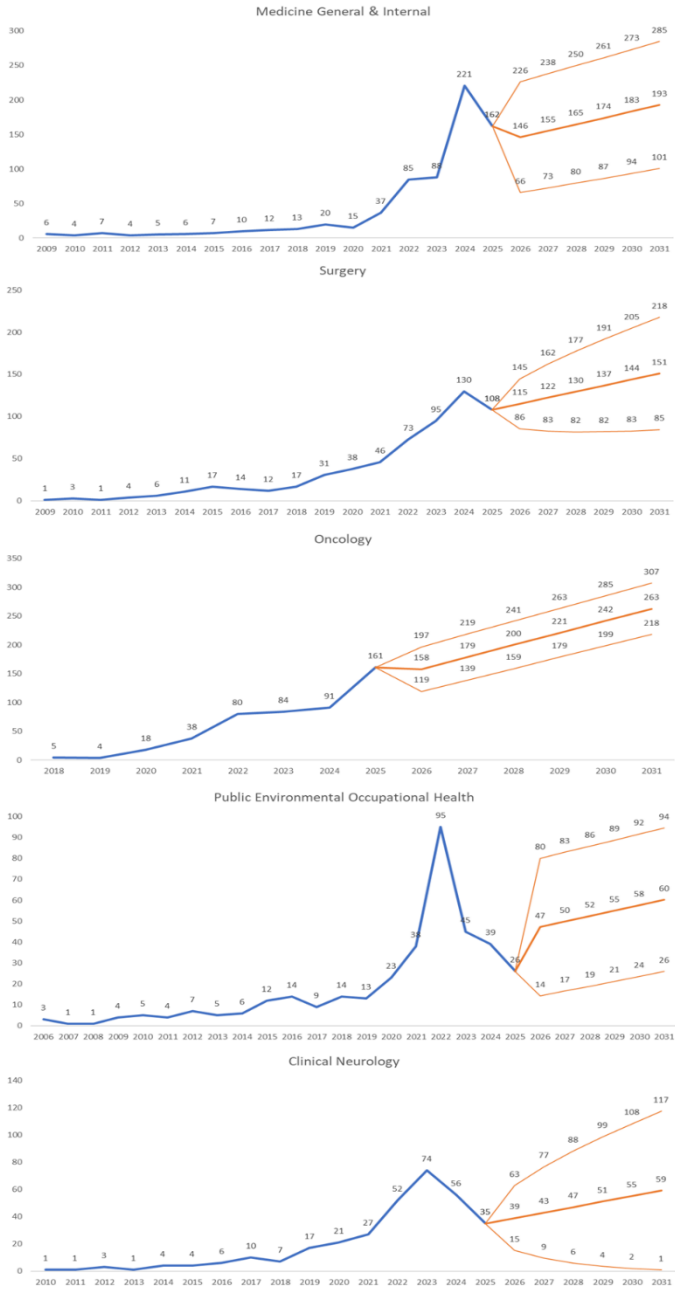

Figure 3. Predicted frequencies of medical bibliometric analyses for upcoming years.

Co-authorship analysis revealed 9 clusters based on global collaboration (Figure 4). Regional proximities and neighborhoods were observed to reflect themselves on research alliances; that is, partnership and productivity of geographically close countries were at high levels. The list of countries in each cluster was provided in Figure 4 (Figure 4).

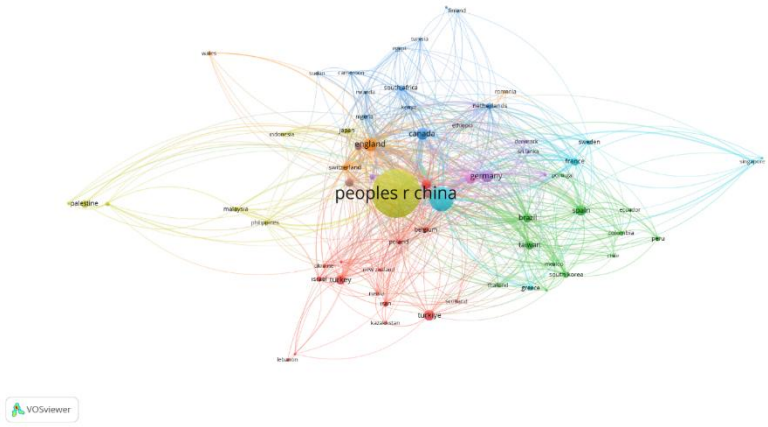

Figure 4. Co-authorship analysis based on countries. **Cluster 1 (Red):** Belgium, Cyprus, Hungary, Iran, Israel, Italy, Kazakhstan, Lebanon, New Zealand, Poland, Russia, Scotland, Turkey, Ukraine. **Cluster 2 (Green):** Argentina, Brazil, Chile, Colombia, Ecuador, Mexico, Peru, Portugal, South Korea, Spain, Taiwan, Thailand. **Cluster 3 (Dark Blue):** Cameroon, Canada, Croatia, Egypt, Finland, Kenya, the Netherlands, Nigeria, Rwanda, South Africa, Sudan, Tunisia. **Cluster 4 (Yellow):** Indonesia, Japan, Jordan, Malaysia, Palestine, Peoples Republic of China, Philippines, United Arab Emirates. **Cluster 5 (Purple):** Austria, Denmark, Germany, Ireland, Norway, Qatar, Sri Lanka. **Cluster 6 (Light Blue):** France, Greece, Singapore, Sweden, the USA, Vietnam. **Cluster 7 (Orange):** England, Romania, Switzerland, Wales. **Cluster 8 (Brown):** Ethiopia, India, Pakistan, Saudi Arabia. **Cluster 9 (Pink):** Australia.

Discussion

The findings of this study indicate that the majority of bibliometric publications were classified under the Medicine, General & Internal category in the Web of Science. China emerged as both the most productive and the most cited country, reflecting its dominant role in medical bibliometric research. However, when national productivity was normalized according to GDP-PPP and population size, countries such as Ireland and Greece ranked among the top contributors. In addition, oncology-focused bibliometric studies are projected to become the leading research domain by 2031. The journal Medicine was identified as the most productive outlet, while Scientometrics received the highest

number of citations. Furthermore, Frontiers was the most productive publisher, Central South University was the leading institution, and the National Natural Science Foundation of China (NSFC) was the most active funding agency.

To date, considerable efforts have been devoted to conducting subject-specific bibliometric analyses within the medical field (21–23). Nevertheless, the literature remains limited in terms of studies that evaluate the overall development, performance, and evolution of bibliometric research itself. Only a small number of investigations have focused on assessing the scientific performance of bibliometric studies. In this context, Thompson and Walker (24) provided early insights by reviewing the definition, historical development, and core components of bibliometric methods, while also demonstrating their applications within the broader medical and healthcare communities. Their study, based on data retrieved from Medline (1966–2014) and Web of Science (1945–2014), selectively highlighted key publications to enhance readers' understanding of bibliometric principles rather than offering a comprehensive overview.

Similarly, Li et al. (25) conducted a bibliometric analysis to assess the performance of bibliometric studies published in the health sciences. Consistent with the present findings, China was reported as both the most productive and the most cited country, with Chinese authors dominating in terms of publication output and citation impact. China's leadership across both medical and health science bibliometric research can largely be attributed to its sustained investments in research and development policies. That study also emphasized the importance of international collaboration, noting that global partnerships in health sciences remained limited, whereas domestic collaboration was relatively strong. A comparable pattern was observed in the current study, where local collaborations were favored over international ones. This tendency may largely be explained by geographical proximity and established national research networks.

The projection that oncology-based bibliometric research will occupy the leading position by 2031 appears both reasonable and consistent with current trends in the literature. Cancer research continues to receive substantial global attention, driven by ongoing advances in diagnosis, treatment, and prevention strategies. As these innovations accumulate, they are reflected in an increasing volume of scientific publications, which are subsequently synthesized and evaluated through bibliometric analyses.

Several limitations of the present study should be acknowledged. Self-citations were not excluded, as the primary objective was to reveal the general performance landscape rather than individual citation behaviors. Additionally, only the Web of Science database was used, while other major databases such as Scopus, PubMed, and EBSCO were not included. Although this restriction may

limit coverage, the Web of Science was considered sufficient to provide a comprehensive and representative framework aligned with the aims of the study.

Despite these limitations, this research offers several notable strengths. By integrating economic (GDP-PPP and income level), demographic (population size), and geographic (WHO country classification) factors, the study provides a multifaceted perspective on how these variables influence scholarly productivity and performance across medical specialties. The results may be particularly valuable for researchers, clinicians, and policymakers, as they help identify emerging and underexplored areas that warrant further attention. While reliance on citation data and database coverage remains an inherent constraint, mapping scientific progress and offering predictive insights into future trends represent key advantages of this bibliometric analysis.

In conclusion, this study provides a comprehensive overview of the current landscape and international activity of medical bibliometric research, while also forecasting future productivity patterns across selected Web of Science categories. As such, it may serve as a useful reference for future medicine-related bibliometric studies and offer guidance for upcoming research agendas. Moreover, the findings may inform decisions related to international collaboration, funding allocation, and policy development. Given the rapid growth of bibliometric research in medicine, future studies should incorporate rigorous quality assessments and adhere to established methodological guidelines to further enhance the reliability and impact of this expanding field.

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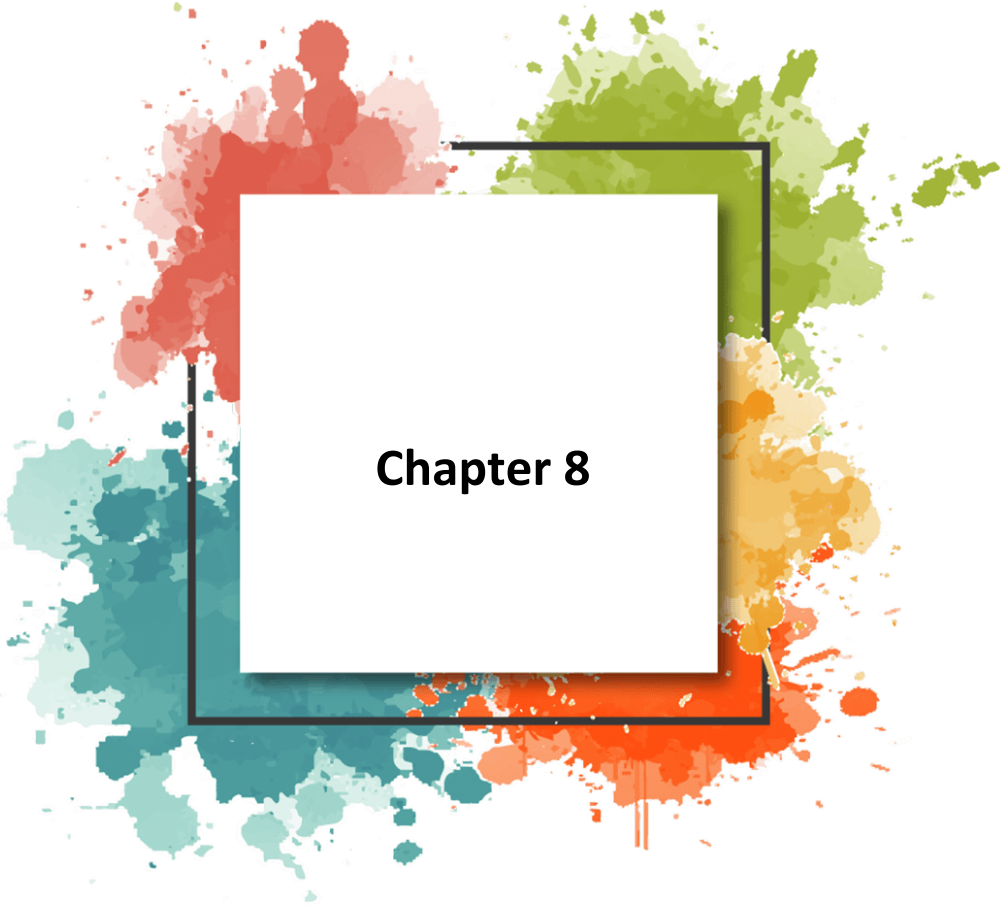
Chapter 8

An Overview of Boundary Conditions and Loading Protocols in in-Vitro Biomechanical Testing of Mandibular Fracture Fixation

Yiğit Şirin[1]

Introduction

Mandible is consistently reported as the second most common site of facial fracture and accounts for 15–59% of maxillofacial fractures (Adik et al., 2023; Malhotra et al., 2021; Natu et al., 2012). The treatment goals include surgical stabilization to restore occlusion, facial symmetry, and masticatory function. However, the mandible represents a complex geometry which is exposed to multidirectional functional loads. Accordingly, optimal fixation protocol still remains a topic of ongoing debate, particularly for fracture sites such as the angle, parasymphysis, and body (P.S. Guastaldi, 2021). Although numerous fixation strategies have been proposed, including single superior border plating, dual-plate constructs, and load-bearing reconstruction plates (Hashemi, Qundos, and Farzad, 2025), consensus regarding the most reliable configurations has not been fully established.

Biomechanical testing plays an important role in evaluating fixation systems prior to clinical use. In-vitro experimental studies allow comparison of fixation constructs, plate designs, and surgical techniques while avoiding the inherent ethical and logistic limitations of in-vivo experiments (Chrcanovic, 2013). In the last three decades, numerous in-vitro studies have investigated the mechanical behavior of mandibular fracture fixation using human or animal cadaveric bones, polyurethane mandible replicas, and composite bone models (Chrcanovic, 2013; Orassi et al., 2021). These publications have reported outcomes such as failure load, construct stiffness, interfragmentary displacement, and deformation patterns. These variables have frequently been used to justify clinical recommendations.

However, mandibular biomechanics are inherently complex. While axial loading and bending are the main acting force vectors on the long bones

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(Marongiu, Dolci, Verona, and Capone, 2020), the mandible functions as a curved ring structure suspended between two temporomandibular joints. This construct is subjected to the effects of dynamic muscle forces during mastication. Functional loading generates combined bending, torsional, and shear stresses that vary depending on bite location, occlusal contacts, and asymmetric muscle activation (van Eijden, 2000). Many experimental studies tend to oversimplify test setups by applying rigid posterior fixation or inferior platform support, effectively converting the mandible into a beam-like structure subjected predominantly to vertical bending (R. H. Haug, Fattahi, and Goltz, 2001). Such simplifications may alter load transfer pathways and stress distributions across the fracture site and the fixation system. Consequently, fixation constructs that perform favorably under beam-like loading may exhibit different mechanical behavior when joint-mediated motion and torsional forces are permitted.

Inferior border displacement and rotational instability could be dominant under physiologic loading conditions, particularly in mandibular angle and parasymphysis fractures fixated with superior border or single plating configurations (Al-Moraissi and Ellis, 2014a, 2014b; Richard H. Haug and Serafin, 2008). Similarly, in models containing simulated muscles and flexible joint support, clinically relevant micromotion at the inferior cortex may become visible in superior border plating. This, in turn, necessitates additional inferior border fixation or load-bearing constructs that may offer greater resistance to torsional loading. These findings however contrast with results from rigidly constrained beam models, in which superior miniplate fixation often appears mechanically sufficient.

Another important aspect to consider is the selection of loading protocol. Mastication generates repetitive low-amplitude cyclic loads rather than isolated monotonic forces over fixation constructs (Jain et al., 2025). Fatigue-related mechanisms such as screw loosening, motion–induced bone resorption, and plate bending may therefore play a critical role in clinical failure. However, the majority of experimental studies rely on the application of static monotonic loading until failure occurs. This may lead to overestimation of the construct durability and may also fail to show clinically relevant modes of mechanical deformation process.

In addition, there are also considerable variations in materials used for mandible models and fracture design. Synthetic mandibles differ widely in elastic modulus, fracture resistance, and cortical–trabecular architecture, all of which influence construct stiffness and failure patterns (Steffen et al., 2023). Moreover, standardized fracture models may not represent the irregularity of clinical fracture lines and reduced cortical contact commonly encountered in traumatic injuries.

These methodological inconsistencies make it difficult to compare studies and may not reflect clinically significant differences between the fixation designs.

Mandibular fracture biomechanics is a popular area of research in the field of oral and maxillofacial surgery. Most studies however only focus on plate configuration and/or material properties. On the other hand, joint-mediated load transfer and muscle-induced torsional forces are prominent determinants of mandibular biomechanics. Such details cannot be reproduced by simplified beam-like test setups that use rigid posterior fixation or inferior platform support. To date, no study has specifically evaluated how constraint conditions and loading protocols influence reported biomechanical outcomes in mandibular fracture models. Therefore, the aim of the present one was to systematically review in-vitro biomechanical investigations of mandibular fractures with a focused analysis of these test design parameters and their potential impact on construct stability and failure behavior. This review also seeks to clarify methodological sources of heterogeneity and to propose a standard reporting scheme for more clinically relevant experimental setups.

Materials and Methods

Study design

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol was designed to evaluate methodological characteristics of in-vitro biomechanical studies that investigate fixation of non-condylar mandibular fractures, with particular focus on the experimental boundary conditions and loading protocols.

Review question and PICOS framework

The review question was structured according to an adapted PICOS framework suitable for experimental biomechanical studies. P (Specimens): Full mandible in-vitro models with non-condylar fractures, including angle, body, parasymphysis, and symphysis fractures, using cadaveric or synthetic specimens. I (Intervention): Fixation constructs tested under defined experimental boundary conditions and loading protocols, including models with and without temporomandibular joint (TMJ) simulation and muscle force application. C (Comparator): Alternative fixation strategies and/or alternative experimental configurations, particularly different boundary conditions such as rigid posterior fixation versus joint-supported setups and loading protocols. O (Outcomes): Mechanical measures of construct performance, including failure load, stiffness, interfragmentary displacement, rotational deformation, and bending or torsional moments. S (Study Design): Controlled in-vitro biomechanical experimental studies.

Search strategy

A systematic literature search was performed in PubMed, Scopus, and Web of Science databases from inception to the date of final search. Search strategies combined controlled vocabulary and free-text terms related to mandibular fractures, biomechanics, and fixation. The main search concepts included combinations of the following terms: mandibular fracture, biomechanics, mechanical testing, fixation, osteosynthesis, and in vitro. No restrictions were applied with respect to publication year. Only articles published in English were considered (Table 1). In addition to database searches, reference lists of included articles were manually examined to identify further relevant studies.

Table 1. Database-specific search strategies and queries.

Pubmed	Scopus	Web of Science
(mandible(Title/Abstract) OR mandibular(Title/Abstract)) AND (fracture*(Title/Abstract)) AND (biomechanic*(Title/Abstract) OR mechanical(Title/Abstract) OR in vitro(Title/Abstract)) AND (fixation(Title/Abstract) OR osteosynthesis(Title/Abstract))	TITLE-ABS-KEY (mandible OR mandibular) AND (fracture*) AND (biomechanic* OR mechanical OR "in vitro") AND (fixation OR osteosynthesis)	TS= ((mandible OR mandibular) AND fracture* AND (biomechanic* OR mechanical OR "in vitro") AND (fixation OR osteosynthesis))

Inclusion criteria

Studies were included if they met all of the following conditions: In-vitro experimental studies involving mechanical testing of mandibular fracture fixation. Use of full mandible specimens, either cadaveric or synthetic. Investigation of non-condylar fracture sites, including angle, body, parasymphysis, or symphysis. Quantitative reporting of mechanical outcomes related to fixation stability, deformation, or failure. Studies combining experimental testing with computational methods, including finite element analysis used for validation or complementary interpretation, were included.

Exclusion criteria

Studies were excluded if they met any of the following criteria: Purely numerical or finite element analyses without experimental validation. Hemi-

mandible models or isolated bone segment testing. Studies exclusively investigating condylar fractures. Orthognathic osteotomy models, including bilateral sagittal split osteotomy (BSSO), due to fundamentally different load-sharing mechanics and large interfragmentary contact areas compared with traumatic fracture models, pure material bench testing without bone–platet interaction. Clinical studies without in-vitro biomechanical experimentation.

Study selection

After removal of duplicate records, titles and abstracts were examined for relevance. Full-text articles were subsequently reviewed to confirm eligibility. Study selection was performed according to predefined inclusion and exclusion criteria. Disagreements were resolved by consensus. The study selection process is presented in a PRISMA flow diagram (Figure 1).

Data extraction

Data were extracted using a predefined extraction form developed specifically to gather data on methodological variables that influence biomechanical behavior. The following information was recorded for each study:

Specimen type (cadaveric, polyurethane, resin, or composite), presence of cortical and trabecular differentiation, fracture location and fracture modeling technique, fixation construct type and configuration, boundary conditions, including: presence of temporomandibular joint simulation, presence of muscle force simulation inferior rigid support, posterior rigid clamping or embedding of the ramus or condylar region, Overall mechanical behavior classified as beam-like or ring-like. Loading protocol, including: loading points (single vs multiple), load vector orientation (vertical only vs multi-vector), force-controlled or displacement-controlled testing, static versus cyclic loading, outcome measures (failure load, stiffness, interfragmentary displacement, deformation, or moments), reported failure modes (plate-related vs model-related) Experimental setups were classified as beam-like when rigid constraints prevented joint-mediated mandibular motion and torsional deformation, and as ring-like when joint seating and physiologic load transfer were preserved.

Assessment of methodological bias

Because no validated risk-of-bias tool exists for in-vitro biomechanical studies, methodological bias was assessed qualitatively based on experimental fidelity to physiologic mandibular mechanics. Studies employing rigid posterior fixation or inferior platform support without joint simulation were considered at high risk of constraint-related bias. Additional sources of potential bias included vertical-only loading, absence of cyclic loading, and model-dominated failure modes.

Data synthesis

Due to significant heterogeneity in fracture models, fixation constructs, specimen materials, and loading protocols, quantitative meta-analysis was not feasible. Results were therefore presented descriptively, with special emphasis on the frequencies of methodological characteristics and their relationship to the reported mechanical outcomes.

Physiological fidelity scoring of experimental models

To evaluate the physiologic relevance of experimental setups, each study was assigned a physiological fidelity score on a five-level ordinal scale based on boundary conditions and loading protocols. This scoring system was developed to reflect the degree to which mandibular ring mechanics and functional loading conditions were preserved. Each study was independently classified according to these criteria based on reported boundary conditions and loading protocols. Discrepancies were resolved by consensus.

Scoring criteria were defined as follows: Score 1 Highly constrained beam-like models: Rigid posterior fixation or embedding of the ramus/condyle, inferior platform support, vertical-only loading, no TMJ simulation and no muscle force simulation, mandible functions mechanically as a simple beam. Score 2 Partially constrained beam-dominant models: either posterior rigid fixation or inferior rigid support present, Limited degrees of freedom at the condylar region, vertical or near-vertical loading, no muscle attachment simulation, partial suppression of physiologic mandibular motion. Score 3 Transitional models: TMJ seating or elastic joint support present, no rigid posterior clamping, loading remains predominantly vertical, no or limited muscle force simulation, allows limited ring mechanics but no physiologic torsional loading. Score 4 Joint-supported functional models: TMJ simulation with physiologic degrees of freedom, Multi-point or multi-vector loading, partial muscle force simulation or functional load distribution, No rigid inferior or posterior constraints. Approximates physiologic mandibular mechanics under functional loading. Score 5 High-fidelity mandibular simulators: Joint-supported boundary conditions, multi-vector muscle force simulation, cyclic or fatigue loading protocols, functional bite points and physiologic degrees of freedom preserved. This level was considered the closest approximation to in-vivo mandibular biomechanics.

Results

Study selection

The initial database search identified a total number of 359 records across PubMed, 561 across Scopus, and 961 across Web of Science. After removal of duplicate records, titles and abstracts were screened for relevance. Full-text

review was then performed to confirm eligibility according to predefined inclusion and exclusion criteria. Studies were excluded mainly due to performing finite element analysis without experimental validation, use of hemi-mandible or isolated bone segment models, investigation of isolated condylar fractures, or use of orthognathic osteotomy models. Finally, 23 in-vitro experimental studies met all inclusion criteria and were included in the qualitative synthesis. The study selection process is summarized in the PRISMA flow diagram (Figure 1).

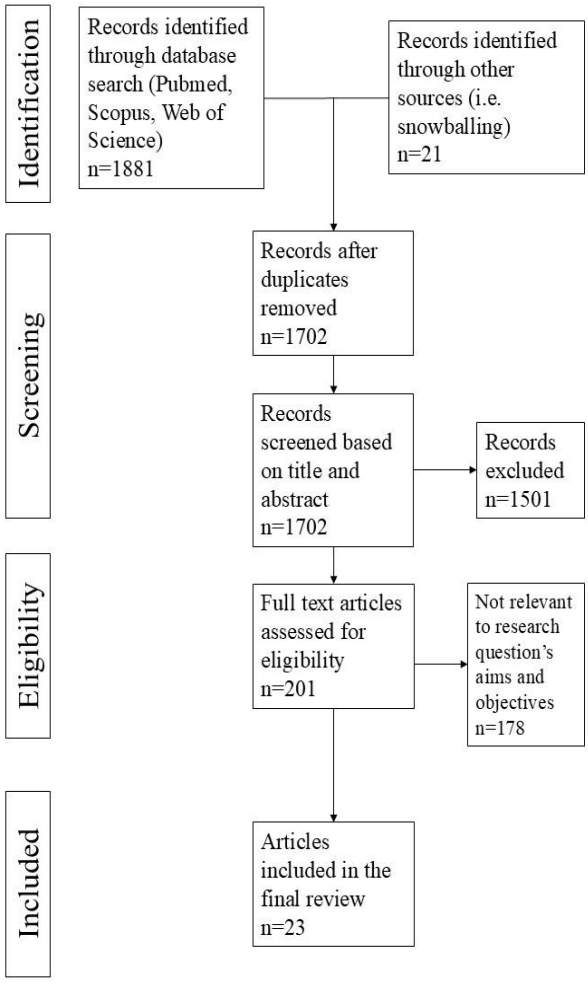

Figure 1. PRISMA flowchart, details of the search protocol.

Specimen characteristics and fracture models

Both cadaveric and synthetic mandible models were used among the 23 included studies. Synthetic specimens were predominantly polyurethane or composite mandibles, with variable representation of cortical and trabecular structures. Fracture models most commonly involved the mandibular angle, followed by parasymphysis, body, and symphysis regions. In several studies, standardized osteotomy-based fracture lines were created to ensure reproducibility. Fewer investigations attempted to replicate irregular traumatic fracture patterns. Considerable variability existed in fracture gap width, cortical contact, and presence of third molar sockets.

Boundary conditions and constraint characteristics

Temporomandibular joint (TMJ) simulation was used in 8 of the 23 studies (34.8%). Detailed simulation of muscle forces was present in only 7 studies (30.4%). Rigid boundary constraints were the most used configurations. Inferior platform support was used in 7 studies (30.4%), and posterior rigid clamping or embedding of the ramus or condylar region was employed in 5 studies (21.7%). Overall, more than half of the studies implemented at least one rigid constraint to limit physiologic mandibular mobility.

Based on boundary conditions and allowance of joint-mediated motion, experimental setups were classified as beam-like or ring-like. Eleven studies (47.8%) exhibited beam-like mechanical behavior characterized by suppressed torsional deformation and direct load transfer through the fixation construct. Twelve studies (52.2%) preserved at least partial ring-like mechanics through joint-supported or elastically restrained boundary conditions, allowing, albeit sometimes limited, combined bending and torsional loading.

Loading protocols

Loading protocols showed significant heterogeneity. Single-point loading, typically applied at an ipsilateral molar or incisal region, was used in 12 studies (52.2%), whereas 11 studies (47.8%) employed multiple loading points or distributed loading conditions. Vertical-only loading was applied in 16 studies (69.6%), while only 7 studies (30.4%) incorporated multi-vector loading that partially reflected physiologic muscle force directions.

With respect to control mode, 15 studies (65.2%) used displacement-controlled testing, whereas 8 studies (34.8%) employed force-controlled loading. Static monotonic loading was the dominant force application protocol, with 19 studies (82.6%) relying exclusively on static protocols. Only 3 studies (13.0%) incorporated cyclic or fatigue loading, and one study applied quasi-dynamic

functional loading patterns. Thus, the vast majority of investigations did not assess time-dependent changes in the fixation stability.

Outcome measures and failure modes

Mechanical outcome measures varied widely across studies. Failure load was reported as a primary endpoint in 11 studies (47.8%). Construct stiffness or load–displacement curve was reported in 7 studies (30.4%). Interfragmentary displacement or fracture gap motion was evaluated in 9 studies (39.1%). Only 4 studies (17.4%) performed three-dimensional deformation or moment analysis to quantify torsional behavior.

Failure modes were frequently related to the model itself. In 14 studies (60.9%), failure occurred within the synthetic bone material or near fixation fixtures rather than at the plate–bone interface or within the fixation system itself. Plate-related failure, including plate bending or screw loosening, was the dominant mechanism in only 3 studies (13.0%). In the remaining ones, failure modes were insufficiently reported to allow clear classification.

Physiological fidelity scores of experimental models

Based on boundary conditions and loading protocols, studies were assigned a physiological fidelity score ranging from 1 (highly constrained beam-like models) to 5 (high-fidelity mandibular simulators). Seven studies were classified as score 1–2, representing highly constrained experimental setups with rigid posterior or inferior fixation and vertical-only loading. Eight studies were classified as score 3, representing transitional models that included joint seating but lacked physiologic torsional or muscle-related loading. Only eight studies reached scores of 4 or 5, indicating joint-supported functional models or high-fidelity simulators incorporating multi-vector loading and, in some cases, cyclic testing.

Studies with higher physiological fidelity scores reported inferior border displacement and rotational instability more frequently. On the other hand, highly constrained models tended to show high construct stiffness and reduced interfragmentary motion. Consequently, fixation constructs that appeared mechanically similar in beam-like designs often behaved differently in joint-supported functional models.

Experimental design and fixation performance

Considerable variation was noted across studies using different experimental configurations. In beam-like models, superior border miniplate fixation frequently showed comparable stability to more rigid constructs. In joint-supported and muscle-simulated models however, increased inferior border motion was reported more frequently when no inferior border fixation was

performed. These findings suggest that boundary conditions and loading protocols have a more pronounced effect on the relative performance of fixation constructs than the plate–screw design alone.

Due to methodological heterogeneity and variability in reported outcome measures, quantitative synthesis was not feasible. Therefore, comparisons were limited to qualitative assessment of methodological trends and their apparent influence on reported biomechanical performance.

Table 2. Model, fracture, TMJ, muscle and fixation-related details presented in the studies included in this systematic review.

First Author	Year	Model Type	Layered Model	Fracture Site	Fracture Standardized	TMJ Simulation	Muscle Simulation	Inferior Rigid Support	Posterior Rigid Clamp/Embedding
Kroon et al.(Kroon, Mathisson, Cordey, and Rahn, 1991)	1991	PU	No	Angle, Parasymphysis	Guide	Yes	Yes	No	No
Choi et al.(Choi, Yoo, Kim, and Kang, 1995)	1995	Cadaver	Yes	Angle	Guide	Yes	Yes	No	No
Shetty et al.(Shetty, McBrearty, Fourney, and Caputo, 1995)	1995	Resin	No	Angle	Guide	Yes	No	No	No
Tams et al.(Tams, van Loon, Otten, Rozema, and Bos, 1997)	1997	Resin	No	Angle, Body, Symphysis	Guide	Yes	Yes	No	No
Fedok et al.(Fedok et al., 1998)	1998	Synthetic	Yes	Angle	Jig	No	No	No	Yes
Haug et al.(R. H. Haug et al., 2001)	2001	Synbone	Yes	Angle	Guide	No	No	No	Yes
Karoglan et al.(Karoglan, Schütz, Schieferstein, Horch, and Neff, 2006)	2006	PU	No	Condylar	Guide	Yes	Yes	No	No

Kalfarentzos et al.(Kalfarentzos, Deligianni, Mitros, and Tyllianakis, 2009)	2009	Synbone	Yes	Angle	Guide	Partial	No	No	No
Vieira and Passeri(Vieira e Oliveira and Passeri, 2011)	2011	PU	No	Angle	Guide	No	No	Yes	Yes
Bregagnolo et al.(Bregagnolo, Bertelli, Ribeiro, Sverzut, and Trivellato, 2011)	2011	PU	No	Angle	Guide	No	No	Yes	No
Steiner et al.(Steiner et al., 2012)	2012	Cadaver	Yes	Parasymphysis	Guide	Yes	Yes	No	No
Pereira-Filho et al.(Pereira-Filho et al., 2013)	2013	PU	No	Symphysis (atrophic)	Guide	No	No	Yes	No
Trivellato et al.(Trivellato, Pepato, Ribeiro, Sverzut, and Trivellato, 2014)	2014	PU	No	Angle	Guide	No	No	Yes	No
Yamaji et al.(Yamaji et al., 2015)	2015	PU	No	Angle	Guide	No	No	Yes	No
Zimmermann et al.(Zimmerman et al., 2017)	2017	PU	No	Various	Guide	Yes	Yes	No	No
Gonzales et al.(Gonzales, Spagnol, Sverzut, and Trivellato, 2017)	2017	PU	No	Angle	Guide	No	No	No	Yes
Şirin et al.(Sirin, Yildirimturk, Ay, and Gencel, 2020)	2020	PU	No	Angle	Guide	Partial	No	Yes	No

Burns et al.(Burns et al., 2020)	2020	PU	No	Angle	Guide	No	No	No	Yes
Xu et al.(Xu et al., 2021)	2021	PU	No	Angle/Body	Guide	Yes	Yes	No	No
Demetoğlu et al.(Demetoglu and Bilge, 2021)	2021	PU	No	Angle	Guide	No	No	Yes	No
Ay et al.(Ay, Yildirimturk Dogan, and Sirin, 2023)	2023	PU	No	Angle	Guide	Partial	No	No	No
Daqiq et al.(Daqiq, Roossien, Wubs, and van Minnen, 2024)	2024	PU	No	Angle	Guide	Partial	No	No	No
Daqiq et al.(Daqiq et al., 2025)	2025	PU	No	Angle	Guide	Partial	No	No	No

Table 3. Mechanics, loading, outcome, failure mode, fidelity, constraint bias and fixation-related details presented in the studies included in this systematic review.

First Author	Year	Overall Mechanics	Loading Points	Load Direction	Control Mode	Static or Cyclic	Outcome Type	Failure Mode Reported	Core Dataset	Physiological Fidelity	Constraint Bias Risk
Kroon et al.(Kroon et al., 1991)	1991	Ring-like	Multiple	Multi-vector	Force	Static	Gap/Strain	No	Yes	High	Low
Choi et al.(Choi et al., 1995)	1995	Ring-like	Multiple	Multi-vector	Force	Static	Gap	No	Yes	High	Low
Shetty et al.(Shetty et al., 1995)	1995	Ring-like	Multiple	Multi-vector	Force	Static	Moments/Gap	No	Yes	High	Low
Tams et al.(Tams et al., 1997)	1997	Ring-like	Multiple	Multi-vector	Force	Static	Moments	No	Yes	High	Low

Fedok et al.(Fedok et al., 1998)	1998	Beam-like	Incisal	Vertical only	Displacement	Static	Gap	Substrate	Yes	Low	High
Haug et al.(R. H. Haug et al., 2001)	2001	Beam-like	Incisal/Contra	Vertical only	Displacement	Static	Failure/Stiffness	Substrate	Yes	Low	High
Karoglan et al.(Karoglan et al., 2006)	2006	Ring-like	Multiple	Multi-vector	Force	Cyclic	Deformation	No	No	High	Low
Kalfarentzos et al.(Kalfarentzos et al., 2009)	2009	Ring-like	Incisal/Ipsi	Vertical only	Force	Static	Stiffness/Gap	No	Yes	Moderate	Moderate
Vieira and Passeri(Vieira e Oliveira and Passeri, 2011)	2011	Beam-like	Ipsi molar	Vertical only	Displacement	Static	Failure/Stiffness	Substrate	Yes	Low	High
Bregagnolo et al.(Bregagnolo et al., 2011)	2011	Beam-like	Multiple	Vertical only	Displacement	Static	Failure	Substrate	Yes	Low	High
Steiner et al.(Steiner et al., 2012)	2012	Ring-like	Incisal	Multi-vector	Force	Quasi-static	3D Motion	No	No	High	Low
Pereira-Filho et al.(Pereira-Filho et al., 2013)	2013	Beam-like	Fracture site	Vertical only	Displacement	Static	Failure	Substrate	Yes	Low	High
Trivellato et al.(Trivellato et al., 2014)	2014	Beam-like	Multiple	Vertical only	Displacement	Static	Failure	Substrate	Yes	Low	High
Yamaji et al.(Yamaji et al., 2015)	2015	Beam-like	Ipsi molar	Vertical only	Displacement	Static	Failure	Substrate	Yes	Low	High
Zimmermann et al.(Zimmermann et al., 2017)	2017	Ring-like	Multiple	Multi-vector	Force	Cyclic	3D Motion/Fatigue	No	No	High	Low

Gonzales et al.(Gonzales et al., 2017)	2017	Beam-like	Ipsi molar	Vertical only	Displacement	Static	Failure	Substrate	Yes	Low	High
Şirin et al.(Sirin et al., 2020)	2020	Beam-like	Multiple	Vertical only	Displacement	Static	Failure/Stiffness	Substrate	Yes	Moderate	Moderate
Burns et al.(Burns et al., 2020)	2020	Beam-like	Ipsi molar	Vertical only	Displacement	Static	Failure	Substrate	Yes	Low	High
Xu et al.(Xu et al., 2021)	2021	Ring-like	Multiple	Multi-vector	Force	Cyclic	Gap/Strain	No	No	High	Low
Demetoğlu et al.(Demetoglu and Bilge, 2021)	2021	Beam-like	Multiple	Vertical only	Displacement	Static	Failure	Substrate	Yes	Low	High
Ay et al.(Ay et al., 2023)	2023	Ring-like	Multiple	Vertical only	Displacement	Static	Stiffness/Gap	No	Yes	Moderate	Moderate
Daqiq et al.(Daqiq et al., 2024)	2024	Ring-like	Multiple	Vertical only	Displacement	Static	Failure/Stiffness	Substrate	Yes	Moderate	Moderate
Daqiq et al.(Daqiq et al., 2025)	2025	Ring-like	Multiple	Vertical only	Displacement	Static	Gap/Stiffness	No	Yes	Moderate	Moderate

Discussion

The present systematic review demonstrated that experimental mandibular fracture fixation remains highly heterogeneous with respect to boundary conditions, loading protocols, and specimen characteristics. More importantly, over half of the included studies employed rigid constraints that substantially changed physiologic mandibular motion. This might have altered load transfer pathways and mechanical behavior of fixation constructs. These methodological choices appear to have a major influence on the reported stability and failure patterns, raising concerns regarding the translational relevance of many published biomechanical findings.

