SUSTAINABLE AND TRENDING STUDIES IN HEALTH SCIENCES

All Sciences Academy

SUSTAINABLE AND TRENDING STUDIES IN HEALTH SCIENCES

Editor
Prof. Dr. Fatih HATİPOĞLU

Sustainable and Trending Studies in Health Sciences
Editor: Prof. Dr. Fatih HATİPOĞLU

Design: All Sciences Academy Design
Published Date: May 2026
Publisher's Certification Number: 72273
ISBN: 978-625-8993-52-3

www.allsciencesacademy.com
allsciencesacademy@gmail.com

CONTENT

Telomere Biology and Genome Stability: Epigenetic and Metabolic Influences on Drug Response and Urinary Tract Infections

Tugba AGBEKTAS[1]
Cemile ZONTUL[2]
Gonca KABAK[3]

[1]Sivas Cumhuriyet University, Yıldızeli Vocational School, Department of Food Processing, Food Technology Program, Sivas, Türkiye. tubaagbektas@cumhuriyet.edu.tr
Orcid ID: 0009-0004-4533-0019
[2]Sivas Cumhuriyet University, Yıldızeli Vocational School, Department of Chemistry and Chemical Processing Technologies, Biochemistry Program, Sivas, Türkiye. cemilezontul@cumhuriyet.edu.tr, Orcid ID: 0000-0002-1436-5145
[3]Department of Medical Biochemistry, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Türkiye. gonca.kabak24@gmail.com, Orcid ID: 0009-0004-2430-5076

ABSTRACT

Telomere biology plays a critical role in maintaining genome stability, regulating cellular aging, and modulating responses to stress, infection, and therapy. Telomere shortening, oxidative DNA damage, and impaired DNA repair mechanisms contribute to genomic instability, cellular senescence, and chronic inflammation. In this context, telomerase reverse transcriptase (TERT) is not only involved in telomere maintenance but also regulates mitochondrial function, metabolic adaptation, oxidative stress responses, and drug resistance. Epigenetic mechanisms, including DNA methylation, histone modifications, non-coding RNAs, and sirtuin-mediated regulation, further influence telomere dynamics and treatment response. In urinary tract infections, telomere dysfunction, epithelial barrier damage, extracellular matrix remodeling, and altered membrane transporter activity may contribute to recurrent infection and variable antibiotic responses. Therefore, the telomere–metabolism–infection network provides a valuable framework for understanding disease progression, therapeutic resistance, and personalized medicine strategies.

Keywords – Telomere biology; genome stability; TERT; epigenetic regulation; oxidative stress; drug resistance; urinary tract infections; personalized medicine

1. INTRODUCTION

Telomeres are specialized nucleoprotein structures located at the ends of eukaryotic chromosomes and are essential for maintaining genome integrity. By protecting chromosome ends from degradation, recombination, and end-to-end fusion, telomeres prevent inappropriate activation of DNA damage responses. In humans, telomeres consist of repetitive TTAGGG sequences and are stabilized by the shelterin complex, which includes TRF1, TRF2, RAP1, TIN2, POT1, and TPP1. Proper telomere maintenance is critical for cellular proliferation, genome stability, and healthy aging, whereas telomere dysfunction contributes to genomic instability, cellular senescence, and cancer development.

2. TELOMERE BIOLOGY AND GENOME STABILITY

Telomeres are specialized nucleoprotein structures located at the ends of eukaryotic chromosomes and play a crucial role in maintaining genome integrity. Human telomeres are composed of repetitive TTAGGG sequences and protect chromosome ends from degradation, recombination, and end-to-end fusion. Their primary function is to prevent chromosome termini from being recognized as DNA double-strand breaks by cellular DNA damage response mechanisms (Blackburn, 2001). Telomeric DNA is protected by a

multiprotein complex known as shelterin. This complex consists of TRF1, TRF2, RAP1, TIN2, POT1, and TPP1 proteins and contributes to chromosome-end stability by promoting the formation of a specialized structure called the T-loop. Among these components, TRF2 plays a particularly important role in preventing chromosomal end-to-end fusions by suppressing the ATM-dependent DNA damage response (de Lange, 2005). The maintenance of telomere integrity is essential for cellular proliferative capacity. Disruption of telomere stability promotes genomic instability and may contribute to the initiation and progression of cancer. In addition, telomere length is widely regarded as an important biological marker of cellular aging (Shay & Wright, 2019).

2.1. Telomere Shortening and Cellular Aging

In somatic cells, telomeres progressively shorten with each cell division as a result of the "end-replication problem" during DNA replication. Recent studies indicate that telomere shortening is associated not only with cellular aging but also with genomic instability, mitochondrial dysfunction, and chronic inflammation. When telomeres reach a critically short length, DNA damage response pathways are activated, leading to cell-cycle arrest and senescence through the p53/p21 and p16INK4a/Rb pathways. Telomere dysfunction has also been linked to cancer, cardiovascular diseases, and neurodegenerative disorders (Victorelli & Passos, 2023). Oxidative stress is another important factor that accelerates telomere shortening. Because telomeric regions are rich in guanine residues, they are particularly vulnerable to oxidative DNA damage. Reactive oxygen species (ROS)-induced damage can impair telomere replication and thereby accelerate cellular aging. Current research further suggests that lifestyle factors, chronic inflammation, and metabolic disorders significantly influence telomere dynamics (Rossiello et al., 2022). During cellular aging, telomere shortening reduces proliferative capacity and contributes to the development of a pro-inflammatory cellular state. Senescent cells secrete various inflammatory mediators, collectively referred to as the senescence-associated secretory phenotype (SASP). This process promotes chronic inflammation and increases the risk of age-related diseases (Campisi, 2013). Telomerase is mainly composed of telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC). TERC serves as a template for the synthesis of telomeric repeats, while TERT provides the reverse transcriptase activity required to add these sequences to chromosome ends. Through this mechanism, telomerase prevents excessive telomere loss during replication and contributes to the maintenance of genomic stability (Shay & Wright, 2019). High telomerase activity in embryonic stem cells, germ cells, and certain adult stem cell populations is essential for preserving long-term proliferative potential. In contrast, the suppression of telomerase activity in

most somatic cells is considered an important tumor-suppressive mechanism that limits uncontrolled cell proliferation. However, telomerase is reactivated in approximately 85–90% of cancer cells, making it one of the key molecular features of cellular immortality (Hanahan, 2022). Recent studies have also demonstrated that telomerase has functions beyond telomere elongation. TERT has been reported to regulate cellular signaling pathways, mitochondrial function, DNA damage responses, and oxidative stress mechanisms. Therefore, telomerase has become an important therapeutic target in both aging biology and cancer research (Shay, 2025).

2.2. Genomic Instability Mechanisms

Genomic instability is characterized by an increased frequency of mutations, deletions, translocations, and chromosomal abnormalities within the cellular genome. Telomere dysfunction is considered one of the major contributors to genomic instability. Critically shortened or dysfunctional telomeres may cause chromosome ends to be recognized as DNA double-strand breaks, thereby triggering end-to-end chromosomal fusions (Artandi & DePinho, 2010). One of the key mechanisms associated with telomere dysfunction is the breakage–fusion–bridge (BFB) cycle. In this process, unprotected chromosome ends fuse with one another, break during mitosis, and subsequently undergo repeated fusion events. As a consequence, extensive chromosomal rearrangements and genetic heterogeneity may arise within cells. These mechanisms are particularly important in tumor initiation and progression. DNA damage response pathways also play a critical role in the maintenance of genome stability. ATM and ATR kinases are activated in response to telomere damage and replication stress. Disruption of the shelterin complex may lead to excessive activation of these signaling pathways, ultimately resulting in cellular senescence or cell death (d'Adda di Fagagna et al., 2003).

2.3. Oxidative Stress and DNA Damage

Oxidative stress is a biological condition that occurs when the production of reactive oxygen species (ROS) exceeds the capacity of cellular antioxidant defense systems. Telomeric DNA is highly vulnerable to oxidative damage because of its high guanine content. Accumulation of ROS can induce single- and double-strand breaks in telomeric regions, thereby accelerating telomere shortening and dysfunction (von Zglinicki, 2002). Mitochondrial dysfunction, chronic inflammation, and exposure to environmental toxins are among the major sources of oxidative stress. Immune cells, particularly those activated during chronic infections, generate large amounts of ROS. This increased oxidative burden disrupts telomere integrity and accelerates cellular aging. Oxidative stress also

interferes with DNA repair mechanisms and thereby contributes to genomic instability. Defects in repair pathways such as base excision repair (BER) and homologous recombination may further aggravate telomere dysfunction. These processes are implicated in the development of age-related pathologies, including cancer, neurodegenerative diseases, and other chronic disorders (Sfeir & de Lange, 2012).

2.4. Epigenetic Regulation and Telomere Dynamics

Telomere biology is regulated not only by genetic mechanisms but also by epigenetic modifications. DNA methylation, histone modifications, and chromatin remodeling are key epigenetic processes that influence telomere stability. The maintenance of heterochromatin structure in telomeric and subtelomeric regions is essential for preserving proper telomere function (Blasco, 2007). Histone deacetylases, particularly SIRT1, play an important role in the regulation of telomeric chromatin. As a NAD^+-dependent deacetylase, SIRT1 is closely associated with DNA damage response pathways, cellular stress adaptation, metabolic regulation, and genome stability during aging (Palacios et al., 2010). MicroRNAs also contribute to the epigenetic regulation of telomere biology. Certain microRNAs suppress telomerase activity by downregulating TERT expression, whereas others regulate the expression of shelterin complex components. Through these mechanisms, microRNAs may directly influence telomere dynamics in aging, cancer, and infection-related processes (Blaudez et al., 2020). The impact of environmental factors on epigenetic regulation suggests that telomere biology is a dynamic and modifiable process. Nutrition, psychological stress, infections, and toxic exposures can significantly affect telomere length and stability. Therefore, telomere biology has become an important field of investigation in personalized medicine, aging research, and biomarker development (Epel & Prather, 2018).