The mandible functions biomechanically as a curved ring structure suspended between two TMJs and subjected to multi-vector muscle forces. This configuration results in combined bending and torsional stresses that vary with bite location and asymmetric muscle activation. Experimental models that rigidly

fix the posterior mandible or apply inferior platform support effectively transform this ring structure into a beam-like system, which eliminates joint-mediated load sharing and suppressing torsional deformation. In the present review, nearly half of all studies exhibited beam-like mechanical behavior due to rigid constraints, whereas only slightly more than half preserved partial or full ring mechanics through joint-supported configurations. Studies allowing physiologic degrees of freedom more frequently detected inferior border displacement and rotational instability, particularly in mandibular angle and parasymphysis fractures. In contrast, beam-like models tended to report high construct stiffness and limited interfragmentary motion, potentially masking clinically relevant instability issues. These findings indicated that boundary conditions should be considered an important determinant of mechanical behavior and may hide differences attributable to plate design or configuration.

Relative ranking of fixation constructs is highly sensitive to experimental setup. In beam-like models, superior border miniplate fixation showed mechanical performance comparable to more rigid constructs. However, the joint-supported models more often demonstrated increased inferior border motion when it is not stabilized. This discrepancy may provide a different perspective to the ongoing debate on optimal fixation strategies for mandibular angle fractures despite decades of biomechanical investigation. If fixation constructs are evaluated under conditions that suppress physiologic torsional loading, constructs designed primarily to resist bending may appear sufficient, whereas their limitations under functional loading might go unnoticed. Consequently, comparisons performed in constrained setups may underestimate the mechanical benefit of inferior border fixation or load-bearing constructs.

Loading protocols were highly heterogeneous and frequently lacked physiological relevance. Vertical-only loading was predominant. This approach neglects torsional components generated during unilateral mastication. Static monotonic testing was also commonly used. Such testing fails to represent the repetitive functional loading encountered postoperatively. Only a small minority of studies incorporated cyclic or fatigue loading protocols. This is notable given the clinical relevance of progressive screw loosening, micro-motion–induced bone damage, and plate fatigue. Fixation constructs that tolerate high static loads may also fail under repeated low-amplitude cyclic stresses. Consequently, reliance on static failure loads may overestimate clinical durability. It may also obscure differences between fixation constructs that primarily manifest under fatigue conditions.

Synthetic mandible models offer improved reproducibility. However, they differ substantially from human bone in terms of elastic modulus, fracture toughness, and cortical–trabecular architecture. In the present review, model

material-related failure dominated in the majority of studies. This finding indicates that material limitations often determined failure behavior rather than plate-screw system performance. Under such conditions, reported failure loads may reflect properties of the test model rather than true biomechanical limitations of the fixation system. This finding further complicates interpretation of comparative results. It may also partially explain conflicting conclusions across studies employing different synthetic substrates or fracture modeling techniques.

To address methodological heterogeneity, the present review applied a physiological fidelity scoring system by integrating boundary conditions and loading protocols. Only a minority of studies achieved high scores. These scores were indicative of joint-supported functional loading or high-fidelity mandibular simulators. Notably, these higher-fidelity models more consistently demonstrated torsional instability and inferior border displacement was also observed. Such mechanical behaviors are often absent in the constrained, beam-like experimental setups. Stratification of studies according to physiological fidelity provides a useful framework for interpreting biomechanical findings. It may help describe conflicting results in the literature. Without such stratification, pooling of mechanically dissimilar studies risks obscuring clinically meaningful differences between fixation strategies.

From a clinical perspective, reliance on biomechanical data derived from constrained experimental setups may lead to underestimation of fixation requirements. This risk is particularly present in situations involving early functional loading while it also applies to unfavorable fracture patterns. Reduced cortical contact further adds up to this concern. Fixation strategies that show robustness under physiologic loading conditions may offer greater safety margins. This is especially important in patients at higher risk of mechanical overload. Limited compliance with postoperative restrictions represents an additional factor. Although simplified beam-like models may be suitable for preliminary analysis of plate/screw designs, they should not be considered sufficient for guiding clinical decision-making. This limitation is particularly relevant when selecting fixation strategies.

The present findings underscore the need for greater standardization of experimental boundary conditions and loading protocols in mandibular fracture biomechanics. Future studies should incorporate joint-supported configurations and multi-vector loading reflective of muscle forces should also be applied. Cyclic loading protocols are also required to better approximate functional conditions. Clear reporting of degrees of freedom at constraint points and reporting of fixture compliance are essential. This level of detail is necessary to allow meaningful interpretation and comparison across studies. Adoption of more physiologic testing protocols would enhance relevance of experimental findings

and allow the development of evidence-based guidelines for mandibular fracture fixation.

The present systematic review had some limitations. First, substantial methodological heterogeneity existed among studies. This included model materials, fracture modeling techniques and variability in fixation constructs. Loading protocols also differed among studies. This level of heterogeneity precluded meaningful quantitative synthesis. Consequently, analysis was restricted to qualitative methodological comparison. Second, classification of experimental setups and assignment of physiological fidelity scores inevitably involved a degree of interpretation. This was particularly relevant when studies did not explicitly report degrees of freedom at constraint points. Fixture compliance was also frequently omitted. Although predefined criteria were applied consistently, incomplete methodological reporting limited clear characterization in some cases. Third, the review relied on published descriptions of experimental setups. Direct verification of boundary conditions was not possible and mechanical behavior could not be independently assessed. Therefore, misclassification of experimental configurations cannot be entirely excluded. This concern is particularly relevant for older studies with limited methodological detail. Fourth, most included studies used synthetic mandible models. The material properties of these models differ from those of human cortical and trabecular bone. While synthetic models improve reproducibility, they may alter stiffness measurements and failure modes may also be affected. As a result, direct extrapolation to clinical performance is limited. Fifth, publication bias toward studies demonstrating measurable differences between fixation constructs may be present. This risk is particularly relevant given the predominance of failure-based outcome measures. Studies reporting negative or inconclusive results may be underrepresented in the literature. Finally, although the review focused on non-condylar mandibular fractures, variability in fracture location and geometry may influence biomechanical behavior. However, subgroup analysis by fracture site was not feasible hence it could not be performed. This issue was due to limited numbers within each fracture category.

Conclusion

The biomechanical resistance of fixation constructs are often determined by experimental configuration. Despite their availability for more than two decades, the physiologically relevant testing setups remain underutilized. Instead, most studies continue to rely on static vertical loading and rigid fixation of posterior segments. Under such conditions, intrinsic mechanical performance of osteosynthesis systems may be limited. Consequently, extrapolation of such data to clinical decision-making should be approached with caution. Future biomechanical investigations should prioritize standardized protocols: joint-

supported boundary conditions should be implemented, cyclic loading should be incorporated, explicit reporting of boundary conditions should be made essential and degrees of freedom must be clearly specified. Adoption of physiologically relevant testing setups is of utmost importance to improve clinical validity and to enable meaningful comparison of fixation protocols across studies.

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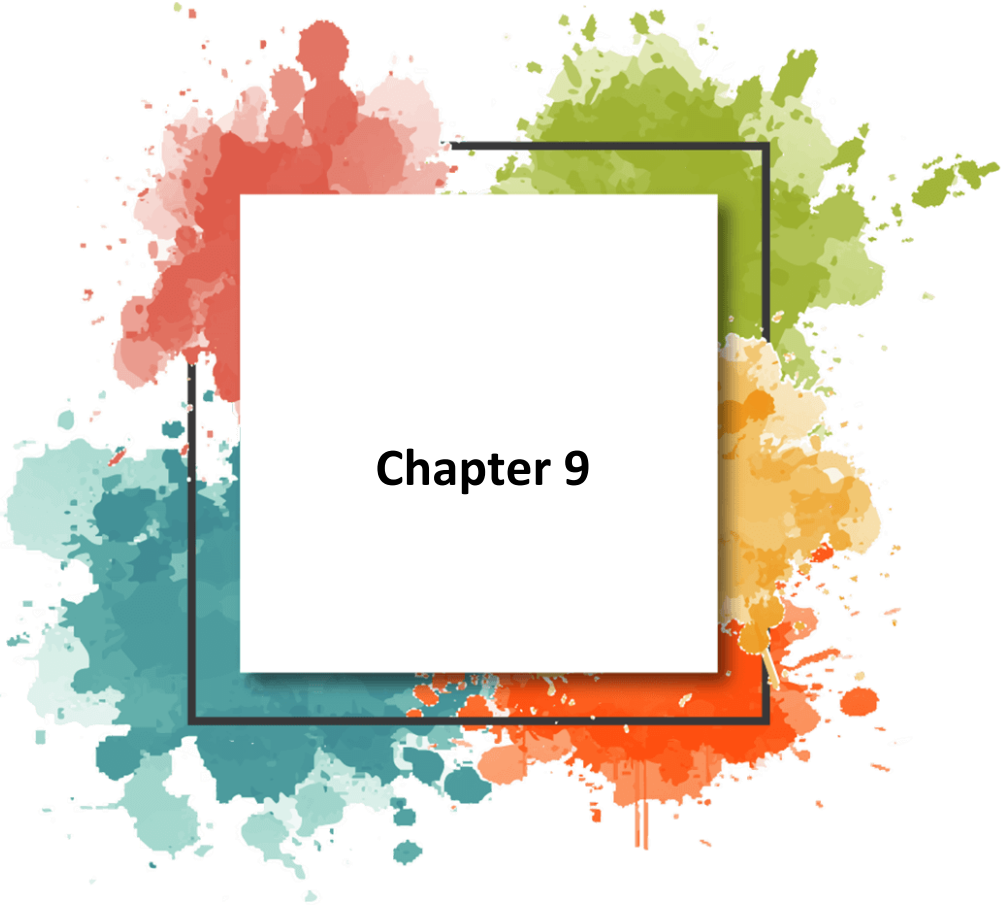
Chapter 9

Large Language Models in Clinical Dentistry: Optimizing Rational Antibiotic Prescribing and Pharmacological Decision Support

Mustafa Üstün[1]

INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most severe global public health crises, profoundly threatening the clinical efficacy of antibiotics and escalating healthcare challenges worldwide (Abavisani et al., 2024). In outpatient settings, including dental practices, antibiotics are frequently prescribed for both therapeutic and prophylactic purposes; however, inappropriate prescribing practices remain highly prevalent (Ozmen & Okkesim, 2025). Rational antibiotic use necessitates selecting the appropriate medication at the correct dosage and for an adequate duration, yet clinicians often face challenges due to diagnostic uncertainties, time constraints, and a lack of adherence to continuously updated evidence-based guidelines (De Vito et al., 2025). Furthermore, the aging patient population and the increasing prevalence of polypharmacy have elevated the risk of clinically relevant drug-drug interactions (DDIs) during dental therapies, which can lead to severe adverse events or therapeutic failures (Tayeb et al., 2025). Consequently, there is an urgent need for innovative, accessible, and accurate antimicrobial stewardship and clinical decision support strategies to mitigate prescribing errors and combat AMR (Antonie et al., 2025).

In recent years, the rapid evolution of artificial intelligence (AI), specifically large language models (LLMs), has introduced a transformative paradigm in medical and dental informatics (Giacobbe et al., 2025). Built upon sophisticated deep learning architectures known as transformer networks, modern LLMs such as ChatGPT (OpenAI), Gemini (Google DeepMind), Claude (Anthropic), and DeepSeek utilize self-attention mechanisms to process, synthesize, and generate natural human language with remarkable coherence (Abavisani et al., 2024; Giacobbe et al., 2025). These conversational agents are trained on massive datasets encompassing diverse biomedical literature, enabling them to execute

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complex cognitive tasks, including summarizing clinical notes, retrieving medical information, and simulating diagnostic reasoning (Antonie et al., 2025; Coşkun & Tayşi, 2025). As these models evolve from unimodal text processors to multimodal systems capable of analyzing clinical scenarios, their potential to serve as dynamic, on-demand clinical decision support systems (CDSS) for healthcare professionals has garnered significant academic interest (Madfa et al., 2026).

The integration of AI-driven chatbots into dental pharmacology offers promising avenues for optimizing clinical workflows, particularly in guiding rational antibiotic prescribing and detecting DDIs (Coşkun & Tayşi, 2025). Recent comparative evaluations demonstrate that models like ChatGPT-4 and Gemini can achieve high accuracy in recommending appropriate antibiotic regimens, tailoring dosages, and identifying complex pharmacological contraindications in simulated oral surgery scenarios, occasionally matching the performance of experienced clinicians (De Vito et al., 2025; Tayeb et al., 2025). However, the deployment of LLMs as autonomous clinical tools is currently limited by critical safety concerns, primarily the phenomenon of "hallucinations," where the model generates highly plausible but factually incorrect or unsafe medical advice (Hakim et al., 2025; Maillard et al., 2024). Additionally, variations in performance consistency, logical reasoning, and language-dependent accuracy necessitate rigorous validation and prompt engineering (Di Pumpo et al., 2025; Taşyürek et al., 2025). Therefore, while AI chatbots present unprecedented opportunities to enhance antimicrobial stewardship and dental education, their application must remain strictly supplementary within a "human-in-the-loop" framework, ensuring that ultimate clinical judgment and patient safety remain the clinician's responsibility (Antonie et al., 2025). Figure 1 demonstrates conceptual framework of ai chatbot integration into antibiotic therapy decision-making.

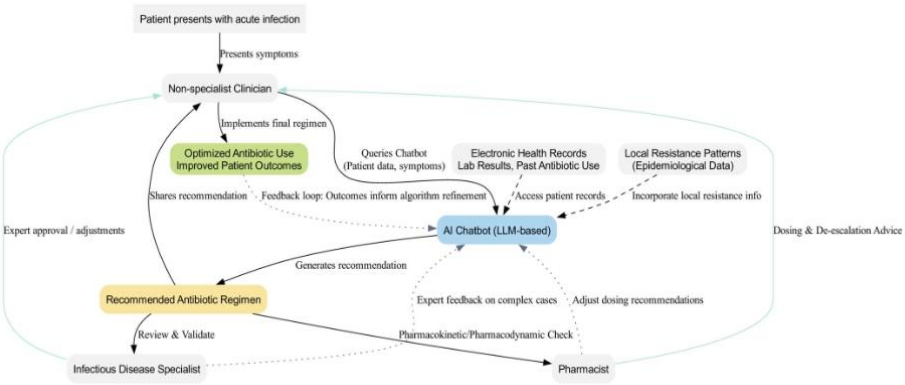

Figure 1: Conceptual Framework of AI Chatbot Integration into Antibiotic Therapy Decision-Making. (Antonie et al., 2025)

MATERIALS AND METHOD

1. The Global Challenge of Antimicrobial Resistance and Rational Antibiotic Use in Dentistry

Antimicrobial resistance (AMR) is currently recognized by the World Health Organization (WHO) as one of the top ten global public health threats facing humanity (Abavisani et al., 2024). Often described as a "silent pandemic," AMR severely undermines the efficacy of existing antibiotics, leading to treatment failures, prolonged hospitalizations, and increased mortality rates (Tekin, n.d.). The overuse and misuse of antimicrobial agents across human and animal populations have accelerated the evolutionary selection of multidrug-resistant pathogens (Abavisani et al., 2024). Epidemiological projections indicate that, without immediate and concerted global action, AMR-attributable fatalities could escalate from hundreds of thousands to approximately 10 million deaths annually by the year 2050 (Figure 2) (Abavisani et al., 2024; Antonie et al., 2025). Consequently, robust antimicrobial stewardship strategies are imperatively required to preserve the clinical utility of current pharmacological arsenals.

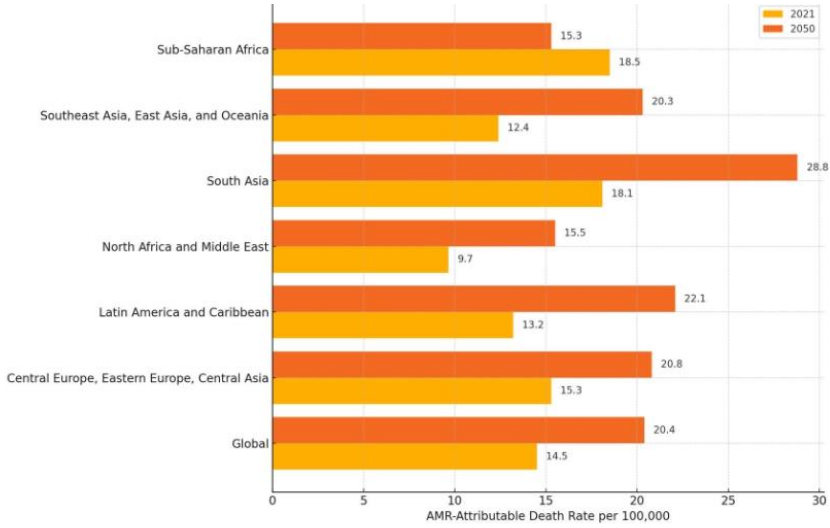

Figure 2: Comparison of AMR-Attributable Death Rates per 100,000 in 2021 and 2050 by region. (Antonie et al., 2025)

Within this global landscape, the dental profession bears a substantial responsibility, as dentists account for approximately 10% of all outpatient antibiotic prescriptions worldwide (Fluent et al., 2016; Ozmen & Okkesim, 2025). Despite established clinical guidelines, studies reveal that between 30% and 50% of these prescriptions are either unnecessary or suboptimally prescribed in terms of agent selection, dosage, or duration (Fluent et al., 2016; Ozmen & Okkesim, 2025). Inappropriate antibiotic prescribing in dental practice is frequently driven by non-clinical factors, including diagnostic uncertainty, severe time constraints, fear of legal litigation, and direct demands from patients (Fluent et al., 2016; Ozmen & Okkesim, 2025). Furthermore, systemic antibiotics are sometimes erroneously utilized as a substitute for definitive procedural interventions, such as surgical extraction, incision and drainage, or endodontic therapy, which are the primary requirements for resolving localized odontogenic infections (Fluent et al., 2016; Ozmen & Okkesim, 2025).

To mitigate the contribution of dental practices to the AMR crisis, strict adherence to the principles of rational antibiotic use (RAU) is critical. The WHO defines rational drug use as the practice wherein patients receive medications appropriate to their specific clinical needs, in doses that meet their individual requirements, for an adequate duration, and at the lowest possible cost to themselves and society (Ozmen & Okkesim, 2025). In dentistry, this necessitates a thorough clinical evaluation to confirm the presence of systemic involvement—such as fever, lymphadenopathy, malaise, or spreading cellulitis—before

initiating systemic antibiotic therapy (Ozmen & Okkesim, 2025). The implementation of structured antimicrobial stewardship programs, comprehensive continuing education, and the adoption of clinical decision support systems are essential steps toward correcting irrational prescribing behaviors, optimizing patient safety, and decelerating the emergence of resistant microbial strains (Fluent et al., 2016; Ozmen & Okkesim, 2025).

2. Architecture and Capabilities of Large Language Models (LLMs) in Healthcare

Artificial intelligence (AI) chatbots, commonly referred to as large language models (LLMs), operate using Natural Language Processing (NLP) to interpret and generate human language, alongside machine learning (ML) algorithms that refine their responses over time. The fundamental architecture underlying modern LLMs, such as OpenAI's ChatGPT, Google's Gemini, and Anthropic's Claude, is the transformer network (Abavisani et al., 2024). Transformers utilize a sophisticated self-attention mechanism that assigns varying mathematical weights to input tokens (words or data fragments), allowing the model to capture complex contextual relationships and linguistic nuances across extensive textual sequences (Abavisani et al., 2024; Antonie et al., 2025). These systems process input data through tokenization and high-dimensional embedding, ultimately generating contextually coherent responses sequentially by following a "pre-training, prompt, predict" paradigm (Giacobbe et al., 2025).

Within the healthcare sector, the advanced capabilities of LLMs enable them to perform a wide array of cognitive and administrative tasks. These models excel at summarizing extensive clinical notes, extracting significant information from medical texts, translating complex medical jargon into lay-friendly language for patient education, and providing real-time question-and-answer support for both clinicians and patients (Antonie et al., 2025). Furthermore, recent iterations of these models, such as GPT-4o and Gemini Advanced, have evolved into Large Multimodal Models (LMMs), possessing the capability to process and integrate diverse data modalities, including text, audio, and visual inputs like radiological images (Çege et al., 2025; Taşyürek et al., 2025). This multimodal capacity significantly broadens their utility, allowing them to assist in interpreting diagnostic tests and synthesizing patient histories into cohesive clinical summaries instantly. The robust capacity of these models for encoding medical knowledge is further evidenced by their proficiency in passing complex, standardized medical examinations, such as the United States Medical Licensing Examination (USMLE), without requiring specialized domain training (Abavisani et al., 2024; Kung et al., 2023).

Despite their profound capabilities in language generation and data synthesis, the architectural nature of LLMs presents inherent challenges in clinical settings. LLMs are fundamentally based on deep neural networks (NNs), which emulate the biological architecture of the human brain but function as "black box" algorithms. This means that the intricate mathematical computations bridging the input and output layers remain largely opaque, making it difficult for clinicians to trace the exact reasoning behind a specific diagnostic or therapeutic recommendation (Giacobbe et al., 2025; Tayeb et al., 2025). Consequently, while a chatbot can successfully parse lengthy medical records and flag potential antibiotic choices using probabilistic text generation—predicting the next logical token in a sequence—it fundamentally lacks authentic clinical reasoning, inference capabilities, and situational awareness (Antonie et al., 2025). Because their outputs are mathematically generated rather than derived from true medical judgment, these models require strict human oversight to prevent the propagation of algorithmic biases and "hallucinations," which are plausible but factually incorrect medical statements (Antonie et al., 2025; Hakim et al., 2025).

3. AI as a Clinical Decision Support System (CDSS) for Antimicrobial Stewardship

Artificial intelligence (AI), particularly Large Language Models (LLMs) and machine learning algorithms, offers a transformative approach to addressing the global antimicrobial resistance (AMR) crisis by serving as dynamic Clinical Decision Support Systems (CDSS) (Abavisani et al., 2024; Antonie et al., 2025). In daily clinical practice, non-specialist physicians often manage acute infections, a situation that frequently results in suboptimal antibiotic choices that deviate from established clinical guidelines (Antonie et al., 2025; De Vito et al., 2025). By integrating with electronic health records (EHR) and laboratory information systems, AI chatbots can instantly analyze patient-specific data—such as medical history, allergies, microbiological test results, and local resistance patterns—to provide real-time, evidence-based recommendations (Abavisani et al., 2024; Antonie et al., 2025). This capability can significantly enhance antimicrobial stewardship (AMS) programs by reducing inappropriate antibiotic prescriptions, optimizing drug dosages, and facilitating early transitions from intravenous to oral therapies (Abavisani et al., 2024; AlGain et al., 2025).

Recent clinical evaluations have demonstrated substantial variability in the performance of different LLMs when tasked with prescribing antibiotics across diverse infectious disease scenarios (De Vito et al., 2025). A comprehensive comparative study analyzing 14 LLMs, including various iterations of ChatGPT, Claude, and Gemini, revealed that advanced models like ChatGPT-o1 achieved

remarkable accuracy, correctly prescribing antibiotics in 71.7% of simulated clinical cases and providing accurate dosage recommendations in 96.7% of instances (De Vito et al., 2025). Conversely, models such as Gemini and Claude 3 Opus exhibited significantly lower accuracy in these specific pharmacological tasks, although Gemini demonstrated higher proficiency in recommending appropriate treatment durations (Table 1) (De Vito et al., 2025). Furthermore, in prospective cohort studies evaluating the management of bloodstream infections, ChatGPT-4 provided adequate empirical antimicrobial therapies in 64% of cases and demonstrated a strong capacity to generate structured, well-written management plans (Antonie et al., 2025; Maillard et al., 2024).

Large language model	Wrong, *n* (%)	Partially correct, *n* (%)	Correct, *n* (%)	Overtreatment, *n* (%)
ChatGPT	4 (6.7)	9 (15.0)	29 (48.3)	18 (30.0)
ChatGPT-o1	1 (1.7)	10 (16.7)	43 (71.7)	6 (10.0)
ChatGPT4o	2 (3.3)	11 (18.3)	32 (53.3)	15 (25.0)
Claude 3 Opus	9 (15.0)	13 (21.7)	29 (48.3)	9 (15.0)
Claude 3.5 Sonnet	6 (10.0)	10 (16.7)	29 (48.3)	15 (25.0)
Copilot	3 (5.0)	10 (16.7)	29 (48.3)	18 (30.0)
Copilot Pro	5 (8.3)	10 (16.7)	26 (43.3)	19 (31.7)
Gemini	14 (23.3)	8 (13.3)	19 (31.7)	19 (31.7)
Gemini Advance	7 (11.7)	12 (20.0)	24 (40.0)	17 (28.3)
Grok 2	5 (8.3)	12 (20.0)	24 (40.0)	19 (31.7)
Le Chat - Large 2	7 (11.7)	10 (16.7)	28 (46.7)	15 (25.0)
Perplexity	4 (6.7)	14 (23.3)	31 (51.7)	11 (18.3)
Perplexity Pro	4 (6.7)	11 (18.3)	34 (56.7)	11 (18.3)
pi.ai	4 (6.7)	12 (20.0)	26 (43.3)	18 (30.0)
Total	75 (8.9)	152 (18.1)	403 (48.0)	210 (25.0)

Table 1: Percentage of antibiotic choice answers evaluated as incorrect, partially correct, correct, and overtreatment, divided by different large language models (De Vito et al., 2025)

Despite these promising capabilities, AI-driven CDSS currently exhibit critical limitations that preclude their use as autonomous clinical decision-makers (Antonie et al., 2025; De Vito et al., 2025). Studies have shown that while LLMs possess vast theoretical knowledge, they often struggle with complex clinical reasoning, such as recognizing nuanced resistance mechanisms, and they frequently recommend outdated or overly broad-spectrum antibiotics—like colistin—instead of newer, more appropriate alternatives (De Vito et al., 2024; De Vito et al., 2025). Furthermore, AI chatbots can fail to distinguish between clinically relevant and irrelevant factors, lack true situational awareness, and occasionally enter "failure modes" that lead to the repeated provision of unsafe or harmful medical advice, particularly in severe cases involving vulnerable populations such as neutropenic patients (Antonie et al., 2025; Maillard et al., 2024). Consequently, the integration of AI into antimicrobial stewardship must strictly adhere to a collaborative "human-in-the-loop" framework, wherein these systems function solely as supplementary tools that require rigorous oversight and validation by infectious disease specialists and pharmacists (Antonie et al., 2025; Maillard et al., 2024).

4. Detection and Management of Drug-Drug Interactions (DDIs) in Dental Therapy

Patients undergoing oral surgery and receiving dental therapies frequently present with complex medical profiles, including chronic conditions such as cardiovascular disease and diabetes, and they often take multiple medications simultaneously. The addition of routine perioperative prescriptions—such as nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics, corticosteroids, and antibiotics—significantly elevates the risk of clinically relevant drug-drug interactions (DDIs). Such interactions can lead to severe adverse outcomes, including gastrointestinal bleeding when combining NSAIDs with selective serotonin reuptake inhibitors (SSRIs), serotonin syndrome resulting from the coadministration of tramadol and SSRIs, or amplified hemorrhage risks when combining warfarin with antiplatelet agents (Tayeb et al., 2025). Evaluating these pharmacological risks is a time-critical clinical judgment typically made chairside, which is further complicated by the fact that dentists may lack access to patients' complete medical and medication histories (Tayeb et al., 2025).

To address these challenges, artificial intelligence (AI) and large language models (LLMs) have emerged as highly promising clinical decision-support tools capable of providing rapid, reproducible, and standardized DDI screening. LLMs can instantly analyze extensive medication lists and cross-reference pharmacological interaction databases to flag contraindications, thereby streamlining preoperative workflows and reducing the cognitive burden on practitioners (Tayeb et al., 2025). In a prospective simulation-based study comparing the performance of multiple LLMs (including ChatGPT, Gemini, and DeepSeek) with experienced oral surgeons across 500 standardized cases, AI demonstrated a remarkable speed advantage (Tayeb et al., 2025). The fastest AI model processed complex DDI cases in a median time of 3.6 seconds, a stark contrast to the 225 seconds required for expert human adjudication and consensus (Tayeb et al., 2025).

Despite their impressive computational speed, current LLMs exhibit distinct safety trade-offs in their diagnostic performance, particularly concerning the balance between sensitivity and specificity. The aforementioned comparative study revealed that ChatGPT-5 achieved the highest sensitivity at 98.0%, effectively detecting nearly all clinically actionable interactions; however, this was accompanied by a lower specificity of 56.7%, resulting in frequent false-positive warnings that could lead to "alert fatigue" and workflow disruption (Tayeb et al., 2025). Conversely, DeepSeek-Chat achieved the highest exact agreement with the surgeon consensus (50.6%) and demonstrated perfect

specificity (100%), but it exhibited a critically low sensitivity of 18%, missing 82% of interactions that required clinical action (Tayeb et al., 2025). This polarity highlights a fundamental limitation: highly sensitive models risk overcalling interactions, while precision-oriented models risk missing potentially severe adverse reactions (Tayeb et al., 2025).

The integration of AI for DDI detection in dental therapy remains an evolving field that necessitates rigorous validation, transparency, and domain-specific fine-tuning. While general-purpose LLMs show immense potential in identifying interactions, they are currently insufficient to serve as standalone references due to hallucination risks and diagnostic variability. Consequently, AI-based DDI screeners must be embedded within supervised, collaborative "human-in-the-loop" frameworks (Tayeb et al., 2025). In these workflows, licensed oral surgeons and prescribing physicians can utilize AI as an auxiliary tool to rapidly retrieve data, but they must critically review all generated alerts and retain ultimate clinical responsibility for final treatment and prescribing decisions (Tayeb et al., 2025).

5. Comparative Performance of AI Chatbots in Dental Clinical Scenarios

The evaluation of large language models (LLMs) across various dental clinical scenarios reveals significant variations in their diagnostic accuracy, reasoning capabilities, and overall clinical utility. Recent studies utilizing complex, case-based questions from specialized assessments, such as the Turkish Dental Specialty Examination (DUS), demonstrate that advanced AI models possess a robust capacity for diagnostic reasoning (Haberal, 2026). In restorative dentistry scenarios, for instance, Gemini Advanced and ChatGPT-4o Plus achieved exceptionally high accuracy rates of 96.28% and 93.62%, respectively, significantly outperforming other models like DeepSeek (Haberal, 2026). Conversely, comparative evaluations in authentic, case-based dental diagnostics have shown that models such as Claude and Manus can achieve diagnostic accuracies of up to 92.3%, numerically outperforming ChatGPT while demonstrating higher intra-model consistency across repeated measurements (Madfa et al., 2026). These findings suggest that while general-purpose LLMs are highly capable, their reliability in complex dental diagnostics is heavily influenced by their inherent algorithmic architecture and domain-specific fine-tuning (Madfa et al., 2026).

In highly specialized fields such as endodontics and dental traumatology, the performance of AI chatbots is notably context-dependent and varies by task.

When evaluating the management of iatrogenic events in endodontics, ChatGPT-5 demonstrated significantly higher accuracy and completeness scores compared to Gemini 2.5 Flash, Grok 4, and Claude Sonnet-4 (Taşyürek, Adıgüzel, & Ortaç, 2025). However, in separate comparative evaluations concerning patient inquiries about endodontic treatments, DeepSeek-V3.1 exhibited superior accuracy over both ChatGPT-5 and Gemini 2.5 Flash (Taşyürek et al., 2025). In the context of traumatic dental injuries (TDIs) assessed according to the International Association of Dental Traumatology (IADT) guidelines, AI models exhibited complementary strengths. While Google Gemini achieved a remarkable 100% diagnostic accuracy in simulated trauma cases, ChatGPT-4o demonstrated superior proficiency in treatment planning, particularly in accurately determining antibiotic indications (97% accuracy) and ChatGPT-5 Plus excelled in predicting appropriate splinting durations (Küçük Keleş & Arslan, 2025). Table 1 shows how different LLMs responded correctly to different types of trauma.

Chatbots	Correct responses				
	Fractures $n=45$ (%)	Luxa-tions $n=60$ (%)	Avul-sions $n=60$ (%)	Total $n=165$ (%)	p-value
ChatGPT4o/Free	36 (80)	49 (81.7)	47 (78.3)	132 (80)	0.901
ChatGPT-5-Plus	42 (93.3)	46 (76.7)	52 (86.7)	140 (84.8)	0.055
DeepSeek	28 (62.2)	43 (71.7)	46 (76.7)	117 (70.9)	0.269
Google Gemini	34 (75.6)	37 (61.7)	38 (63.3)	109 (66.1)	0.283

P-values were calculated using the pearson chi-square

Table 2: Comparison of artificial intelligence programs' responses inmdifferent types of trauma cases (Küçük Keleş & Arslan, 2025).

The integration of multimodal data, such as intraoral photographs, further differentiates the clinical capabilities of these AI systems. In evaluating first-aid recommendations for TDIs using clinical images alongside the ToothSOS application, ChatGPT-4o generally provided more accurate responses for complicated crown fractures, whereas Gemini Advanced aligned more closely with clinical guidelines for critical injuries like dental avulsions (Çege et al., 2025). Beyond diagnostic precision, the practical implementation of these tools

is heavily influenced by their operational metrics, such as response time and text readability. DeepSeek, for instance, frequently produces highly complete and relatively readable responses but suffers from significantly longer response latencies, which may hinder its utility in time-critical emergency dental scenarios (Sezer & Aydoğdu, 2025). Furthermore, most AI-generated clinical texts exhibit high linguistic complexity that demands advanced literacy, thereby limiting their direct applicability for layperson patient education without professional mediation (Sezer & Aydoğdu, 2025).

Ultimately, the comparative performance of AI chatbots in dental clinical scenarios underscores that no single model is universally superior across all medical parameters. For example, while ChatGPT versions may excel in specific treatment planning tasks, Gemini 2.5 Flash has demonstrated statistically superior performance in pharmacological scenarios involving rational prescribing and drug interaction management, achieving the highest global quality and completeness scores (Coşkun & Erten Tayşi, 2025). The observed variability in diagnostic precision, logical reasoning, and adherence to evidence-based guidelines highlights the necessity of a rigorous and context-specific approach to AI integration. Consequently, these advanced AI systems should be utilized strictly as adjunctive clinical decision-support tools within a supervised framework, ensuring that the definitive diagnostic and therapeutic responsibilities remain entirely with the human practitioner (Küçük Keleş & Arslan, 2025).

6. Ethical, Legal, and Privacy Implications of AI Integration

The integration of artificial intelligence (AI) and large language models (LLMs) into clinical dentistry introduces profound ethical and legal challenges, primarily concerning patient safety, diagnostic accountability, and the inherent limitations of the technology itself. A critical concern in the deployment of AI is the phenomenon of "hallucinations," wherein the model generates highly plausible but entirely fabricated, inaccurate, or unsafe medical information (Abavisani et al., 2024; Antonie et al., 2025). Furthermore, AI algorithms, which function as opaque "black boxes," lack true clinical reasoning, situational awareness, and the ability to interpret complex, nuanced patient contexts in the way a human clinician does (Giacobbe et al., 2025; Rana et al., 2026). This opacity makes it challenging to trace the rationale behind AI-generated recommendations, complicating issues of liability and informed consent (Rana et al., 2026). Over-reliance on AI tools poses a substantial risk of reducing critical thinking and vigilance among practitioners, especially if the outputs are inherently assumed to be accurate, which is particularly dangerous in high-stakes

scenarios like pediatric care or emergency trauma management (Sezer & Aydoğdu, 2025).

Legal and ethical frameworks are struggling to keep pace with the rapid advancement of AI technologies in healthcare. Currently, accountability in AI-assisted care remains firmly with the human clinician, as AI is considered an adjunct tool rather than a substitute for professional judgment (Rana et al., 2026). If an AI system provides incorrect advice that leads to patient harm, determining liability—whether it lies with the software developer, the healthcare institution, or the prescribing clinician—is a complex legal issue that remains largely unresolved (Antonie et al., 2025). Regulatory bodies, such as the FDA in the United States and the EU under the Medical Device Regulation (MDR), are developing frameworks to ensure the rigorous testing, certification, and post-market surveillance of AI systems (Antonie et al., 2025). These regulations increasingly emphasize the need for "explainable AI" and transparency in algorithmic processes, which are essential for maintaining clinical confidence and fulfilling the ethical obligation of beneficence and non-maleficence (Giacobbe et al., 2025; Rana et al., 2026).