3. TELOMERASE COMPLEX AND TERT-MEDIATED CELLULAR REGENERATION

Telomerase is a ribonucleoprotein enzyme complex responsible for maintaining telomere length and preserving chromosomal stability. The core components of this complex are telomerase reverse transcriptase (TERT) and the telomerase RNA component (TERC). TERC provides the RNA template required for the synthesis of telomeric repeats, whereas TERT catalyzes the addition of these repeat sequences to chromosome ends through its reverse transcriptase activity. In this way, telomerase counteracts progressive telomere shortening and contributes to the maintenance of genome integrity. In addition to its catalytic function, the structural domains of telomerase play important roles in enzyme activity and telomere binding. The TEN domain,

in particular, is one of the major functional regions involved in regulating the interaction between telomerase and telomeric DNA (Liu et al., 2024). Moreover, shelterin-associated proteins such as TPP1 and ACD are involved in recruiting telomerase to chromosome ends. TPP1 is especially important because it enhances telomerase processivity and promotes efficient telomere elongation (Tornesello et al., 2023).

3.1. TERT Activation and Transcriptional Control

The regulation of telomerase activity is primarily determined by the expression level of telomerase reverse transcriptase (TERT). In normal somatic cells, TERT expression is highly restricted or nearly absent; however, in many cancer cells, TERT is reactivated, allowing cells to escape replicative senescence and acquire unlimited proliferative capacity. Therefore, TERT activation is considered one of the key molecular mechanisms underlying cellular immortality in cancer. Among the mechanisms responsible for TERT activation, TERT promoter mutations are particularly important. These mutations generate novel binding sites for ETS family transcription factors, thereby enhancing TERT transcription in various malignancies (Tornesello et al., 2023). TERT promoter mutations are frequently detected in tumors such as glioblastoma, melanoma, hepatocellular carcinoma, and bladder cancer. The most common hotspot mutations occur at the C228T and C250T regions, both of which significantly increase TERT promoter activity and telomerase expression (Liu et al., 2016). TERT transcription is controlled by a complex regulatory network involving multiple transcription factors. Positive regulators such as c-Myc, NF-κB, and SP1 bind to the TERT promoter and enhance gene expression. In particular, the c-Myc/MAX complex activates TERT transcription by binding to E-box sequences within the promoter region. SP1 contributes to basal transcriptional activity through its interaction with GC-rich promoter regions, whereas NF-κB may increase TERT expression through inflammation- and tumor-associated signaling pathways. Additional transcription factors, including STAT3, HIF-1α, and AP-1, have also been implicated in the regulation of TERT expression (Cong & Shay, 2008; Shay & Wright, 2019). In contrast, tumor suppressor proteins such as p53 and WT1 negatively regulate TERT expression. p53-mediated repression helps maintain telomerase activity under strict control during cellular stress and DNA damage responses. Loss or impairment of these suppressive mechanisms in cancer cells allows persistent TERT activation and supports malignant progression. Furthermore, oncogenic signaling pathways such as PI3K/AKT/mTOR and MAPK may indirectly promote TERT transcription and telomerase activity (Liu et al., 2013; Shay & Wright, 2019). Epigenetic mechanisms also play a critical role in the regulation of TERT expression. DNA methylation, histone acetylation, histone methylation, and chromatin

remodeling can alter the chromatin structure of the TERT promoter and thereby influence its transcriptional activity. In cancer cells, epigenetic reprogramming of the TERT promoter is frequently associated with active chromatin marks. For example, histone modifications such as H3K4me3 and H3K9ac may support transcriptional activation, particularly in mutant TERT alleles (Dogan & Forsyth, 2024). Recent studies have shown that TERT promoter hypermethylation may paradoxically be associated with increased TERT expression in certain cancer types. This phenomenon may be explained by methylation-mediated inhibition of repressor binding sites within the promoter region. In addition, chromatin remodeling complexes and non-coding RNAs have been reported to contribute to TERT regulation. MicroRNAs, particularly miR-138 and miR-491, can suppress telomerase activity by reducing TERT mRNA stability and expression (Liu et al., 2004; Dogan & Forsyth, 2024).

3.2. TERT and Cancer Metabolism

TERT not only maintains telomere length but also exerts important regulatory effects on cellular metabolism. In cancer cells, TERT activation is closely associated with glycolytic reprogramming, mitochondrial function, and the oxidative stress response. These effects enhance the energy-producing capacity of tumor cells and support their proliferation, invasion, and survival (Palamarchuk et al., 2023). In particular, TERT has been shown to regulate mitochondrial function and control the generation of reactive oxygen species (ROS), thereby reducing oxidative cellular damage. In cancer cells, TERT activation also supports aerobic glycolysis, commonly known as the “Warburg effect.” In this metabolic state, cells preferentially rely on glycolysis for energy production even in the presence of oxygen, while generating biosynthetic intermediates required for rapid proliferation. TERT has been reported to increase glucose uptake by enhancing the expression of glycolytic enzymes and promoting lactate production (Choi et al., 2023). Furthermore, TERT may protect mitochondrial DNA from oxidative damage and improve the efficiency of ATP production. Recent studies have indicated that TERT contributes to tumor progression through interactions with Wnt/β-catenin and MYC-related metabolic pathways. Activation of Wnt/β-catenin signaling increases TERT expression, whereas elevated TERT levels further enhance β-catenin-mediated transcription, thereby establishing a positive feedback loop. Similarly, the MYC oncoprotein activates the TERT promoter and supports cellular growth and metabolic reprogramming (Choi et al., 2023). In addition, TERT may influence fatty acid metabolism and amino acid biosynthesis. In particular, it has been suggested to contribute to the metabolic demands of cancer cells through the regulation of glutamine metabolism. This role of TERT in metabolic adaptation enables tumor cells to better respond to the tumor

microenvironment and may increase their metastatic potential (Palamarchuk et al., 2023).

3.3. Effects of Telomerase on Drug Resistance

High telomerase activity is closely associated with the development of resistance to chemotherapy and radiotherapy in cancer cells. Cells with elevated TERT expression are more resistant to DNA damage and can evade apoptotic signaling pathways (Yan et al., 2023). Telomerase activity contributes to cellular survival under genotoxic stress by enhancing the efficiency of DNA repair mechanisms. In particular, TERT has been linked to DNA damage response pathways mediated by ATM/ATR and DNA-PK. TERT also suppresses cell death by increasing the expression of anti-apoptotic proteins. Anti-apoptotic molecules such as BCL-2 and survivin have been shown to be activated through TERT-related mechanisms, thereby reducing the effectiveness of chemotherapeutic agents. Moreover, high telomerase activity in cancer stem cells is considered one of the major contributors to tumor recurrence, progression, and treatment resistance (Yan et al., 2023). Therefore, telomerase inhibitors and TERT-targeted therapies represent important strategies in current oncological research. In recent years, telomerase-based immunotherapies and nanotechnology-assisted approaches have attracted increasing attention (Siteni et al., 2024). Telomerase inhibitors such as imetelstat are being evaluated in clinical trials for various hematological malignancies. In addition, TERT peptide-based cancer vaccines and CAR-T cell therapies are considered promising therapeutic approaches. Nanoparticle-based drug delivery systems aim to selectively transport telomerase inhibitors to tumor tissues. These systems may reduce systemic toxicity while improving therapeutic efficacy. Furthermore, experimental studies using CRISPR/Cas9-based gene editing technologies to suppress TERT expression have gained momentum and may provide new opportunities for targeted cancer therapy in the future (Siteni et al., 2024).

3.4. TERT-Associated Signaling Pathways

TERT is involved in several cellular signaling pathways, including PI3K/AKT, NF-κB, and Wnt/β-catenin, all of which regulate cell proliferation, inflammation, survival, and stress adaptation. Activation of the PI3K/AKT pathway enhances telomerase activity by promoting TERT phosphorylation and facilitating its nuclear translocation. Similarly, the NF-κB pathway can stimulate TERT transcription under inflammatory conditions, thereby supporting tumor-promoting processes. The Wnt/β-catenin pathway is another important regulator of TERT expression. Upon activation, β-catenin cooperates with TCF/LEF transcription factors to

induce TERT promoter activity. In turn, TERT may further strengthen Wnt signaling, creating a reciprocal regulatory loop that enhances cellular proliferation. This bidirectional interaction has been particularly associated with the development of aggressive phenotypes in stem-like tumor cells. Beyond its nuclear functions, TERT also exerts regulatory effects within mitochondria. Mitochondrial TERT has been reported to preserve mitochondrial DNA stability, reduce reactive oxygen species production, and support cellular energy metabolism. These non-canonical functions of TERT have attracted considerable attention in recent years because of their relevance to aging, neurodegenerative disorders, and cancer biology. TERT-mediated mitochondrial protection may enhance antioxidant defense mechanisms, increase cellular tolerance to stress, and suppress apoptosis-related signaling pathways.

4. TELOMERE-PROTECTIVE COMPLEXES AND DNA REPAIR

4.1. Shelterin Complex and Protection of Chromosome Ends

The shelterin complex is a specialized nucleoprotein structure composed of six core proteins: TRF1, TRF2, RAP1, TIN2, POT1, and TPP1. This complex protects telomeres from being recognized as DNA damage sites. TRF1 and TRF2 bind to double-stranded telomeric DNA, whereas POT1 binds to the single-stranded 3′ overhang. TIN2 acts as a central bridging protein by connecting TRF1, TRF2, and the POT1–TPP1 subcomplex. Shelterin is essential for maintaining genome stability because it prevents chromosome ends from activating DNA damage response pathways. Disruption of shelterin components may activate ATM- and ATR-mediated signaling, leading to chromosomal fusions, cellular senescence, or genomic instability. TRF1 plays a major role in telomere replication by preventing replication fork stalling within telomeric regions. In this way, TRF1 suppresses telomere fragility and supports accurate DNA replication. Recent studies suggest that altered TRF1 expression in cancer cells may influence telomere stability and tumor progression. TRF2 is a key protective factor that prevents telomeres from being recognized as DNA double-strand breaks. It suppresses ATM kinase activation and contributes to the formation of the T-loop structure, thereby protecting chromosome ends. Loss of TRF2 can result in telomere fusion through non-homologous end joining, leading to genomic instability. Therefore, TRF2 is regarded as a critical regulator of both aging and cancer biology. RAP1 contributes to telomere homeostasis through its interaction with TRF2. Although RAP1 does not directly bind telomeric DNA, it localizes to telomeres via TRF2. Its functions include regulation of telomere length, control of homologous recombination, and modulation of inflammatory signaling pathways. RAP1 has also been implicated in metabolic regulation and NF-κB-associated inflammation.

POT1 binds to the single-stranded telomeric 3′ overhang and forms a heterodimer with TPP1. This complex protects telomere ends and suppresses ATR-dependent DNA damage signaling. TPP1, encoded by the ACD gene, is also essential for recruiting telomerase to telomeres. The POT1–TPP1 complex enhances telomerase processivity and contributes to telomere length regulation. Mutations in POT1 have been associated with several hematological malignancies and solid tumors by promoting telomere dysfunction and genomic instability. TIN2 serves as a central adaptor protein within the shelterin complex. It directly interacts with TRF1, TRF2, and TPP1, thereby maintaining the structural stability of the complex. Recent evidence indicates that TIN2 is not merely a structural component but also actively participates in telomeric DNA organization and T-loop formation. TIN2 deficiency can activate both ATM- and ATR-dependent DNA damage responses, resulting in impaired cell growth and chromosomal abnormalities. Recent studies further suggest that the shelterin complex may be organized into distinct subcomplexes, such as TRF1–TIN2–TPP1–POT1 and TRF2–RAP1. This organization is thought to play an important role in regulating telomeric chromatin architecture.

4.2. CST Complex, Replicative Stress, and DNA Repair Mechanisms

The CST complex, composed of CTC1, STN1, and TEN1 proteins, is an important nucleoprotein complex involved in telomere replication. It contributes to telomere integrity particularly during late replication by stabilizing the DNA polymerase α-primase complex. Because telomeric regions are rich in guanine, they are prone to forming secondary DNA structures such as G-quadruplexes. These structures may cause replication fork stalling. The CST complex helps resolve such structures, promotes replication continuity, and prevents telomere fragility. In addition to its telomeric functions, CST also operates at genome-wide sites of replicative stress and supports the restart of DNA synthesis.

CTC1, the largest subunit of the CST complex, plays a central role in maintaining telomere stability and genome integrity. It binds to single-stranded DNA regions formed during replication and participates in the replicative stress response. Mutations in CTC1 have been linked to telomere biology disorders such as Coats plus syndrome, dyskeratosis congenita, and bone marrow failure. These mutations may cause telomere shortening, chromosomal instability, and accumulation of DNA damage. Replicative stress occurs when replication forks slow down or stall during DNA synthesis. Telomeres are among the most vulnerable genomic regions to replicative stress because of their repetitive sequence composition and secondary structures. The CST complex functions in coordination with the ATR/CHK1 pathway to promote the restart of stalled replication forks. If

single-stranded DNA regions generated during replicative stress are not properly repaired, chromosomal breaks and cell death may occur.

4.3. DNA Double-Strand Breaks and Repair Mechanisms

DNA double-strand breaks are among the most severe forms of DNA damage threatening genome integrity. Cells repair these lesions mainly through homologous recombination and non-homologous end joining. Double-strand breaks occurring at telomeric regions are particularly important because inappropriate repair may result in chromosomal end-to-end fusions. Shelterin and CST complexes suppress inappropriate DNA repair responses by preventing telomeres from being interpreted as DNA breaks. Under replicative stress, double-strand breaks activate ATM and ATR kinases, which regulate cell-cycle checkpoints. If the damage cannot be repaired, cells may undergo senescence or apoptosis.

4.4. Alternative Lengthening of Telomeres

Alternative lengthening of telomeres is a telomerase-independent mechanism that maintains telomere length through homologous recombination-based processes. ALT is observed in certain cancer types, including osteosarcoma, glioblastoma, and soft tissue sarcomas. ALT-positive cells typically exhibit high genomic instability and increased sister chromatid exchange. SLX4IP is one of the regulatory proteins involved in ALT activity. It contributes to the maintenance of ALT by regulating homologous recombination-mediated telomere elongation. Loss of SLX4IP suppresses ALT activity, induces telomere dysfunction, and reduces tumor cell proliferation. Therefore, SLX4IP is considered a potential therapeutic target in ALT-positive cancers.

5. EPIGENETIC AND METABOLIC REGULATION OF CELLULAR AGING

5.1. Sirtuins, Epigenetic Regulation, and Cellular Aging

Telomeres and subtelomeric regions are characterized by compact heterochromatin structures. Epigenetic modifications such as H3K9 trimethylation and histone hypoacetylation are important for maintaining telomeric silencing. Disruption of these histone modifications has been associated with reduced telomere stability and increased genomic instability. Sirtuin family proteins regulate chromatin organization primarily through histone deacetylation. These mechanisms help maintain compact chromatin architecture at telomeric regions and suppress inappropriate DNA damage responses. Loss of telomeric heterochromatin is considered one of the major epigenetic features of aging. SIRT1 is one of the best-characterized members

of the NAD^+-dependent class III histone deacetylase family. It regulates chromatin structure and gene expression through the deacetylation of histones such as H1K26, H3K9, and H4K16. SIRT1 also interacts with DNA repair proteins, p53, and FOXO transcription factors, thereby regulating cellular stress responses. In terms of telomere stability, SIRT1 contributes to the preservation of shelterin proteins and the maintenance of telomeric chromatin integrity. During aging, reduced SIRT1 expression is associated with chromatin disorganization, DNA damage accumulation, and cellular senescence. NAD^+ metabolism plays a central role in cellular energy homeostasis and epigenetic regulation. Since SIRT1 activity depends directly on NAD^+ availability, changes in cellular energy status can significantly affect sirtuin function. Age-related decline in NAD^+ levels is linked to mitochondrial dysfunction, increased oxidative stress, reduced SIRT1 activity, and activation of inflammatory pathways. In particular, the AMPK–SIRT1–PGC-1α axis is considered a key regulatory system controlling mitochondrial biogenesis and energy metabolism.

5.2. Epigenetic Aging Biomarkers

Epigenetic aging refers to biological aging processes that can be assessed through DNA methylation patterns and chromatin alterations. Biomarker systems known as epigenetic clocks are used to estimate biological age independently of chronological age. SIRT1 activity is strongly associated with epigenetic aging. Reduced SIRT1 expression is linked to telomere shortening, increased inflammation, and accumulation of DNA damage. Moreover, age-related changes in histone acetylation patterns contribute to the development of age-associated diseases. SIRT1 also has important immunomodulatory effects. It suppresses proinflammatory cytokine production by deacetylating the p65 subunit of NF-κB. During bacterial and viral infections, changes in SIRT1 activity may influence the magnitude of the immune response. In chronic inflammatory conditions, decreased SIRT1 activity is associated with increased oxidative stress, mitochondrial dysfunction, and cellular aging. These mechanisms may contribute to progressive epithelial cell damage, particularly in chronic infections and urinary tract infections. SIRT1 activators have therefore been proposed as potential therapeutic agents for controlling infection-related inflammation.

5.3. Agmatine Metabolism and Cellular Defense Mechanisms

Agmatine is a biogenic amine synthesized from L-arginine by arginine decarboxylase. It plays a role in cellular stress responses, signal transduction, and energy metabolism. As an early intermediate in the polyamine biosynthetic pathway, agmatine is linked to the synthesis of putrescine, spermidine, and spermine. It can also be degraded by agmatinase into

putrescine and urea, thereby contributing to nitrogen balance and metabolic homeostasis. Recent literature indicates that agmatine is not only an intermediate metabolite but also a rapidly modulated signaling molecule under cellular stress conditions. Polyamines such as putrescine, spermidine, and spermine are essential for DNA stability, RNA translation, and chromatin organization. Agmatine functions both as a precursor and as a regulatory metabolite within this system. Polyamine metabolism is directly associated with cellular proliferation and aging. Increased spermidine levels have been shown to activate autophagy and influence epigenetic regulation by altering histone acetylation. This is particularly important for cellular stress responses and aging biology. Agmatine may also affect energy metabolism by modulating mitochondrial membrane potential and regulating oxidative stress responses. By reducing ROS production, it may limit cellular damage. Activation of the PGC-1α signaling pathway, which is associated with mitochondrial biogenesis, supports the functional connection between polyamine metabolism and cellular energy regulation.