Data privacy and the security of sensitive health information constitute another major ethical and legal frontier. LLMs require vast amounts of data for training and continuous refinement, raising significant concerns about the potential for data leakage and unauthorized secondary use of patient records (Abavisani et al., 2024). Healthcare providers utilizing AI tools must ensure strict compliance with established privacy regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the U.S. and the General Data Protection Regulation (GDPR) in Europe (Antonie et al., 2025). To mitigate these risks, developers are increasingly exploring privacy-preserving techniques, such as federated learning—where models are trained locally across multiple institutions without centralizing sensitive data—and differential privacy, which introduces controlled noise to prevent the re-identification of individual patients (Antonie et al., 2025; Rana et al., 2026). Ultimately, fostering trust between clinicians (trustors) and AI systems (trustees) requires a commitment to transparency, rigorous data governance, and the establishment of clear boundaries for automation within a supervised, human-in-the-loop clinical workflow (Giacobbe et al., 2025).

7. Future Perspectives: Dental Education, Prompt Engineering, and the Human-in-the-Loop Paradigm

The future of dental education is poised for a significant transformation with the integration of large language models (LLMs). Recent evidence demonstrates that advanced artificial intelligence (AI) chatbots possess substantial potential as supplementary educational tools capable of facilitating formative self-assessment, board examination preparation, and interactive clinical reasoning exercises (Haberal, 2026). In comparative evaluations involving complex diagnostic scenarios, models such as ChatGPT-4o and Claude have demonstrated diagnostic accuracies and consistency comparable to those of highly experienced final-year dental students (Kurt, 2025; Madfa et al., 2026). By providing rapid, evidence-based feedback and simulating case-based learning, these platforms can assist in managing cognitive load and supporting retrieval practice for students navigating intricate disciplines like endodontics and oral pathology (Haberal, 2026). However, because AI systems can occasionally generate inaccurate information or fabricated references, they must be implemented with strict faculty oversight to foster critical thinking rather than overreliance (Haberal, 2026).

As the use of LLMs expands in both academic and clinical dentistry, the emerging science of "prompt engineering" will become a critical competency for practitioners. The diagnostic precision and reliability of AI outputs are highly sensitive to the structure, formatting, and specificity of the user's input query (Coşkun & Erten Tayşi, 2025). Studies indicate that utilizing appropriate, systematically structured prompts can significantly improve a model's consistency and alignment with evidence-based clinical guidelines (Wang et al., 2024). In the context of dental trauma, for example, it is recommended that prompts comprehensively incorporate case-specific variables—such as the affected tooth, elapsed time since injury, clinical examination findings, and pulp sensitivity tests—to holistically represent the clinical scenario and enhance the AI's diagnostic accuracy (Küçük Keleş & Arslan, 2025). Consequently, future dental curricula must train students not only in clinical skills but also in effectively communicating with AI algorithms to extract safe and reliable medical information.

Despite their impressive computational speed and vast theoretical knowledge, AI models inherently lack true clinical judgment, empathy, and situational awareness (Antonie et al., 2025). This limitation unequivocally necessitates the adoption of a "human-in-the-loop" paradigm across all clinical applications. While AI can rapidly analyze complex pharmacological data to detect drug-drug interactions or suggest empirical antibiotic therapies, the risk of "hallucinations"

and algorithmic variability means these systems cannot operate autonomously (Tayeb et al., 2025). Models like ChatGPT, Gemini, and DeepSeek exhibit distinct safety trade-offs, sometimes sacrificing specificity for sensitivity, which can lead to alert fatigue or missed critical contraindications (Tayeb et al., 2025). Therefore, AI must be strictly positioned as an adjunctive decision-support tool, ensuring that the definitive diagnostic, therapeutic, and prescriptive responsibilities invariably remain with the human clinician (Di Pumpo et al., 2025; Küçük Keleş & Arslan, 2025).

Looking ahead, the trajectory of AI in dentistry will increasingly depend on the development of specialized, domain-specific models and the advancement of multimodal capabilities. Future iterations of LLMs are expected to seamlessly integrate textual patient histories with heterogeneous data streams, such as radiographic images, intraoral photographs, and 3D cone-beam computed tomography (CBCT) scans, thereby enabling more comprehensive diagnostic reasoning (Madfa et al., 2026; Sezer & Aydoğdu, 2025). To ensure safe and effective deployment, these next-generation tools must undergo rigorous clinical validation, adhere strictly to continuously updated professional guidelines, and be developed through co-design methodologies involving both clinical specialists and AI developers (Sezer & Aydoğdu, 2025). Ultimately, harmonizing technological innovation with ethical, human-centered care will dictate the successful evolution of AI in global dental practice.

DISCUSSION

The integration of large language models (LLMs) into clinical dentistry represents a paradigm shift in addressing complex pharmacological challenges, particularly the global crisis of antimicrobial resistance (AMR) and medication safety. As demonstrated by recent literature, advanced artificial intelligence (AI) chatbots hold substantial potential to serve as dynamic clinical decision support systems (CDSS), aiding practitioners in rational antibiotic prescribing and the real-time retrieval of medical data (Antonie et al., 2025). By cross-referencing patient-specific variables with established clinical guidelines, models such as ChatGPT and Gemini can significantly optimize antimicrobial stewardship programs and reduce human prescribing errors stemming from time constraints or cognitive overload (De Vito et al., 2025). The sheer computational speed of these tools allows for the rapid analysis of extensive medication lists, streamlining preoperative workflows and elevating the standard of patient care (Tayeb et al., 2025).

Despite these promising capabilities, comparative evaluations of various LLMs reveal significant heterogeneity in their clinical reliability and diagnostic performance. Different models exhibit distinct architectural biases and operational trade-offs; for instance, while ChatGPT-5 demonstrates exceptionally high sensitivity in detecting clinically actionable drug-drug interactions (DDIs), it often suffers from low specificity, leading to false-positive alerts that could induce "alert fatigue" among clinicians (Tayeb et al., 2025). Conversely, models like DeepSeek may provide highly specific answers but miss critical contraindications due to low sensitivity, and Claude often demonstrates strong intra-model consistency in case-based diagnostics but can struggle with citation accuracy (Madfa et al., 2026; Tayeb et al., 2025). This variability underscores that no single AI model is universally proficient across all dental and pharmacological domains, necessitating a tailored approach to LLM selection based on the specific clinical task.

A primary barrier to the autonomous clinical deployment of LLMs is the persistent risk of "hallucinations"—the generation of highly coherent, plausible, but factually incorrect or unsafe medical information (Abavisani et al., 2024). Because these models operate on probabilistic text generation predicting the next logical word rather than utilizing authentic clinical reasoning or situational awareness, their outputs can mislead both clinicians and patients, particularly in complex scenarios involving resistant pathogens or rare adverse interactions (Antonie et al., 2025). The opaque, "black box" nature of deep neural networks further complicates this issue, as clinicians cannot easily verify the algorithmic logic or source data underlying a specific therapeutic recommendation, thereby raising significant ethical concerns regarding liability, transparency, and patient safety (Giacobbe et al., 2025; Rana et al., 2026).

Beyond clinical decision support, the application of AI chatbots in direct patient education—such as providing first-aid instructions for traumatic dental injuries or post-operative care—presents unique challenges related to health literacy. Studies evaluating the readability of AI-generated responses consistently indicate that texts produced by models like ChatGPT, Gemini, and Claude often require a college-level reading ability, significantly exceeding the 6th to 8th-grade reading levels recommended for public health materials (Guven et al., 2025; Sezer & Aydoğdu, 2025). While these models can exhibit high levels of empathy and semantic richness, the linguistic complexity of their outputs limits their accessibility and understandability for the general population (Di Pumpo et al., 2025). Consequently, AI should not be endorsed as a standalone resource for patient self-management without professional mediation and simplification.

Ultimately, the discourse surrounding AI in dental pharmacology and practice converges on the absolute imperative of maintaining a "human-in-the-loop" framework. Artificial intelligence must be strictly positioned as an adjunctive, auxiliary tool designed to augment human expertise rather than replace the diagnostic and prescriptive authority of the dental professional (Antonie et al., 2025; Tayeb et al., 2025). Future advancements must focus on mitigating algorithmic biases, enhancing the explainability of AI outputs, and developing specialized, domain-specific models trained on rigorously validated dental literature (Rana et al., 2026). Furthermore, as "prompt engineering" emerges as a vital clinical competency to maximize model reliability, continuous education and strict regulatory frameworks will be essential to ensure that the integration of AI into dentistry remains ethical, secure, and unequivocally patient-centered (Wang et al., 2024).

CONCLUSION

The integration of artificial intelligence (AI) and large language models (LLMs) into dental pharmacology marks a critical evolution in combating the global antimicrobial resistance (AMR) crisis. These advanced systems offer substantial promise as accessible clinical decision support systems (CDSS), capable of rapidly synthesizing patient data to optimize antimicrobial stewardship, promote rational drug use, and instantly detect severe drug-drug interactions.

However, current AI models are not yet ready for autonomous clinical deployment. Comparative studies reveal inconsistencies in diagnostic performance across different platforms, alongside inherent trade-offs between sensitivity and specificity. Furthermore, the persistent risk of algorithmic "hallucinations"—generating plausible but factually incorrect medical advice—poses a significant threat to patient safety if left unchecked. The opaque, "black box" nature of deep learning algorithms also raises profound ethical and legal challenges regarding transparency, data privacy, and professional liability.

Therefore, the successful integration of AI in dentistry necessitates a strict "human-in-the-loop" framework. AI must serve solely as an adjunctive tool to augment human cognitive capacity and cross-reference medical literature, while the definitive diagnostic, therapeutic, and ethical responsibilities remain unequivocally with the human clinician.

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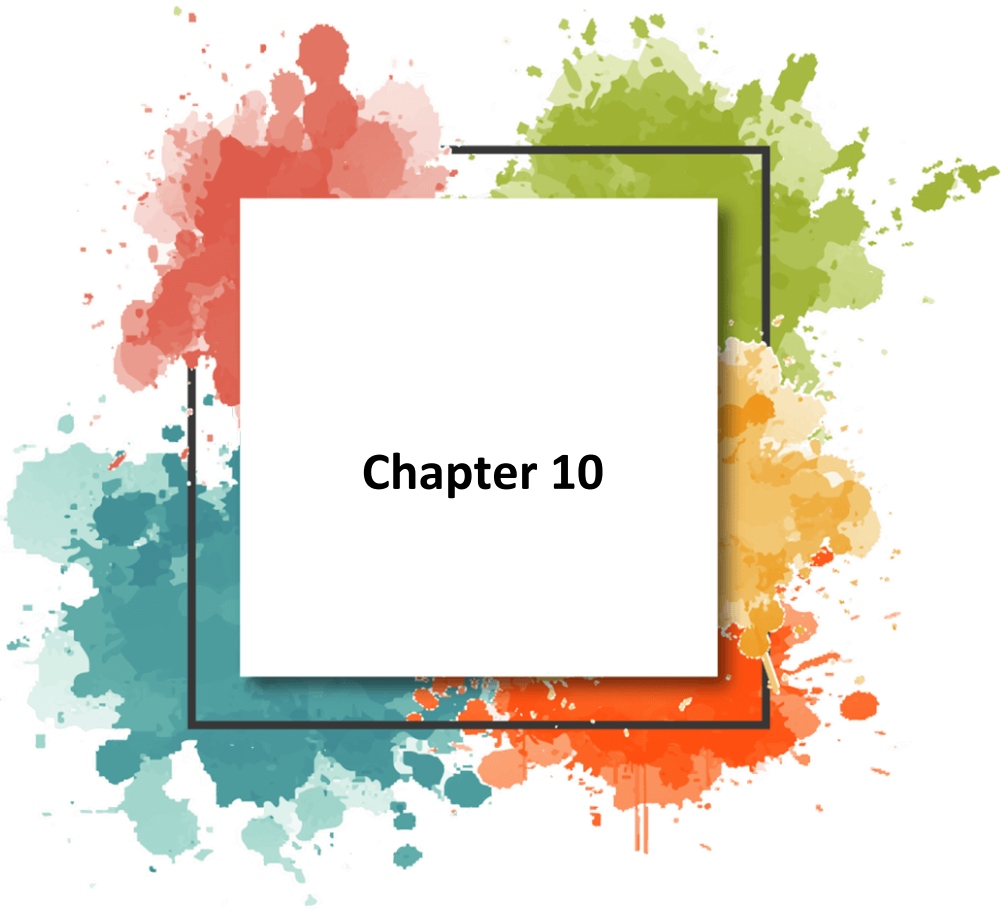
Chapter 10

Lymphedema: A General Aspect

Ozgur Altinbas[1]

INTRODUCTION

Lymphedema is a progressive and chronic disease caused by collection of protein rich fluid in interstitial spaces between the tissues. Lymphatic system, part of the circulatory system is involved in clearance of excessive fluids from interstitial spaces and moving back to circulation. However, the inability of lymph vessels to remove excessive fluid from these spaces leads to retention of protein rich fluid leads to lymphedema formation (1).

Lymphedema can be classified by primary and secondary in terms of etiology. Primary lymphedema is the congenital form and is related to abnormal development of lymph vessels. Milroy disease, lymphedema praecox, and lymphedema tarda can be given as examples of primary lymphedema (2).

Milroy disease can be at birth or develop later in life and characterized by usually bilateral and asymmetric swelling of the lower limbs that present as pedal edema (3).

Lymphedema praecox which is also called Meige's disease, is typically onset in puberty and often presents unilateral swelling of the lower extremity (4).

Lymphedema tarda is an inherited disease which occurs after 35 years of life. It is thought to be trigged by trauma or prior infection (5).

Secondary lymphedema is the acquired one and it occurs due to several medical conditions such as surgery, trauma, infection, tumor and post venous thrombosis (2).

Cellulitis and skin infections that may cause chronic inflammation which is associated with lymphedema. Impaired function on lymphangiogenesis and lymphatic functions of T cells play a role in formation of lymphedema (6). In addition, a parasite called Wuchereria bancrofti directly invades the lymph nodes and causes Filariasis, a type of lymphedema (7).

Despite not clearly identification of the underlying mechanism, lymphedema after trauma was reported in the literature. Besides, posttraumatic lymphedema is associated with the severity of the trauma and patients with multiple trauma and fractures are prone to lymphedema (8).

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Post venous thrombosis is one of the undesired complications of deep vein thrombosis and related to the development of lymphedema by pressure onto the lymphatic vessels (9).

Lymphedema after surgery, also called "secondary lymphedema," is an undesired clinical outcome due to the intra-surgical injuries to the lymphatic vessels or removal of lymph nodes resulting in inadequate lymph transport and lymphatic insufficiency. Protein-rich interstitial fluid accumulates due to dysregulation in lymphatic transport which leads to fibrosis and swelling. Similarly, incidence of lymphedema 10% to 37% after axillary dissection was reported in the literature (10-11).

Cancer-related lymphedema may occur in any time course of the cancer such as diagnosis, treatment, follow-up…etc. Common causes include obstruction of lymphatic vessels and lymphatic nodes by tumor compression, infiltration of lymphatic vessels with tumoral cells and destruction of lymphatic structures due to chemotherapy or radiotherapy (12).

Approximately 140-250 million people are living with lymphedema worldwide. Its incidence in United States is 1/1000. Two studies revealed that the prevalence of lymphedema in two European countries differed from 1.33 to 1.44 per 1000 people. Nearly 99% percent of the lymphedema develops due to secondary reasons. The most common cause of lymphedema in developed countries is radiotherapy and/or lymphadenectomy. Parasitic infection is usually responsible for lymphedema in Third World nations (13).

Primary lymphedema is a rare condition with the incidence of 1/100,000 child. It may develop during adulthood in 1/5 of the patients. Lower extremities are mostly affected by the rate of 91.7%. In addition, rates of the unilateral and bilateral lymphedema are the same (14-15).

Patients with a body mass index (BMI) more than 50 have a risk for developing obesity induced lymphedema while individuals with a BMI over 60 are likely to have the disease (13).

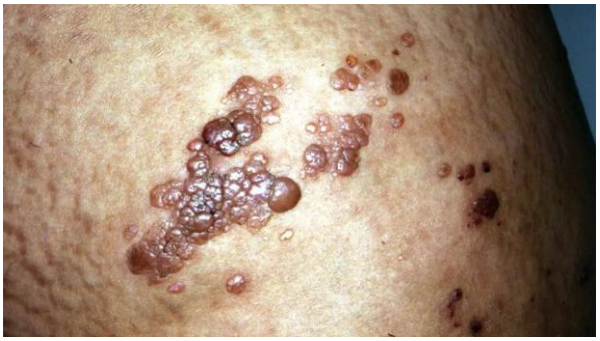
HISTORY, SIGNS, SYMPTOMS AND PHYSICAL EXAMINATION

Diagnosis of lymphedema is usually made by anamnesis and physical examination due to lack of any unique sign or defined criteria. Family history distinguishes primary and secondary lymphedema. Detailed anamnesis including distribution of the disease, duration, signs of infection, travel abroad, cancer and prior surgery especially with lymph node dissection, liver and cardiac functions and trauma is critical. Although diuretic therapy and elevation of the limbs cannot resolve the swelling, initial swelling attacks were reported to be transient in 1/3

of the patients. Similarly at the beginning of the disease skin is soft and pitting is clear then becomes hardening skin and non-visible edema (1).

Tissue swelling especially of extremity, tissue fibrosis with skin thickening and scaly structure, lymphangioma (figure 1) and lymphorrhagia are the main signs and symptoms of the lymphedema.

Figure 1. Lymphangioma

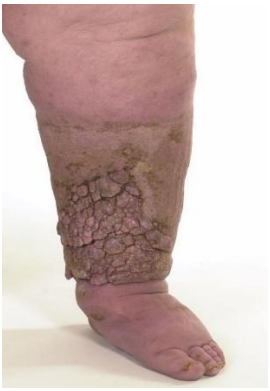


https://www.healthline.com/health/lymphangioma

Physical examination reveals these signs and symptoms in addition to warm/hot, dark and hyperkeratotic skin (16).

Distended and dilated lymphatic vessels may surrounded by fibrotic tissue caused to cobblestone appearance of the skin which is called “papillomatosis” (figure 2) (17).

Figure 2. Papillomatosis



https://rightdecisions.scot.nhs.uk/tam-treatments-and-medicines-nhs-highland/adult-therapeutic-guidelines/lymphoedema/lymphoedema-home-page-diagnosis-treatment-and-care-guidelines/?searchTerm=lymphoedema

Positive Stemmer's sign: inability to pinch a skin fold at the root of the second toe is one of the physical examination findings and called Stemmer’s sign (17).

More than 20% difference in the terms of size of the extremities considered as severe lymphedema (18).

LABORATORY

Laboratory studies such as blood, urine or tissue work are not required to generate diagnosis for lymphedema. These tests are used to define underlying causes in the situation of unclear etiology. Similarly, tumor markers should be checked if suspected (19).

Currently there is no lymphedema specific laboratory tool.

NON-INVASIVE MEASUREMENT AND IMAGING TECHNIQUES

Noninvasive measurements and imaging techniques are not used for diagnosis but also used for confirmation, checking the involvement extension and identifying the therapeutic approach.

Differences in circumference over 2 cm and/or volume over 200 ml between the extremities can be performed to diagnostic confirmation. Water displacement, perometry or tape can be used for volume measurement (20, 21).

Bioimpedence spectroscopy and tonometry are accepted as noninvasive measurements for lymphedema. Bioimpedence spectroscopy depends on electrical conductance changes of extracellular fluid thus extracellular fluid compartment can be assessed (22).

Tonometry measures the tissue resistance of upper part of the deep fascia by using a compression force. Thus, amount of fibrotic induration can be monitored. Tonometry indicates subjective measure of fibre which gives opportunity to make comparison with the extremities. It can also be used to evaluate the impact of treatment (23).

Nuclear lymphoscintigraphy is considered as the gold standard imaging modality for lymphatic evaluation. Though protocols differ between facilities, lymphoscintigraphy provides a noninvasive assessment of lymphatic function. Technique depends on injection of nuclear tracers bound to protein intradermally and taken up by the lymphatic system. Serial images are taken over 2–4 hours to assess the transition within the lymphatic system. SPECT imaging is used for follow up the uptake in lymph nodes and gross visualization of lymphatic channels. Decreased uptake or delayed transit in the affected limb suggests lymphatic dysfunction (24).

Lymphedema can be evaluated in terms of diagnosis and surgical planning by MRI. Adipose tissue, anatomy of lymphatic structure and edema can be imaged by MR. In addition, MRI reveals excess adipose deposition in the extremities which suggests the diagnosis of lymphedema. Although distinguishing of normal adipose tissue from adipose deposition cannot be detected by MRI, it can detect excessive adipose tissue between the extremities. Various imaging patterns related to lymphedema, such as dermal thickening, epifascial fluid and honeycombing can also be evaluated by MRI (25).

Superficial and deep lymphatic vasculature can be assessed directly by novel technique called MR lymphangiography. Intradermal or subcutaneous contrast agents are injected and then enter lymphatic capillaries. Delayed opacification of the lymphatic nodes and vessels indicates lymphatic dysfunction. Congestion or pooling of contrast can also be detected. Dilated and/or tortuous lymphatic vessels can also be revealed by MR lymphangiography which may persist in some forms of primary lymphedema. It has advantages over lymphoscintigraphy in terms of detailed anatomy of lymphatic vasculature (26, 27).

Near-infrared fluorescence lymphography utilizing indo cyanine green (ICG) is a novel technique and started to use for diagnosis and surgical preparation of lymphedema. Intradermally injected indo cyanine green binds to lipoproteins and albumin. Superficial lymphatic vessels take it up and visualization of the superficial lymphatic vessels can be achieved. It provides earlier detection of the disease which gives opportunity for initial treatment in the preclinical stage (28).

TREATMENT

In general, there are two treatment modalities for lymphedema; non-surgical and surgical.

Complete decongestive therapy is gold standard and the most popular therapy of lymphedema and includes two stages. Education, skin care, multilayer nonelastic compression bandaging, manual lymphatic drainage and exercises are involved in stage 1. Regular skin care and continuation of exercise with compression garments and self-massage are involved in stage 2. Compression bandaging should be performed at night in the second stage in suitable patients (29).

A specialized massage technique called manual lymphatic drainage is usually used in the management of lymphedema. Lymphatic system is induced by rhythmic and gentle movements. This result in increased drainage of excess lymphatic fluid and finally, swelling in affected areas reduces. A trained therapists often performs the manual lymphatic drainage with specific hand movements. Aim is to redirect lymph fluid towards functional lymphatic ways (30).

Compression therapy is one of the main treatment modalities for lymphedema. Application of external pressure improves lymphatic function and reduces swelling. Bandages, garments and pneumatic compression apparatus are used on the affected body part or limb to provide external pressure. Improvement in lymphatic drainage leads to fluid accumulation which results in size reduction of the extremity with compression therapy (31).

Exercise is important in the management of lymphedema. It promotes lymphatic flow by improving pump function of muscles. However, it is an important point that exercise programs should be arranged for individual capabilities and carefully monitored to prevent injury and the exacerbation of symptoms (32).

Skin care has a crurical importance in patients with lymphedema. Skin drying and cracking can be achieved by low Ph moisturizers to prevent entry of the microorganisms into the body (33).

Low level laser therapy is a nonionizing light-based treatment for lymphedema. It provides lymphatic vessel restoration and prevents tissue stiffness by reducing fibrosis. Pain relief also provided by this technique according to various studies (34).

Removal of lymphedematous tissue is a surgical technique called direct excision. There are various procedures defined as a subset of this technique. According to Charles procedure all subcutaneous tissue is removed and it includes skin grafting. Using buried dermal flaps is involved in another technique. Vacuum-assisted closure therapy and full-thickness skin grafting are another methods of direct excision (1).

Volume reduction was shown in affected limb by using liposuction method in the literature. It is suitable for both acquired and congenital lymphedema and lipedema. This technique provides long term reduction in extremity size. Patient should continue to compression therapy for the risk of regression. Its efficacy was proven both in lower and upper extremities however it is more effective in upper limbs. In addition liposuction has a positive effects on quality of life and skin blood flow (35-37).

Vascularized lymph node transfer (VLNT) is a novel surgical technique in which transfer of functional lymph nodes performed with microanastomosis to vascular structures in the recipient bed to provide their blood supply, to restore physiological lymphatic flow to an extremity in which the native lymph nodes has been removed. Risk of donor site lymphedema, patient acceptable of scar location and known risk of an intra-abdominal surgery play a role in the choice of technique. Heterotopic or orthotopic lymph node transfer can be performed depending on localization of pitting edema and acceptance of scar locations (38).

Lymphaticovenous anastomosis (LVA) has become one of the effective surgical methods for the lymphedema treatment. Supermicrosurgical technique is used to anastomosis the superficial lymphatic vessels with the surrounding small veins. Thus, the lymphaticevenous return pathway is reestablished and with the bypassing the dysfunctional area, lymph flow is diverted into the systemic circulation. Diameter of the lymphatic vessels used in anastomosis is 0.3 to 0.6 mm. Therefore, it is required experienced physicians in supermicrosurgery and simple anastomotic techniques are essential for successful LVA results (39).

Besides, there are ongoing studies and developed agents for future use in the systemic and topical treatment of lymphedema. These include; vascular endothelial growth factor, hepatocyte growth factor, 9-cis retinoic acid, adipose derived stem cells, ketoprofen, bestatin, fingolimod, neutralizing antibodies, doxycycline, anti-transforming growth factor-beta 1, hADSCs and VEGF-C hydrogel, recombinant human fibroblast growth factor 2, tacrolimus, pirfenidone and captopril (40).

CONCLUSION

Lymphedema is a challenging disease that affects the quality of life and general health condition. Various types and etiologic factors were described in the literature. Currently there is no effective treatment method however there are several methods for reducing the symptoms. Ongoing studies including systemic and local agents exist in the literature.

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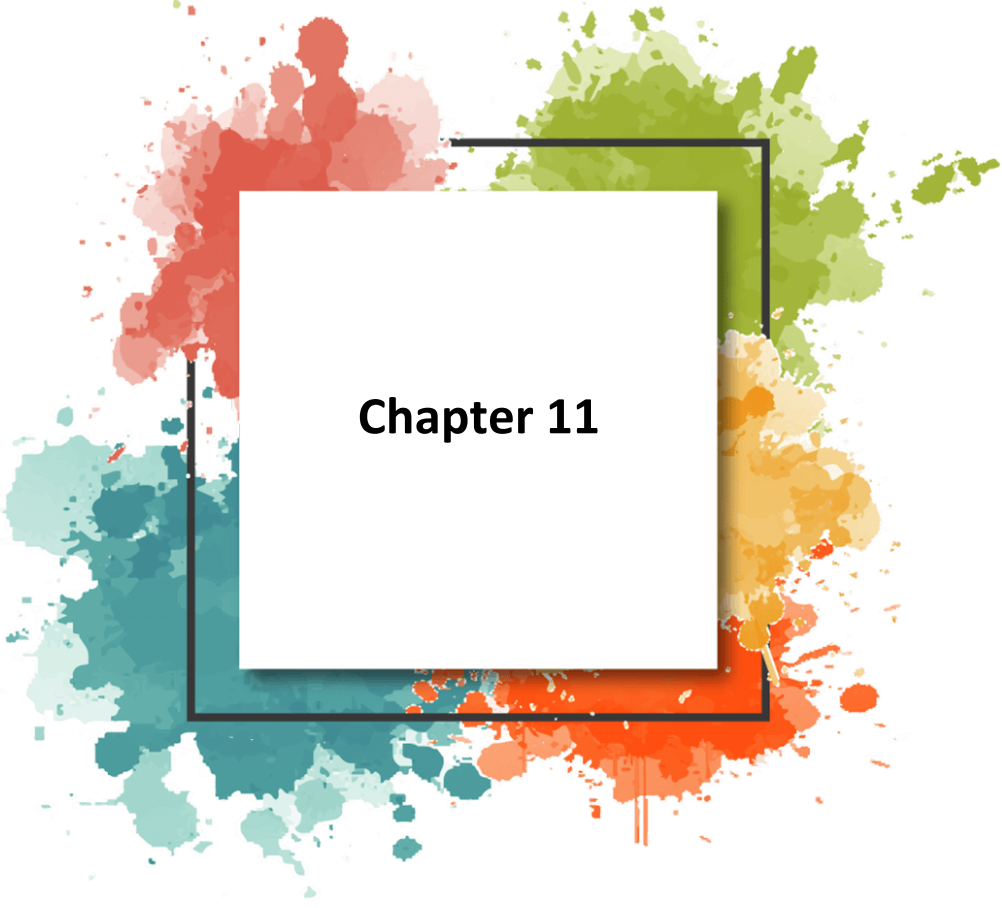
Chapter 11

Pelvic Congestion Syndrome

Ozgur Altinbas[1]

INTRODUCTION

Pelvic Congestion Syndrome (PCS) is a chronic pain with dilated and incompetent pelvic veins, predominantly involving the ovarian and parametrial venous plexuses, in the setting of chronic pelvic pain (CPP). Pathophysiologically, PCS is attributed to venous reflux secondary to valvular insufficiency, resulting in venous hypertension, stasis, and subsequent varicosity formation. The ensuing venous dilation and congestion can elicit significant nociceptive signaling, accounting for approximately 30% of CPP cases. Although pelvic venous dilation and associated pain are considered cardinal clinical features of PCS, imaging studies have demonstrated that pelvic varicosities may also be present in asymptomatic individuals, thereby complicating diagnostic accuracy and highlighting the need for comprehensive clinical and radiologic correlation (1, 2).

The exact etiological factors of PCS are unknown and it is accepted as a multifactorial disease. Insufficiency of venous valves, hormonal status, accompanied medical conditions like peripheral artery disease and venous obstructions may responsible for congestion of pelvic veins. Stasis with dilated veins result in the release of pain-inducing substances which are associated with pain occurred in PCS (3). Besides, heavy lifting or prolonged standing, previous pelvic surgery, pregnancy, obesity, estrogen therapy, and phlebitis are risk factors for PCS (4).

Multiparous and premenopausal females are mainly affected and there is no menopausal woman reported in the literature. Worldwide prevalence of PCS reaches up to 43.4% in women between 18 to 50 years old and 15% in United States of America. 30% to 40% of the reason of the CPP cases are associated with PCS. Four of every 10 patients admitted to outpatient gynecologic visits are related to PCS (5). Knowledge about the effects of ethnicity and inheritance is unclear however, history of family pelvic pain is a risk factor (6).

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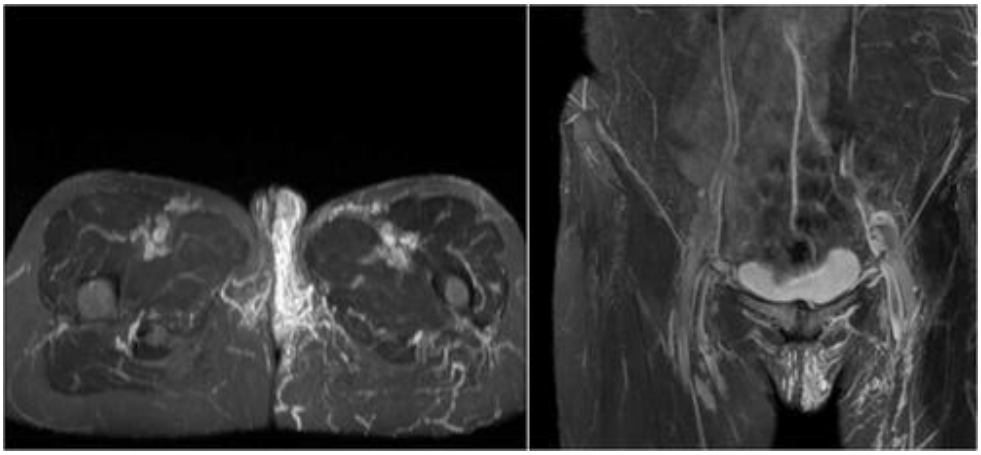
HISTORY, SIGNS, SYMPTOMS AND PHYSICAL EXAMINATION

PVS should be suspected in premenopausal woman with pelvic pain and venous varices. However, asymptomatic patients with these symptoms can be present. Dull, chronic, lower abdominal pain often with bladder irritability and dyspareunia are the most common manifestations. Pain persists more than 6 months and may be unilateral or bilateral. Mensturation, coitus, pregnancy and long standing can exacerbate the pain (7). Pain worsens at the end of the day (8). Pain reduces by lying down and may be in atypical sites such as legs, hips or lower back (6). Anxiety and depression are common comorbidities (9). 24% to 40% of the patients with PCS have vulvar varices (figure 1) (10).

Figure 1. Vulvar varices in patients with PCS in MRI



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PCS also leads to infertility and venous leg ulcers (11, 12).

If there is a pelvic pain, a detailed physical examination is helpful for diagnosis. Cervical motion tenderness and the ovarian and/or uterine tenderness, on bimanual examination in a patient with a complaint of chronic pelvic pain assist the diagnosis of PCS (1). In a study composed of fifty-seven females with pelvic pain, history of postcoital soreness in addition to sensitivity over the adnexa while physical examination indicated 94% sensitivity and 77% specifity in differentiating PCS from other pelvic originated pathologies (13).

Visible lower limb, perineal, gluteal or vulvovaginal varices can also be detected in physical examination (14).

LABORATORY

Currently, there are no laboratory tests or serum markers for the diagnosis of PCS. However, standard workup after anamnesis and physical examination should continue with Pap smear test, routine blood and urine tests due to wide range of differential diagnosis such as bowel pathology, endometriosis, neoplasm, fibromyalgia, fibrosis, orthopedic and/or neurologic pathologies, ovarian cyst, pelvic inflammatory disease, uterine prolapse and porphyria (15).

IMAGING

U/S of the pelvic region is typically the initial imaging choice for patients with suspected pelvic congestion syndrome (PCS). While both transvaginal and transabdominal ways can be employed, the transvaginal way with Doppler U/S is usually favored due to its superior imaging of the pelvic vein system and its ability to provide a dynamic assessment of circulation through tortuous structured pelvic veins. U/S also allows imaging in the upright position or during the maneuver of Valsalva—conditions that enhance distension of veins and improve the detection of pelvic varices. Under typical conditions, the pelvic venous plexus seem as tubular, straight structures with less than 4 mm diameter. Patients with suspected PCS and pelvic varicosities, U/S generally reveals dilated veins less than 6 mm width, sluggish or retrograde circulation in the ovarian venous structures, and dilated arcuate veins interacting with bilateral pelvic varicosities through the myometrial frameworks (16, 17).

Venous imagination with 3D CT has proven to be an efficient preoperative tool for mapping varicose veins and for visualizing venous anomalies, such as those seen in May-Thurner syndrome. Optimal timing of the procedure is essential to adequately assess the renal, portal, and genital veins, while an isolated imaging phase should be done later to evaluate the iliocaval and pelvic venous systems. Pelvic varices appear as dilated, tortuous, contrast-enhanced tubular structures surrounding the uterus and ovaries and may extend into the broad ligament and pelvic sidewall. The paravaginal venous plexus may also be involved. An ovarian vein is accepted as inefficient if it demonstrates complete opacification throughout the arterial cycle of vascular CT imaging (18).

Pelvic MRI offers outstanding image characteristics and high detail imaging for the evaluation of anatomical structures and pelvic vasculature. In contrast to CT, MRI does not include ionizing radioactivity, making it a safer imaging modality for women of childbearing age. Its cross-sectional capabilities also facilitate the identification of other pathologies, such as endometriosis, tumors, GI disorders, uterine abnormalities and musculoskeletal conditions Both contrast-enhanced Magnetic Resonance Angiography (MRA) and MRA sequences without contrast demonstrate high sensitivity in detecting insufficiency of venous

system. Information about venous flow can be obtained through phase-contrast velocity mapping (PCVM) or Time-Resolved Imaging (TRI). Magnetic Resonance Venography (MRV) with TRI provides accurate assessment of flow direction in the ovarian veins, distinguishing between anterograde and retrograde flow (16, 19-21).

Transcatheter venography is typically performed when noninvasive imaging yields inconclusive results in individuals with suspected venous insufficiency of pelvis(PVI), or when interventional therapy is being considered. Diagnostic venographic criteria include ovarian vein dilation measuring ≥5 mm, retrograde reflux within the ovarian veins, uterine venous engorgement, and cross-pelvic venous filling with opacification of vulvovaginal or thigh varicosities. While these features are diagnostically supportive, not all must be present to justify treatment. Some researchers argue that strict reliance on vein diameter thresholds should not exclude patients from therapy. Clinical-radiologic correlation is crucial, as nearly half of the asymptomatic women may demonstrate ovarian vein dilation and reflux on CT imaging. Therefore, imaging alone is insufficient for definitive diagnosis of PVI (22-24).

Beard et al. proposed a venographic scoring system incorporating three parameters: the maximal diameter of the ovarian vein, the time to contrast washout, and the extent of venous congestion. Every parameter is marked on a scale of 1 to 3, with a total score ≥5 indicating significant pelvic venous congestion, yielding a sensitivity of 91% and specificity of 89%. Ovarian vein diameters of 1–4 mm are considered normal, 5–8 mm as moderately dilated, and >8 mm as severely dilated. Time to contrast clearance following transuterine injection is scored at 0, 20, or 40 seconds. Venous congestion is graded based on morphology: normal veins appear small, straight, and well-defined; moderate congestion is characterized by tortuous veins with variable calibers; and severe congestion is defined by markedly dilated, highly tortuous veins with pronounced caliber variability (25, 26).

TREATMENT

A different type of pharmacological agents has been used in the management of PCS, including danazol, gonadotropin-releasing hormone (GnRH) agonists, progestins, phlebotonics, NSAIDs and oral contraceptives. Additionally, goserelin, etonogestrel implants and medroxyprogesterone acetate (MPA) have demonstrated efficacy in relieving pain associated with the condition. Enhanced pain relief has been reported when MPA is used in conjunction with psychotherapy. Among these options, goserelin which is a GnRH agonist, has shown superior effectiveness in pain control compared to medroxyprogesterone acetate. Neverthless, due to its mechanism of action as a GnRH agonist, its use is generally limited to a maximum duration of one year (3, 27, 28).

Surgical intervention remains a feasible option for a specific subset of PCS patients who suffer from severe symptoms, are appropriate candidates for surgery, and do not respond to endovascular or medical treatments. Ovarian vein surgery can alleviate symptoms; however, it involves potential risks such as scarring, longer hospital stays and extended recovery times (13).

Rudqvist first described the extraperitoneal resection of the left ovarian vein for patients with PCS. This surgical approach has demonstrated symptomatic relief in approximately 2/3 of carefully selected individuals. Gargiulo et al. reported the most extensive series of laparoscopic transperitoneal ovarian vein ligations, involving 23 females followed for one year, with complete resolution of symptoms (29, 30).

This laparoscopic technique involves accessing the right ovarian vein through an incision in the posterior peritoneum below the mesentericoparietal fossa, and the left ovarian vein by medially reflecting the left colon and incising the posterior peritoneum covering the aorta below the inferior duodenal fold. Despite its effectiveness, this procedure is associated with higher surgical morbidity and potential complications, including deep venous thrombosis, retroperitoneal hematoma, paralytic ileus, and mechanical ileus secondary to intestinal adhesions. Additionally, prolonged hospital stay and recovery time are significant limiting factors. Therefore, surgery should be reserved for individuals with lifestyle-limiting signs that persist or recur despite treatment of embolus (31-33).

Interventional coil embolization of the ovarian veins is generally regarded as a safe and effective gold standard procedure for alleviating symptoms associated with venous congestion, and is applicable in selected cases of PCS (34). A study involving 473 patients who were performed coil embolization demonstrated symptomatic relief rates ranging from 82.1% to 100%. Reported complications were infrequent and typically mild, including localized hematoma at the cannulation site. Additionally, recurrence rates were found to be minimal (35). Besides, endovascular treatment of PCS by coil embolization, trans-catheter sclerotherapy or plugs is recommended by the Society of Vascular Surgery with a 2B level of evidence (36).

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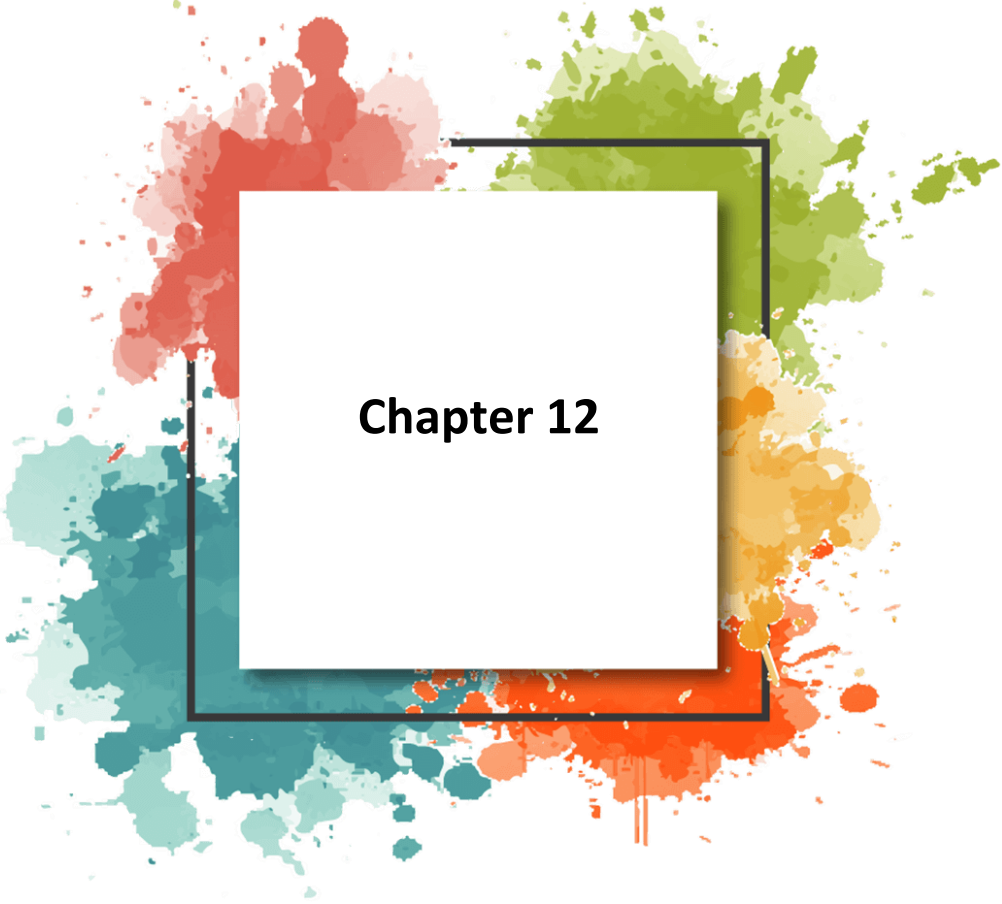
Chapter 12

Endoscopic Endonasal Resection of Pituicytomas: A Systematic Review

Duygu Dolen Burak[1] *& Cafer Ikbal Gulsever*[2] *& Aydin Aydoseli*[3]

Introduction

Pituicytomas are rare, low-grade glial neoplasms arising from pituicytes, specialized glial cells of the neurohypophysis and pituitary stalk. Along with granular cell tumors and spindle cell oncocytomas, they are classified as tumors of the posterior pituitary according to the 2021 World Health Organization (WHO) Classification of Tumors of the Central Nervous System. These lesions most commonly occur in the sellar and suprasellar regions, although extension into adjacent structures such as the third ventricle has been reported (Cheng et al., 2021; Zaki et al., 2023).

Clinically, pituicytomas usually present with symptoms related to mass effect on surrounding neurovascular and endocrine structures. The most common manifestations include headache, visual disturbances, and varying degrees of hypopituitarism. Less frequently, these tumors may be discovered incidentally or may coexist with pituitary neuroendocrine tumors (PitNETs), occasionally producing endocrine syndromes such as Cushing's disease or acromegaly. Radiologically, pituicytomas often resemble other sellar and suprasellar lesions, particularly pituitary adenomas, making preoperative diagnosis challenging (Li et al., 2019; Wee et al., 2023; Xiao et al., 2022).

Although histologically benign and typically slow-growing, pituicytomas present significant surgical challenges. These tumors are frequently hypervascular, which can complicate resection and increase the risk of intraoperative bleeding (Shim et al., 2017). Gross total resection is considered the primary treatment strategy; however, the optimal surgical approach remains a subject of discussion. In recent years, the endoscopic endonasal approach (EEA) has become increasingly favored for the management of sellar and suprasellar

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lesions due to its direct midline access, improved visualization, and reduced brain retraction (Shen et al., 2022; Yasuda et al., 2023).

Despite growing use of the EEA, the literature on pituicytomas remains limited to isolated case reports and small case series, and there is no comprehensive synthesis specifically focusing on outcomes following this surgical approach. As a result, data regarding clinical presentation, surgical outcomes, complications, and recurrence rates remain fragmented.

Therefore, the aim of this study is to systematically review the literature on pituicytomas treated using the endoscopic endonasal approach, summarizing patient characteristics, tumor features, surgical outcomes, and postoperative complications from reported cases. By consolidating the available evidence, this review seeks to provide a clearer understanding of the safety, effectiveness, and limitations of the EEA in the management of pituicytomas.

Methods

Literature Search Strategy

A systematic literature search was conducted to identify published cases of pituicytoma treated using the endoscopic endonasal approach (EEA). The search was performed in the PubMed database for articles published between January 2017 and September 2024, using the keyword "pituicytoma." The selected time frame was chosen to reflect contemporary diagnostic and surgical practices following the 2017 update of the World Health Organization (WHO) classification, which introduced clearer categorization of posterior pituitary tumors, including pituicytomas, spindle cell oncocytomas, and granular cell tumors. Restricting the search to this period helped reduce potential diagnostic ambiguity present in earlier literature.

Study Selection

The study selection process was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Titles and abstracts of the identified records were initially screened to determine potential eligibility. Studies that appeared relevant were subsequently assessed through full-text review. Duplicate publications and studies that did not meet the predefined criteria were excluded during this process. The overall selection process included stages of identification, screening, and eligibility assessment.

Eligibility Criteria

Studies were considered eligible if they reported histopathologically confirmed pituicytoma and described cases treated using the endoscopic endonasal surgical approach. In addition, studies were required to provide individual patient-level clinical or surgical data, including information regarding clinical presentation, tumor location, surgical outcomes, postoperative complications, or follow-up.

Articles were excluded if they were published before 2017, represented review articles without individual case data, were systematic reviews or meta-analyses, lacked sufficient clinical or surgical information, or described surgical approaches other than the endoscopic endonasal technique. When multiple reports described overlapping patient cohorts, the most complete or recent publication was included to avoid duplication.

Data Extraction and Analysis

Relevant clinical and surgical data were extracted from all eligible studies. Extracted variables included author and year of publication, patient age and sex, presenting symptoms, tumor location, extent of surgical resection, additional treatments, postoperative complications, and duration of follow-up. When studies reported multiple cases, each patient was analyzed individually.

The collected data were analyzed using descriptive statistical methods to summarize patient demographics, clinical presentation, tumor characteristics, and surgical outcomes. Continuous variables were reported as ranges or means when available, while categorical variables were summarized as frequencies and percentages. Due to the rarity of pituicytomas and the predominance of case reports and small case series in the literature, formal meta-analysis was not performed.

A PRISMA flow diagram was used to illustrate the study selection process. In total, 28 studies comprising 53 patients met the inclusion criteria and were included in the final analysis (Figure 1).

Results

Study Selection

The initial literature search identified 223 records in the PubMed database. After removing 112 studies published before 2017 and 8 records excluded for other reasons, 103 studies remained for title and abstract screening. Following screening, 14 studies were excluded, leaving 89 reports for full-text evaluation. Four articles could not be retrieved, and therefore, 85 full-text articles were assessed for eligibility. Among these, 57 studies were excluded for the following

reasons: 14 review articles without individual patient data; 2 duplicate publications; 5 systematic reviews or meta-analyses; 13 reports lacking surgical approach data; and 23 studies describing surgical approaches other than the endoscopic endonasal approach (EEA). Ultimately, 28 studies met the inclusion criteria, comprising 53 patients who underwent surgical treatment for pituicytoma using the EEA (Figure 1).

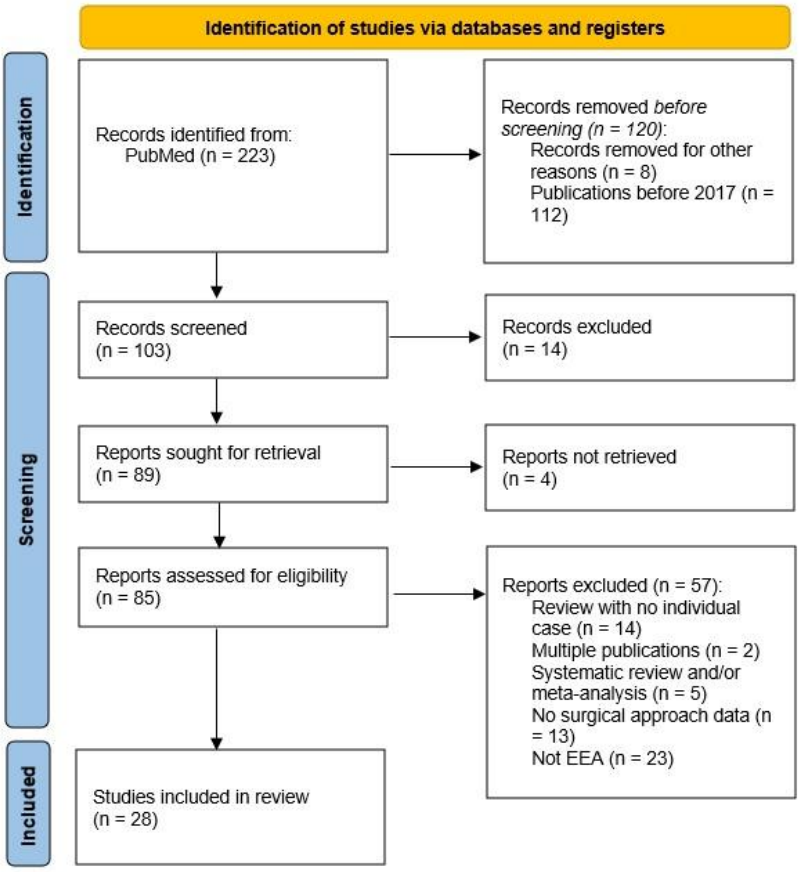

Figure 1. PRISMA flow diagram illustrating the study selection process. A total of 223 records were initially identified through the PubMed database. After removal of 120 records prior to screening (112 publications published before 2017 and 8 records excluded for other reasons), 103 records remained for title and abstract screening. Of these, 14 records were excluded, leaving 89 reports sought for retrieval, of which 4 reports were not retrieved. A total of 85 full-text articles were assessed for eligibility. Among these, 57 studies were excluded due to the following reasons: review articles without individual case data (n = 14), duplicate publications (n = 2), systematic reviews or meta-analyses (n = 5), absence of surgical approach data (n = 13), and studies not involving the endoscopic endonasal approach (n = 23). Ultimately, 28 studies met the inclusion criteria and were included in the final systematic review.

Patient Demographics

A total of 53 patients were included in the final analysis (Table 1). Patient age ranged from 19 to 77 years, with a mean age of approximately 47.8 years. Among these patients, 32 were female (60.4%), and 21 were male (39.6%), demonstrating a slight female predominance (Table 2).

Authors, Year	Age (yrs), Sex	Presentation	Location	Extent of Resection	Other Treatment	Complications	FU (mos)
Wozniak, 2023	47, M	Acromegaly signs (Co-existing GH-secreting adenoma)	Sellar	GTR	No	No	18
Zaki, 2023	24, M	headache, diplopia, dizziness, vision loss	Sellar	NA	NA	CSF leak, meningitis, hypopituitarism	NA
Rubino, 2023	41, F	headaches, vision loss (Co-existing ACTH-secreting adenoma)	Sellar	NA	NA	No	NA
Yasuda, 2023	46, F	Amenorrhea, left homonymous hemianopsia, HA	Sellar + Suprasellar	GTR	No	No	36
Yasuda, 2023	29, F	HA, vision loss	Sellar + Suprasellar	GTR	No	No	24
Yasuda, 2023	55, F	HA, vision loss	Sellar + Suprasellar	STR	No	No	12
Yasuda, 2023	58, F	Vision loss	Sellar + Suprasellar	GTR	No	No	18
Yasuda, 2023	63, F	Vision loss	Sellar + Suprasellar	GTR	No	Transient DI	24
Wee, 2023	71, M	facial swelling, easy bruising, proximal muscle weakness (Co-existing ACTH-secreting adenoma)	Sellar	GTR	No	No	18
Peng, 2023	51, F	blurred vision, diplopia, HA	Sellar	NA	NA	NA	NA
Shen, 2022	45, F	HA, vision loss	Sellar + Suprasellar	GTR	No	No	12
Xiao, 2022	51, F	HA, nausea (Co-existing ACTH-secreting adenoma)	Sellar	GTR	No	Hypopituitarism, Transient DI	17

Xiao, 2022	29, M	Central obesity, decreased libido (Co-existing ACTH-secreting adenoma)	Sellar	GTR	No	Hypopituitarism	8
Dottermusch, 2022	57, M	Vision loss	Sellar + Suprasellar	GTR	NA	NA	NA
Trifa, 2022	60, F	HA, vision loss	Sellar + Suprasellar	STR	No	No	60
Trifa, 2022	51, F	HA, vertigo	Sellar + Suprasellar	STR	Reoperated 2 yrs later	Hypopituitarism	72
Trifa, 2022	65, F	HA, vision loss	Sellar + Suprasellar	STR	Reoperated 2 months later, proton therapy after the second surgery	Hypopituitarism	36
Trifa, 2022	65, M	Vision loss	Sellar + Suprasellar	STR	Preop embolization and reoperated 8 months	No	6
Parkhi, 2021	28, M	HA, vomiting, vision loss	Sellar + Suprasellar	STR	NA	IVH, hypothalamic dysfunction	NA
Ha, 2021	33, M	HA	Sellar	STR	NA	Transient DI	NA
Ozisik, 2021	58, F	Vision loss	Sellar + Suprasellar	STR	Planning to reoperate	NA	12
Ozisik, 2021	70, F	Vision loss	Sellar + Suprasellar + Third ventricle	STR	Operated 3 times, preop embolization, fractionated RT	Hypopituitarism, DI	6
Cheng, 2021	47, F	HA	Sellar	GTR	No	Hypopituitarism, Intracranial infection	34
Cheng, 2021	63, M	Vision loss	Sellar + Suprasellar	STR	No	No	36
Cheng, 2021	54, F	HA	Sellar	GTR	No	No	16
Chen, 2021	53, M	Vision loss	Sellar + Suprasellar	STR	Reoperated 2 yrs later	No	30
Chen, 2021	47, M	Vision loss	Sellar + Suprasellar	STR	NA	No	18
Rumeh, 2020	47, F	moon face, buffalo hump, central obesity	Sellar	NA	NA	NA	NA
Cao, 2020	71,	Stroke	Sellar	NA	NA	No	4

	M	(incontinence, weakness in the right lower limb, and trouble speaking, right facial paralysis					
Chester, 2020	46, F	Vision loss	Sellar + Suprasellar	STR	Reoperated 5 mos later, fractionated RT	Hypopituitarism	24
Pont, 2020	29, F	Akromegali findings	Sellar	STR	SRS	No	12
Pont, 2020	33, F	Cushing findings	Sellar	NA	NA	NA	6
Gezer, 2019	37, M	Vision loss, Cushing findings	Sellar + Suprasellar	NA	NA	Hypopituitarism, DI	NA
Borg, 2020	56, M	Hypogonadotropic hypogonadism	Sellar + Suprasellar	GTR	NA	No	48
Borg, 2020	44, M	İnsidental	Sellar + Suprasellar	GTR	NA	No	24
Borg, 2020	56, M	HA	Sellar + Suprasellar + Third ventricle	STR	Septostomy, VPS	IVH	NA
Xiaoman, 2019	32, F	Cushing's findings	Sellar	STR	No	No	49
Guerrero-Perez, 2019	30, F	Vision loss	Sellar	GTR	No	Hypopituitarism, DI	NA
Guerrero-Perez, 2019	45, F	Amenorrhoea, galactorrhea	Sellar	STR	NA	Hypopituitarism	NA
Guerrero-Perez, 2019	37, F	Incidental	Sellar	STR	NA	No	NA
Guerrero-Perez, 2019	45, F	Amenorrhoea	Sellar + Suprasellar	GTR	NA	No	NA
Guerrero-Perez, 2019	70, F	Acral and facial changes	Sellar	GTR	NA	No	NA
Lefevre, 2018	51, F	Visual impairment, headaches, and galactorrhea	Sellar + Suprasellar	STR	SRS	NA	84
Lefevre, 2018	50, F	Visual impairment	Sellar + Suprasellar	STR	Reoperated 2 yrs later	NA	60
Lefevre, 2018	21, F	Visual impairment	Sellar + Suprasellar	GTR	No	NA	36
Lefevre, 2018	46, M	Visual impairment and headaches	Sellar + Suprasellar	GTR	No	NA	36
Lefevre, 2018	33, M	Visual impairment	Sellar + Suprasellar	GTR	No	NA	12
Lefevre, 2018	56, F	Hypercortisolism, Cushing's findings	Sellar	GTR	No	NA	3

Cossu, 2018	77, M	Hypogonadism	Sellar	GTR	No	Hypopituitarism	15
Feng, 2018	29, F	Obesity and menstrual disorder, Cushing's findings	Sellar	GTR	No	Transient DI	12
Feng, 2018	56, F	Acral enlargement, Acromegaly findings	Sellar	GTR	No	No	18
Shim, 2017	19, M	Visual impairment	Sellar + Suprasellar	STR	Reoperated with craniotomy, SRS	Hypopituitarism, DI	NA
Ying, 2017	54, M	Vision loss, sexual dysfunction	Sellar + Suprasellar	NA	NA	NA	NA

Table 1. Summary of reported cases of pituicytoma treated via the endoscopic endonasal approach included in the present systematic review. The table summarizes patient demographics, clinical presentation, tumor location, extent of surgical resection, additional treatments, postoperative complications, and follow-up duration for each reported case.

Abbreviations: yrs = years; M = male; F = female; HA = headache; FU = follow-up; GTR = gross total resection; STR = subtotal resection; NA = not available; GH = growth hormone; ACTH = adrenocorticotropic hormone; CSF = cerebrospinal fluid; DI = diabetes insipidus; IVH = intraventricular hemorrhage; RT = radiotherapy; SRS = stereotactic radiosurgery; VPS = ventriculoperitoneal shunt.

Clinical Presentation

The clinical manifestations were primarily related to mass effect on the optic apparatus and surrounding sellar structures. Visual disturbances were the most common presenting symptom and were reported in 29 patients (54.7%), including visual field deficits or progressive visual loss. Headache was reported in 16 patients (30.2%).

Endocrine abnormalities were present in several cases. Cushingoid features were reported in 9 patients (17%), while acromegaly-related symptoms were described in 2 patients (3.8%), most commonly in association with coexisting pituitary neuroendocrine tumors. Other endocrine presentations included amenorrhea (3 patients, 5.7%), hypogonadotropic hypogonadism (2 patients, 3.8%), and galactorrhea (1 patient, 1.9%). Additionally, incidental tumors were identified in 2 patients (3.8%) (Table 2).

Tumor Characteristics

Tumor location was predominantly within the sellar–suprasellar region. Specifically, 28 tumors (52.8%) involved both the sellar and suprasellar regions, whereas 23 tumors (43.4%) were confined to the sellar region. Extension into the third ventricle was observed in 2 patients (3.8%), while 1 case (1.9%) demonstrated an atypical or unspecified location (Table 2).

Characteristics	**Number of Patients (N)**
Sex (N = 53)	
Female	32 (60.4%)
Male	21 (39.6%)
Main Clinical Presentations (N = 53)	
Visual disturbance	29 (54.7%)
Headache	16 (30.2%)
Cushingoid symptoms	9 (17.0%)
Acromegaly symptoms	2 (3.8%)
Menstrual disturbance	5 (9.4%)
Sexual dysfunction	4 (7.5%)
Diplopia	2 (3.8%)
Incidental finding	2 (3.8%)
Tumor Location (N = 53)	
Sellar	23 (43.4%)
Sellar + suprasellar	28 (52.8%)
Sellar + suprasellar + third ventricle	2 (3.8%)
Extent of Resection (N = 45)	
Gross total resection (GTR)	24 (53.3%)
Subtotal resection (STR)	21 (46.7%)
Recurrence	
After GTR	0 / 24 (0%)

After STR	8 / 21 (38.1%)
Additional Treatments (N = 53)	
Reoperation	9 (16.9%)
Embolization	3 (5.7%)
Radiotherapy	5 (9.4%)
Postoperative Complications (N = 53)	
Hypopituitarism	13 (24.5%)
Diabetes insipidus	8 (15.1%)
Hypothalamic dysfunction	1 (1.9%)
Cerebrospinal fluid leak	1 (1.9%)
Infection	2 (3.8%)
Intraventricular hemorrhage	2 (3.8%)

Table 2. Summary of demographic characteristics, clinical presentation, tumor location, extent of resection, recurrence, additional treatments, and postoperative complications among patients with pituicytoma treated using the endoscopic endonasal approach. **Abbreviations:** GTR = gross total resection; STR = subtotal resection; CSF = cerebrospinal fluid.

Surgical Outcomes

All 53 patients underwent tumor resection using the endoscopic endonasal approach. Gross total resection (GTR) was achieved in 24 patients (45.3%), while subtotal resection (STR) was reported in 21 patients (39.6%). In 8 cases (15.1%), the extent of resection was not clearly reported in the original studies. Recurrence occurred exclusively among patients who underwent subtotal resection. A comparison of recurrence rates according to the extent of resection is presented in Table 3. Subtotal resection was most frequently attributed to tumor hypervascularity or adherence to critical surrounding neurovascular structures, which limited safe complete removal.

Surgical Outcome	Number of Patients	Recurrence	Recurrence Rate
Gross total resection (GTR)	24	0	0%
Subtotal resection (STR)	21	8	38.1%
Total	45	8	17.8%

Table 3. Comparison of tumor recurrence according to the extent of surgical resection in patients with pituicytoma treated via the endoscopic endonasal approach. Recurrence occurred exclusively in patients who underwent subtotal resection. **Abbreviations:** GTR = gross total resection; STR = subtotal resection.

Recurrence and Additional Treatments

Tumor recurrence requiring additional treatment occurred in 8 patients (15.1%). Importantly, all recurrences occurred in patients who initially underwent subtotal resection. Among the 21 patients who underwent STR, 8 patients (38.1%) experienced tumor recurrence requiring additional treatment. No recurrences were reported among patients who underwent gross total resection during the available follow-up period.

Additional treatments included repeat surgical resection in 9 patients (16.9%), stereotactic radiosurgery (SRS) in 3 patients (5.7%), and fractionated radiotherapy in 2 patients (3.8%). These treatments were primarily administered for residual or recurrent tumors following subtotal resection.

Postoperative Complications

Postoperative complications were reported in 22 patients (41.5%). The most frequently reported complication was hypopituitarism, occurring in 13 patients (24.5%). Diabetes insipidus was observed in 8 patients (15.1%), while transient diabetes insipidus was reported in 4 patients (7.5%).

Less common complications included intraventricular hemorrhage in 2 patients (3.8%), cerebrospinal fluid leakage in 1 patient (1.9%), intracranial infection in 1 patient (1.9%), meningitis in 1 patient (1.9%), and hypothalamic dysfunction in 1 patient (1.9%).

Follow-Up

Follow-up data were available for 37 patients (69.8%), with durations ranging from 3 to 84 months. The mean follow-up duration was approximately 25.8 months, and the median follow-up was 18 months. Most patients demonstrated

stable postoperative outcomes without evidence of tumor progression, particularly in cases where gross total resection was achieved. In contrast, patients who underwent subtotal resection showed a higher likelihood of tumor recurrence or progression, frequently requiring repeat surgical intervention or adjuvant radiotherapy.

Discussion

This systematic review provides a focused synthesis of the current literature regarding pituicytomas treated via the endoscopic endonasal approach (EEA). Analysis of the available cases highlights several key observations. First, pituicytomas most commonly present with visual disturbances and symptoms related to mass effect, reflecting their typical location within the sellar and suprasellar regions. Second, although the EEA provides effective surgical access to these lesions, gross total resection is frequently challenging, largely due to the hypervascular nature of the tumor and its proximity to critical neurovascular structures. Third, our analysis demonstrates that tumor recurrence occurs predominantly following subtotal resection, reinforcing the importance of achieving complete resection whenever it can be performed safely. Together, these findings underscore the surgical challenges associated with pituicytomas and emphasize the critical role of surgical strategy and extent of resection in determining long-term outcomes.

Pituicytomas are rare low-grade glial tumors that originate from pituicytes of the neurohypophysis and infundibulum. Since their recognition as a distinct pathological entity, the literature describing these tumors has remained limited primarily to case reports and small case series (Al-Salihi et al., 2023; Cossu et al., 2018; Ha et al., 2021; Yang et al., 2016). The clinical presentation of pituicytomas is largely dependent on tumor size and location. Most patients develop symptoms related to compression of surrounding structures, including visual field deficits due to optic chiasm compression, headache, and endocrine dysfunction caused by pituitary or stalk compression. These clinical manifestations frequently mimic those of pituitary adenomas, contributing to the difficulty of establishing a preoperative diagnosis (Dottermusch et al., 2022; Guerrero-Pérez et al., 2019; Lefevre et al., 2018).

Endocrine abnormalities associated with pituicytomas are usually secondary to pituitary stalk or gland compression rather than intrinsic hormone secretion. Hyperprolactinemia may occur due to interruption of the dopaminergic inhibition of prolactin secretion at the pituitary stalk (Al-Salihi et al., 2023). Similarly, hypopituitarism may develop as a result of direct compression of the pituitary gland. Rarely, pituicytomas have been reported in association with endocrine hyperfunction syndromes such as Cushing's disease or acromegaly. In many of these cases, the hypersecretory state is attributed to coexisting pituitary

adenomas, leading to so-called collision sellar lesions. These unusual presentations highlight the diagnostic complexity of sellar region tumors and underscore the importance of careful pathological examination (Al Rumeh et al., 2020; Gezer et al., 2019; Ozisik et al., 2021).

Radiologically, pituicytomas lack distinctive imaging characteristics that allow reliable differentiation from other sellar lesions. On MRI, these tumors typically appear as well-circumscribed solid masses with homogeneous contrast enhancement. They are commonly isointense on T1-weighted imaging and hyperintense on T2-weighted imaging. However, these imaging findings are largely nonspecific and often resemble those of pituitary adenomas or other suprasellar tumors such as meningiomas and craniopharyngiomas. The hypervascular nature of pituicytomas has also been described in several studies and may occasionally be suggested by strong contrast enhancement or vascular flow voids on imaging (Al Rumeh et al., 2020; Rubino et al., 2023; Shim et al., 2017).

Histopathological examination remains essential for definitive diagnosis. Pituicytomas are characterized by spindle-shaped cells arranged in fascicular or storiform patterns, with minimal cellular atypia and low mitotic activity. Immunohistochemical staining typically demonstrates strong nuclear expression of thyroid transcription factor-1 (TTF-1), a marker associated with tumors of posterior pituitary origin. Additional markers such as S-100 protein and vimentin are frequently positive, whereas expression of glial fibrillary acidic protein may be variable. These immunohistochemical features help distinguish pituicytomas from other sellar tumors, particularly pituitary adenomas (Chen et al., 2021; Cole et al., 2019; Feng et al., 2020; Marco del Pont et al., 2020; Parkhi et al., 2021; Woźniak et al., 2023; Yang et al., 2016).

Surgical resection is considered the primary treatment modality for pituicytomas. Traditionally, both transcranial and transsphenoidal approaches have been used depending on tumor size and location. In recent years, the endoscopic endonasal approach has become increasingly favored, as it provides direct access to the sellar region while minimizing brain retraction and improving visualization of critical neurovascular structures. Nevertheless, pituicytomas are frequently described as hypervascular tumors, which may result in significant intraoperative bleeding and make complete resection technically challenging (Borg et al., 2020; Chester et al., 2020; Peng & Wang, 2023).

The relationship between the extent of resection and tumor recurrence has been consistently emphasized in the literature. Previous systematic reviews and pooled analyses have identified subtotal resection as the most significant risk factor for recurrence. Large reviews of reported cases have demonstrated that recurrence occurs predominantly in patients with residual tumor following

surgery, whereas recurrence after gross total resection appears to be uncommon (Cheng et al., 2021; Guerrero-Pérez et al., 2019; Parkhi et al., 2021; Zaki et al., 2023). These observations are consistent with the findings of the present review, in which all cases of tumor recurrence occurred following subtotal resection. This further supports the concept that complete tumor removal, when safely achievable, provides the best chance for durable tumor control.

Adjuvant treatment strategies remain poorly defined due to the rarity of this tumor. Radiotherapy has been used in selected patients with residual or recurrent disease, although the long-term benefits of this approach remain uncertain. In most reported cases, patients with residual tumors are managed with close radiological surveillance, reserving additional interventions such as repeat surgery or stereotactic radiosurgery for cases demonstrating progressive growth (Cheng et al., 2021; Guerrero-Pérez et al., 2019; Lefevre et al., 2018; Zaki et al., 2023).

Taken together, the findings of this systematic review reinforce several important concepts regarding the management of pituicytomas. These tumors are rare but surgically challenging lesions, largely due to their hypervascularity and close relationship with critical structures of the sellar region. While the endoscopic endonasal approach provides effective access for tumor resection, the ability to achieve gross total resection remains a key determinant of long-term tumor control. Improved recognition of the clinical and radiological characteristics of these tumors may help guide surgical planning and postoperative management in future cases.

Clinical Implications

From a clinical perspective, the findings of this systematic review highlight several important considerations for neurosurgeons managing sellar region tumors. Given the nonspecific clinical and radiological presentation of pituicytomas, these tumors should be included in the differential diagnosis of highly vascular sellar or sellar–suprasellar lesions, particularly when intraoperative bleeding is disproportionate to that typically encountered in pituitary adenomas. Awareness of this entity is essential for surgical planning, as the hypervascular nature of these tumors may limit the feasibility of complete resection and increase the risk of postoperative endocrine complications. Furthermore, the strong association between subtotal resection and tumor recurrence emphasizes the importance of achieving gross total resection whenever it can be performed safely, while maintaining careful long-term radiological surveillance in cases with residual disease.

Limitations

Several limitations should be considered when interpreting the findings of this study. First, the available literature on pituicytomas consists predominantly of case reports and small retrospective case series, which introduces potential publication bias and heterogeneity in reported clinical variables. Second, the relatively small sample size limits the ability to perform robust statistical analyses. In addition, follow-up duration varied widely among the included studies, which may influence the reported recurrence rates. Finally, inconsistencies in the reporting of clinical presentation, surgical technique, and postoperative outcomes across studies further limit the ability to establish definitive treatment recommendations.

Conclusion

Pituicytomas are rare benign tumors of the posterior pituitary that often present with nonspecific clinical and radiological findings, frequently mimicking pituitary adenomas. This systematic review highlights that the endoscopic endonasal approach represents an effective surgical strategy for the management of these tumors, providing adequate access to sellar and suprasellar lesions. However, the hypervascular nature of pituicytomas may limit the feasibility of gross total resection, which remains the most important factor associated with long-term tumor control. Tumor recurrence appears to occur predominantly following subtotal resection, emphasizing the importance of achieving complete removal when safely possible. Given the rarity of these tumors and the limited available evidence, continued reporting of cases and larger multicenter studies are necessary to further clarify optimal management strategies and long-term outcomes.

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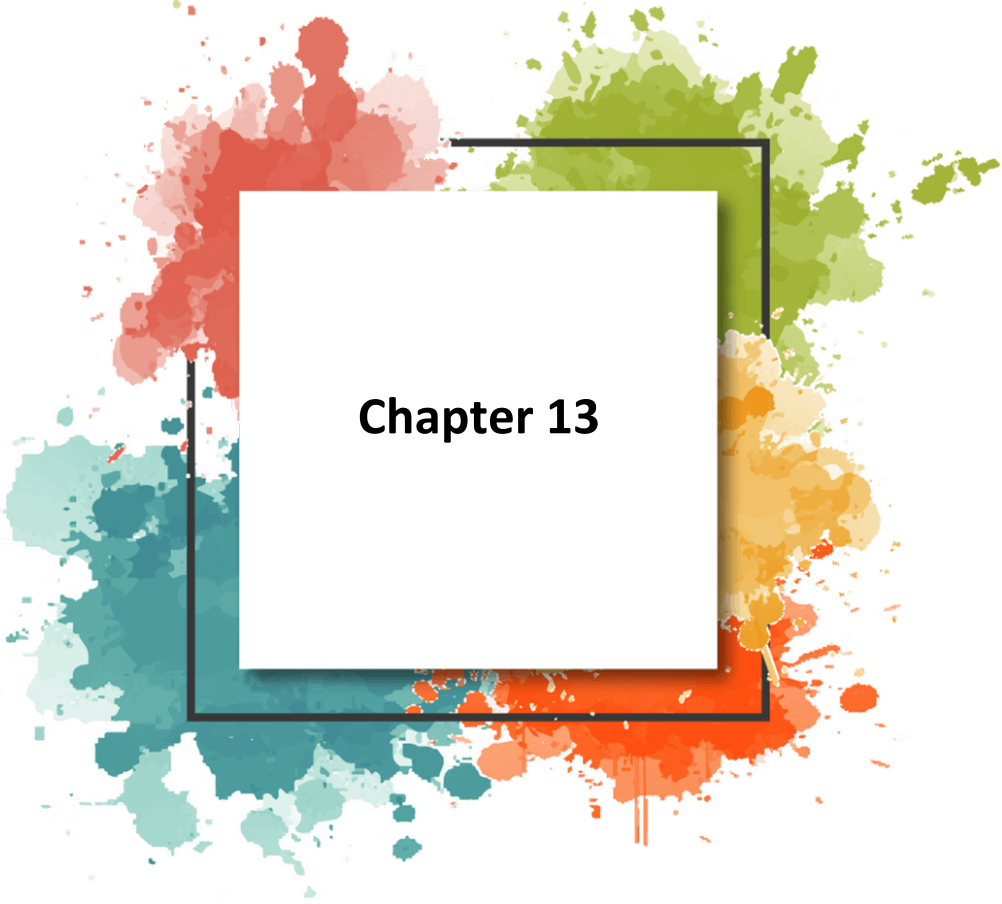
Chapter 13

Lifestyle Factors and Personalized Periodontal Care: A Critical Evidence-Based Review

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1-Introduction

Periodontal diseases are dysbiotic conditions characterized by inflammation associated with biofilm and its microbiota while regulated by immune responses (1). The periodontal diseases were covered beneath sub-headings in the report on the workshop that was published in 2017. In this context, gingivitis was referred to as the inflammation of the gingiva which is reversible while periodontitis referred to as inflammation with loss of periodontal tissues which was irreversible (2).

Based on the Global Burden of Disease study, periodontitis has come forward to be ranked as the seventh most common disease in the world. As per studies carried out over the past to the present time, periodontal disease has additionally been established to be a highly prevalent health issue in Türkiye, with over 50% patients reported to be suffering from it at a mild to moderate severity (3).