6. INFECTION, INFLAMMATION, AND URINARY TRACT PATHOPHYSIOLOGY

6.1. Antimicrobial Effects of Agmatine

Agmatine may exert antimicrobial effects by interfering with bacterial growth and communication mechanisms. This effect is associated with suppression of biofilm formation and reduction of bacterial virulence. In Gram-negative bacteria, agmatine may contribute to growth inhibition by disrupting membrane stability and activating cellular stress responses. In urinary tract infections, bacteria adapt to the host environment by reorganizing their metabolic pathways. Polyamine metabolism and agmatine conversion may enhance bacterial stress tolerance and survival. On the host side, agmatine may modulate inflammatory responses by regulating nitric oxide synthesis and cytokine production. Thus, agmatine can be considered a potential immunometabolic defense modulator.

6.2. Matrix Metalloproteinases and Tissue Remodeling

Matrix metalloproteinase-9 is a zinc-dependent endopeptidase that plays an important role in the degradation of basement membrane components, particularly type IV collagen. MMP-9 is synthesized as an inactive proenzyme and becomes activated through proteolytic cleavage. This activation commonly occurs in inflammatory environments through the action of other proteases or oxidative stress. Structurally, MMP-9 contains a catalytic domain, a zinc-binding region, and a hemopexin-like C-terminal domain. These domains determine substrate specificity and extracellular

matrix binding. Current studies indicate that MMP-9 is not only involved in tissue degradation but also participates in cell migration, angiogenesis, and immune regulation. Tissue inhibitor of metalloproteinase-1 is one of the main endogenous inhibitors of MMP-9. TIMP-1 binds to MMP-9 and blocks its proteolytic activity, thereby preserving extracellular matrix integrity. The balance between MMP-9 and TIMP-1 is crucial for determining the extent of tissue remodeling and inflammation. A shift toward MMP-9 activity may lead to excessive matrix degradation and chronic tissue damage, whereas TIMP-1 predominance may promote fibrotic processes. During inflammation, immune cells, particularly neutrophils and macrophages, increase MMP-9 production. Although this response may contribute to pathogen clearance, uncontrolled MMP-9 activity can cause tissue injury. MMP-9 is induced by proinflammatory cytokines such as TNF-α and IL-1β and, together with ROS production, accelerates extracellular matrix degradation. In chronic inflammatory conditions, increased MMP-9 activity may drive pathological tissue remodeling.

6.3. Extracellular Matrix Degradation in Bacterial Infections

During bacterial infections, both pathogens and the host immune response contribute to extracellular matrix degradation. MMP-9 facilitates basement membrane breakdown at sites of bacterial invasion and supports inflammatory cell migration. However, some bacteria can manipulate MMP activity to overcome tissue barriers and promote the spread of infection. This process may increase tissue damage, particularly in chronic infections. The urothelium forms the first line of defense against urinary tract infections. This barrier is protected by tight junction proteins such as claudins and occludins, as well as by the glycosaminoglycan layer. Increased MMP-9 activity may disrupt urothelial barrier integrity and facilitate bacterial adhesion. TIMP-1 attempts to counterbalance this process by preserving epithelial structure. In recurrent urinary tract infections, an imbalance in the MMP-9/TIMP-1 axis may increase urothelial permeability and contribute to chronic infection.

6.4. Nuclear Stress Proteins and Cellular Adaptation

The nuclear stress response is an adaptive system activated in response to environmental stressors such as DNA damage, oxidative stress, hypoxia, and infection. Its main purpose is to maintain genomic integrity and determine the appropriate cellular outcome, including repair, cell-cycle arrest, senescence, or apoptosis. When DNA damage occurs, ATM and ATR kinases are activated, initiating cell-cycle checkpoint responses. In this process, transcription factors such as p53 regulate the expression of stress response genes and guide cellular adaptation. Nuclear protein 1 and NUPR-

like proteins are small intrinsically disordered proteins involved in cellular stress responses. Members of the NUPR family function as transcriptional coregulators and facilitate adaptation to cellular stress. These proteins are particularly involved in survival responses in pancreatic and epithelial cells. They have also been associated with suppression of apoptotic signaling and activation of DNA repair mechanisms.

6.5. Cellular Defense and Transcriptional Reprogramming

Under stress conditions, cells reorganize their gene expression profiles and initiate survival-oriented transcriptional programs. The NUPR family contributes to this process by interacting with transcription factors and modulating gene expression without directly altering chromatin structure. This reprogramming includes activation of antioxidant defense genes, metabolic adaptation, and adjustment of cell-cycle checkpoints. Under chronic stress, such adaptations may lead to persistent phenotypic changes. Following DNA damage, cells either activate repair pathways or enter permanent cell-cycle arrest, known as senescence. NUPR proteins may indirectly influence these cellular decision-making processes. NUPR2 is considered a stress adaptation protein related to the NUPR family and may enhance cellular resilience, particularly under chronic inflammatory conditions. Increased oxidative stress and DNA damage during infection may trigger NUPR2-mediated adaptive responses. In chronic inflammation, NUPR2 may support stress tolerance by reprogramming cellular metabolism and suppressing apoptosis. This mechanism may contribute to the persistence of infection, especially in epithelial tissues.

6.6. Membrane Transporters, Drug Response, and Kidney Physiology

Organic cation transporters are integral membrane proteins that mediate the transport of many endogenous and exogenous cationic compounds across cell membranes. These transporters are particularly important in the distribution and elimination of drugs in kidney, liver, and intestinal epithelial tissues. OCT proteins have broad substrate specificity and can transport numerous drugs, toxins, and metabolites into cells, thereby contributing to renal excretion processes. OCT2, encoded by the SLC22A2 gene, is highly expressed in the basolateral membrane of renal proximal tubular cells. It plays a major role in the renal uptake of platinum-based chemotherapeutics such as cisplatin, as well as metformin and several antibiotics. OCT2-mediated transport represents one of the initial steps determining urinary drug excretion. Therefore, alterations in OCT2 function can directly influence plasma drug concentrations and toxicity risk. In kidney injury, OCT2 expression may change and significantly affect drug elimination. The

pharmacokinetics of antibiotics includes absorption, distribution, metabolism, and excretion. The kidney is the principal organ responsible for eliminating many hydrophilic antibiotics. Transporters such as SLC22A2 may facilitate the entry of aminoglycosides and certain antibiotics into renal cells, thereby influencing both therapeutic efficacy and potential toxicity. Consequently, antibiotic dosing may vary according to renal function and transporter expression. OCT2-mediated drug uptake is also a key mechanism of nephrotoxicity, particularly for agents such as cisplatin. Excessive accumulation of these drugs in proximal tubular cells can lead to mitochondrial dysfunction, ROS production, and cell death. Polymorphisms in SLC22A2 may alter OCT2 function and contribute to interindividual differences in drug response, especially for drugs such as metformin and cisplatin.

6.7. Relationship Between Telomere Dynamics and Urinary Tract Infections

Urothelial cells constitute the primary epithelial barrier of the urinary tract and provide the first line of defense against pathogens. Telomere shortening and DNA damage accumulation in these cells may reduce proliferative capacity and impair epithelial integrity. Telomere dysfunction can induce replicative senescence, thereby limiting urothelial regeneration. Senescent urothelial cells may also develop a senescence-associated secretory phenotype and secrete proinflammatory cytokines. This inflammatory environment may increase susceptibility to urinary tract infections. Chronic and recurrent urinary tract infections create a continuous inflammatory and oxidative stress environment, which may accelerate telomere shortening. Reactive oxygen species damage telomeric DNA because of its guanine-rich structure. Persistent immune cell activation, increased ROS production, and overload of DNA repair systems may further promote telomere erosion. As a result, premature aging and reduced regenerative capacity may occur in urothelial cells. Uropathogenic bacteria, particularly Escherichia coli, can contribute to DNA damage through adhesion, invasion, and toxin release. Bacterial toxins and ROS generated during the inflammatory response may increase DNA double-strand breaks and promote genomic instability.

7. EPIGENETIC MECHANISMS IN DRUG AND ANTIBIOTIC RESPONSE

7.1. Epigenetic Mechanisms and Drug Resistance

DNA methylation is one of the major epigenetic mechanisms regulating gene expression through the addition of methyl groups to cytosine residues, especially within CpG islands. This process is generally associated with gene

silencing and contributes to long-term reprogramming of cellular responses. In cancer and chronic infection, DNA methylation is an important determinant of drug response. Hypermethylation of tumor suppressor genes may reduce apoptotic responses and contribute to chemotherapy resistance. Similarly, methylation-mediated repression of DNA repair genes may alter cellular sensitivity to drug-induced DNA damage. In infectious diseases, methylation changes in host immune response genes may impair pathogen clearance and increase the risk of chronic infection. Histone modifications such as acetylation, methylation, and phosphorylation also regulate gene expression by altering chromatin structure. Histone acetylation generally opens chromatin and enhances transcription, whereas deacetylation is associated with gene repression. Histone modifications contribute to drug resistance by reprogramming stress response genes and DNA repair mechanisms. Cancer cells may use histone-modifying enzymes to develop adaptive resistance to chemotherapy. Non-coding RNAs, including miRNAs, lncRNAs, and siRNAs, are important regulatory molecules that do not encode proteins but control gene expression. These RNA species are involved in the regulation of telomerase activity and telomere stability. Telomere-related RNAs such as TERC and TERRA contribute to telomere structure maintenance and telomerase regulation. miRNAs may indirectly control telomere elongation by targeting TERT expression. In the context of drug resistance, non-coding RNAs can influence treatment outcomes by regulating stress adaptation in both cancer cells and infection-related processes. Telomerase, composed of the TERT/TERC complex, extends the replicative lifespan of cells by preventing telomere shortening. In cancer cells, elevated telomerase activity is a major mechanism underlying unlimited proliferative capacity. Increased telomerase activity contributes to chemotherapy resistance because maintenance of telomere stability can suppress DNA damage-induced apoptosis. This is particularly relevant for DNA-damaging chemotherapeutic agents. Telomerase activity may also support adaptive drug resistance by modulating cellular stress responses.