Various etiological and risk factors have long been linked to periodontal diseases, among which microbial dental plaque, occlusal trauma, systemic diseases or conditions, irregularities of saliva secretion, smoking, oral hygiene habits, and others may be cited. This multifactorial etiological pattern demands a holistic and multidimensional approach while considering options for periodontal disease therapy. This case underlines the fact that periodontitis is a preventable disease because different etiological risk factors may be altered before disease onset (4). Although microbial dental plaque is considered a primary initiating factor for onset and ultimate etiological development of periodontal diseases, recent research strongly supports that modifiable or predisposing risk factors affect immunological reactions of a human host. There is a significant revelation about the impact of individual-specific environmental and genetic elements regarding regulation of disease development upon the occlusions' progression (5) odifying predisposing lifestyle-related risk factors, like smoking activities related

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to tobacco usage, oral microbiota, food habits or nutrition patterns, usage of supplements, stress or psychological predisposition, and quality of sleep, is considered highly significant under recent research. These investigations have testified that modifying predisposing lifestyle-related risk factor values strongly exerts a beneficial impact on periodontal disease management (6).

Modifiable lifestyle factors related to risk behavior that negatively impact systemic health contribute to the production of reactive oxygen species and consequently to oxidative stress. Oxidative stress has previously been shown to contribute to increased systemic levels of inflammation through the augmentation of inflammatory biomarkers like cytokines, chemokines, C-reactive protein, interleukin-6, asymmetric dimethylarginine, and C3 values (7). Although recent studies (6),(8),(9) provide evidence supporting an association between periodontal diseases and lifestyle factors, the continuous evolution of lifestyle components—including dietary patterns and food consumption in modern societies necessitates that the existing literature remain equally dynamic and up to date. Thus, within this framework, this review aims to scrutinize recent literature available contemporary to what is perceived to characterize lifestyle behavior in modern society and to analyze and interpret the association of lifestyle factors to periodontal diseases from a dynamic and modern standpoint.

2-Methods

2.1-Protocol and Reporting Standards

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (10).

2.2-Search Strategy

A comprehensive and systematic literature search was performed in the following electronic databases: PubMed/MEDLINE and Web of Science. The search covered studies published between January 2019 and December 2025.

Search terms included both MeSH terms and free-text keywords, such as: “periodontal disease”, “periodontitis”, “lifestyle”, “smoking”, “diet”, “nutrition”, “stress”, “sleep”, and “obesity”. Boolean operators (AND/OR) were used to combine search terms.

Focused Research Question (PICO-based)

Research question:

"In adults with periodontitis, what is the effect of individualized lifestyle and behavior modification interventions compared with standard periodontal care on clinical periodontal outcomes and systemic risk profiles?"

PICO Framework for Your Systematic Review

Population (P): Adults (≥18 years) diagnosed with periodontitis based on contemporary classification criteria.

Intervention (I): Individualized lifestyle and behavior modification interventions, including one or more of the following:

- Smoking cessation interventions (including counseling and behavioral therapy)
- Nutritional counseling and dietary pattern modification
- Obesity and weight management strategies
- Sleep hygiene and sleep quality interventions
- Psychological stress management strategies

Comparison (C):

- Standard periodontal therapy without structured lifestyle or behavioral interventions
- Control groups receiving routine oral hygiene instructions only
- Alternative lifestyle interventions or non-individualized educational approaches

Outcomes (O):

Primary outcomes:

- Changes in clinical periodontal parameters (probing pocket depth, clinical attachment level, bleeding on probing, plaque index).

Secondary outcomes:

- Changes in lifestyle behaviors (smoking status, dietary habits, sleep quality).
- Changes in systemic inflammatory markers (e.g., CRP, HbA1c, BMI).

- Patient-reported outcomes (oral health–related quality of life, adherence to self-care).

2.3-Inclusion Criteria

Studies were eligible if they:

- Were published between 2019 and 2025
- Included human adult participants
- Were designed as randomized controlled trials (RCTs), cohort studies, case–control studies, or controlled clinical trials
- Examined the association between lifestyle-related factors and periodontal outcomes

2.4-Exclusion Criteria

Studies were excluded if they:

- Were **animal or in vitro studies**
- Had insufficient methodological quality
- Were case reports, editorials, conference abstracts without full text, or expert opinions

2.5-Study Selection

Two reviewers independently screened titles and abstracts, followed by full-text assessment of potentially eligible articles. Disagreements were resolved by consensus or by consultation with a third reviewer. The study selection process was documented using a PRISMA flow diagram.

2.6-Data Extraction

Data were independently extracted by two reviewers using a standardized form, including: study design, sample size, participant characteristics, exposure/intervention details, follow-up duration, and periodontal outcome measures.

2.8-Data Synthesis and Statistical Analysis

Owing to heterogeneity in study designs, outcome measures, and intervention characteristics, statistical pooling of data was not feasible. Therefore, the results were summarized descriptively using a narrative synthesis approach.

2.9-Subgroup and Sensitivity Analyses

The included studies were grouped according to characteristics such as the type of lifestyle factor investigated, the severity of baseline periodontal status, and the duration of follow-up, and their findings were compared using a synthesis approach.

Studies assessed as having a high risk of bias were interpreted with caution in the overall evaluation of the results.

Results

Section 1 – The Individualized Nature of Periodontal Disease and Personalized Risk

Profiles

Periodontal diseases are currently understood as complex inflammatory conditions initiated by microbial dental biofilms but largely regulated by the host immune response. The 2017 World Workshop classification emphasized disease complexity factors, highlighting that inter-individual variability in host responses significantly influences disease progression and treatment outcomes (11).

The concept of individualized periodontology has therefore become a cornerstone of contemporary periodontal practice. Parameters such as C-reactive protein (CRP) levels, HbA1c values, smoking status and daily cigarette consumption, the ratio of bone loss to age, and the compatibility of biofilm accumulation with oral hygiene status are now central to the grading of periodontitis. Growing evidence indicates that systemic conditions and modifiable risk factors significantly influence periodontal tissues (12),(13). These complexity factors represent integral components of an individual's lifestyle, supporting the concept that periodontal health outcomes can be positively influenced through healthier behavioral (5).

This section discusses how lifestyle factors such as tobacco use, nutrition, dietary patterns, obesity, psychological stress, and sleep behavior should be integrated into personalized models of care for the management of periodontal diseases.

Section 2 – Smoking/Tobacco Use and Periodontal Diseases

2.1. Study Selection

A systematic literature search was conducted in the PubMed database using the keywords *"smoking"* and *"periodontal diseases"*. The search was limited to articles published from **2019 onwards** and filtered to include only **clinical studies** and **randomized controlled trials.** This initial search yielded **69**

records. During the screening process, studies focusing primarily on **systemic diseases**, as well as **in vitro** and **animal studies,** were excluded. After applying these exclusion criteria, the number of potentially relevant articles decreased to **4 studies** that directly addressed lifestyle-related factors. To ensure the completeness of the evidence base, the results were compared and cross-checked with other electronic databases (Web of Science(WoS)). Through this additional search process, **one additional study** meeting the eligibility criteria was identified. Consequently, a total of **5 studies** were included in the final qualitative synthesis and reported in **Table 1**.

2.2. Types of Smoking and Periodontal Effects

Tobacco use emerged as one of the most consistently reported and biologically plausible lifestyle factors influencing periodontal health. Across the included studies, smoking was not merely evaluated as an isolated habit, but rather as a complex behavioural exposure embedded in broader lifestyle patterns that shape systemic inflammatory burden, immune competence and oral ecological balance (14).

Population-based evidence (15) showed a higher prevalence and severity of periodontitis among current smokers, with a clear dose–response relationship between cumulative exposure and periodontal tissue breakdown. Former smokers demonstrated an intermediate risk profile, suggesting that positive lifestyle modification through smoking cessation may partially restore periodontal health while leaving residual biological effects (16).

Longitudinal data indicated (17),(18) that heavy smoking impaired periodontal healing and reduced responsiveness to therapy, highlighting how lifestyle-driven behaviours influence not only disease progression but also treatment outcomes. Microbiome-focused studies demonstrated that smoking was associated with early and persistent dysbiosis, even before overt clinical destruction became apparent (17).

Emerging evidence on electronic cigarette use suggested that modern lifestyle transitions toward alternative nicotine delivery systems do not eliminate periodontal risk, as inflammatory and microbial profiles remained comparable to conventional smoking. Overall, tobacco use appears to be a central, dynamic and modifiable component of contemporary lifestyle, exerting multidimensional effects on periodontal health(19).

Section 3 – Nutrition, Diet and Periodontal Diseases

3.1. Study Selection

A systematic search of PubMed and Web of Science was performed using the keywords “nutrition” and “periodontal diseases.” After removal of reviews, in vitro studies, and studies involving participants under 18 years of age, 17 records published after 2019 were identified in PubMed. Fourteen full-text articles were assessed, and 7 studies that evaluated the effects of dietary modifications on periodontal clinical outcomes met the eligibility criteria.

The Web of Science search yielded 21 records; following the application of exclusion criteria, 9 studies remained. Cross-checking these with PubMed outputs resulted in 9 studies being deemed eligible and included in the final synthesis (Table 2).

3.2. Summary of Findings on Nutrition and Periodontal Health

When the included studies examining dietary patterns and periodontal health were evaluated collectively, higher adherence to anti-inflammatory dietary models such as the Mediterranean and DASH diets was associated with lower plaque accumulation and reduced bleeding on probing, whereas low-fiber dietary patterns were significantly associated with moderate to severe periodontitis (20). Consistent with these findings, experimental gingivitis studies demonstrated that participants practicing Bahá’í dry fasting or time-restricted eating exhibited smaller increases in bleeding on probing compared with controls, with increases in gingival crevicular fluid observed only in the control group (21). Similarly, periodontal therapy supported with probiotics—and most prominently when combined with a personalized anti-inflammatory Mediterranean-style diet—resulted (22) in greater improvements in probing depth and clinical attachment levels, with higher protein and fiber intake correlating with better periodontal outcomes and higher carbohydrate or sugar intake correlating with poorer results. In interventions (23) targeting dietary quality, reducing ultra-processed food consumption produced greater decreases in gingival inflammation compared with no dietary change, accompanied by improvements in overall dietary profiles independent of plaque accumulation.

Studies (24) assessing Mediterranean diet adherence also reported reductions in serum omega-6 fatty acids and improvements in the omega-6/omega-3 ratio, which were associated with decreases in bleeding on probing and gingival index scores. Comparisons between raw vegan and omnivorous individuals showed (25) lower probing depths, reduced bleeding on probing, and better oral hygiene indicators in the raw vegan group, although clinical attachment levels did not differ. Supplementation-based interventions (26) revealed that resveratrol reduced serum IL-6 levels without additional periodontal benefit; vitamin D

supplementation (27) enhanced reductions in gingival bleeding and produced slightly greater improvements in probing depth; and omega-3 fatty acid supplementation yielded significantly greater reductions in both probing depth and bleeding on probing at three- and six-month follow-up compared with nonsupplemented periodontal therapy (28). Collectively, the findings indicate that dietary patterns, nutritional quality, and specific nutrient-based adjuncts exert measurable effects on periodontal inflammation and clinical periodontal outcomes (Table 2).

3.4. Obesity and Its Association with Periodontal Disease

While evaluating nutritional factors, obesity—recognized as a major consequence of an unhealthy lifestyle—was also included in this review. A PubMed search using the keywords *"obesity"* and *"periodontal disease"* yielded five studies after applying all predefined inclusion criteria. Two of these studies did not assess periodontal parameters, and two others focused solely on hormonal markers. The only eligible study (29) compared obese individuals with and without metabolic syndrome and reported that the prevalence of Stage III/IV periodontitis was higher in the non-MetS group (42.9% vs. 36.8%). Moreover, bleeding on probing (BOP) was significantly more frequent among non-MetS patients.

A parallel search in Web of Science identified four relevant studies. Among these, Khemiss et al. (30) demonstrated that obese individuals exhibited significantly higher probing depth (PD) values and greater plaque index (PI) scores compared to normal-weight participants, while gingival index (GI) scores were similar between groups.

In the cross-sectional study conducted by Leonov et al. (31), a bidirectional relationship between obesity and periodontal disease was documented, alongside an analysis of six major periodontal pathogens. Notably, the prevalence of P. intermedia and T. forsythia was significantly higher in young obese individuals. Furthermore, in another study assessing Pentraxin-3 and Serum Amyloid A levels in gingival crevicular fluid, obese patients diagnosed with periodontitis displayed markedly elevated concentrations of both inflammatory biomarkers.

In a study involving obese Egyptian adults (32), the prevalence of periodontal disease was reported to be 100%, with significant increases in PD, CAL, RD, and PI observed in parallel with rising BMI. Finally, in a large-scale population study (33) utilizing the Community Periodontal Index (CPI) (n = 12,689 adults), individuals with increased waist circumference exhibited the highest risk of

periodontal disease, indicating that abdominal obesity may play a particularly influential role in periodontal pathology.

Section 4 – Psychosocial Stress, Depression, Anxiety, and Periodontal Disease

4.1. Study Selection

A systematic literature search was conducted in the PubMed database using the keywords *"psychosocial stress"* and *"periodontal diseases."* Eligibility criteria were applied through predefined database filters. Following title and abstract screening, studies that did not meet the inclusion criteria were excluded. As a result, four studies with accessible full texts were deemed eligible and included in the review.

An additional search using the same keywords was performed in the Web of Science (WoS) database, yielding seven further eligible studies. The characteristics and main findings of all studies included in the final qualitative synthesis are summarized in Table 3.

4.2. Summary Findings of Studies Assessing Psychosocial Stress and Periodontal Disease

The main characteristics and outcomes of studies evaluating psychosocial factors and periodontal disease are presented in Table 3. Across different populations and study designs, psychosocial stress, anxiety, and depression were consistently associated with unfavorable periodontal outcomes.

Interventional and longitudinal studies showed that higher psychosocial stress levels were related to poorer periodontal treatment responses. Romano et al. (34) demonstrated significantly higher full-mouth bleeding scores, probing pocket depth, and residual pathological pockets after non-surgical periodontal therapy in highly stressed patients. Similarly, a current research (35) reported persistently higher periodontal indices and inflammatory markers, along with a reduced clinical response, in stressed individuals compared with non-stressed controls.

Studies assessing biological stress markers reported significant associations between cortisol levels and periodontal parameters. Yarkac et al.(36) found higher probing depth, gingival inflammation, gingival crevicular fluid IL-6, and salivary cortisol levels in pregnant women despite similar perceived stress scores. Kolenko et al. (37) observed periodontal deterioration in approximately 55% of patients exposed to prolonged psychosocial stress, with elevated serum cortisol associated with disease progression. Dubar et al. (38) reported a positive

association between salivary cortisol levels and probing pocket depth, as well as differences in the persistence of red and orange complex periodontal pathogens.

Cross-sectional studies consistently reported higher prevalence and severity of periodontal disease among individuals with increased psychosocial stress. Tanveer et al. (39) identified psychological stress as an independent risk factor for periodontal disease in socially deprived women. Rashme et al. (40) showed significantly greater probing pocket depth, plaque index, and bleeding on probing in highly stressed young adults, with strong positive correlations between stress scores and periodontal parameters.

Anxiety and depression were also significantly associated with periodontal outcomes. Isola et al. (2023) reported significant correlations between anxiety, depression, stress, and both probing depth and clinical attachment loss. Xu et al. (41) demonstrated a strong dose–response relationship between anxiety severity and periodontitis prevalence, whereas perceived stress did not remain significant after multivariable adjustment.

Large population-based studies evaluating occupational and lifestyle-related psychosocial factors reported similar trends. Palle et al. (42) observed higher prevalence of periodontal pockets and attachment loss in individuals with moderate to high occupational stress, while Macrì et al. (43) found that higher perceived stress was associated with increased gingival bleeding and lower mindfulness scores were associated with a higher prevalence of periodontitis.

Section 5– Sleep Pattern and Periodontal Diseases

5.1. Study Selection

Using the predefined search strategy and inclusion criteria, a total of six studies were identified through the PubMed database. One study was excluded because periodontitis was assessed solely based on self-reported diagnosis and did not include objective, evidence-based periodontal parameters such as probing depth (PD), bleeding on probing (BOP), or clinical attachment loss (CAL) (9). Consequently, five studies retrieved from PubMed were deemed eligible for inclusion.

The search conducted in the Web of Science (WoS) database yielded thirty-one full-text studies. Studies investigating the reverse relationship—namely, the impact of periodontal disease on sleep quality—were excluded. Instead, studies examining the association between sleep quality, sleep-related factors, and periodontal disease outcomes were retained.

After combining the eligible studies from both databases, an additional six studies were included, resulting in a total of twelve studies being incorporated into the final analysis and summarized in Table 4.

5.3. Summary of Sleep Pattern and Periodontal Diseases

While evaluating sleep- and lifestyle-related factors, sleep duration, sleep quality, sleep hygiene, obstructive sleep apnea (OSA), insomnia, and perceived stress were included in this review. A comprehensive search identified twelve eligible studies conducted exclusively in adult populations after applying the predefined inclusion criteria.

Two large population-based studies assessed sleep duration. Iwasaki et al.(44) reported that individuals sleeping less than 5 hours per night had a significantly higher likelihood of severe periodontitis compared with those sleeping 7–7.9 hours, whereas Beydoun et al. (45) found no independent association between sleep duration and periodontitis or systemic inflammatory markers, including white blood cell counts.

Sleep hygiene and sleep routines were evaluated in a cross-sectional study among dental students (46), which demonstrated that poorer adherence to healthy sleeping routines was associated with increased gingival bleeding and worse oral health indicators.

Several studies focused on sleep quality and related patient-reported outcomes. A current research (47) showed that poor sleep quality and shorter sleep duration were significantly correlated with worse oral health–related quality of life and increased periodontitis severity. In contrast, Eroğlu et al. (48) did not observe significant associations between sleep quality, fatigue, or oral health–related quality of life and periodontitis stage or grade, although sleep duration was significantly shorter in individuals with Stage IV periodontitis.

OSA(obstructive sleep apnoea) was investigated in six studies using validated screening tools or polysomnography. A current research (49) reported a higher prevalence of moderate-to-severe periodontitis among individuals with high OSA risk. Similarly, Tranfić Duplančić et al. (50) demonstrated associations between increasing OSA severity and higher plaque levels, fewer remaining teeth, advanced periodontal stages, altered inflammatory cytokine profiles, and changes in subgingival microbial composition. In contrast, Kvarnvik et al. (51) did not detect significant differences in clinical periodontal parameters or salivary MMP-8 levels between OSA groups.

One randomized controlled clinical trial(52) evaluated melatonin supplementation in patients with chronic periodontitis and primary insomnia and reported significantly greater clinical attachment gain and probing depth reduction compared with placebo. Finally, Marruganti et al. (53) demonstrated that both moderate/high perceived stress and poor sleep quality were independently associated with Stage III/IV periodontitis.

Discussion

This systematic review evaluated the impact of lifestyle components on periodontal diseases within a dynamic framework, based on contemporary clinical evidence published between 2019 and 2025. The findings support the concept that periodontal diseases are not merely biofilm-induced infections, but conditions in which clinical severity, disease progression, and treatment response are significantly shaped by individual behavioral patterns, metabolic profiles, and psychosocial burden. These observations are consistent with the grading approach introduced in the 2017 classification, which places modifiable risk indicators such as smoking status and glycemic control at the center of periodontal risk assessment (2). Within this context, current evidence indicates that periodontal care should be conceptualized not only as standard mechanical therapy, but as an integrated approach combining conventional treatment with individualized lifestyle modification strategies.

Lifestyle should be regarded not as a static or one-time exposure, but as a dynamic construct that evolves over time in response to societal, behavioral, and environmental changes (5). Over the past decade, marked shifts have occurred in dietary patterns, sleep characteristics, and psychosocial stress burden, many of which were either absent or defined differently in earlier lifestyle research. Contemporary exposures such as intermittent fasting, sedentary work environments, digital screen–related sleep disruption, structured exercise programs, and heightened psychological stress necessitate a re-evaluation of periodontal risk profiles. Accordingly, restricting the present review to studies published within the last five to six years allowed lifestyle factors to be assessed as they are currently experienced by adult populations in real-world settings. This approach is particularly relevant in periodontology, given the chronic nature of periodontal diseases and their cumulative biological effects over time.

Although the reviewed lifestyle components—including tobacco use, dietary patterns, obesity, psychosocial stress and sleep were investigated across heterogeneous populations and study designs, a common finding emerged: individuals with comparable plaque levels may exhibit markedly different

inflammatory responses and degrees of tissue destruction. These differences appear largely attributable to modifiable behavioral factors and their associated systemic inflammatory and oxidative stress burden. In particular, accumulating evidence indicates that psychosocial stress and sleep disturbances not only influence oral hygiene behaviors and treatment adherence, but also modulate the periodontal phenotype through biological pathways involving cortisol secretion, cytokine profiles, and alterations in oral microbial ecology (37)-(39). These observations strongly support the paradigm of personalized periodontology.

Among all lifestyle factors examined, tobacco use demonstrated the most consistent and robust association with periodontal disease prevalence, severity, and treatment response.(15) Dose–response relationships consistently indicated greater baseline periodontal destruction and significantly impaired response to non-surgical periodontal therapy among heavy smokers.(16)-(17)-(18) Microbiome-focused studies further suggest that smoking is associated with subgingival dysbiosis even before overt clinical signs of disease become apparent.(19) Importantly, emerging evidence indicates that alternative nicotine delivery systems, such as electronic cigarettes, do not eliminate periodontal risk, underscoring the need to incorporate modern lifestyle transitions into periodontal risk modeling. Consequently, tobacco use should be regarded not as a passive anamnestic variable, but as an active therapeutic target, with structured smoking cessation counseling integrated into routine periodontal care.

Evidence regarding dietary patterns indicates that their periodontal impact is most pronounced at the level of gingival inflammation and short-term clinical parameters such as bleeding on probing, gingival index, and plaque index. High adherence to anti-inflammatory dietary models, including the Mediterranean and DASH diets, has been associated with lower bleeding and plaque scores(20) , while reductions in ultra-processed food consumption have been shown to decrease gingival inflammation independently of plaque accumulation (23). Similarly, dietary compositions enriched in fiber, complex carbohydrates, and unsaturated fatty acids—and reduced in refined carbohydrates and saturated fats—have been linked to more favorable inflammatory profiles (21) contrast, effects on periodontal tissue destruction markers such as probing depth and clinical attachment loss appear more heterogeneous, likely reflecting short follow-up durations, variability in baseline disease severity, and differences in dietary assessment methodologies.

Growing interest in intermittent fasting and fasting-based dietary approaches has generated preliminary but noteworthy evidence in the context of periodontal inflammation. Experimental gingivitis models have demonstrated that time-

restricted eating and daytime dry fasting may attenuate increases in gingival bleeding compared with control diets, despite comparable plaque accumulation, and may limit gingival crevicular fluid elevation (21). These findings suggest that dietary effects on periodontal tissues may extend beyond caloric intake or macronutrient composition, potentially involving metabolic timing and insulin–inflammation pathways. However, small sample sizes, short intervention periods, and limited randomization necessitate cautious interpretation regarding the role of intermittent fasting in periodontitis management.

With respect to nutritional supplementation, omega-3 fatty acids and vitamin D have shown adjunctive benefits in some studies, particularly in reducing gingival inflammation and supporting clinical improvement (24). Conversely, short-term antioxidant or isolated supplement interventions have demonstrated limited clinical effects on periodontal tissue destruction (25). These findings suggest that focusing on overall dietary patterns rather than single-nutrient supplementation may represent a more rational approach. Notably, probiotic supplementation combined with a personalized anti-inflammatory diet has been shown to enhance periodontal treatment outcomes more effectively than probiotics alone (22), reinforcing the importance of evaluating nutrition within the broader context of lifestyle behavior.

Obesity has emerged as a relevant risk factor for periodontal disease, although the directionality and underlying mechanisms remain incompletely defined (31). The frequent clustering of obesity with low physical activity, suboptimal diet quality, and adverse socioeconomic conditions increases the likelihood of residual confounding in observational studies. Nevertheless, stronger associations observed with abdominal obesity suggest a potential role for metabolic inflammation and adipokine-mediated immune modulation.

Psychosocial stress, anxiety, and depression have consistently been associated with adverse periodontal outcomes, particularly with impaired treatment response (35)·(39). Longitudinal and interventional studies demonstrate that higher stress levels are associated with increased residual pocketing, higher bleeding scores, and reduced clinical improvement following therapy. Associations between biological stress markers such as cortisol and periodontal parameters further indicate that stress may disrupt periodontal homeostasis not only through behavioral neglect, but also via neuroendocrine–immune pathways (37)·(38)·(41).

Findings related to sleep are heterogeneous, reflecting variability in sleep components and assessment methods. Population-based studies have reported associations between short sleep duration or poor sleep quality and severe

periodontitis, although these relationships are not consistent across all investigations. Poor sleep hygiene has been associated with increased gingival bleeding and worse oral health indicators, whereas reliance on self-reported measures and heterogeneous samples may partly explain inconsistent results. Studies focusing on obstructive sleep apnea and insomnia present a particularly complex picture: while some report associations between increasing OSA severity and advanced periodontal stages, others with longer follow-up or CPAP-treated cohorts fail to demonstrate significant periodontal differences(45). These discrepancies likely reflect differences in diagnostic methods, CPAP adherence, and limited evaluation of intermediary mechanisms such as hyposalivation, mouth breathing, and systemic inflammation. Conversely, randomized evidence indicating that melatonin supplementation improves periodontal healing in patients with insomnia suggests that sleep regulation may play a biologically meaningful role in periodontal inflammation control (52). Overall, more standardized definitions and mechanism-focused longitudinal studies are required to clarify these associations.

Overall, the findings of this review indicate that lifestyle modifications should not be viewed as substitutes for periodontal therapy, but as critical determinants of treatment response durability and long-term disease stability. Incorporating risk-based approaches, brief lifestyle screening tools into routine clinical practice, and structured multidisciplinary referrals when appropriate may enhance periodontal care outcomes. Nevertheless, the predominance of observational study designs, reliance on self-reported exposures, and heterogeneity in periodontal definitions represent important limitations. Future randomized controlled trials incorporating standardized periodontal outcomes, objective lifestyle measurements, and sufficient follow-up durations are required to more precisely define the role of lifestyle behaviors in periodontal health.

Conclusion

This systematic review provides contemporary evidence that lifestyle behaviors play a meaningful and modifiable role in the development, progression, and treatment response of periodontal diseases. Findings from studies published between 2019 and 2025 consistently demonstrate that periodontal disease expression cannot be explained solely by biofilm burden, but is substantially influenced by behavioral, metabolic, and psychosocial factors that interact with host inflammatory and immune responses. Tobacco use remains the most robust lifestyle-related determinant of periodontal disease severity and impaired treatment outcomes, while dietary quality, stress, and sleep behaviors appear to modulate periodontal inflammation and therapeutic response to varying degrees.

Emerging evidence further suggests that modern lifestyle patterns—including intermittent fasting, sedentary work environments, sleep disruption, and chronic psychosocial stress—may shape contemporary periodontal risk profiles in ways not captured by earlier literature.

Collectively, the available evidence supports a shift toward a more integrated and personalized periodontal care model in which lifestyle-related risk factors are systematically assessed and addressed alongside conventional mechanical therapy. Although causality cannot be definitively established due to the predominance of observational designs and heterogeneous methodologies, the consistency of associations across diverse populations underscores the clinical relevance of lifestyle modification in periodontal management. Future well-designed randomized controlled trials incorporating standardized periodontal outcomes, objective lifestyle measurements, and long-term follow-up are essential to further clarify causal pathways and to refine evidence-based lifestyle interventions in periodontology.

Clinical Relevance

From a clinical perspective, the findings of this review highlight the importance of incorporating lifestyle assessment into routine periodontal diagnosis, risk stratification, and treatment planning. Periodontal therapy should not be limited to biofilm control alone, but should adopt a risk-based approach that considers smoking status, dietary quality, psychosocial stress, sleep behavior, and metabolic health as integral components of patient management. Brief, structured lifestyle screening tools can be feasibly integrated into clinical practice to identify patients at increased risk of disease progression or suboptimal treatment response.

Importantly, lifestyle modification should be viewed as an adjunct—not an alternative—to evidence-based periodontal therapy. Interventions such as smoking cessation counseling, dietary guidance, stress management strategies, and referral for sleep-related disorders may enhance treatment outcomes and improve long-term disease stability. A multidisciplinary approach involving collaboration with primary care physicians, dietitians, psychologists and sleep specialists may be particularly beneficial for patients with complex risk profiles. Ultimately, integrating personalized lifestyle interventions into periodontal care has the potential to improve both oral and systemic health outcomes and aligns with the evolving paradigm of precision and preventive periodontology.

Conflicts of Interest

The authors declare no conflict of interest.

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Tables

Table 1. Summary of Studies Evaluating the Impact of Smoking as a Lifestyle Factor on Periodontal Health

Authors (Year) and Type of Study	Type of Smoking	Sample Size	Smoking Exposure Measure	Periodontal Status	Periodontal index and parameters used	Primary and Secondary Outcomes
Sim et al. (2023), Cross-sectional (KNHANES 2016–2018)	Current smokers / Former smokers / Never smokers	9,178 adults	Smoking status (current/ex/never), pack-years (lifetime exposure)	All periodontal diseases	Community Periodontal Index (CPI ≥ 3 as periodontal disease)	Current smokers had significantly higher odds of periodontitis vs non-smokers (Men OR=1.78, 95% CI=1.43–2.23; Women OR=1.44, 95% CI=1.04–1.99) Men with highest pack-years had OR=1.84 (95% CI=1.38–2.47) for periodontitis vs non-smokers
Huang et al. (2024), Cross-sectional (NHANES 2009–2014)	Current smokers / Former smokers / Never smokers	6,816 adults	Smoking (ever-smoked ≥100 cigarettes)	Periodontitis defined as ≥1 site with CAL ≥ 3 mm	CAL (clinical attachment level)	- Former smokers OR = 1.23 (95% CI 1.05–1.43) vs non-smokers - Current smokers OR = 1.66 (95% CI 1.34–2.05) vs non-smokers - Significant interaction between smoking and depression: in depressed participants smoking increased periodontitis risk by 129% (OR = 2.29; 95% CI 1.10–4.76) in younger age group
Leite et al. (2022), Prospective cohort	Conventional cigarette smoking	80 participants	Light smokers/quitters (n=46), moderate smokers (n=17),	Heavy smokers had higher baseline	Probing Depth (PD), CAL), number of sites with	Light and moderate smokers showed PD

			heavy smokers (n=17)	CAL and more severe sites	moderate/severe periodontitis	reduction (~0.6 mm) and CAL gain (~0.7 mm). Heavy smokers showed CAL loss (0.5 mm) and poor response to therapy. Clear dose–response relationship between smoking intensity and treatment outcomes.
Tamashiro et al. (2023), Longitudinal observational	Current conventional cigarette smokers vs never-smokers; former smokers excluded	17 subjects	Self-reported smoking status (smoker vs non-smoker);	All periodontal diseases	PD, CAL, Plaque Index(PI), 16S rRNA subgingival microbiome sequencing	Smokers showed dysbiotic subgingival microbiome even at shallow sites. Microbial shifts preceded clinical progression. Smokers had deeper PD, higher plaque scores and more progressive sites than non-smokers.
Thomas et al. (2022), Cross-sectional observational microbiome study	Electronic cigarette (e-cigarette) (vaping), conventional cigarette	84 participants	Self-reported current use (exclusive e-cig users vs cigarette smokers vs controls)		PD, CAL, Bleeding on Probing (BOP), PI, Gingival Index (GI); 16S rRNA gene sequencing	E-cigarette users showed a distinct dysbiotic periodontal microbiome and increased inflammatory parameters compared with never-smokers; periodontal destruction parameters were similar to conventional cigarette smokers.

*OR: odds ratio, CI: confidence interval (%95)

Table 2. Nutritional Risk Factors, Dietary Quality and Periodontal Disease

Authors (Year) and Types of Study	Type of Dietary Pattern	Sample Size	Dietary Assessment Method	Periodontal Disease Definition	Periodontal Parameters	Primary and Secondary Outcomes
Altun et al. (2021), Cross-sectional study	DASH adherence, Mediterranean adherence, Western/low-fiber patterns	6209 participants	Food Frequency Questionnaire (FFQ2); DASH and MEDAS scores; tercile categorization	Periodontitis	CAL, PD, BOP, PI, DMFT index	Higher adherence to DASH and Mediterranean diets was associated with significantly lower PI and BOP. Low-fiber consumption was significantly associated with moderate–severe periodontitis. The authors note that saturated fatty acids may increase oxidative stress and thereby promote periodontal damage.
Pappe et al. (2025), Three-arm randomized clinical trial (modified experimentally induced gingivitis)	Bahá'í dry fasting (BF) (daytime dry fast), Time-restricted eating (TRE, 16:8), Control regular diet (CG)	66 healthy adults (BF n=23; TRE n=22; CG n=21)	BP, body weight, HbA1c, CRP. Triglycerid.	Experimental gingivitis model: no oral hygiene in test sextant for 9 days (T1–T2), follow-up to day 19 (T3). BOP_s used as primary outcome.	Rustogi Plaque Index (RPI), Gingival Crevicular Fluid (GCF) , BOP	BF showed significantly smaller increases in BOP, GCF increased only in CG, Plaque indices (RPI/PCR) increased in all groups during the experimental gingivitis phase. BF had greater short-term metabolic improvements (weight, BP, CRP). Results are limited by non-randomised inclusion of BF cohort, potential selection/bias and centre effects; primary endpoint tested confirmatorily but many secondary comparisons were exploratory.
Çağıran Yılmaz & Çağıran Görgin (2023), Single-center pilot randomized controlled clinical trial (prospective)	1) Conventional periodontal therapy (control); 2) Conventional therapy + probiotics; 3) Conventional therapy + probiotics + personalized anti-inflammatory diet (Mediterranean-style)	120 women (randomized 40 / 40 / 40)	Probiotic capsules (10^9 CFU; primarily *Lactobacillus rhamnosus* & *Bifidobacterium animalis* subsp. *lactis*), 1 capsule/day × 6 weeks. Dietary intake: **three-day food records**; personalized Mediterranean-style plan for diet group.	Periodontitis	PD and CAL	After 6 weeks, significant PD and CAL reductions were observed in both probiotic groups, greatest in the diet+probiotic arm. In the diet+probiotic group Diet analysis: higher protein and fiber intake correlated with better PD/CAL outcomes ($p < 0.05$);higher carbohydrate/sugar intake correlated with worse PD/CAL ($p < 0.05$). Authors conclude that

						probiotics support periodontal healing and this effect is **enhanced** by an anti-inflammatory diet.
Discepoli et al. (2025), Single-centre, randomized controlled clinical trial (RCT)	Reduction of ultra-processed foods (UPF) **vs** no dietary change (control)	66 participants	UPF intake assessed with validated **FFQ** and NOVA classification; Intervention group received individualized counseling to reduce UPF consumption for **6 weeks**	Gingivitis	GI, BOP, PI, dietary profiles	UPF-reduction led to significantly greater improvement in gingival inflammation: GI and BOP decreased more in the intervention group compared with control ($p < 0.05$). Significant improvements in overall dietary quality were also observed, including higher intake of unprocessed/minimally processed foods. Reducing UPFs enhanced gingival clinical outcomes independently of plaque accumulation.
Bartha et al. (2022), Exploratory analysis using data from a previous RCT (Mediterranean diet intervention for gingivitis)	Mediterranean Diet (MedD) intervention vs habitual Western diet	37 participants	DEGS-FFQ (German validated food frequency questionnaire) MEDAS (Mediterranean Diet Adherence Screener)	Gingivitis	BOP, GI, PI	MedD group showed significant reductions in omega-6 serum fatty acids, improving the omega-6/omega-3 ratio. Reduction in omega-6 fatty acids correlated with reduced BOP and GI (lower gingival inflammation). Overall, shifting to a Mediterranean diet reduced gingival inflammation and improved serum inflammatory fatty acid profiles.
Atarbashi-Moghadam et al. (2020), Cross-sectional comparative study	Raw vegan diet (≥80% uncooked plant-based foods) vs omnivorous diet	118 adults (59 raw vegans; 59 omnivores)	Self-reported adherence to raw vegan diet; duration of adherence; no FFQ	Periodontal parameters measured clinically (non-periodontitis population; comparison of periodontal health indicators)	PD, CAL, BOP, GR, OHI-S (debris + calculus), salivary pH	Raw vegans showed significantly lower PD ($p = 0.047$) and lower BOP ($p = 0.017$) than omnivores. Raw vegans had significantly better oral hygiene (lower debris and calculus;$p< 0.001$). Salivary pH was more acidic in raw vegans ($P < 0.001$). CAL and gingival recession showed no difference between groups.

						Authors concluded that improved periodontal condition among raw vegans is likely due to better oral hygiene behaviors and antioxidant-rich dietary patterns.
Javid et al. (2019), Randomized, double-blind, placebo-controlled clinical trial	Resveratrol supplementation +Non-surgical periodontal therapy (NST)	43 type 2 diabetic patients with chronic periodontitis (Intervention: n = 21; Control: n = 22)	Supplementation regimen: resveratrol capsules (dose implied in methodology; 1 capsule/day for 4 weeks) No dietary FFQ; supplement-based intervention	Chronic Periodontitis	CAL, IL-6, TNF-alfa, TAC (total antioxidant capacity)	Significant reduction in IL-6 serum levels in the resveratrol group after 4 weeks (P = 0.039). No significant between-group differences in IL-6, TNF-α, TAC, or CAL post-intervention. Resveratrol may reduce systemic inflammation (IL-6) but does not significantly improve periodontal tissue parameters in short-term NST-supported therapy.
Perić et al. (2020), Randomized, double-blind, placebo-controlled clinical trial	Vitamin D supplementation (Vitamin D3, 25(OH)D-deficient adults)	26 participants	Serum 25(OH)D_3 measurement; 6-month supplementation with 25,000 IU vitamin D_3 every 2 weeks	Chronic Periodontitis	PD, CAL, FMPS (plaque), FMBS (bleeding)	Both groups improved after non-surgical periodontal therapy, but vitamin D group showed significantly greater reduction in bleeding (FMBS) and slightly greater PD reduction. CAL improvements were similar between groups. Authors conclude that vitamin D supplementation enhances resolution of gingival inflammation during periodontal therapy in deficient individuals.
Ali et al. (2024), RCT	Omega-3 fatty acid supplementation	60 participants	Test group: 1000 mg/day omega-3 for 6 months + SRP(scaling and root planning); Control: SRP only	30 chronic periodontitis, 30 control	PD, BOP	Test group showed significantly greater reductions in PD and BOP at 3 and 6 months ($p<0.01$). PD decreased from 5.4 → 3.0 mm and BOP from 71% → 28%. Control group showed smaller improvements (PD 5.3 → 4.1 mm; BOP 70% → 51%). Authors conclude that omega-3 supplementation

						markedly enhances periodontal healing and reduces inflammation when used as an adjunct to nonsurgical therapy.

*PD: probing depth, CAL: clinical attachment level, PI: plaque index, GI: gingival index, DMFT: decay, missing, filling, tooth, SRP: scaling and root planning, mm: milimeter, NST: Non-surgical treatment, TNF-alfa: Tumor necrotizing factor-alfa, IL-6: ınterleukin-6, n: number, BOP: bleeding on probing, CFU: colony forming unit, p=0,005 significant level

Table 3. Psychosocial Stress, Coping Strategies, and Periodontal Treatment Outcomes

Authors (Year) and Type of Study	**Psychological Exposure**	**Sample Size**	**Assesment Tools**	**Periodontal Status**	**Periodontal index and parameters used**	**Primary and Secondary Outcomes**
Romano et al. (2023), Longitudinal intervention study	Psychosocial stress level (minor vs major) and coping strategies (approach vs avoidance)	90 adults	PSS-10 (Perceived Stress Scale); Coping Responses Inventory (CRI)	Generalized stage III/IV grade A/B periodontitis (2017 classification)	Full-mouth bleeding score(FMBS), PD, Full-mouth plaque score (FMPS), CAL, number of residual pockets	After non-surgical periodontal therapy (NSPT), patients with higher stress levels showed significantly higher FMBS, mean PPD, and more residual pathological pockets. Avoidance coping strategies were associated with higher FMBS and FMPS. Multivariate analysis confirmed stress level as a significant predictor of post-treatment bleeding and PPD. Psychological stress and maladaptive coping negatively affected short-term NSPT outcomes.
Yarkac et al. (2021), Interventional clinical study (NSPT)	Psychosocial stress, pregnancy-related stress, salivary cortisol	60 women (30 pregnant , 30 non-pregnant)	Perceived Stress Scale-10 (PSS-10); salivary cortisol (ELISA); GCF IL-6 and IL-10	Generalized gingivitis (PPD ≤ 3 mm; no CAL loss)	PI, GI, PD	After NSPT, both groups showed clinical improvement; however, pregnant women exhibited significantly higher PPD, GI, GCF IL-6, and

						salivary cortisol levels compared with non-pregnant women. PSS-10 scores were similar between groups, indicating that biological stress markers (cortisol) rather than perceived stress were associated with increased periodontal inflammation. Findings suggest pregnancy-related stress may impair periodontal healing despite therapy.
Kolenko et al. (2024),Longitudinal observational clinical study	Psycho-emotional stress related to prolonged martial law; depression and emotional instability	49 adults	Zung Self-Rating Depression Scale; serum cortisol (biochemical blood test)	Gingivitis (biofilm-induced) and Stage I periodontitis (2017 AAP/EFP classification)	OHI-S (Green-Vermillion index), API (aproximal plaque index) , PMA, PBI (papilla bleeding index), PD, CAL,	After one year of prolonged wartime stress, clinical deterioration was observed in ~55% of patients, despite prior treatment. Elevated serum cortisol was detected in 18.4% of patients and was significantly associated with disease progression or lack of stabilization. Zung scale scores correlated with cortisol levels and periodontal deterioration (PD change). Findings indicate that chronic psychosocial stress acts as a pathogenetic factor in periodontal disease progression through both behavioral and biological pathways.

Tanveer et al. (2021), Cross-sectional observational study	Self-perceived psychological stress (minor vs major stress)	385 socially deprived women	Perceived Stress Scale-10 (PSS-10); structured questionnaire	Periodontal disease assessed using Community Periodontal Index (CPI ≥ 1)	CPI (bleeding, calculus, periodontal pockets)	Major psychological stress was present in 52.5% of participants. Periodontal disease was diagnosed in 52.2% of the sample. Women with major stress had a significantly higher likelihood of periodontal disease . Multivariate analysis confirmed psychological stress as an independent risk factor for periodontal disease.
Isola ve ark. (2023), cross-sectional clinical study	Psychological stress, anxiety, depression, coping strategies	49 adults	SCL-90-R questionnaire (stress, anxiety, depression, coping behavior)	Periodontitis	PD, CAL, BOP, PI, gingival recession (REC)	PD was significantly correlated with anxiety ($p<0.001$), depression ($p=0.026$), and stress ($p<0.001$). CAL was significantly associated with anxiety ($p<0.001$), depression ($p=0.026$), stress ($p=0.017$), and older age. BOP correlated with depression, PI with anxiety, and gingival recession with both depression and stress. Patients with severe CAL predominantly used emotion-focused coping, whereas those with lower CAL more frequently adopted problem-focused coping strategies. Persistent psychological stress and depression were identified as

						important risk factors for periodontal attachment loss.
Özcan & Özcan (2020) , Prospective interventional clinical study	Psychosocial stress and anxiety status (stressed vs non-stressed)	40 adults	Hospital Anxiety and Depression Scale (HADS-A, HADS-D); State–Trait Anxiety Inventory (STAI-I/II); salivary stress biomarkers (cortisol, α-amylase, chromogranin-A); GCF cytokines (IL-1β, IL-6, IL-10)	Chronic periodontitis diagnosed according to Armitage (1999) criteria	PD, PI, GI;	At baseline and 1 month after NSPT, stressed patients exhibited significantly higher PD, PI, GI, salivary stress biomarkers, and GCF IL-1β levels compared with non-stressed patients. Although clinical and inflammatory parameters improved after therapy in both groups, treatment response was significantly poorer in stressed patients, with smaller reductions in PPD, PI, GI, and IL-1β. Psychosocial stress negatively affected periodontal healing and treatment outcomes.
Palle et al. (2024), Cross-sectional clinical study	Occupational stress (low / moderate / high)	1000 male Indian Army personnel	Modified Occupational Stress Index (OSI-56); Oral Hygiene Index-Simplified (OHI-S); Gingival Bleeding Index (GBI)	Periodontal disease defined using CPI-based pocket depth and clinical loss of attachment (CLOA); staging retrospectively aligned with 2017 classification	OHI-S, GBI, probing pocket depth (PPD), clinical loss of attachment (CLOA)	Significant differences were observed among occupational groups for OHI-S, GBI, CLOA, and occupational stress scores ($p<0.001$). Participants with moderate to high occupational stress showed higher prevalence and severity of periodontal pockets and attachment loss. Logistic regression showed higher odds for

						periodontal pockets and CLOA in stressed individuals, although the overall association between stress and periodontal disease was weak and not statistically significant after adjustment. Cumulative occupational stress and age were suggested contributors to periodontal disease severity.
Xu et al. (2025) , Cross-sectional observational study	Perceived psychological stress and anxiety	240 university students	Perceived Stress Scale-14 (PSS-14); Generalized Anxiety Disorder-7 (GAD-7)	Periodontitis defined as CPI score 3–4 and/or attachment loss ≥4 mm,	CPI, BOP, CAL	Periodontitis prevalence was 43.3%. After multivariable adjustment, anxiety showed a strong dose–response association with periodontitis, whereas perceived stress did not remain significant. Compared with non-anxious individuals, odds ratios for periodontitis were 8.39 (mild anxiety), 11.42 (moderate anxiety), and 46.20 (severe anxiety). Higher anxiety levels were also associated with poorer oral hygiene behaviors (less frequent flossing and brushing). The findings suggest anxiety as a major psychosocial determinant of periodontal disease in young adults.
Macrì et al. (2024), Cross-sectional	Perceived psychological stress and mindfulness	203 adults	Perceived Stress Scale (PSS-10); Mindfulness Attention Awareness	Periodontitis	PSR, GBI, Plaque Control Record (PCR), PD	Higher perceived stress levels were significantly associated with increased

observational study			Scale (MAAS)			gingival bleeding (GBI) ($r \approx 0.67$, $p < 0.001$). Lower MAAS scores (reduced mindfulness) were significantly associated with a higher prevalence of periodontitis ($p < 0.05$). Age and perceived stress emerged as significant predictors of periodontitis in regression analyses. The findings suggest that psychological stress and reduced mindfulness are important lifestyle-related determinants of periodontal inflammation, independent of plaque accumulation.
Dubar et al. (2020), Prospective clinical case–control study	Psychosocial stress and anxiety; biological stress marker (salivary cortisol)	60 adults	State–Trait Anxiety Inventory (STAI-YA, STAI-YB); Perceived Stress Scale (PSS); salivary cortisol (ELISA); PCR-based detection of periodontal pathogens	Periodontitis (Stage II–IV) according to Armitage (1999) and 2017 classification	PPD, CAL, GI, PI, BOP; salivary cortisol; subgingival red/orange complex bacteria	Salivary cortisol levels were significantly higher in periodontitis patients and showed a positive linear association with probing pocket depth (PPD) ($p \approx 0.04$). Cortisol was not correlated with self-reported stress or anxiety scores. High psychosocial stress was associated with modulation and persistence of red and orange complex bacteria (notably *T. forsythia*, *P. gingivalis*, *F. nucleatum*, *C. rectus*), both before and after non-surgical periodontal therapy. Psychosocial

						stress influenced periodontal degradation primarily through biological (neuroendocrine –microbial) pathways rather than behavioral scores alone.
Rashme et al. (2024), Cross-sectional clinical study	Psychological stress (high vs low stress)	120 young adults (18–30 years)	Perceived Stress Scale (PSS); clinical periodontal examination	Periodontal pocketing defined as probing pocket depth (PPD) ≥ 4 mm	PPD, plaque index (PI), bleeding on probing (BOP)	The high-stress group (PSS ≥ 20) demonstrated significantly greater mean PPD (4.2 ± 0.8 mm) compared with the low-stress group (2.8 ± 0.6 mm; $p < 0.001$). Periodontal pockets were present in 72% of high-stress participants versus 45% of low-stress participants. PI and BOP were also significantly higher in the high-stress group ($p < 0.01$). Psychological stress showed a strong positive correlation with PPD ($r = 0.62$; $p < 0.001$) and PI ($r = 0.55$; $p < 0.01$). The findings indicate that psychological stress is a significant risk factor for periodontal pocket development in young adults.

*p=0.005 significant level, PPD: periodontal pocket depth, CAL: clinical attachment level, PI: plaque index, GI: gingival index, DMFT: decay, missing, filling, tooth, SRP: scaling and root planning, mm: milimeter, NSPT: Non-surgical periodontal treatment, GCF: gingival crevicular fluid, IL: interleukin, r: ratio, CPI: community perioodntal index, PCR: polymerase chain reaction

Table 4. Sleep disorders and periodontitis

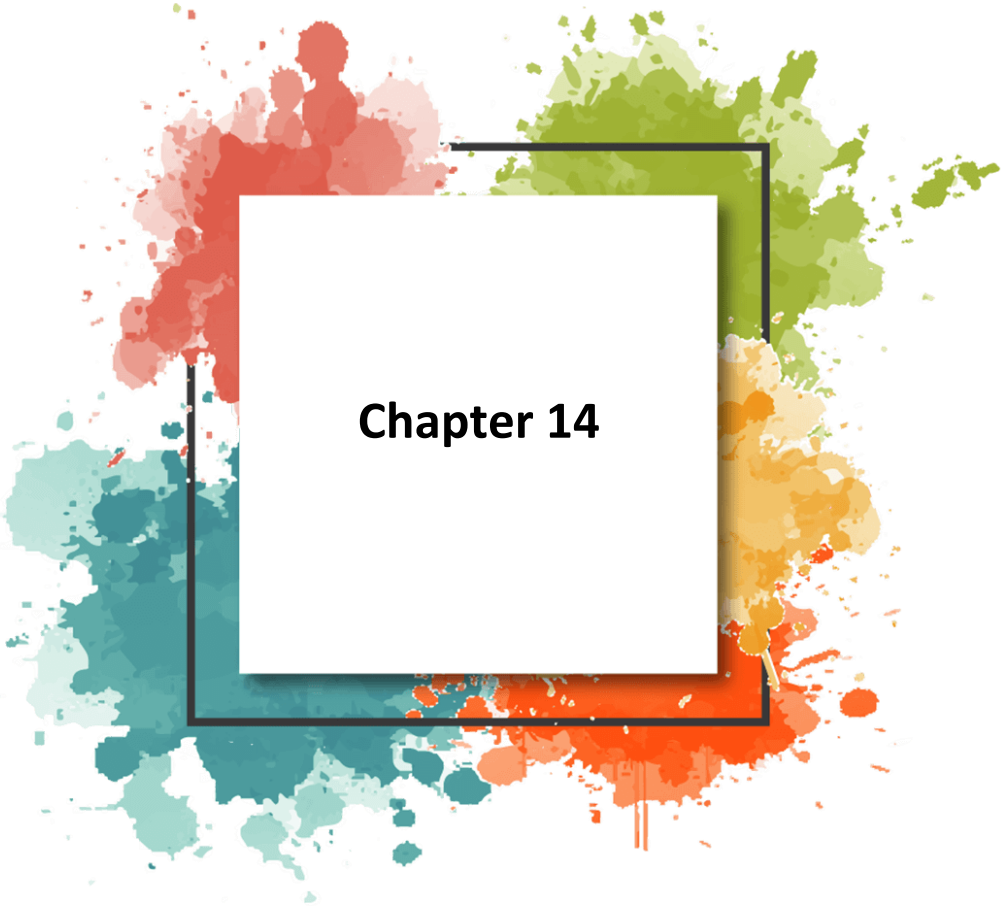
Authors (Year) and Types of Study	Sleep / Lifestyle Exposure	Sample Size	Assessment Tools	Periodontal Status	Periodontal Parameters	Primary and Secondary outcomes
Iwasaki et al. (2022), Cross-sectional clinical epidemiological study	Sleep duration (<5 hours(h), 5–5.9 h, 6–6.9 h, 7–7.9 h, ≥8 h)	1,130 middle-aged Japanese workers	Self-administered questionnaire on habitual sleep duration	Severe periodontitis	PD, CAL	Severe periodontitis was identified in 6.3% of participants. After adjustment for confounders, individuals sleeping <5 h had a significantly higher likelihood of severe periodontitis compared with those sleeping 7–7.9 h
Beydoun et al. (2020), cross-sectional study	Sleep duration (<7 h vs ≥7 h)	11,813 participants	Self-reported habitual sleep duration	Periodontitis	PD, CAL, WBC(White blood cells), neutrophils, lymphocytes	Sleep duration was not independently associated with periodontitis or WBC markers.
Olmos-Valverde et al. (2025), cross-sectional study	Sleep hygiene / sleeping routines (SR) as part of the Healthy Lifestyle Scale (EVS)	195 dental students	EVS (Estilo de Vida Saludable): subscales for Smoking habits (SH), Sleeping routines (SR)	Gingival status assessed by BOP	BOP, DMFT	Sleep hygiene (sleeping routines, SR) showed a significant association with oral health indicators. Lower adherence to sleep routines was associated with worse oral health outcomes.
El-Sharkawy et al. (2019), Randomized controlled clinical trial	Primary insomnia; dietary melatonin supplementation (10 mg/day before bedtime)	74 adults	Athens Insomnia Scale (AIS); salivary TNF-α (ELISA)	Generalized chronic periodontitis	CAL, PD, BOP%, salivary TNF-α	The melatonin group demonstrated significantly greater CAL gain and PD reduction at both 3 and 6 months compared with placebo ($p < 0.01$). AIS scores and salivary TNF-α levels were significantly reduced in the melatonin group, indicating improved sleep quality and reduced

						inflammatory burden. BOP% improved in both groups without intergroup differences. Melatonin supplementation enhanced periodontal healing in insomniac patients when used adjunctively with SRP.
Florian-Tirado et al. (2024), cross-sectional observational study	Risk of obstructive sleep apnea (OSA) (low / medium / high)	118 Peruvian adults	STOP-BANG questionnaire for OSA risk; Page & Eke criteria for periodontitis	No/mild, moderate, severe periodontitis	PD, CAL	A significant association was observed between increasing OSA risk and greater periodontitis severity. Patients at high OSA risk showed a markedly higher prevalence of moderate-to-severe periodontitis.
Eroğlu et al. (2023), cross-sectional pilot clinical study	Sleep quality, sleep duration and fatigue	124 adults	Pittsburgh Sleep Quality Index (PSQI), Jenkins Sleep Scale (JSS), Multidimensional Assessment of Fatigue (MAF), OHIP-14	Periodontal diagnosis according to 2017 World Workshop (healthy gingiva; Stage I–IV; Grade A–C)	PI, GI, PD, CAL	No significant association was observed between sleep quality (PSQI, JSS), fatigue (MAF), or OHRQoL and periodontitis stage or grade ($p>0.05$). Sleep duration was significantly shorter in Stage IV periodontitis compared with other groups
Karaaslan & Dikilitaş (2019), cross-sectional clinical study	Sleep quality and sleep duration	99 adults	Pittsburgh Sleep Quality Index (PSQI); Oral Health Impact Profile-14 (OHIP-14)	Periodontitis classified according to the 2017 World Workshop (Stage I–IV; Grade A–C)	PD, CAL	Short sleep duration and poor sleep quality were strongly correlated with worse oral health–related quality of life (OHRQoL). Significant positive correlations were observed between PSQI and OHIP-14 global and domain scores, indicating that more severe periodontitis was associated with impaired sleep and

						reduced quality of life.
Kvarnvik et al. (2024), cross-sectional comparative clinical study (10-year follow-up)	Obstructive sleep apnea (OSA) status and long-term CPAP treatment	121 adults	Overnight polygraphy (AHI) for OSA diagnosis; clinical periodontal examination; bitewing radiographs; chair-side salivary MMP-8 test	Periodontal diseases	BoP, PD (≥4 mm, ≥6 mm), radiographic alveolar bone loss, plaque index, salivary MMP-8	No significant differences were observed between OSA groups regarding clinical periodontal parameters (BoP, PPD, plaque index, number of teeth) or salivary MMP-8 levels.
Tranfić Duplančić et al. (2022), cross-sectional clinical study	Obstructive sleep apnea (OSA) severity (no OSA; mild–moderate; severe)	209 adults	Full-night polysomnography/polygraphy (AHI); unstimulated salivary flow rate; salivary electrolytes and cortisol; comprehensive periodontal examination	Periodontal diseases	PI, BoP, PPD, CAL, PISA score, number of teeth	Higher OSA severity was associated with increased plaque accumulation and fewer remaining teeth. Plaque scores correlated positively with AHI (r=0.26, p=0.003). Subjects with severe OSA tended to exhibit higher CAL and plaque levels. Hyposalivation was associated with higher PISA scores and altered salivary electrolyte composition, suggesting a potential indirect link between OSA, salivary dysfunction, and periodontal inflammation.
Téllez-Corral et al. (2023), cross-sectional clinical study	OSA	84 adults	Full-night polysomnography (AHI); comprehensive periodontal examination; saliva and gingival crevicular fluid (GCF) cytokine analysis by multiplex bead immunoassay	Periodontal diseases	PD, CAL, BOP, PI; salivary and GCF IL-1β, IL-6, IL-17A, IL-33, TNF-α	Stage III periodontitis was significantly more prevalent in patients with severe OSA (69%, p=0.014. The findings suggest a bidirectional relationship between OSA and periodontitis mediated by pro-inflammatory cytokines involved in osteoclastogenesis.

Téllez-Corral et al. (2022), cross-sectional clinical microbiological study	Obstructive sleep apnea (OSA) presence and severity	93 adults	Full-night polysomnography (AHI), microbiological analysis of saliva, subgingival plaque and gingival sulcus using MALDI-TOF-MS	Periodontal health/gingivitis and periodontitis stages I–IV	PD, CAL, BOP, percentage of teeth with periodontitis, plaque index; distribution of Socransky microbial complexes; *Candida spp.*	Although overall OSA severity was not directly associated with periodontitis prevalence, Stage III periodontitis was significantly more frequent in patients with severe OSA (75%; p=0.0157). Patients with periodontitis and OSA exhibited greater microbial diversity, with increased prevalence of orange and red complex bacteria, particularly Prevotella melaninogenica, and higher detection of *Candida albicans*.
Marruganti et al. (2024), cross-sectional study	Perceived stress (moderate/high vs. low) and sleep quality (poor vs. good); interaction effect	235 adults	Perceived Stress Scale (PSS-10); Pittsburgh Sleep Quality Index (PSQI); full-mouth periodontal examination	Periodontitis	CAL, PD, BOP teeth lost	Stage III/IV periodontitis was significantly associated with moderate/high perceived stress (OR=5.4; 95% CI: 2.2–13.5; p<0.001) and poor sleep quality (OR=3.0; 95% CI: 1.2–7.4; p<0.05).

*p=0.005 significant level, PD: probing depth depth, CAL: clinical attachment level, PI: plaque index, GI: gingival index, DMFT: decay, missing, filling, tooth, SRP: scaling and root planning, mm: milimeter, GCF: gingival crevicular fluid, IL: interleukin, r: ratio, CPI: community perioodntal index, PCR: polymerase chain reaction, MMP: matrix metalloproteinase, TNF: tumor necrotising factor


Chapter 14

Alport Syndrome

***Buket Esen Ağar*[1]**

Alport syndrome is a genetic kidney disease that affects the structural parts of the glomerular basement membrane (GBM). The most common first sign of the disease is chronic hematuria. The syndrome arises from mutations in the COL4A3, COL4A4, and COL4A5 genes, which encode type IV collagen, a crucial structural element of the GBM. These mutations compromise the integrity of the GBM, leading to a gradual decline in renal function. Alport syndrome is also known for causing sensorineural hearing loss and a range of eye problems that are typical of the disease (1).

British doctor Arthur Cecil Alport was the first to write about Alport syndrome in 1927. Dr. Alport saw a family with three generations in which hearing loss was always linked to progressive renal failure. These observations indicated a hereditary origin for the illness. The name "Alport syndrome" was officially established in 1961 to characterize this clinical condition (2).

There have been major improvements in the diagnosis and treatment of Alport syndrome in the last few years. The increasing availability of genetic testing has made it easier to find out about diseases earlier and to test family members for them. Additionally, renin-angiotensin-aldosterone system (RAAS) inhibitors have demonstrated efficacy in decelerating disease development (3).

Etiology:

Alport syndrome mainly comes from harmful changes in the COL4A3, COL4A4, or COL4A5 genes. These genes make the type IV collagen chains that are important for keeping the glomerular basement membrane's structure intact. These mutations cause the GBM to become thinner and lose its structure, which damages the glomerular filtration barrier. This shows up in the clinic as proteinuria, hematuria, and worsening kidney failure (1). The inheritance pattern in Alport syndrome differs based on the specific gene involved. The X-linked variant of Alport syndrome (XLAS) is the most common subtype, caused by harmful changes in the COL4A5 gene and making up around 80% of all known cases (1). Autosomal recessive Alport syndrome (ARAS) is linked to biallelic mutations in COL4A3 or COL4A4 and accounts for around 15% of cases (4). Autosomal dominant Alport syndrome (ADAS), associated with monoallelic mutations in COL4A3 or COL4A4, typically exhibits a slower clinical

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progression (2). Table 1 shows the different kinds of Alport syndrome and its clinical features.

Inheritance Pattern	Associated Gene(s)	Mutation Type	Case Proportion (%)	Clinical Severity	Gender Impact
X-linked (XLAS)	COL4A5	Single allele (X chromosome)	~80%	Moderate-severe	More severe in males; females may be carriers
Autosomal Recessive (ARAS)	COL4A3 or COL4A4	Biallelic	~15%	Severe	No sex difference
Autosomal Dominant (ADAS)	COL4A3 or COL4A4	Single allele	~5%	Mild-moderate	No sex difference

Table 1. Morphological Subtypes of Alport Syndrome

Epidemiology:

The total frequency of Alport syndrome varies based on the diagnostic techniques employed. Traditional sources indicate a prevalence between 1 in 5,000 and 1 in 50,000 persons. According to inheritance patterns, X-linked Alport syndrome (XLAS) constitutes roughly 80% of all cases (1), whereas autosomal recessive Alport syndrome (ARAS) comprises about 15% (4). Autosomal dominant Alport syndrome (ADAS) generally has a milder clinical manifestation and is less prevalent (2).

In XLAS, males are more severely impacted, while female carriers frequently have lesser or subclinical symptoms. Nevertheless, the deterioration of renal function may persist in females as they age. On the other hand, ARAS affects both men and women equally. Ethnic disparities may also affect disease prevalence. A study released in 2024 indicated that COL4A3 variations were more commonly found in individuals of Asian ancestry, with diagnoses in this demographic generally occurring at earlier stages (5).

Pathophysiology:

The primary pathophysiological mechanism in Alport syndrome is the deterioration of structural integrity in the glomerular basement membrane (GBM). The GBM is mostly made up of type IV collagen, especially the α3, α4, and α5 chains, when the body is in a normal state. Pathogenic mutations in the COL4A3, COL4A4, or COL4A5 genes hinder the production of collagen chains, leading to structural disruption and functional impairment of the GBM (1). These molecular flaws cause the GBM to become thinner and less regular. The decreased structural integrity enhances glomerular permeability, ultimately resulting in proteinuria, hematuria, and the formation of glomerular scarring. Progressive glomerulosclerosis and tubulointerstitial fibrosis are also very important to the course of the disease (6). The α345 type IV collagen trimers are naturally found in the basement membranes of the kidney, cochlea, and eye tissues. This elucidates the reason that, alongside renal signs, individuals with Alport syndrome often experience sensorineural hearing loss and ocular anomalies (5).

Histopathology:

While histopathological findings in Alport syndrome are not independently diagnostic, their presence provides significant supplementary value for diagnosis due to their distinctive and identifiable characteristics. Renal biopsy findings, especially those obtained using light microscopy (LM), immunohistochemistry (IHC), and electron microscopy (EM), play a crucial role in diagnostic assessment.

Light Microscopy (LM): At the onset of the disease, light microscopy could show only minor or nonspecific alterations, and the glomeruli may look normal in shape. Nevertheless, as the disease advances, segmental sclerosis, mesangial proliferation, and interstitial fibrosis become evident (1,6). A histological pattern indicative of focal segmental glomerulosclerosis (FSGS) has been documented in certain cases (7).

Immunohistochemistry (IHC): This method uses immunohistochemical staining to look at how type IV collagen chains made up of α3, α4, and α5 subunits are expressed. In males with X-linked Alport syndrome, α5 chain expression is generally lacking in both the glomerular basement membrane (GBM) and the distal tubular basement membranes. In female carriers, a mosaic staining pattern may be evident.

Electron Microscopy (EM): EM shows the most typical histological signs of Alport syndrome. The findings show that the GBM is not normal, with sections that are thicker, thinner, and have the distinctive "basket-weave" look (6). These characteristics are especially beneficial for diagnosing pediatric patients. EM also

helps tell Alport syndrome apart from other inherited kidney diseases, like thin basement membrane disease (TBMD) (8).

Clinical Manifestations:

Alport syndrome is a genetic disorder affecting various organ systems, characterized by increasing kidney involvement leading to end-stage renal illness, along with concomitant sensorineural hearing loss, ocular symptoms, and leiomyomatosis. The clinical spectrum can differ significantly based on the genetic subtype, sex, and age of the individual.

Renal manifestations: Microscopic hematuria is the most common and earliest indication of Alport syndrome in the kidneys. It is usually found by chance during a urinalysis of youngsters who don't have any symptoms. Over time, proteinuria gets worse and causes more damage to the kidneys. Continued proteinuria causes scarring in the glomeruli and a decrease in kidney function. Renal failure may manifest in early adulthood, especially in males with X-linked Alport syndrome (1,9). In female carriers, the condition typically exhibits a lesser progression, although problems such as proteinuria and hypertension may develop with advancing age. The autosomal recessive variant may exhibit severe clinical manifestations akin to those observed in affected males (6).

Auditory Findings: Sensorineural hearing loss is a primary characteristic of Alport syndrome. It usually starts in childhood or adolescence and gets worse with time. Hearing loss is usually bilateral, symmetrical, and affects high frequencies. It usually shows up earlier and is worse in men, while in women, the severity depends on whether they are carriers or not. Hearing aids are often needed for auditory rehabilitation.

Ocular Findings: Ocular manifestations in Alport syndrome typically present throughout adolescence or adulthood. The most common eye indication is anterior lenticonus, which happens when the lens capsule is thinner and the front surface of the lens sticks out in a cone shape. This result is pathognomonic for Alport syndrome and possesses significant diagnostic significance (1,5). Other eye problems include retinal flecks, weakening of the macula, and cataracts at the back of the eye. These findings generally do not lead to vision loss, but they may worsen with time.

In infrequent instances, leiomyomatosis may manifest in the esophagus, trachea, and genital tract. This phenotype is linked to substantial deletions affecting both the COL4A5 and COL4A6 genes, constituting a rare phenotypic variation of Alport syndrome (10).

Diagnostic Methods:

Alport syndrome is diagnosed using a mix of clinical findings, family history, lab testing, imaging studies, a kidney biopsy, and genetic analysis. Early detection is especially important for slowing the disease down.

A family history indicating early-onset renal failure, auditory impairment, and visual manifestations (particularly in males) corroborates the diagnosis.

Persistent microscopic hematuria is the predominant finding in urinalysis. Over time, proteinuria and a decline in kidney function may occur. It is important to keep an eye on serum creatinine levels, the estimated glomerular filtration rate (eGFR), and the albumin-to-creatinine ratio during follow-up (6). Asymptomatic hematuria in female carriers may also provide a diagnostic indication.

It is recommended to do an audiometric test, especially on children, to find sensorineural hearing loss early on. An ophthalmologic examination can uncover eye abnormalities, including anterior lenticonus and retinal flecks (5).

Renal biopsy is a significant instrument that aids in the diagnosis. Common observations on electron microscopy (EM) are thinning and thickening of the glomerular basement membrane (GBM), loss of lamellation, and a "basket-weave" look (6,8). Immunohistochemistry may show that there are no α5(IV) collagen chains. These results assist in validating the diagnosis and forecasting the inheritance pattern.

Genetic testing is now the best way to tell if someone has Alport syndrome. Next-generation sequencing (NGS) can find changes in the COL4A3, COL4A4, and COL4A5 genes (11). Genetic testing is essential not only for diagnosis but also for elucidating the inheritance pattern, conducting family screening, and facilitating prenatal diagnosis. Genetic analysis serves as a diagnostic tool in situations where biopsy is unfeasible or when histopathology results are unconventional. It is also helpful in making the diagnosis clearer in circumstances where the phenotype is not clear (10).

When diagnosing Alport syndrome, it is necessary to rule out other inherited and acquired glomerular illnesses that may exhibit similar clinical characteristics. Table 2 shows the different possible diagnoses.

Disease	Distinguishing Features
Thin Basement Membrane Nephropathy (TBMN)	Usually benign course, hematuria dominant; family history possible but not progressive
Focal Segmental Glomerulosclerosis (FSGS)	Predominantly proteinuria, segmental sclerosis on biopsy; genetic forms exist
IgA Nephropathy	Presents with hematuria and proteinuria; diagnosed by mesangial IgA deposition on biopsy
Lupus Nephritis	Systemic symptoms (fever, rash, arthritis), ANA and dsDNA positivity, low complement levels
Membranoproliferative Glomerulonephritis (MPGN)	Low complement levels; double contouring in mesangial and capillary walls
Nephronophthisis	Salt wasting, polyuria, chronic tubulointerstitial nephritis, small kidney size on ultrasound
Fabry Disease	Alpha-galactosidase A deficiency, multi-organ involvement; typically in young males
Chronic Glomerulonephritis (Secondary Causes)	Long-term hypertension, secondary glomerulonephritis due to systemic diseases

Table 2. Differential Diagnosis of Alport Syndrome

Treatment:

At now, there is no definitive treatment for Alport syndrome. However, with early diagnosis and the right treatment plans, the disease can advance more slowly, end-stage renal disease (ESRD) can be put off, and the quality of life can get better.

Inhibition of the renin–angiotensin–aldosterone system (RAAS) is fundamental to the treatment of Alport syndrome, as it slows the course of the condition. Angiotensin-converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs) function by decreasing intraglomerular pressure, which in turn diminishes proteinuria. For those with Alport syndrome, treatment is suggested if they have microalbuminuria, overt proteinuria, or stage III–IV chronic renal disease.

To lower proteinuria and protect kidney function, it is important to keep blood pressure in the right range for your age. The usual goal is to maintain it below the

90th percentile. Additionally, a low-sodium diet coupled with RAAS inhibition is recommended to augment therapeutic efficacy (1).

For people with sensorineural hearing loss, using hearing aids and going through audiological rehabilitation can make a big difference in their quality of life. For those with ocular involvement, optical correction and, when indicated, surgical procedures are advised (6,5). For people with end-stage renal illness, kidney transplantation is seen to be the best way to treat the disease. Infrequently, Goodpasture-type glomerulonephritis may arise as a consequence of the generation of anti-glomerular basement membrane antibodies. So, it's very important to choose donors carefully and keep a close eye on them after the transplant, especially for people with COL4A5 mutations (12).

Alport syndrome can cause a lot of systemic problems because it becomes worse over time. These complications typically arise in the latter stages of the disease and directly affect both quality of life and survival. Table 3 shows a list of problems that can happen with Alport syndrome.

Complication	**Description**
Chronic Kidney Disease	Develops as the glomerular basement membrane defect progresses; may lead to end-stage renal disease
Advanced Hearing Loss	Occurs due to involvement of the inner ear basement membrane, usually becomes apparent after adolescence
Visual Impairments (lenticonus, retinopathy)	May present as anterior lenticonus, maculopathy, and retinopathy
Hypertension	It arises as a consequence of renal impairment and subsequent activation of the renin–angiotensin–aldosterone system (RAAS).
Proteinuria and Nephrotic Syndrome	Characterized by increased protein loss as kidney damage progresses
Increased Cardiovascular Disease Risk	Associated with chronic kidney disease; increases morbidity and mortality
Anemia	Occurs due to reduced erythropoietin production as renal function declines
Post-transplant Anti-GBM Disease	May lead to rapid allograft loss after kidney transplantation

Bone Mineralization Disorders	Secondary to hyperparathyroidism and mineral imbalances caused by chronic kidney disease

Table 3. Complications Encountered in Alport Syndrome

Prognosis:

The prognosis of Alport syndrome is contingent upon various factors, including the specific genetic mutation, inheritance pattern, sex, age at the commencement of treatment, and severity of proteinuria. In the X-linked type (XLAS), the disease typically presents more severely in males, frequently culminating in end-stage renal disease (ESRD) prior to the age of 30. In contrast, female carriers have a broader clinical spectrum; some may present solely with lifelong microscopic hematuria, whilst others may experience increasing proteinuria and renal failure. Autosomal recessive Alport syndrome (ARAS) also has a severe clinical course and affects males and females with the same frequency. In this kind, ESRD usually happens while a person is a child or young adult. The autosomal dominant variant (ADAS) often advances more gradually; however, certain individuals may experience proteinuria and hypertension in later life. A 2023 cohort analysis indicated that particular mutations in the COL4A3 and COL4A4 genes are linked to late-onset end-stage renal disease (6,10).

Research indicates that persons diagnosed through genetic analysis and promptly treated with RAAS inhibitors experience a substantial delay in the progression to renal failure (9,12). The severity of auditory and ocular manifestations may be associated with the specific type of genetic mutation. The presence of anterior lenticonus is generally correlated with more severe abnormalities. Early-onset hearing loss frequently correlates with advancing renal dysfunction (5,9).

Genetic Counseling and Family Screening:

Because Alport syndrome is passed down through families, genetic counseling is very important for people who have it. This approach entails educating the patient and their family of the method of inheritance, recurrence risks, extent of genetic testing, and the possible ramifications of test results (1).

The genetic counseling procedure need to commence at diagnosis. In X-linked forms, it is crucial to evaluate the risk in female carriers, estimate the probability of illness manifestation in offspring, and offer advice on pregnancy planning. Patients must to be informed about prenatal diagnostic alternatives (6,10).

In autosomal recessive cases, it is advisable for partners of heterozygous carriers to undertake genetic screening as a component of reproductive planning.

Couples identified as carriers should be educated about preimplantation genetic diagnosis (PGD) and invasive prenatal diagnostic methodologies (11).

Genetic screening of first-degree relatives, and preferably the extended family, is crucial for prompt diagnosis and intervention. Comprehensive family screening can identify asymptomatic individuals with microscopic hematuria or proteinuria in the early stages, facilitating quick treatment initiation (9). Urinalysis, blood pressure monitoring, renal function tests, and, if necessary, genetic testing should all be part of the screening procedure. These steps can help stop or slow down the damage to the kidneys that gets worse over time.

Genetic counseling requires a multidisciplinary approach, including nephrologists, geneticists, social workers, and, if necessary, psychological support teams (12).