7.2 Epigenetic Factors in Antibiotic Resistance

Antibiotic resistance is shaped not only by genetic mutations but also by epigenetic mechanisms. Bacteria can regulate gene expression in response to environmental stress through DNA methylation and chromatin-like structures. On the host side, epigenetic changes may indirectly influence antibiotic efficacy by suppressing or overactivating immune response genes. In chronic infections, epigenetic reprogramming may contribute to the development of bacterial persister phenotypes. In this state, bacteria do not necessarily inactivate antibiotics directly; rather, they temporarily enter a dormant-like condition that allows them to survive antimicrobial treatment.

8. SYSTEMS BIOLOGY AND PERSONALIZED MEDICINE APPROACHES

8.1. Systems Biology Perspective on the Telomere–Metabolism–Infection Network

Systems biology aims to understand cellular behavior by evaluating biological processes as interconnected networks rather than isolated molecules or pathways. Telomere biology, metabolic reprogramming, and infection responses are closely linked, particularly in urinary tract infections and chronic inflammatory conditions. Therefore, modern research increasingly investigates these processes through multi-omics integration and computational modeling. The multi-omics approach involves the integrated analysis of genomic, epigenomic, transcriptomic, proteomic, and metabolomic data. This approach allows researchers to identify dynamic interactions within biological systems. Processes such as telomere shortening, DNA damage response, and metabolic stress are regulated not only at the genetic level but also at the levels of protein expression and metabolite production. For example, telomere dysfunction may increase NAD^+ consumption by affecting cellular energy metabolism, while also altering inflammatory gene expression. Understanding these complex interactions requires multi-omics integration.

8.2. Transcriptomic and Proteomic Analyses

Transcriptomic analysis reveals which genes are actively expressed in a cell, whereas proteomic analysis identifies the functional protein products of these genes. In telomere biology, the expression levels of TERT, shelterin proteins, and DNA damage response proteins are major determinants of cellular aging. During inflammation and infection, NF-κB signaling, interferon-response genes, and cytokine expression profiles may change substantially. At the proteomic level, these changes are associated with increased MMP-9, TIMP-1, and other inflammatory proteins. Therefore, integrated transcriptomic and proteomic analysis provides a more accurate understanding of disease mechanisms. In urinary tract infections, the transcriptomic profile of urothelial cells may help determine the severity of bacterial invasion and the risk of chronicity. Metabolomic analysis involves the systematic study of metabolites, which represent the final products of cellular processes. Telomere shortening and cellular aging are closely associated with major metabolic pathways, including NAD^+, ATP, ROS, and polyamine metabolism. Reduced NAD^+ levels can decrease SIRT1 activity, thereby affecting epigenetic regulation and accelerating cellular aging.

Similarly, increased oxidative stress metabolites can enhance DNA damage and accelerate telomere shortening. In infection processes, metabolomic profiling can reveal how pathogens reprogram host metabolism. For example, bacteria may alter amino acid and iron metabolism to gain a survival advantage, contributing to chronic infection.

8.3. Artificial Intelligence-Assisted Biomarker Analysis

Artificial intelligence and machine learning methods are increasingly used for the analysis of multi-omics data. These approaches can identify disease-associated biomarkers from high-dimensional biological datasets. Complex relationships among telomere biology, metabolic networks, and infection processes are difficult to fully resolve using conventional statistical methods alone. AI-based algorithms can analyze these multivariable systems and identify novel biomarkers for early diagnosis and prognosis. In chronic urinary tract infections, AI-based models may help predict recurrence risk and support the development of personalized treatment strategies. In addition, integrated evaluation of telomere length, inflammatory markers, and metabolic profiles may enable the generation of individualized risk scores.

8.4. Clinical Translation and Personalized Medicine

One of the main goals of systems biology is to translate basic scientific findings into clinical practice. The relationship among telomere biology, metabolism, and infection provides a strong foundation for personalized medicine. For example, individuals with marked telomere shortening may benefit more from therapies targeting oxidative stress and inflammation. Similarly, polymorphisms in transporter genes such as SLC22A2 may require individualized drug dosing. In infectious diseases, the patient's metabolic and epigenetic profile may directly influence antibiotic response. Therefore, future treatment strategies are expected to be shaped not only by the characteristics of the pathogen but also by host biology. This approach may enable the development of more effective, targeted, and safer treatment protocols, particularly for chronic diseases such as recurrent urinary tract infections.

9. CONCLUSION

In conclusion, telomere biology, genome stability, epigenetic regulation, metabolic adaptation, and infection-related immune responses are closely interconnected biological processes. Telomere shortening and telomere dysfunction contribute to genomic instability, cellular senescence, oxidative stress, and impaired tissue regeneration. These mechanisms are particularly

important in cancer progression, drug resistance, aging-related cellular decline, and chronic inflammatory conditions. TERT and telomerase activity play central roles not only in telomere maintenance but also in cellular metabolism, mitochondrial function, oxidative stress regulation, DNA damage responses, and treatment resistance. In cancer cells, TERT activation supports unlimited proliferation, metabolic reprogramming, and resistance to chemotherapy and radiotherapy. Therefore, telomerase and TERT-associated pathways represent important therapeutic targets in modern oncology. In addition, epigenetic mechanisms, including DNA methylation, histone modifications, non-coding RNAs, and sirtuin-mediated regulation, significantly influence telomere dynamics, cellular aging, immune responses, and drug sensitivity. Metabolic regulators such as NAD^+, SIRT1, polyamines, and agmatine further connect telomere biology with cellular defense mechanisms, inflammation, and infection-related tissue damage. In the context of urinary tract infections, telomere shortening, oxidative stress, epithelial barrier dysfunction, extracellular matrix remodeling, and altered drug transporter activity may contribute to recurrent infection and variable treatment responses. These findings suggest that recurrent urinary tract infections should not be evaluated only as microbial events, but also as conditions shaped by host genomic stability, epigenetic status, metabolic profile, and immune regulation. Overall, the telomere–metabolism–infection network provides a promising framework for understanding disease progression, therapeutic resistance, and personalized treatment strategies. Future studies integrating genomics, epigenomics, transcriptomics, proteomics, metabolomics, and artificial intelligence-based biomarker analysis may provide new insights into early diagnosis, recurrence prediction, and individualized therapeutic approaches.

REFERENCES

Abu-Zeinah, G., et al. (2020). Role of POT1 in human cancer. Cancers, 12(10), 2739.

Aguado, J., d'Adda di Fagagna, F., & Wolvetang, E. (2023). Epigenetic hallmarks of aging. Nature Aging, 3(9), 1056–1072.

Artandi, S. E., & DePinho, R. A. (2010). Telomeres and telomerase in cancer. Carcinogenesis, 31(1), 9–18.

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. Cell Research, 21(3), 381–395.

Baylin, S. B., & Jones, P. A. (2016). Epigenetic determinants of cancer. Nature Reviews Cancer, 16(9), 574–586.

Blackburn, E. H. (2001). Switching and signaling at the telomere. Cell, 106(6), 661–673.

Blaudez, D., Botta, A., & Brondello, J. M. (2020). Telomere and telomerase regulation by microRNAs. Frontiers in Genetics, 11, 606.

Brew, K., & Nagase, H. (2010). The tissue inhibitors of metalloproteinases (TIMPs): An ancient family with structural and functional diversity. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1803(1), 55–71.
Brunet, A., Sweeney, L. B., Sturgill, J. F., et al. (2004). Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science, 303(5666), 2011–2015.
Cai, S. W., & Chen, Y. (2023). Emerging roles of CST complex in genome-wide replication rescue. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1878(2), 188890.
Campisi, J. (2013). Aging, cellular senescence, and cancer. Annual Review of Physiology, 75, 685–705.
Casadesús, J., & Low, D. A. (2013). Epigenetic gene regulation in bacterial systems. Microbiology and Molecular Biology Reviews, 77(3), 472–495.
Chang, H. H. Y., Pannunzio, N. R., Adachi, N., & Lieber, M. R. (2017). Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nature Reviews Molecular Cell Biology, 18(8), 495–506.
Chen, Y., et al. (2015). SLC22A2 polymorphisms and metformin pharmacokinetics. Clinical Pharmacology & Therapeutics, 97(6), 558–568.
Ciarimboli, G. (2012). Membrane transporters as mediators of cisplatin effects and side effects. Scientifica, 2012(1), 473829.
Ciccia, A., & Elledge, S. J. (2010). The DNA damage response: Making it safe to play with knives. Nature, 408(6881), 759–770.
Cong, Y. S., & Shay, J. W. (2008). Whole regulation of human telomerase. Nature Reviews Genetics.
Covarrubias, A. J., Perrone, R., Grozio, A., & Verdin, E. (2021). NAD+ metabolism and its roles in cellular processes during ageing. Nature Reviews Molecular Cell Biology, 22(2), 119–141.
d'Adda di Fagagna, F., et al. (2003). A DNA damage checkpoint response in telomere-initiated senescence. Nature, 426(6963), 194–198.
de Lange, T. (2005). Shelterin: The protein complex that shapes and safeguards human telomeres. Genes & Development, 19(18), 2100–2110.
Deng, Y., Zhang, Y., Zhou, J., & Wang, X. (2023). CTC1 and human telomere biology disorders. International Journal of Molecular Sciences, 24(11), 9384.
Dogan, F., & Forsyth, N. R. (2024). TERT promoter methylation is oxygen-sensitive and regulates telomerase activity. Biomolecules, 14(1), 131.
Eisenberg, T., Knauer, H., Schauer, A., Büttner, S., Ruckenstuhl, C., Carmona-Gutierrez, D., ... & Madeo, F. (2009). Induction of autophagy by spermidine promotes longevity. Nature Cell Biology, 11(11), 1305–1314.
Epel, E. S., & Prather, A. A. (2018). Stress, telomeres, and psychopathology: Toward a deeper understanding of a triad of early aging. Annual Review of Clinical Psychology, 14, 371–397.
Esteller, M. (2011). Non-coding RNAs in human disease. Nature Reviews Genetics, 12(12), 861–874.
Giacomini, K. M., et al. (2010). Membrane transporters in drug development. Nature Reviews Drug Discovery, 9(3), 215–236.
Hamburg, M. A., & Collins, F. S. (2010). The path to personalized medicine. New England Journal of Medicine, 363(4), 301–304.
Hanahan, D. (2022). Hallmarks of cancer: New dimensions. Cancer Discovery, 12(1), 31–46.

Harley, C. B., Futcher, A. B., & Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. Nature, 345(6274), 458–460.

Harman, A. H., & Bryan, T. M. (2024). Telomere maintenance and the DNA damage response: A paradoxical alliance. Frontiers in Cell and Developmental Biology, 12, 1472906.

Hasin, Y., Seldin, M., & Lusis, A. (2017). Multi-omics approaches to disease. Genome Biology, 18, 83.

Hassanshahi, A., Soti, M., Ranjbar, H., Razavinasab, M., Pirmoradi, Z., Kohlmeier, K. A., Janahmadi, M., & Shabani, M. (2023). Perspectives on agmatine neurotransmission in acute and chronic stress-related conditions. Current Neuropharmacology, 23(15), 1560–1574.

Horvath, S., & Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nature Reviews Genetics, 19(6), 371–384.

Janovič, T., Perez, G. I., Boelting, G., & Schmidt, J. C. (2025). TRF1 and TRF2 form distinct shelterin subcomplexes at telomeres. Cell Reports, 44, 116178.

Jones, P. A. (2012). Functions of DNA methylation. Nature Reviews Genetics, 13(7), 484–492.

Kahraman, N., et al. (2019). Polyamine metabolism and bacterial stress response. Microbiology Research, 10(2), 112–120.

Kallingal, A., Krzemieniecki, R., Maciejewska, N., Brankiewicz-Kopcińska, W., & Baginski, M. (2024). TRF1 and TRF2: Pioneering targets in telomere-based cancer therapy. Journal of Cancer Research and Clinical Oncology, 150(7), 353.

Kauppinen, A., Suuronen, T., Ojala, J., Kaarniranta, K., & Salminen, A. (2013). Antagonistic crosstalk between NF-κB and SIRT1 in the regulation of inflammation and metabolic disorders. Cellular Signalling, 25(10), 1939–1948.

Koepsell, H. (2020). Organic cation transporters in health and disease. Pharmacological Reviews, 72(1), 253–322.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell, 128(4), 693–705.

Laube, G., & Bernstein, H. G. (2017). Agmatine: Multifunctional arginine metabolite and magic bullet in clinical neuroscience? Biochemical Journal, 474(16), 2619–2640.

Laube, G., & Bernstein, H. G. (2017). Agmatine: Multifunctional arginine metabolite and magic bullet in clinical neuroscience? Biochemical Journal, 474(16), 2619–2640.

Lautrup, S., Sinclair, D. A., Mattson, M. P., & Fang, E. F. (2023). NAD+ in brain aging and neurodegenerative disorders. Nature Aging, 3(1), 16–28.

Libbrecht, M. W., & Noble, W. S. (2015). Machine learning applications in genetics. Nature Reviews Genetics, 16(6), 321–332.

Liu, L., Saldanha, S. N., Pate, M. S., Andrews, L. G., & Tollefsbol, T. O. (2004). Epigenetic regulation of human telomerase reverse transcriptase promoter activity during cellular differentiation. Genes, Chromosomes and Cancer, 41(1), 26–37.

Liu, M., Zhang, Y., Jian, Y., Gu, L., Zhang, D., Zhou, H., & Xu, Z.-X. (2024). The regulations of telomerase reverse transcriptase (TERT) in cancer. Cell Death & Disease, 15(90).

Liu, T., Yuan, X., & Xu, D. (2016). Cancer-specific telomerase reverse transcriptase (TERT) promoter mutations: Biological and clinical implications. Genes, 7(7), 38.

Lovejoy, C. A., Li, W., Reisenweber, S., Thongthip, S., Bruno, J., De Lange, T., ... & ALT Starr Cancer Consortium. (2012). Loss of ATRX, genome instability, and an altered DNA damage response are hallmarks of the alternative lengthening of telomeres pathway. PLoS genetics, 8(7), e1002772.

Madeo, F., Eisenberg, T., Pietrocola, F., & Kroemer, G. (2018). Spermidine in health and disease. Science, 359(6374), eaan2788.

McClintock, B. (1941). The stability of broken ends of chromosomes in Zea mays. Genetics, 26(2), 234–282.

Moinard, C., Cynober, L., & de Bandt, J. P. (2005). Polyamines: Metabolism and implications in human diseases. Clinical Nutrition, 24(2), 184–197.

Molderings, G. J., Heinen, A., Menzel, S., & Göthert, M. (2004). Agmatine, a metabolic derivative of arginine and decarboxylation product, is a signalling molecule in the nervous system. Pharmacology & Therapeutics, 104(3), 215–234.

Morrissey, K. M., et al. (2013). The role of OCT2 in renal drug handling. Drug Metabolism and Disposition, 41(3), 585–593.

Nagase, H., Visse, R., & Murphy, G. (2019). Structure and function of matrix metalloproteinases and TIMPs. Cardiovascular Research, 121(1), 22–37.

Pabla, N., & Dong, Z. (2008). Cisplatin nephrotoxicity: Mechanisms and renoprotection. Kidney International, 73(9), 994–1007.

Palacios, J. A., et al. (2010). SIRT1 contributes to telomere maintenance and augments global homologous recombination. Journal of Cell Biology, 191(7), 1299–1313.

Palamarchuk, A. I., Kovalenko, E. I., & Streltsova, M. A. (2023). Multiple actions of telomerase reverse transcriptase in cell death regulation. Biomedicines, 11(4), 1091.

Patti, G. J., Yanes, O., & Siuzdak, G. (2012). Innovation in metabolomics. Nature Reviews Molecular Cell Biology, 13(4), 263–269.

Pegg, A. E. (2016). Functions of polyamines in mammals. Journal of Biological Chemistry, 291(29), 14904–14912.

Podlevsky, J. D., et al. (2021). TIN2 is an architectural protein that facilitates TRF2-mediated interactions on telomeric DNA. Nucleic Acids Research, 49(22), 13000–13015.

Poole, L. A., et al. (2021). Telomere maintenance mechanisms and SLX4IP in ALT cancers. Cell Reports

Reyes, J. C., et al. (2014). The stress response protein NUPR1: A transcriptional regulator in cancer and stress adaptation. Oncogene, 33(32), 4220–4229.

Reyes, J. C., et al. (2021). NUPR1 as a stress response regulator in cancer and inflammation. Cells, 10(10), 2700.

Rossiello, F., Jurk, D., Passos, J. F., & d'Adda di Fagagna, F. (2022). Telomere dysfunction in ageing and age-related diseases. Nature cell biology, 24(2), 135-147.

Sahai, E., & Astsaturov, I. (2021). Extracellular matrix dynamics in infection and inflammation. Nature Reviews Molecular Cell Biology, 22(11), 734–748.

Satriano, J. (2004). Agmatine: At the crossroads of the arginine pathways. Annals of the New York Academy of Sciences, 1030, 34–43.

Scarpulla, R. C. (2011). Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. Biochimica et Biophysica Acta, 1813(7), 1269–1278.
Sfeir, A., & de Lange, T. (2012). Removal of shelterin reveals the telomere end-protection problem. Science, 336(6081), 593–597.
Shah, P., & Swiatlo, E. (2008). A multifaceted role for polyamines in bacterial pathogens. Molecular Microbiology, 68(1), 10–20.
Shay, J. W. (2025). Role of telomeres and telomerase in aging and cancer. Nature Reviews Molecular Cell Biology, 26(1), 15–29.
Shay, J. W., & Wright, W. E. (2019). Telomeres and telomerase: Three decades of progress. Nature Reviews Genetics, 20(5), 299–309.
Siteni, S., Grichuk, A., & Shay, J. W. (2024). Telomerase in cancer therapeutics. Cold Spring Harbor Perspectives in Medicine, 16(12), a041703.
Stewart, J. A., Wang, F., Chaiken, M. F., Kasbek, C., Chastain, P. D., Wright, W. E., & Price, C. M. (2012). Human CST promotes telomere duplex replication and general replication restart after fork stalling. The EMBO journal, 31(17), 3537-3549.
Stewart, J. A., Wang, F., Chaiken, M. F., Kasbek, C., Chastain, P. D., & Price, C. M. (2024). Human CST promotes telomere duplex replication and general replication restart after fork stalling. Nature Communications, 15, 2418.
Tornesello, M. L., Cerasuolo, A., Starita, N., Amiranda, S., Bonelli, P., Tuccillo, F. M., Buonaguro, F. M., Buonaguro, L., & Tornesello, A. L. (2023). Reactivation of telomerase reverse transcriptase expression in cancer: The role of TERT promoter mutations.
Vandooren, J., Van den Steen, P. E., & Opdenakker, G. (2013). Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): The next decade. Critical Reviews in Biochemistry and Molecular Biology, 48(3), 222–272.
Vandooren, J., Van den Steen, P. E., & Opdenakker, G. (2017). Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9. Critical Reviews in Biochemistry and Molecular Biology, 52(4), 393–401.
Vaquero, A., Scher, M., Erdjument-Bromage, H., Tempst, P., Serrano, L., & Reinberg, D. (2007). SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation. Nature, 450(7168), 440–444.
Vaziri, H., Dessain, S. K., Eaton, E. N., et al. (2001). hSIR2SIRT1 functions as an NAD-dependent p53 deacetylase. Cell, 107(2), 149–159.
Victorelli, S., & Passos, J. F. (2023). Telomeres and cell senescence—size matters not. EBioMedicine, 91, 104553.
Vogel, C., & Marcotte, E. M. (2012). Insights from proteomics. Nature Reviews Genetics, 13(4), 227–232.
von Zglinicki, T. (2002). Oxidative stress shortens telomeres. Trends in Biochemical Sciences, 27(7), 339–344.
von Zglinicki, T. (2002). Oxidative stress shortens telomeres. Trends in Biochemical Sciences, 27(7), 339–344.
Worthen, R. J., et al. (2015). Polyamine metabolism in bacterial survival and virulence. Frontiers in Cellular and Infection Microbiology, 5, 84.
Yan, S., Lin, S., Qiu, H., Wang, X., He, Y., Wang, C., & Huang, Y. (2023). Regulation of telomerase towards tumor therapy. Cell & Bioscience, 13(228).