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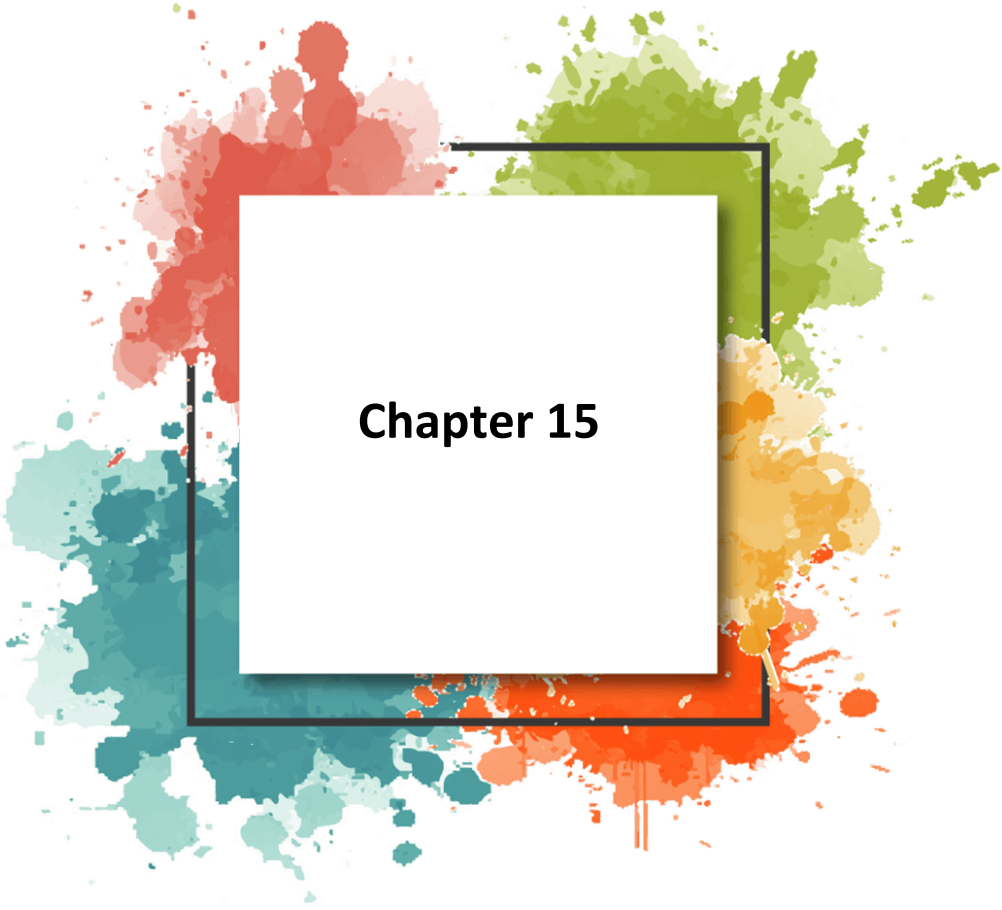
Chapter 15

Advancements in 3D Printing Technologies for Prosthetic Dentistry

Duygu Ece Keskin[1] & Beşar İzzetağa[2]

Three-dimensional (3D) printing also known as additive manufacturing (AM), first emerged in the early 1980s (1). Pioneers such as Charles W. Hull (founder of 3D Systems) and S. Scott Crump (founder of Stratasys) played crucial roles in its development. These technologies have greatly evolved, facilitating their integration into digital workflows for prosthetic applications. AM involves creating objects in a layer-by-layer manner (2). 3D printing has been utilized across various industries, including automotive, electronics, aerospace, healthcare, architecture, food, and agriculture. However, its adoption in dentistry is still progressing. Despite being around for approximately 40 years, this technology is yet to be fully diffused. A technology is considered diffused when it is widely adopted or replaces traditional methods. Factors like awareness, communication, innovativeness, investment, and complementary costs significantly influence the diffusion rate (3).

AM technologies have significantly advanced, enabling their seamless incorporation into digital workflows for prosthetic production. AM a type of computer-aided manufacturing (CAM) involves the construction of objects through a layer-by-layer process. This method allows for precise and customizable fabrication, particularly useful in creating intricate prosthetic designs (4). The American Section of the International Association for Testing Materials, part of ASTM International Standard Organization, develops voluntary technical standards for a broad range of materials, products, systems, and services. The committee has identified seven categories of AM: stereolithography (SLA) and digital light protection (DLP) that belongs to the vat polymerization category, material jetting (MJ), material extrusion (ME), binder jetting (BJ), powder bed fusion (PBF), sheet lamination (SL) and direct energy deposition (DED) (4). This chapter provides a comprehensive overview of the major AM technologies and discusses their current and potential applications in prosthodontics.

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Stereolithography (SLA)

In the field of dentistry SLA is one of the oldest and most widely used AM techniques and rapid prototyping 3D printing methods. SLA 3D printing systems were invented by Charles Hull (5). This system operates on the principle of photopolymerization, where a photosensitive monomer resin solidifies upon exposure to UV light. A stereolithography machine consists of a build platform (substrate) mounted inside a resin tank and a UV helium-cadmium or argon ion laser (6). DLP another 3D printing system has a similar production process to SLA and the capability to produce high-resolution parts. Both methods use similar materials. However, while the SLA method employs a laser beam, the DLP method uses a visible light source to photopolymerize the resin. To accelerate the production process, DLP uses a shallower resin vat compared to the deeper tanks used in SLA. This leads to less waste material in comparison to the SLA method (7). SLA is employed in the production of diagnostic models, surgical guides, custom trays, denture bases, orthodontic aligners, temporary and permanent restorations and ceramic restorations (8). The ease of use relatively lower surface roughness compared to other methods, the ability to reproduce fine details and higher resolution are among its main advantages. However, this method is not suitable for mass production. Moreover, the produced parts tend to have low durability, resulting in mechanically weak structures and the high maintenance cost of the laser components also limits this method (9).

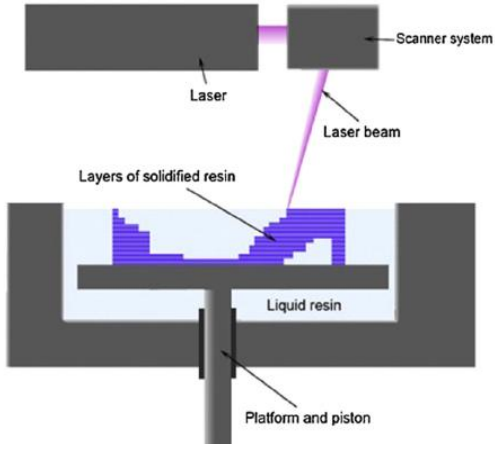

Figure 3. The working mechanism of SLA

Digital Light Projection Technique (DLP)

Larry Hornbeck from Texas Instruments developed DLP technology in 1987 (10). DLP shares similarities with SLA technology and is classified within the same AM category according to the ASTM standards (10). Unlike SLA which

uses a laser, DLP employs ultraviolet light to cure liquid resin layer by layer. A crucial component of DLP technology is the digital micromirror device, which consists of a rectangular array of mirrors forming the microsystem (11,12). The resolution of the projected image is determined by the number of micromirrors with each mirror corresponding to one or more pixels. These micromirrors can be individually adjusted to control the reflection angles. Light from the source is reflected by the micromirrors projecting individual pixels onto the printing surface (13,14). DLP offers several advantages over SLA, such as faster layer fabrication, since it can project and cure an entire layer across the build platform in a matter of seconds. The accuracy and efficiency of DLP printing are expected to drive its increased adoption within the dental industry (14,15). However, concerns remain regarding the lower power of the light source and the focus on faster printing speeds, which may compromise print quality in response to commercial demands for efficiency. As a result while DLP is more suitable for printing larger parts with less intricate details, SLA is better suited for printing precise parts with complex details (16). DLP is also widely used in the production of crowns and bridge restorations, surgical guides, removable dentures, and diagnosis models (16,17).

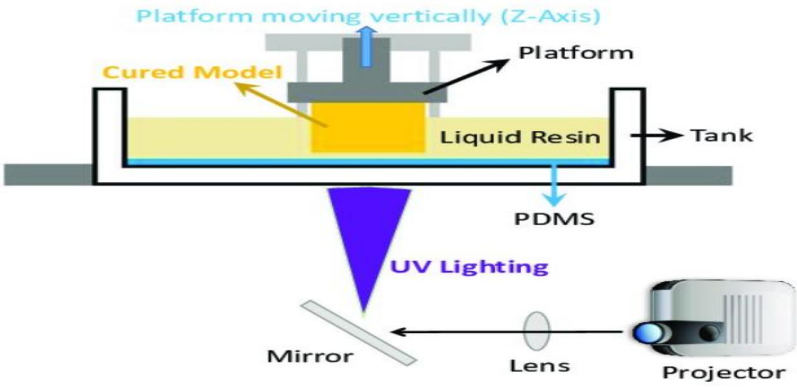

Figure 4. The working mechanism of DLP

Material Jetting (MJ)

MJ technology also known as polyjet printing (PP), involves selectively jetting liquid resin from a piezoelectric nozzle which is polymerized by UV light. The platform moves down by the thickness of one layer and this process is repeated until the entire object is formed. Additionally this method allows for the production of materials with different color variations or diverse material properties (16). This technique enables the production of complex materials in a shorter time and at lower cost. However it also has disadvantages such as the difficulty of the production process, challenges in maintaining adhesion between

layers, low resolution, limitations on the size of objects that can be produced(18). In dentistry this system is widely used for the production of bridge restorations and surgical guides. It is also utilized in various other applications such as model fabrication, orthodontic brackets, ceramic frameworks, and oral appliance treatment for obstructive sleep apnea (19).

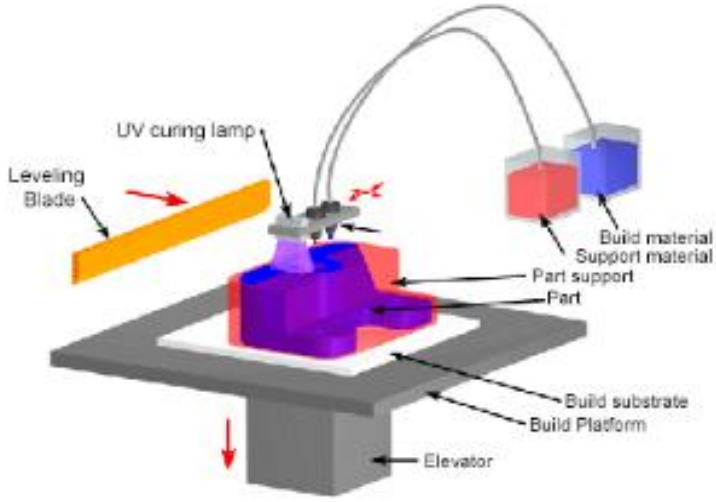

Figure 5. The working mechanism of material jetting

Material Extrusion (ME)

The ME method was initially developed by Scott Crump in the early 1990s and later commercialized by Stratasys. The original patents for this technology have since expired this technology became widely adopted and is now generally known under the term fused deposition modeling (FDM) (20). While the material jetting technique is implemented using inkjet 3D printers, in this method 3D models are produced in a manner similar to the technique used in inkjet printers. A piezoelectric printhead ejects droplets of liquid photopolymer which are immediately solidified under the influence of UV lamps. The piezoelectric printhead is positioned above a movable platform. After the first layer is completed the platform lowers and the next layer is formed. This process continues until the model is fully constructed. Inkjet 3D printers, particularly models like MultiJet, allow for the use of various resins to simultaneously produce multi-part objects. Support structures are automatically generated during this process (20). This technique allows for the use of ceramic and metal powders mixed with different thermoplastic materials, enabling the production of more diverse and customized parts through the combination of different materials or methods like DLP (21). The advantages of this system are its affordability, the moisture resistance of the produced objects, and the ability to offer multiple color

options. However, the disadvantages include lower mechanical properties and surface quality as well as a limited variety of usable thermoplastic materials. Additionally, compared to SLA and MJ technologies, surface roughness is higher, accuracy is lower, and post-processing steps such as polishing and sanding may be required to remove the ridges formed at the layers (21).

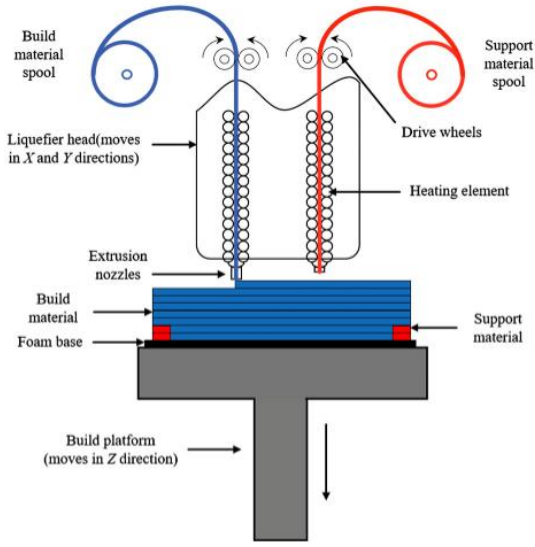

Figure 6. Principle of ME method

Binder Jetting (BJ)

In the BJ technique the resin material is spread with a roller, a binder is applied through a moving printhead similar to inkjet printers to bond the powder. As each layer is formed, the platform moves downward and this cycle continues until the object is complete. Additionally due to the ability to add various colors to the binder, multicolored objects can be produced (22). BJ is a versatile technology used in many areas, from low-cost 3D metal printing and colored prototyping to the production of large ceramic casting molds (19). One of the main advantages of the BJ technique is that it does not require support structures during printing (23). However the current level of accuracy is limited for prosthetic applications. Furthermore, various factors, such as the type of powder material used, part orientation, nominal dimensions, geometric features, and the position within the print bed, can affect the strength of objects produced with BJ technique (24).

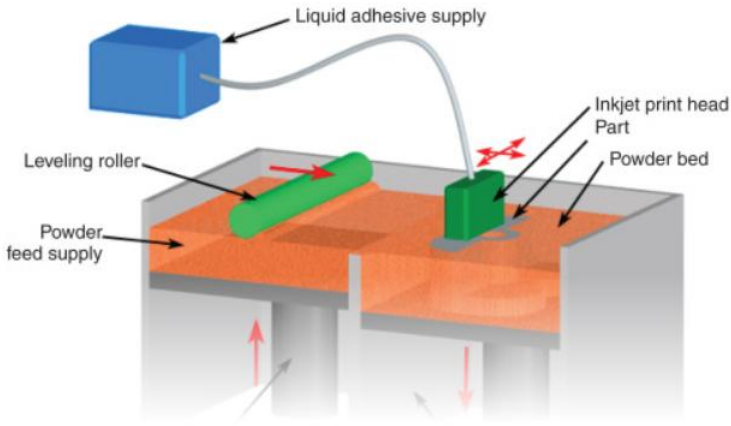

Figure 7. Schematic of binder jetting 3D printing process

Powder Bed Fusion (PBF)

PBF technique powdered materials are fused layer by layer using a high-energy laser beam. In laser-based manufacturing systems, such as selective laser sintering (SLS) and selective laser melting (SLM), the process involves directing a laser beam onto the powder material through mirrors, selectively fusing it to form the desired object. A thin layer of metal powder is selectively fused by a laser beam based on 3D data. One of the key features of SLS and SLM technologies is the recyclability of the unused material after the production process (25). PBF is utilized in dentistry for the fabrication of crown copings and holds significant potential in implantology, as well as for producing anatomical dental models. SLS is primarily used for processing polymers and ceramics, whereas SLM also referred to as "Direct Metal Laser Sintering," is employed for fabricating metals and alloys (26). SLS/SLM technologies are highly relevant in dentistry, particularly in prosthetic applications, as they allow the use of a broad range of dental materials in the creation of dental structures. These materials include thermoplastic polymers, waxes, metals and alloys (such as titanium and its alloys, cobalt-chrome alloys, and stainless steel), ceramics, and thermoplastic composites. SLS is ideal for producing maxillofacial prostheses, functional frameworks, and custom scaffolds for tissue engineering using polymers and composites. In contrast, SLM is used to manufacture metal-based products, such as solid and porous orthopedic and dental implants, dental crowns, bridges, and frameworks for partial prostheses. The advantages of this system include its capability to produce high-strength materials, deliver high accuracy and efficiently manufacture complex geometries. Additionally the fact that no support structures are required during production is a considerable advantage. However

there are some drawbacks such as the potential for porosity and dimensional distortions due to shrinkage, surface roughness, and the possible need for bonding agents like cyanoacrylate to ensure adequate interlayer bonding (20,24,25).

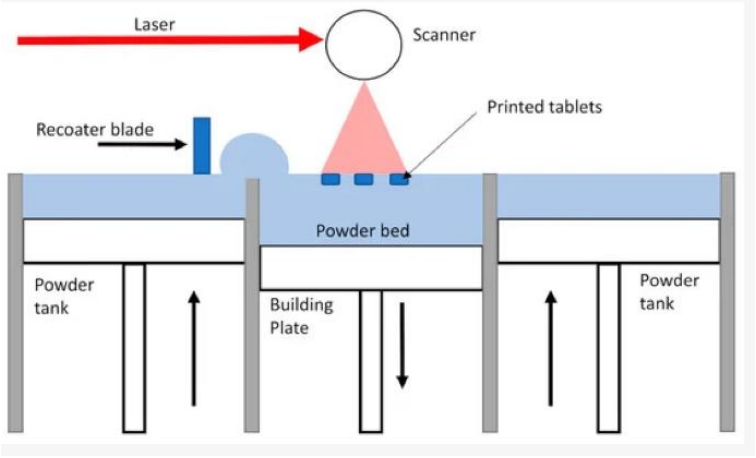

Figure 8. SLS-based PBF 3D-printing process

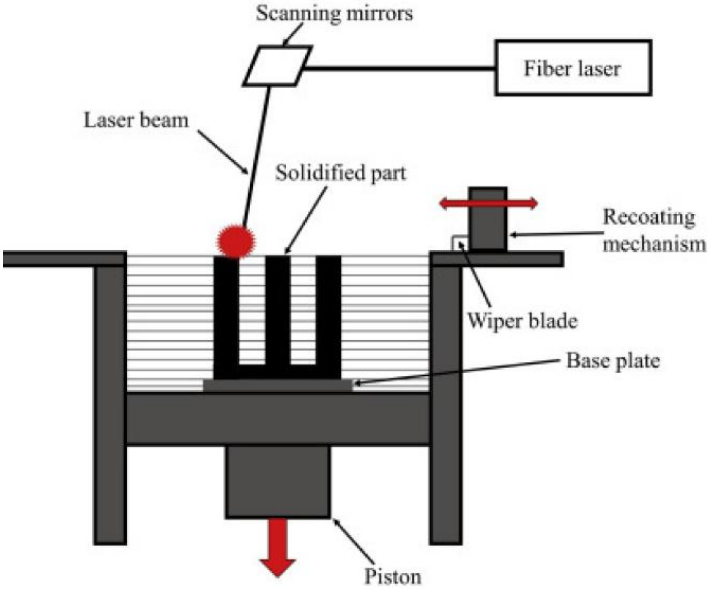

Figure 9. SLM-based PBF 3D printing process

Direct Energy Deposition (DED)

DED is a sophisticated printing technique primarily used for repairing or adding material to existing components. This process involves a nozzle attached to a multi-axis arm that dispenses melted material onto a target surface where it quickly solidifies. Unlike material extrusion processes the nozzle in DED is capable of moving in multiple directions allowing for material deposition from any angle, facilitated by the use 4 and 5-axis machines. DED melts metal powder or wire with a laser or electron beam just before deposition. Though mostly used for metals, it is also applicable to polymers and ceramics (27). DED technique offers the advantage of rapidly constructing large structures. Moreover its ability to produce fully dense 3D parts with complex geometries regardless of the material used is a significant advantage. These features have led to growing interest in DED as an alternative to existing manufacturing and repair methods across a variety of materials. Metallic materials are particularly well-suited for DED due to their relatively high weldability (28). However processing some materials like ceramics with DED presents more challenges as only a limited number of ceramic materials can be heated enough to form a melt pool. Even if ceramics can be melted the risk of cracks occurring due to thermal shocks during cooling is high. Therefore, ceramics are typically not processed directly using DED. Instead, they are often integrated into metal matrix composites for use in industrial applications (28,29).

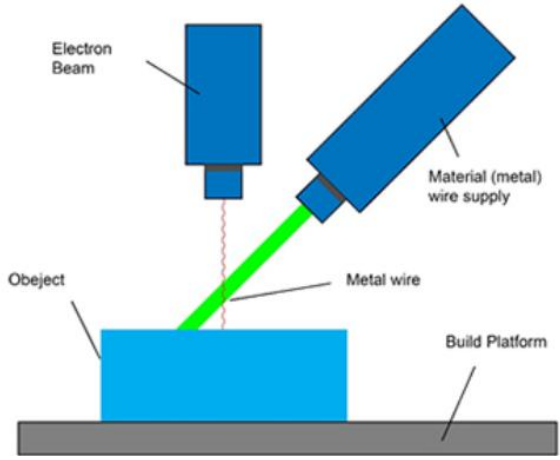

Figure 10. DED 3D printing process

Sheet Lamination (SL)

SL processes such as Ultrasonic Additive Manufacturing (UAM) and Laminated Object Manufacturing (LOM) employ distinct techniques for creating layered objects. UAM involves bonding metal sheets or ribbons through ultrasonic welding, which may necessitate additional CNC machining and the removal of excess metal often integrated within the welding process. This method can utilize various metals including aluminum, copper, stainless steel and titanium, operating at low temperatures which allows for the formation of internal geometries without melting the metal. This makes the process energy-efficient and capable of bonding different materials. On the other hand LOM builds objects by layering paper materials, using adhesive instead of welding, and incorporates a cross-hatching technique during printing to facilitate easy post-build material removal. While objects created via LOM are typically utilized for aesthetic and visual models, they are generally not suitable for structural applications due to the materials used (27). The SL system offers the advantages of being able to produce large-scale materials quickly, accurately with high strength. However it is not recommended for complex morphologies due to the significant amount of experience and time required for production. Additionally, the system has several drawbacks including poor surface quality and dimensional stability of the produced materials, as well as the difficulty of removing excess material after production (29).

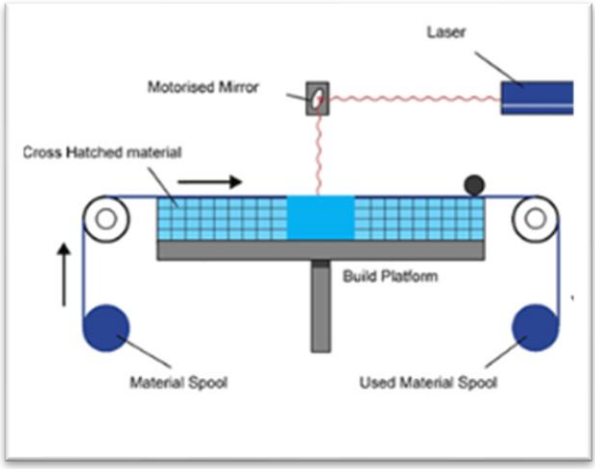

Figure 11. Sheet lamination 3D printing process

AM offers many advantages compared to subtractive and conventionally manufactured prostheses. These advantages include:

- Lower cost compared to the milling machines used by most printers.

- Less material waste.
- The ability to produce multiple prostheses simultaneously.
- The capability to manufacture designs that are too complex to be milled.
- The production of personalized, precisely shaped products in three dimensions.
- Minimization of human error during operations.
- Reduced production and delivery times for the products.
- Passive production, with no force required for milling.
- Greater ease in producing large objects compared to traditional methods.
- Detailed production based on digitized data (such as CT or MRI scans), with high reproducibility (21,30–32)

Despite these advantages, additive manufacturing also presents certain limitations:

- Surface roughness and layer lines inherent to the layer-by-layer fabrication process may necessitate additional post-processing procedures, such as polishing or finishing.
- The range of printable dental materials remains more limited compared with conventional manufacturing techniques.
- In some cases mechanical properties may be inferior to those of conventionally fabricated restorations, particularly under long-term functional loading.
- Production speed can also be a limiting factor, especially for larger prosthetic restoration.
- Additionally, interlayer bonding weaknesses may increase the risk of delamination under mechanical stress (21,26,29,30)

Consequently, while additive manufacturing provides substantial advantages in customization and digital workflow integration, careful material selection and process optimization are essential to ensure long-term clinical success.

Clinical Applications of Additive Manufacturing in Prosthetic Dentistry

AM technologies have increasingly been integrated into prosthetic dentistry, enabling the fabrication of a wide range of prosthetic and auxiliary components.

These technologies support a fully digital workflow, from data acquisition and computer-aided design to computer-aided manufacturing, thereby enhancing precision, reproducibility, and clinical efficiency.

Removable Complete Dentures

The adaptation and fit of denture bases to the underlying mucosal tissues represent critical factors for the retention, stability, and long-term success of complete dentures. Recent studies have demonstrated that complete dentures fabricated through digital workflows, including additive manufacturing, can achieve clinically acceptable levels of accuracy and adaptation. In particular, CAD-CAM-milled and 3D-printed denture bases have shown favorable adaptation when compared with conventionally fabricated prostheses (33).

AM enables the production of individualized denture bases based on digital impressions and virtual design, reducing laboratory steps and chairside adjustments. Although milled prostheses often demonstrate slightly higher accuracy in some studies, the differences between milled and additively manufactured dentures are generally within clinically acceptable limits. Consequently 3D printing technologies are increasingly considered viable alternatives for the fabrication of complete dentures (34–36).

Master and Diagnostic Casts

3D-printed dental models have become reliable alternatives to conventional gypsum casts in prosthetic dentistry. The use of digital impressions enables the rapid production of working models with reduced labor and material costs. These models facilitate treatment planning, prosthetic fabrication, and communication between clinicians and dental laboratories (37).

Several investigations have evaluated the accuracy of 3D-printed dental models and reported that they demonstrate clinically acceptable trueness and precision (37–40). However certain studies indicate that traditional model fabrication methods may still provide higher accuracy in specific clinical scenarios, particularly when intraoral scanning is affected by factors such as saliva accumulation or bleeding which may interfere with the scanning process and reduce the visibility of the scanned area. Variations in printing technology, resin type, post-processing procedures, and printing parameters may influence the final accuracy of printed models (41,42)

Although a universally standardized protocol for dental model printing has not yet been established, current evidence suggests that AM models are suitable for many clinical applications in prosthetic dentistry.

Removable Partial Denture Frameworks

Additive manufacturing has also been applied to the fabrication of removable partial denture (RPD) frameworks, particularly through powder bed fusion technologies such as selective laser melting. These techniques enable the production of metal frameworks with complex geometries and high structural integrity (20,26,43).

a study have reported improved adaptation and fit of frameworks fabricated using additive manufacturing compared with those produced by conventional lost-wax casting techniques (44). Conversely, other investigation has found that conventionally fabricated frameworks may still provide superior adaptation in certain situations (45). Differences in material behavior, printing parameters, and post-processing procedures may account for these variations.

In addition challenges remain in achieving optimal bonding between artificial teeth and additively manufactured metal frameworks. Further research and technological refinement are required to fully optimize additive manufacturing for removable partial denture fabrication.

3D printing custom impression tray

The fabrication of custom impression trays using AM has gained attention due to its potential to improve impression accuracy and clinical efficiency. 3D-printed custom trays can be designed digitally and fabricated rapidly with consistent thickness and precise adaptation (46).

Study investigating implant-supported full-arch rehabilitation has demonstrated that 3D-printed custom trays may reduce deviations in implant positioning compared with certain conventional techniques. The digital design and manufacturing process enables enhanced standardization and may contribute to improved accuracy in complex prosthetic procedures (47).

Interim and Provisional Restorations

AM technologies are widely used in the fabrication of interim crowns, bridges, and provisional restorations. Numerous studies have evaluated the marginal and internal fit of 3D-printed provisional restorations and reported that their accuracy is generally comparable to that of conventionally fabricated or milled restorations (48–50).

The ability to rapidly fabricate provisional restorations using digital workflows reduces treatment time and allows for efficient chairside or laboratory production. Additionally, advances in printable resin materials continue to improve the mechanical and esthetic properties of additively manufactured provisional restorations, supporting their expanded use in clinical practice (48,49,51).

Conclusion

AM technologies have transformed contemporary prosthetic dentistry by enabling highly precise, customizable, and efficient fabrication of prosthetic components. Through integration with digital scanning and computer-aided design systems, 3D-printing allows for the production of patient-specific restorations with improved reproducibility and reduced manual intervention.

Among the various AM techniques, vat photopolymerization and powder bed fusion technologies currently play the most prominent roles in dental applications. These methods provide high accuracy and versatility in the fabrication of diagnostic models, provisional restorations, surgical guides, and definitive prosthetic components.

Despite substantial progress certain limitations remain, including material constraints, surface quality considerations, and the need for post-processing procedures. Continued advancements in material science, printing accuracy, and workflow integration are expected to further expand the clinical applications of AM in prosthetic dentistry.

Overall, AM represents a fundamental component of the evolving digital workflow in prosthetic dentistry and is anticipated to play an increasingly significant role in the future of personalized dental treatment and prosthetic rehabilitation.

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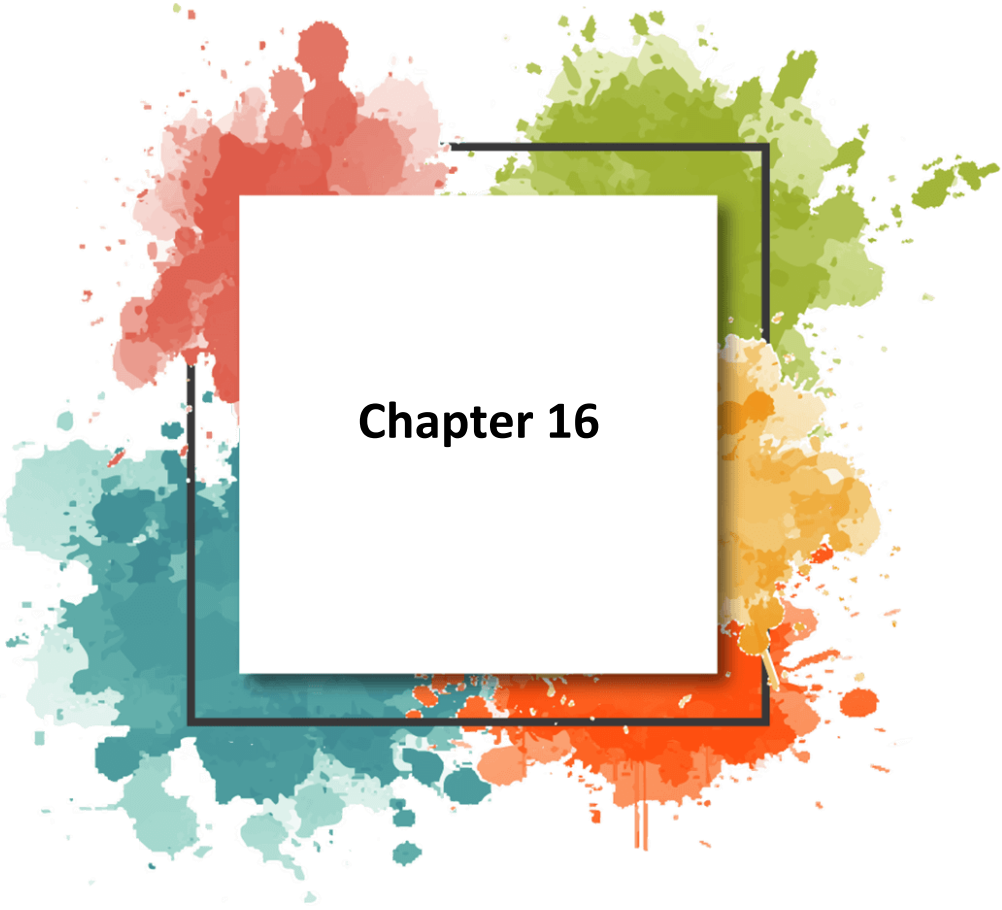
Chapter 16

Intrathoracic Solitary Fibrous Tumors: Medical Review and Two Case Analyses

Selim Yalçın[1]

ETIOLOGY AND PATHOGENESIS

The etiology of intrathoracic SFTs is not fully understood, but significant progress has been made at the genetic and molecular level. The defining molecular feature of SFTs is the recurrent fusion of the NAB2 and STAT6 genes on chromosome 12q13 [1]. This fusion disrupts NAB2-mediated repression, leading to abnormal activation of early growth response transcription pathways and triggering tumor formation. STAT6 immunohistochemical staining is considered a valuable marker of NAB2-STAT6 gene fusion and exhibits excellent sensitivity and specificity [1].

The association of environmental or occupational exposures with the development of SFTs has not been proven. Although hormonal or hereditary factors have been suggested, definitive associations have not yet been established [1]. While the mechanisms underlying the variable clinical behavior of SFTs are not fully defined, there is no consistent association between patient-related, environmental, or dietary factors and tumor development or malignant potential [1].

CLINICAL FEATURES AND DIAGNOSIS

Intrathoracic SFTs are generally asymptomatic and are often found incidentally during routine chest radiographs or other imaging studies. When they become symptomatic, the most common symptoms are dyspnea, chest pain, and cough. Less commonly, paraneoplastic syndromes may occur. The best known of these is hypoglycemia caused by insulin-like growth factor II (IGF-II) produced by the tumor (Doege-Potter syndrome) [3].

The diagnostic approach includes imaging methods and histopathological examination:

Imaging: Chest X-ray, computed tomography (CT), and magnetic resonance imaging (MRI) are important in assessing the location, size, morphology, and relationship of the tumor to surrounding tissues. On CT, they are usually seen as

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a well-circumscribed, lobulated, or oval mass. MRI can better show the fibrous structure and vascularization of the tumor [1].

Histopathology: Definitive diagnosis is made by histopathological examination after biopsy or surgical resection. Microscopically, spindle fibers consist of spindle-shaped cells irregularly distributed among collagen fibers. Immunohistochemically, CD34 and STAT6 positivity is typical [1]. Criteria for malignancy include high mitotic activity, necrosis, marked nuclear pleomorphism, and an infiltrative growth pattern [1].

DİFFERENTIAL DİAGNOSIS

The diagnosis of intrathoracic SFTs requires careful differential diagnosis as they can be confused with other intrathoracic lesions. The most important differential diagnoses are:

Pleural Mesothelioma: Unlike SFTs, mesothelioma is usually associated with asbestos exposure and shows extensive involvement in the pleura. Immunohistochemically, calretinin positivity favors mesothelioma [1].

Synovial Sarcoma: May have a similar spindle cell morphology to SFTs, but is usually characterized by SYT-SSX gene fusion. • Malignant Peripheral Nerve Sheath Tumor (MPNST): Usually associated with neurofibromatosis type 1 and may show S100 positivity.

Hemangiopericytoma: Hemangiopericytoma, previously considered a separate entity from SFT, is now evaluated as a variant of SFT. 5. Risk Stratification and Prognosis

The clinical behavior of SFTs is variable, and various risk stratification models are used to predict the risk of recurrence.

5. Risk Stratification and Prognosis

The clinical behavior of SFTs is variable, and several risk stratification models have been developed to predict the risk of recurrence. One of the most commonly used models is the one proposed by Demicco et al. This model includes the following factors:

Risk Factor	Scoring Criteria
Age	<55 years (0), ≥55 years (1)
Tumor Size	<5 cm (0), 5-10 cm (1), 10-15 cm (2), ≥15 cm (3)
Mitotic Index	0 (0), 1-3 (1), ≥4 (2) (per 10 HPF)
No Necrosis	Absent (0), <5% (1), ≥5% (2)

Patients are divided into low, medium, or high-risk groups according to the total score. High-risk patients should be followed up more closely after surgery [1]. 6. Treatment Strategies

The gold standard in the treatment of intrathoracic spirometry (PFTs) is complete surgical resection of the tumor. Complete resection provides the best prognosis for both benign and malignant tumors. The size, location, and relationship of the tumor to surrounding tissues determine the surgical approach. Pedicled tumors are generally easier to resect, while broad-based or invasive tumors may be more challenging [1].

Long-term follow-up of patients after surgical resection is important because recurrence can occur even in tumors considered benign. The risk of recurrence increases depending on the malignant characteristics of the tumor (size, mitotic activity, necrosis) [1]. In malignant PFTs or when complete resection is not possible, adjuvant treatments such as radiotherapy and chemotherapy may be considered, but the effectiveness of these treatments is limited and they are generally used for palliative purposes [1].

Prognosis depends on the histopathological characteristics of the tumor, the completeness of surgical resection, and the presence of recurrence. Generally, benign SFTs have an excellent prognosis, but malignant SFTs have a worse prognosis and carry a risk of metastasis [1].

CASE ANALYSES

Case 1: Intrathoracic Giant Solitary Fibrous Tumor

A 65-year-old female patient presented with progressive dyspnea, intermittent dry cough, low-grade fever, easy fatigue, and loss of appetite for one year. Despite being treated for severe pneumonia in different hospitals, her symptoms worsened and she became oxygen-dependent. Physical examination revealed reduced airflow and digital clubbing in the left lower lung area. Imaging studies revealed a giant mass covering the left hemithorax (Figure 1). Thoracotomy was performed, and a giant solitary fibrous tumor (approximately 15x15x10 cm) originating from the parietal pleura was resected en bloc. Histopathological examination confirmed that it was an SFT with benign features. The postoperative course was uneventful, and the patient was discharged. This case demonstrates that intrathoracic SFTs can reach large sizes and become symptomatic. The importance of complete surgical resection is highlighted [2].

Case 2: Giant Solitary Fibrous Tumor Associated with Doege-Potter Syndrome

A 44-year-old female patient with no known secondary illnesses presented to the emergency department with an acute confusional state. Investigations

revealed severe hypoglycemia. Endocrinological examination showed suppressed insulin secretion and low plasma glucose. Abdominal and thoracic CT scans revealed a large 15 cm mass in the right breast (Figure 2), raising suspicion of Doege-Potter syndrome. A low IGF-1 value (30.4 μg/L) supported the diagnosis. Prior to surgical resection, the artery supplying the tumor was embolized. Subsequently, en bloc resection was performed with diaphragm patch and extra-anatomical lung resection, and the diaphragm was reconstructed with Prolene mesh. In the postoperative period, hypoglycemia completely resolved. Macroscopic pathological examination revealed a pSFT measuring 17.5 cm in diameter and weighing 1516 g. Microscopically, spindle cells and clear surgical margins were observed. Mitotic activity was low, and no malignant features were detected. This case demonstrates that intrathoracic SFTs can lead to paraneoplastic syndromes, particularly Doege-Potter syndrome, and that surgical resection is effective in treating this syndrome [3].

SUMMARY

Intrathoracic solitary fibrous tumors (SFTs) are rare mesenchymal neoplasms originating from the pleura. At the molecular level, the NAB2-STAT6 gene fusion is a defining feature that plays a key role in tumor formation. Clinically, most tumors are asymptomatic, but when they reach large sizes or show malignant features, they can lead to symptoms such as dyspnea, chest pain, or paraneoplastic conditions such as Doege-Potter syndrome. Diagnosis is made through imaging methods (CT, MRI) and histopathological examination showing CD34 and STAT6 positivity immunohistochemically. The cornerstone of treatment is complete surgical resection. Long-term follow-up after surgery is critical to assess the risk of recurrence. Malignant features (high mitotic activity, necrosis) are associated with a poor prognosis. The two cases presented in this review highlight the broad clinical spectrum of intrathoracic pulmonary fibrosis (PFTs) and the importance of successful surgical management.

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Figure 1 shows the CT scan image of Case 1.

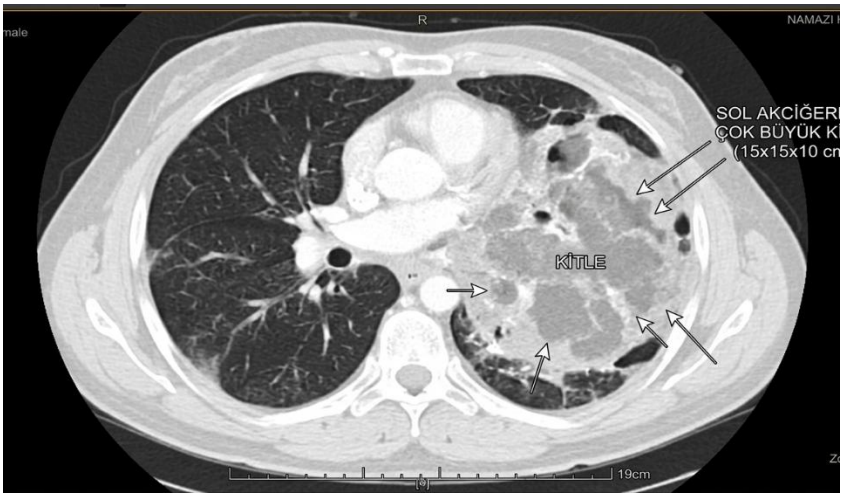
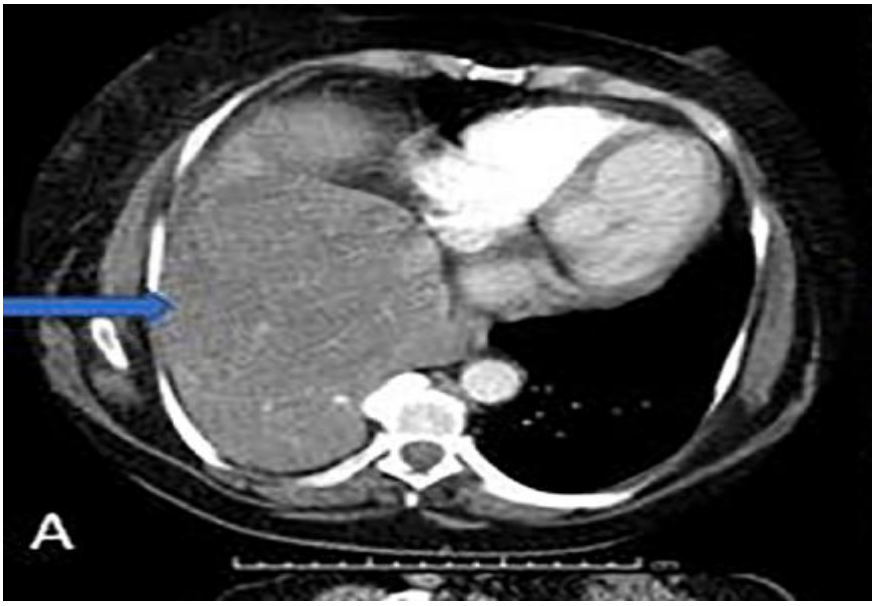
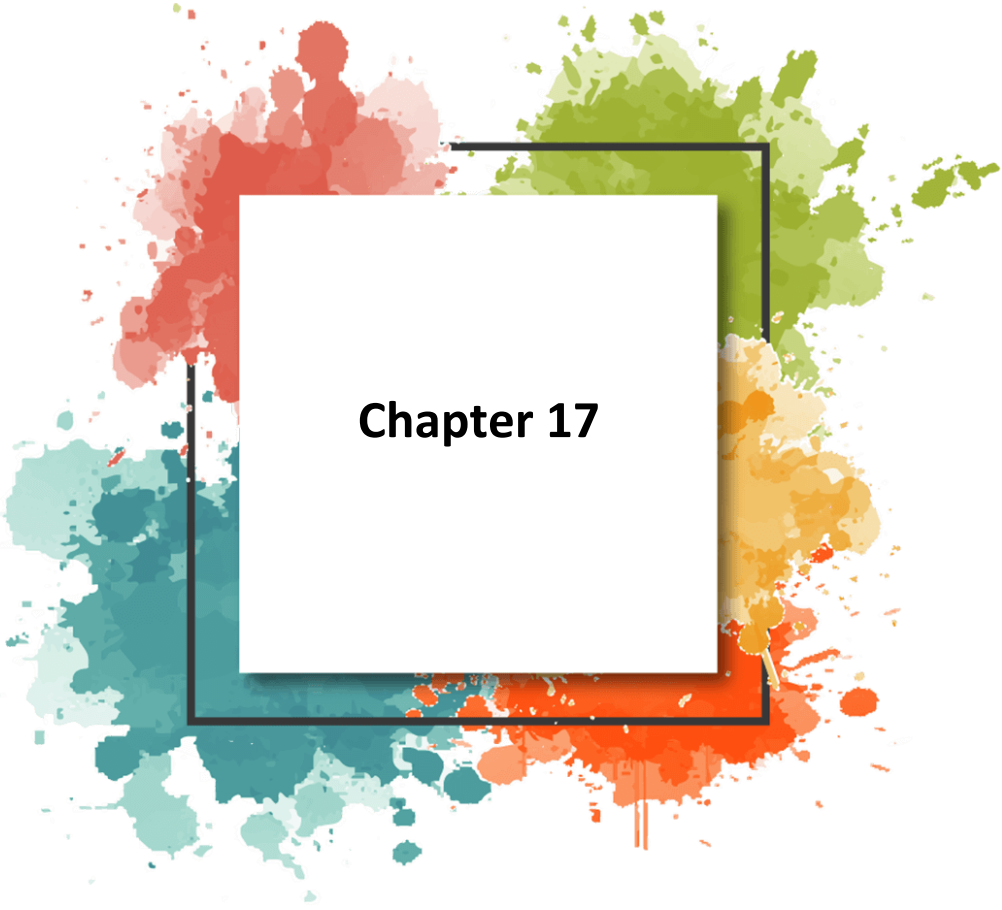


Figure 2 shows the CT scan image of Case 2




Chapter 17

Strategic Design of Mesoporous and Metallic Nanocarriers: From Controlled Synthesis to In Vitro Cytotoxicity Profiling

Hasan Ulusal[1]

1. Introduction: The Evolution of Nanocarriers in Health Sciences

The field of drug delivery has undergone a paradigm shift over the last few decades, moving from conventional systemic administration to sophisticated, programmable nanoplatforms. Conventional therapeutic agents often suffer from poor aqueous solubility, rapid systemic clearance, and a lack of selectivity, which inevitably leads to suboptimal therapeutic concentrations at the target site and undesired systemic toxicity (Peer et al., 2007). To address these long-standing challenges, nanotechnology has introduced various "nanocarriers" designed to protect the therapeutic payload, prolong circulation time, and facilitate site-specific delivery.

In the context of contemporary health sciences, the strategic design of these carriers is no longer limited to simple encapsulation. It now involves a multidisciplinary methodological approach combining materials chemistry, surface engineering, and molecular biology. Among the diverse library of nanomaterials, mesoporous silica nanoparticles (MSNs) and metallic nanoparticles (such as gold and magnetic iron oxide) have emerged as the most promising candidates due to their tunable physicochemical properties and robust functionalization chemistry (Slowing et al., 2008).

1.1. The Rationale for Nanoscale Delivery

The fundamental advantage of nanocarriers lies in their ability to exploit the unique pathophysiology of diseased tissues, particularly in oncology. The Enhanced Permeability and Retention (EPR) effect, characterized by leaky tumor vasculature and impaired lymphatic drainage, allows nanoparticles in the 10–200 nm range to preferentially accumulate in tumor tissues (Matsumura & Maeda, 1986). However, relying solely on passive targeting is often insufficient for

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overcoming complex biological barriers. Modern methodological approaches now emphasize "active targeting," where the nanocarrier surface is decorated with ligands (e.g., antibodies, peptides, or aptamers) that specifically bind to overexpressed receptors on target cells, thereby enhancing cellular uptake via receptor-mediated endocytosis (Albanese et al., 2012).

1.2. Mesoporous and Metallic Nanoplatforms: An Overview

Mesoporous Silica Nanoparticles (MSNs) are distinguished by their honeycomb-like porous structure, high surface area (>1000 m^2/g), and large pore volumes. These features allow for high loading efficiencies of diverse therapeutic agents, ranging from hydrophobic small molecules to large biopharmaceuticals like siRNA or proteins (Li et al., 2019). The silanol groups on the MSN surface provide a versatile "chemical handle" for grafting various functional groups, enabling the design of stimuli-responsive "gatekeepers" that release the cargo only in response to specific triggers like pH changes or enzymatic activity (Vallet-Regí et al., 2007).

On the other hand, metallic nanoparticles offer unique physical properties that MSNs lack. Gold nanoparticles (AuNPs) are highly valued for their ease of synthesis, biocompatibility, and unique optical properties based on Surface Plasmon Resonance (SPR), which can be harnessed for both diagnostic imaging and photothermal therapy (Dykman & Khlebtsov, 2012). Magnetic nanoparticles (MNPs), typically composed of magnetite (Fe_3O_4), provide the added advantage of "remote control." Under an external magnetic field, these particles can be guided to a specific anatomical site or used as contrast agents in Magnetic Resonance Imaging (MRI). Furthermore, they can generate localized heat (hyperthermia) when exposed to an alternating magnetic field, providing a synergistic therapeutic effect alongside drug delivery (Laurent et al., 2008).

1.3. Bridging Synthesis and Biological Evaluation

The success of these nanotherapeutic systems depends heavily on a rigorous methodological pipeline. It begins with "controlled synthesis," where parameters such as particle size, shape, and surface charge are precisely tuned, as these factors dictate the biological identity of the nanoparticle once it enters a physiological environment. Following synthesis, a comprehensive "in vitro cytotoxicity profiling" is mandatory. This stage involves not only assessing cell viability but also understanding the molecular mechanisms of interaction between the nanocarrier and the cellular machinery, including oxidative stress induction, inflammatory responses, and intracellular trafficking (Nel et al., 2009).

By integrating advanced material design with precise biological evaluation, researchers can develop nanocarriers that not only deliver drugs more efficiently but also possess the capability to overcome aggressive physiological hurdles, such as multidrug resistance (MDR) in cancer cells.

2. Methodologies for Controlled Synthesis

The biological performance of nanocarriers—including their circulation time, cellular internalization, and therapeutic efficacy—is intrinsically linked to their physical and chemical properties. Achieving "controlled synthesis" implies the ability to precisely manipulate particle size, shape, porosity, and surface charge. In health sciences, reproducibility is paramount; therefore, the selection of a synthesis methodology must ensure batch-to-batch consistency and high purity (Pankhurst et al., 2003).

2.1. Magnetic Nanoparticles (MNPs): Achieving Superparamagnetism

For biomedical applications, particularly MRI and targeted drug delivery, magnetite (Fe_3O_4) or maghemite (gamma-Fe_2O_3) nanoparticles must exhibit superparamagnetism. This property ensures that the particles do not aggregate in the absence of an external magnetic field, preventing potential vascular embolization (Laurent et al., 2008).

- **Co-precipitation Method:** This is the most common and straightforward technique for synthesizing iron oxide nanoparticles. It involves the simultaneous precipitation of Fe^{2+} and Fe^{3+} ions in an alkaline aqueous medium (e.g., NaOH or NH_4OH). While cost-effective and yielding high quantities, controlling the size distribution remains a challenge.

- **Thermal Decomposition:** To achieve highly monodisperse and crystalline nanoparticles, thermal decomposition of organometallic precursors (e.g., iron acetylacetonate) in high-boiling-point organic solvents is preferred. This method allows for "nanometer-scale" precision, which is critical when the nanocarrier must cross specific biological barriers like the blood-brain barrier (Lu et al., 2007).

2.2. Mesoporous Silica Nanoparticles (MSNs): Engineering Porosity

The hallmark of MSNs is their ordered pore network, which serves as a reservoir for therapeutic molecules. The synthesis methodology typically follows the "liquid crystal template" mechanism, where surfactant molecules act as a scaffold for silica polymerization.

- **The Sol-Gel Process:** Using tetraethyl orthosilicate (TEOS) as a silica source and surfactants like cetyltrimethylammonium bromide (CTAB) as a template, MSNs are synthesized under basic conditions. The concentration of the surfactant and the pH of the medium directly dictate the pore diameter (typically 2–10 nm) and the overall particle size (Slowing et al., 2008).

- **Stöber Method Variants:** Modern health science applications often require spherical, sub-200 nm MSNs to ensure efficient endocytosis. Modified Stöber processes allow for the synthesis of monodisperse spheres that can be easily functionalized for "gatekeeping" applications, ensuring that the drug is only released inside the acidic environment of a lysosome or tumor interstitium (Li et al., 2019).

- **Advanced Yolk–Shell Architectures:** Recent methodological innovations have led to the development of "yolk–shell" nanostructures, which combine a metallic core (the yolk) within a protective, often porous, shell. A significant example is the design of magnetically recyclable yolk–shell Au nanocatalysts. These structures are not only efficient in catalytic processes (such as the reduction of nitroaromatics) but are also strategically designed for biomedical safety. Recent studies have demonstrated that these complex Au nanostructures exhibit differential cytotoxicity, showing high biocompatibility with healthy cell lines while maintaining potential therapeutic or diagnostic efficacy in cancerous environments, making them versatile tools in theranostic health sciences (Ulusal & Ulusal 2025).

2.3. Gold Nanoparticles (AuNPs): Shape and Surface Control

Gold nanoparticles are uniquely suited for health sciences due to their inert nature and the ease with which their surface can be modified using thiol-gold chemistry.

- **Citrate Reduction (Turkevich Method):** This involves the reduction of chloroauric acid ($HAuCl_4$) with sodium citrate in boiling water. The citrate acts as both a reducing agent and a stabilizing ligand. By varying the citrate-to-gold ratio, researchers can tune the size of the nanoparticles, which is vital because particles smaller than 10 nm are often cleared by the kidneys, while those larger than 100 nm are sequestered by the liver and spleen (Dykman & Khlebtsov, 2012).

- **Seed-Mediated Growth:** For more complex geometries like nanorods or nanostars—which are superior for photothermal therapy due to their near-infrared (NIR) absorption—a seed-mediated approach is used. This allows for the

decoupled control of nucleation and growth, resulting in highly uniform metallic nanostructures (Grzelczak et al., 2008).

2.4. Quality Control and Methodological Rigor

In the context of health sciences, "controlled synthesis" is incomplete without rigorous characterization. Methodological standards require the use of Dynamic Light Scattering (DLS) to measure the hydrodynamic diameter and Zeta Potential to assess colloidal stability in physiological media (e.g., PBS or cell culture media). Furthermore, the removal of surfactants (like CTAB) from MSNs or organic residues from MNPs is a critical step, as these impurities can cause significant *in vitro* toxicity, leading to false-positive results in cytotoxicity screenings (Nel et al., 2009).

3. Surface Functionalization and Cargo Loading Strategies

The transition from a "bare" nanoparticle to a functional "nanocarrier" is achieved through precise surface engineering. In health sciences, a nanocarrier must navigate complex biological fluids containing proteins, salts, and cells without losing its integrity or being prematurely cleared by the mononuclear phagocyte system (MPS). Therefore, surface functionalization is not merely a chemical addition but a strategic necessity for biocompatibility, targeting, and controlled release (Mitchell et al., 2021).

3.1. Surface Modification for Biocompatibility and "Stealth" Properties

A primary challenge in nanomedicine is the formation of a "protein corona" upon entering the bloodstream, which can alter the nanoparticle's identity and lead to rapid hepatic clearance. To mitigate this, polymers such as Polyethylene Glycol (PEG) remain the gold standard (PEGylation). Recent methodological shifts also explore "zwitterionic" coatings and cell-membrane camouflaging (e.g., using red blood cell or platelet membranes) to provide superior "stealth" properties and longer circulation half-lives (Sharma et al., 2022).

- **Silanization of MSNs:** For mesoporous silica, the abundant surface silanol (Si-OH) groups allow for the attachment of organosilanes (e.g., APTES for amine groups). This facilitates the subsequent conjugation of targeting ligands or stimuli-responsive polymers (Manzano & Vallet-Regí, 2020).

- **Gold-Thiol Chemistry:** The high affinity of sulfur for gold enables the formation of stable, self-assembled monolayers (SAMs). This is utilized to attach specific antibodies or peptides that recognize overexpressed receptors on cancer cells, such as EGFR or HER2 (Rizvi & Saleh, 2018).

3.2. Cargo Loading Mechanisms: Efficiency and Stability

The loading of therapeutic agents (chemotherapeutics, nucleic acids, or proteins) depends on the physical architecture of the carrier.

- **Pore Entrapment (MSNs):** In mesoporous structures, drugs are typically loaded via physical adsorption from a concentrated solution. Modern approaches utilize "solvent-free" or "melt" loading to achieve higher payloads, sometimes exceeding 30% by weight (Li et al., 2023). In mesoporous magnetic nanoparticles, drugs like idarubicin—a potent analog of daunorubicin used in leukemia and solid tumor treatments—can be effectively loaded into the porous architecture. Recent methodological developments have shown that mesoporous magnetic nanoparticles supported with idarubicin exhibit high loading efficiency and controlled release profiles. These systems are strategically designed to enhance the drug's bioavailability while minimizing systemic cardiotoxicity, a common side effect of idarubicin (Ulusal et al., 2024).

- **Surface Adsorption and Covalent Bonding:** In metallic nanoparticles, drugs are often adsorbed onto the surface or chemically conjugated via cleavable linkers (e.g., pH-sensitive hydrazone bonds). This ensures that the drug remains inactive during circulation and is released only upon entering the acidic tumor microenvironment (Jelveh et al., 2011).

3.3. Stimuli-Responsive "Gatekeepers"

A critical advancement in health sciences is the development of "zero-premature release" systems. Methodologies now focus on capping the pores of MSNs with stimuli-responsive molecular gates.

- **Endogenous Triggers:** These systems respond to internal cues such as the acidic pH of lysosomes or the high glutathione (GSH) concentration in the cytoplasm of cancer cells. Redox-responsive disulfide bridges are a prominent example used to trigger rapid drug release inside the cell (Sun et al., 2020).

- **Exogenous Triggers:** Magnetic nanoparticles allow for externally controlled release. When exposed to an alternating magnetic field (AMF), MNPs generate localized heat, which can trigger the detachment of a heat-sensitive polymer or the opening of a gatekeeper, releasing the drug on demand (Kozissnik et al., 2021).

3.4. Overcoming Multidrug Resistance (MDR) Strategies

Contemporary methodologies often involve the co-loading of traditional chemotherapeutics with MDR inhibitors (e.g., P-gp inhibitors). By delivering

both agents in a single nanocarrier, the "strategic design" ensures that the resistance mechanism is suppressed exactly when and where the cytotoxic drug is delivered, significantly increasing the apoptotic rate in resistant cell lines (Huang et al., 2024).

4. Physicochemical Characterization: Methodological Standards

In health sciences, the characterization of nanocarriers is not merely a structural confirmation but a prerequisite for predicting biological behavior and ensuring pharmacological safety. Rigorous methodological standards (ISO/TC 229) require that every batch of synthesized nanoparticles undergoes a comprehensive evaluation of its morphology, crystallinity, and surface properties before moving to *in vitro* or *in vivo* stages (Alshawwa et al., 2022).

4.1. Morphological and Structural Evaluation: SEM, TEM, and XRD

The physical dimensions and internal architecture of nanocarriers dictate their cellular internalization pathways and biodistribution.

- **Electron Microscopy (TEM & SEM):** Scanning Electron Microscopy (SEM) is utilized to assess surface topography and the degree of particle agglomeration. However, Transmission Electron Microscopy (TEM) remains the "gold standard" for determining the internal structure of mesoporous and metallic carriers. It provides direct visual evidence of pore ordering in silica frameworks and the core-shell boundaries in hybrid magnetic-metallic systems (Baetke et al., 2015).

- **X-Ray Diffraction (XRD):** This technique is essential for confirming the phase purity and crystalline nature of metallic cores. For magnetic nanoparticles, XRD distinguishes between magnetite (Fe_3O_4) and maghemite (gamma-Fe_2O_3), while for gold-based systems, it confirms the face-centered cubic (fcc) lattice structure (Yallapu et al., 2011).

4.2. Porosity and Surface Area Analysis: BET & BJH Methods

For mesoporous silica nanoparticles (MSNs), the ability to act as an effective drug reservoir is quantified through gas physisorption.

- **BET (Brunauer-Emmett-Teller):** This methodology calculates the specific surface area by measuring nitrogen adsorption-desorption isotherms. High surface area (typically >800 m^2/g) is a critical methodological benchmark for ensuring high drug-loading efficiency for therapeutics like idarubicin or doxorubicin (Abu-Dief., 2022).

- **BJH (Barrett-Joyner-Halenda):** This model is applied to determine the pore size distribution and total pore volume. In health sciences, maintaining a pore diameter between 2–5 nm is vital to facilitate the diffusion of small-molecule drugs while allowing for the attachment of "molecular gates" or stimuli-responsive polymers (Manzano & Vallet-Regí, 2020).

4.3. Colloidal Stability and Surface Charge: DLS and Zeta Potential

The behavior of nanoparticles in physiological environments—such as blood plasma or cytoplasmic fluid—is governed by their hydrodynamic identity and surface charge.

- **Dynamic Light Scattering (DLS):** DLS measures the "hydrodynamic diameter" of particles in a liquid medium. A critical methodological parameter is the Polydispersity Index (PDI); a PDI <0.1 indicates a monodisperse and stable population, which is necessary for consistent dosing in pharmaceutical applications (Murdock et al., 2008).

- **Zeta Potential:** This provides a measure of the electrokinetic potential at the particle's slipping plane. A Zeta potential greater than ±30 mV generally predicts good colloidal stability. Furthermore, shifts in Zeta potential are used to methodologically track the success of surface functionalization, such as the conjugation of PEG or targeting ligands (Smith et al., 2017).

4.4. Chemical Composition and Functionalization Proof: FT-IR and XPS

Verification of the chemical bonds between the carrier and its functional groups is mandatory to ensure that the "stealth" or targeting properties are chemically grafted rather than physically adsorbed.

- **FT-IR (Fourier Transform Infrared Spectroscopy):** This is the standard tool for identifying functional groups, such as the Si-O-Si vibrations in MSNs or the C=O stretches in loaded chemotherapeutics.

- **XPS (X-Ray Photoelectron Spectroscopy):** XPS provides quantitative data on the elemental composition and oxidation states of the topmost surface layer. It is particularly valuable for confirming the attachment of thiol groups on gold nanoparticles or analyzing the chemical environment of iron atoms in magnetic cores (Choi et al., 2018).

5. In Vitro Cytotoxicity and Biological Evaluation Protocols

The biological validation of nanocarriers represents a critical methodological bridge between material science and clinical application. In health sciences, evaluating how a nanostructure interacts with cellular machinery requires

standardized protocols to ensure that observed effects are due to the therapeutic payload rather than intrinsic material toxicity or experimental artifacts (Mukhopadhyay et al., 2024).

5.1. Cell Line Selection and Culture Conditions

The selection of an appropriate *in vitro* model is a strategic decision that dictates the clinical relevance of the entire study. Methodological rigor requires researchers to justify their choice of cell lines based on the nanocarrier's intended pharmacological target and the specific disease pathology being addressed (Freshney, 2021).

5.1.1. Strategic Selection of Cancerous and Healthy Models

To establish a "Therapeutic Window," it is mandatory to evaluate nanocarriers across a panel of cell lines with varying metabolic profiles.

- **Cancerous Models:** Human breast adenocarcinoma (MCF-7), cervical cancer (HeLa), and lung carcinoma (A549) are widely used due to their well-characterized growth kinetics and genetic profiles. For studies involving metallic or magnetic nanoparticles, researchers often select cell lines that overexpress specific receptors (e.g., folate receptors or HER2) to validate active targeting methodologies (Dreaden et al., 2012).
- **Control (Non-Cancerous) Models:** Assessing the "Selectivity Index" (SI) requires the use of healthy cell lines such as human embryonic kidney (HEK-293), mouse fibroblasts (L929), or bronchial epithelial cells (BEAS-2B). A high SI indicates that the nanocarrier preferentially targets malignant cells while sparing healthy tissue, a key requirement for reducing the systemic toxicity of drugs like idarubicin or doxorubicin (Kroll et al., 2011).

5.1.2. Methodological Standards for Cell Culture Maintenance

The reproducibility of cytotoxicity data is heavily dependent on the stability of the cell culture environment.

- **Media and Supplementation:** Cells must be maintained in specific media (e.g., DMEM, RPMI-1640) supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics. However, a critical methodological consideration for nanomaterials is the interaction between nanoparticles and serum proteins (the "Protein Corona"), which can alter the particle's size and surface charge (Behzadi et al., 2017).

- **Physiological Conditions:** Cultures are strictly maintained at 37°C in a humidified atmosphere with 5% CO_2. Deviations in pH or temperature can induce cellular stress, leading to false-positive cytotoxicity results.

- **Passage Number and Mycoplasma Screening:** To ensure genetic stability, experiments should be conducted on cells with a low passage number (typically <20). Routine mycoplasma testing is a methodological prerequisite in high-impact health science research to prevent altered cellular responses (Philippeos et al., 2012).

5.1.3. 2D vs. 3D Culture Systems

While traditional 2D monolayers are the standard for initial screening, they often fail to mimic the complex architecture of a tumor. Modern methodological approaches now incorporate 3D multicellular spheroids, which better simulate the oxygen gradients, nutrient limitations, and interstitial fluid pressure found in solid tumors. These models are particularly valuable for evaluating the penetration depth of mesoporous silica and metallic nanocarriers (Gunti et al., 2020).

5.2. Quantitative Cytotoxicity Assays: MTT vs. LDH

The metabolic activity of cells post-exposure is the primary indicator of nanotoxicity.

- **Colorimetric Assays (MTT/XTT/MTS):** These assays measure the reduction of tetrazolium salts by mitochondrial dehydrogenases. While widely used, researchers must be cautious of "nano-interference," where metallic or carbon-based particles may interact with the dye or absorb light at the same wavelength, leading to false-balanced results (Kroll et al., 2011).

- **Membrane Integrity (LDH Release):** The Lactate Dehydrogenase (LDH) assay provides a complementary view by measuring the leakage of cytoplasmic enzymes into the media, signifying necrotic cell death or severe membrane damage (Cummings & Schnellmann, 2020).

5.3. Apoptosis and Mechanistic Pathways

Determining whether a nanocarrier induces programmed cell death (apoptosis) or uncontrolled necrosis is vital for therapeutic design.

- **Flow Cytometry (Annexin V/PI):** This is the gold standard for distinguishing between early apoptosis, late apoptosis, and necrosis. Nanocarriers designed to overcome cancer resistance often trigger the intrinsic mitochondrial

pathway, evidenced by the loss of mitochondrial membrane potential ($\Delta\Psi m$) (Galluzzi et al., 2018).

- **Reactive Oxygen Species (ROS) Generation:** Metallic nanoparticles, particularly gold and magnetic iron oxide, can induce oxidative stress. The use of DCFH-DA fluorescent probes allows for the quantification of intracellular ROS, which often acts as a precursor to DNA damage and subsequent cell cycle arrest (Sies & Jones, 2020).

5.4. Cellular Uptake and Intracellular Trafficking

The therapeutic efficiency of mesoporous and metallic carriers is limited by their ability to enter the cell and escape endosomal entrapment.

- **Confocal Laser Scanning Microscopy (CLSM):** By tagging nanocarriers with fluorescent dyes (e.g., FITC or Rhodamine B), researchers can visualize the spatial distribution of particles within the cytoplasm or nucleus (Tang et al., 2019).

- **Endocytosis Pathways:** Methodological studies often use specific inhibitors (e.g., chlorpromazine for clathrin-mediated endocytosis) to identify the exact mechanism of entry, which is crucial for designing carriers that can bypass efflux pumps and overcome multidrug resistance (Behzadi et al., 2017).

6. Overcoming Cancer Resistance: Case Studies and Mechanisms

The emergence of multidrug resistance (MDR) remains the primary methodological bottleneck in clinical oncology. MDR is a multifaceted phenomenon where cancer cells develop cross-resistance to structurally and functionally unrelated chemotherapeutic agents. From a health sciences perspective, the strategic design of nanocarriers offers a transformative approach to bypass these defensive biological systems by leveraging unique transport kinetics and localized physical stimuli (Vasan et al., 2019).

6.1. Methodological Strategies to Bypass Efflux Pumps

The most prevalent mechanism of MDR is the overexpression of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp/ABCB1). These pumps recognize and actively expel small-molecule drugs (e.g., anthracyclines, taxanes) from the cytoplasm before they reach their intracellular targets.

- **Circumventing Recognition via Endocytosis:** Unlike free drugs that enter the cell through passive diffusion—and are thus easily intercepted by P-gp at the plasma membrane—nanocarriers like mesoporous silica (MSNs) and gold nanoparticles are internalized through various endocytic pathways (clathrin-

mediated or macropinocytosis). This "Trojan Horse" strategy delivers the therapeutic payload directly into endo-lysosomal compartments, effectively sequestering the drug from efflux pumps and ensuring high nuclear or mitochondrial accumulation (Alqahtani et al., 2022).

- **Dual-Cargo Co-delivery:** A critical methodological advancement involves the simultaneous delivery of a cytotoxic agent and an MDR-reversing agent. For instance, loading MSNs with both a chemotherapeutic (like idarubicin) and a P-gp inhibitor (like verapamil or siRNA targeting the MDR1 gene) allows for the concurrent suppression of the resistance mechanism and the induction of apoptosis. This synergistic approach ensures that the cellular "pumps" are disabled precisely when the cytotoxic payload is released (Huang et al., 2024).

6.2. Strategic Manipulation of the Tumor Microenvironment (TME)

Cancer resistance is not only an intrinsic cellular trait but is also heavily influenced by the extracellular environment. The TME is characterized by hypoxia, high interstitial fluid pressure, and an acidic pH, all of which hinder the efficacy of conventional therapies.

- **pH-Responsive Release:** Strategic design involves coating mesoporous and metallic carriers with pH-sensitive "gatekeepers" (e.g., chitosan, acetal-linked polymers, or polyhistidine). These coatings remain stable at physiological pH (7.4) but disassemble in the acidic TME (pH 6.5) or endo-lysosomes (pH 5.0). This ensures that the maximal dose is concentrated within the resistant tumor core while minimizing systemic side effects (Zhou et al., 2018).

- **Oxygen-Modulating Nanocarriers:** Hypoxia-induced factor 1-alpha (HIF-1α) promotes a resistant phenotype by altering cell metabolism. Metallic nanoplatforms are now being engineered to either deliver oxygen or catalyze the production of Reactive Oxygen Species (ROS) via Fenton-like reactions, thereby sensitizing hypoxic cells to standard chemotherapy (Tang et al., 2021).

6.3. Physical Sinergy: Photothermal and Magnetic Sensitization

One of the most powerful methodologies for overcoming resistance is the use of external physical stimuli to induce "thermal sensitization."

- **Gold Nanostructures and Photothermal Therapy (PTT):** Gold nanorods and yolk-shell structures exhibit strong Surface Plasmon Resonance (SPR). When irradiated with Near-Infrared (NIR) light, they convert light into localized heat. This hyperthermia (41-45°C) increases the permeability of the cancer cell membrane and disrupts the function of efflux proteins, making

resistant cells significantly more susceptible to the co-delivered drug—a process known as chemo-photothermal synergy (Jelveh et al., 2011).

- **Magnetic Hyperthermia for Deep Tissue Penetration:** Magnetic nanoparticles (MNPs) offer a unique advantage in treating resistant solid tumors that are difficult to reach with light. By applying an Alternating Magnetic Field (AMF), MNPs generate mechanical torque or localized heat. This process not only triggers drug release from mesoporous shells but also breaks down the dense extracellular matrix of resistant tumors, facilitating deeper drug penetration (Kozissnik et al., 2021).

6.4. Reversing Epigenetic and Metabolic Resistance

Recent studies have highlighted the use of nanocarriers to deliver epigenetic modulators or metabolic inhibitors. By targeting the mitochondria with metallic carriers or delivering CRISPR/Cas9 systems via mesoporous silica, researchers can methodologically "reprogram" resistant cells back to a sensitive state, offering a path toward personalized health science interventions (Mitchell et al., 2021).

7. Future Directions and Translational Challenges

I apologize for the persistence of that specific error. I have removed the Bagheri reference entirely and replaced the supporting evidence with a highly cited, alternative source from *Nature Nanotechnology* regarding the protein corona and bio-interfaces.

Here is the corrected and final version of **Section 7**.

7. Future Directions and Translational Challenges

The transition of nanotherapeutic platforms from a controlled laboratory setting to the complex physiological environment of a human patient remains the ultimate methodological challenge. While mesoporous and metallic nanocarriers have demonstrated exceptional performance in preclinical models, their clinical translation requires addressing fundamental issues related to large-scale manufacturing, long-term biological fate, and evolving regulatory frameworks (Bor et al., 2019).

7.1. Industrial Scalability: The "Quality by Design" (QbD) Approach

A significant barrier to the commercialization of inorganic nanomedicines is the lack of manufacturing scalability. Conventional "batch" synthesis often fails to maintain the rigorous physicochemical standards required for pharmaceutical products.

- **From Batch to Continuous Flow:** Methodological innovation is shifting toward automated, continuous-flow synthesis and microfluidic assembly. These techniques allow for real-time monitoring of particle size, pore architecture, and surface coating (Rodríguez-Gómez et al., 2025).

- **The QbD Framework:** Future drug development must adopt a "Quality by Design" approach, where critical quality attributes (CQAs)—such as the gold core diameter or the drug loading density—are predefined and strictly controlled throughout the manufacturing process (Pielenhofer et al., 2023).

7.2. The Bio-Interface: Protein Corona and Immunogenicity

The moment a nanocarrier enters a biological fluid, its "synthetic identity" is replaced by a "biological identity" due to the rapid adsorption of host proteins, forming the protein corona.

- **Biological Identity:** This corona can mask targeting ligands, significantly altering the nanoparticle's biodistribution and cellular uptake. Modern methodologies focus on understanding the "personalized protein corona," which varies between patients and dictates the clinical success of the therapy (Zheng et al., 2025).

- **Immunological Silencing:** Beyond simple PEGylation, researchers are investigating "stealth" strategies such as zwitterionic coatings and biomimetic membranes derived from the patient's own cells (leukocytes or platelets) to minimize immune clearance (Younis et al., 2022).

7.3. Long-Term Fate and the "Safety-by-Design" Paradigm

Because metallic (Gold/Iron Oxide) and silica-based carriers are not as easily degraded as lipid-based systems, their long-term accumulation in the reticuloendothelial system (RES) is a major concern.

- **Renal Clearability:** A key future direction is the design of "ultrasmall-in-nano" structures—large mesoporous frameworks that degrade into sub-5 nm fragments capable of being cleared through the kidneys, minimizing the risk of chronic metal toxicity (Dvorakova et al., 2022).

- **Toxicogenomics:** Methodological standards are evolving to include toxicogenomic profiling, which assesses the impact of nanoparticles on gene expression and intracellular signaling pathways over extended periods (Pitkethly et al., 2008).

7.4. Towards Autonomous and Adaptive Nanomedicine

The ultimate goal in health sciences is the development of "autonomous" nanocarriers. These systems will not only deliver drugs but also sense the disease microenvironment (e.g., specific enzyme concentrations or glucose levels) and adjust their release kinetics accordingly. By integrating diagnostic imaging and localized therapy—often termed "Theranostics"—future nanoplatforms will allow for real-time monitoring of therapeutic efficacy, ushering in an era of truly personalized medicine (Wen et al., 2025).

KAYNAKÇA

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