Yang, R., Han, Y., Guan, X., Hong, Y., Meng, J., Ding, S., Long, Q., & Yi, W. (2023). Regulation and clinical potential of telomerase reverse transcriptase (TERT/hTERT) in breast cancer. Cell Communication and Signaling, 21(218).

Yang, Y., Liu, Y., Wang, Y., Chao, Y., Zhang, J., Jia, Y., Tie, J., & Hu, D. (2022). Regulation of SIRT1 and its roles in inflammation. Frontiers in Immunology, 13, 831168.

Yu, X., Liu, M.-M., Zheng, C.-Y., Liu, Y.-T., Wang, Z., & Wang, Z.-Y. (2023). Telomerase reverse transcriptase and neurodegenerative diseases. Frontiers in Immunology, 14, 1165632.

Zeman, M. K., & Cimprich, K. A. (2014). Causes and consequences of replication stress. Nature Cell Biology, 16(1), 2–9.

Zhang, D., Li, J., & Li, T. (2022). Agmatine mitigates palmitate-induced mitochondrial and metabolic dysfunction in microvascular endothelial cells. Human & Experimental Toxicology, 41, 1–13.

Zhang, Y., Hou, K., Tong, J., Zhang, H., Xiong, M., Liu, J., & Jia, S. (2023). The altered functions of shelterin components in ALT cells. International Journal of Molecular Sciences, 24(23), 16830.

From Gut to Brain: A Bibliometric Analysis of Microbiota Research in Alzheimer's Disease

Erhan BALLI[1]

Rahmi YILDIZ[2]

1- Agri Ibrahim Cecen University, Department of Pharmacy, Pharmacy Services Program, 04100, Agri, Turkey
2- Van Yuzuncu Yil University, Vocational School of Gevas, Division of Veterinary, Van, Turkey

ABSTRACT

Objectives: The aim of this study was to systematically evaluate the global research landscape on the relationship between gut microbiota and Alzheimer's disease using bibliometric methods. It aimed to identify publication trends, key contributors, collaboration networks, and emerging research themes in this rapidly evolving field.
Methods: This bibliometric analysis included studies (2009–2026) retrieved from the Web of Science Core Collection using the terms "Gut AND (Microbiota OR Microbiome) AND Alzheimer". After PRISMA-guided screening, 3,129 publications were analyzed using VOSviewer (v1.6.20) with text mining and visualization.
Results: A total of 3,129 publications were included, accumulating 67,191 citations and yielding an H-index of 164. Publication output and citation frequency increased markedly after 2017. China and the USA were the most productive countries, while the USA demonstrated the highest citation impact. Leading institutions included the University of California system, Harvard University, and the Chinese Academy of Sciences. Keyword analysis identified Alzheimer's disease, gut microbiota, gut–brain axis, neuroinflammation, and oxidative stress as dominant themes.
Discussion: These findings indicate that research on the gut microbiota–Alzheimer's disease axis is rapidly expanding and interdisciplinary. The focus on mechanistic pathways and microbiota-targeted interventions highlights translational potential, but further multi-omics studies and well-designed clinical trials are needed.

Key Words: Alzheimer's disease; gut microbiota; microbiome; gut–brain axis; bibliometric analysis.

INTRODUCTION

Alzheimer's disease is the most common neurodegenerative disorder in the elderly and is characterized by progressive deterioration in memory, language, executive functions, and activities of daily living (Tenchov et al., 2024). Alzheimer's disease (AD) is the most prevalent cause of dementia worldwide and is recognised as a major public health concern due to its escalating incidence, progressive nature, and the paucity of effective therapeutic interventions available ("2024 Alzheimer's disease facts and figures," 2024). Although the pathogenesis of Alzheimer's disease has been extensively studied, classical hypotheses focusing on amyloid-β and tau proteins fail to explain all its biological and clinical aspects (Dias & Socodato, 2025). This situation has increased interest in researching new mechanisms that may play a role in the development and progression of the disease.

The gut microbiota is the entire collection of microorganisms that colonize the gastrointestinal tract; this community plays important roles in metabolism, immune response, and maintaining health, and is shaped by environmental and genetic factors (Sokol, 2019).In recent years, the gut microbiota has emerged as a significant regulator of central nervous system functions via the gut–brain axis. It affects the immune system, metabolic processes and neuroinflammation, and is closely related to brain function and behaviour (Cryan & Dinan, 2012). It has been reported that the microbiota plays a critical role in the pathogenesis of neurodegenerative diseases by interacting with the immune and nervous systems in complex ways (Fung et al., 2017).

A growing number of experimental and clinical studies suggest that imbalances in the gut microbiota may be associated with the development and progression of Alzheimer's disease (Zhao et al., 2025). In particular, it has been suggested that changes in microbiota composition may increase systemic inflammation, disrupt blood-brain barrier integrity, and affect the production of neuroactive metabolites (Kowalski & Mulak, 2019; Vogt et al., 2017). These findings have led to the relationship between gut microbiota and Alzheimer's disease becoming an interdisciplinary and rapidly developing field of research.

The rapid increase in the number of publications in this field makes it difficult to comprehensively evaluate literature and systematically identify research trends. Bibliometric analysis is a powerful method that enables the quantitative evaluation of scientific output, the identification of influential authors and institutions, the mapping of collaboration networks, and the identification of research foci and future trends (Donthu et al., 2021). Visualization-based software, such as VOSviewer, makes it possible to present complex bibliometric data through understandable network maps (van Eck & Waltman, 2010).

This study analyzed publications on gut microbiota and Alzheimer's disease published between 2009 and 2026 using bibliometric methods. The aim of the study is to reveal the global outlook of this research field, identify prominent research themes, and shed light on future research directions.

MATERIALS AND METHODS

Data Collection Source and Search Strategy

The data used in the bibliometric study were obtained from the Web of Science (WOS) database on January 21, 2026. The selected period covers the years 2009 to 2026. The query used includes the terms “Gut and (Microbiota and Microbiome) and Alzheimer*” in the titles, abstracts, and keywords of all documents in the database. This query searched for all publications containing the words “gut,” “microbiota,” or ‘microbiome’ and

the term “Alzheimer's” in any form (e.g., Alzheimer's, Alzheimers, Alzheimer's, etc.).
Documents were first filtered according to language criteria, and only studies written in English were included. Subsequently, a restriction was applied based on document type; not only original research articles but also review articles were included in the evaluation. The PRISMA 2020 flow diagram (Page et al., 2021) showing the information flow at different stages of the analysis is presented in Figure 1.
Citation information (e.g., author(s), document title, and publication year), bibliographic information (e.g., institutional information, categories, and publisher), abstract and keywords (e.g., author keywords and index keywords), and other features were obtained for quantitative and qualitative analysis using document export settings in the WOS database.

Data Analysis

In this bibliometric study, collaboration networks, research focuses, and current trends in the fields of gut microbiota and Alzheimer's disease were analyzed using VOSviewer (version 1.6.20, Leiden University, Netherlands). VOSviewer is an effective software for visualizing and interpreting large-scale bibliometric data (van Eck & Waltman, 2010; Yildiz et al., 2024).
The data was systematically collected from the WOS Core Collection database and analyzed together with full records and citation references. Country/region and institutional collaborations, author contributions, and keyword co-occurrence analyses were performed using VOSviewer.
Publication and citation trends were evaluated using Microsoft Office Excel 2019. Findings were presented using data visualization methods such as network and density maps.

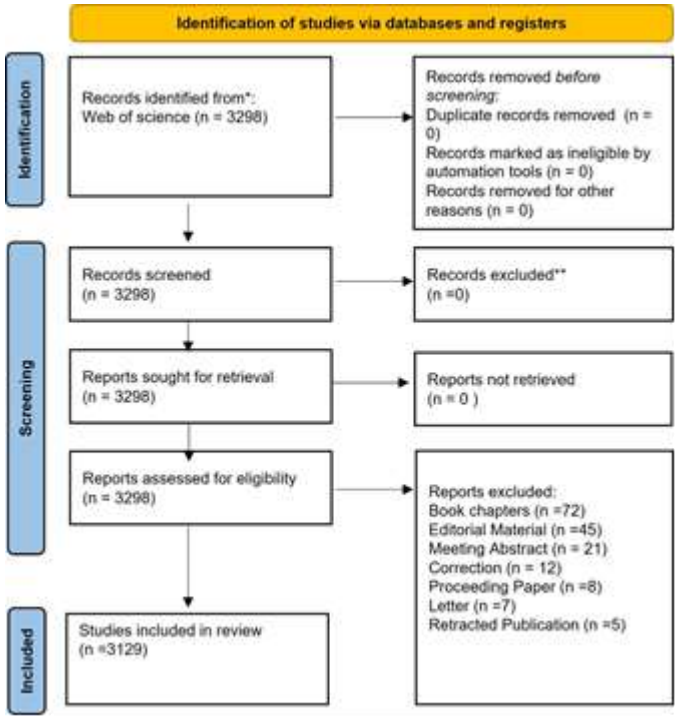


Figure 1. Flowchart of literature review and study selection according to the PRISMA 2020 statement.

RESULTS

A total of 3,129 published articles obtained from the WOS database were included in the study. These articles received a total of 67,191 citations, and when self-citations were excluded, the number of citations was determined to be 64,384. The H-index is 164. A significant upward trend in both the number of citations and the number of studies conducted has been observed, particularly since 2019. The distribution of studies and citations to articles by year is shown in Figure 2.

The research areas with the highest proportion of publications were Neuroscience (27.5%), Molecular Biology (13.94%), Pharmacology (11.26%), Nutrition and Dietetics (9.91%), and Microbiology (8.57%), respectively. The distribution of publications by research area is presented in Table 1.

When countries are ranked by number of publications, China ranks first (n = 1117; 35.72%), followed by the USA (n = 728; 23.28%), Italy (n = 246; 7.87%), and India (n = 232; 7.42%). The top 25 countries in this ranking are listed in Table 2. Looking at the citation network, citation density is observed in China and the USA (Figure 4). The VOSviewer network map for authors with the most publications and citations is shown in Figure 5.

When bibliographic coupling is evaluated by country, USA and China are seen to have higher representation among the leading countries in this field. When examining the priority status of universities and research institutions in terms of publication numbers, the University of California System (2.78%), the University of Texas System (2.27%), Harvard University (2.24%), and the Chinese Academy of Sciences (2.05%) were identified as the leading institutions. Accordingly, the top 25 institutions determined based on their connections are shown in Table 3.

In the evaluation based on Web of Science indexes, it was determined that most articles were in the Science Citation Index Expanded (SCI-Expanded) category (93.41%), followed by the Emerging Sources Citation Index (ESCI) (6.52%) and Social Sciences Citation Index (SSCI) (6.36%) categories (Table 4).

When examining the publisher distribution, Elsevier ranked first with 19.97%, followed by MDPI (16.97%), Springer Nature (12.97%), Frontiers Media SA (11.89%), and Wiley (6.71%); these five publishers account for approximately 68.5% of all publications (Table 5).

Analysis based on selected keywords revealed that keywords such as “Alzheimer's disease,” “gut microbiota,” “gut microbiome,” “gut-brain axis,” “microbiota,” and “microbiome” are among the subject areas forming the largest clusters in the literature and having the most connections (Figure 3).

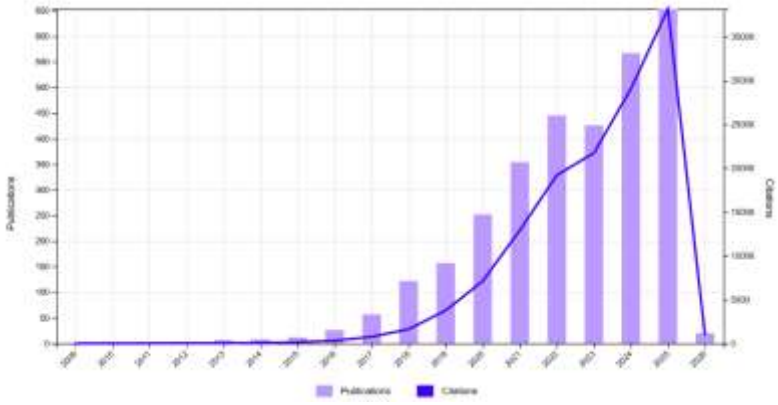


Figure 2. Publication and citation frequency of studies on the relationship between Alzheimer's disease and gut microbiota according to the WOS database between 2009 and 2026 (last accessed: January 21, 2026)

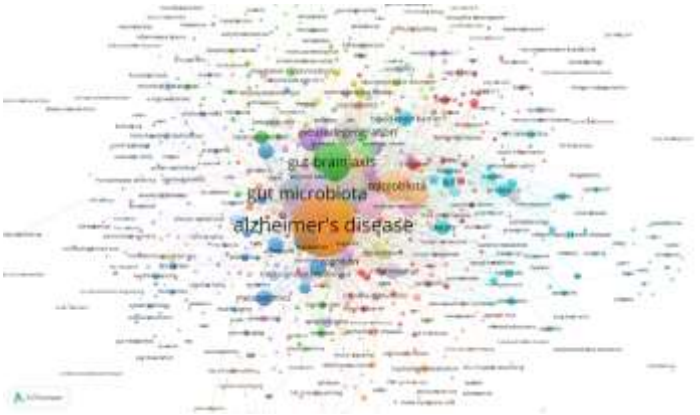


Figure 3. A bibliometric analysis of studies examining the relationship between Alzheimer's disease and gut microbiota between 2009 and 2026 based on the WOS database, showing a visualization map of keywords. *(Connection lines indicate co-occurrence relationships within articles. Keywords represented by larger circles or font sizes appear more frequently in the literature.)*

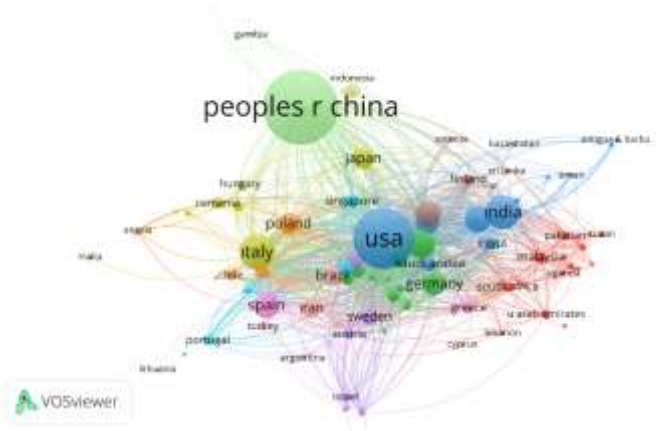


Figure 4. A visualization map of the citation network among countries producing publications on the relationship between Alzheimer's disease and gut microbiota between 2009 and 2026, according to the WOS database. *(Collaboration between countries is shown by lines connecting them. Thicker lines indicate stronger collaboration. Countries with larger circles or font sizes have a higher level of international collaboration.)*

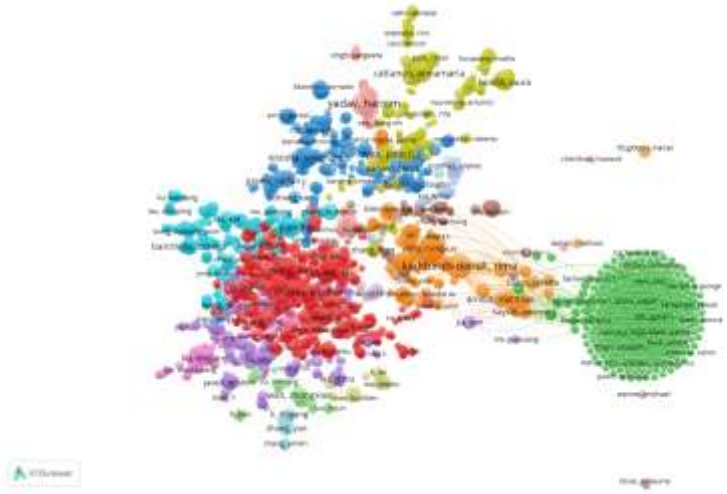


Figure 5. According to the WOS database, the authors with the most publications and citations on the relationship between Alzheimer's disease and gut microbiota between 2009 and 2026.

Table 1. Distribution of publications examining the relationship between Alzheimer's disease and gut microbiota in the WOS database between 2009 and 2026, by subject area

Web of Science Categories	Record Count	% Of 3.127
Neurosciences	860	27.502
Biochemistry Molecular Biology	436	13.943
Pharmacology Pharmacy	352	11.257
Nutrition Dietetics	310	9.914
Microbiology	268	8.571
Geriatrics Gerontology	233	7.451
Food Science Technology	222	7.099
Clinical Neurology	215	6.876
Cell Biology	203	6.492
Immunology	189	6.044
Chemistry Multidisciplinary	173	5.532
Medicine Research Experimental	140	4.477
Multidisciplinary Sciences	124	3.965
Psychiatry	113	3.614
Chemistry Medicinal	100	3.198
Gastroenterology Hepatology	75	2.398
Chemistry Applied	72	2.303

Medicine General Internal	72	2.303
Endocrinology Metabolism	65	2.079
Biotechnology Applied Microbiology	56	1.791
Integrative Complementary Medicine	49	1.567
Biology	47	1.503
Agriculture Multidisciplinary	36	1.151
Polymer Science	32	1.023
Behavioral Sciences	31	0.991

Showing 25 Out Of 103 Entries

Table 2. Distribution of countries conducting academic studies on the relationship between Alzheimer's disease and gut microbiota between 2009 and 2026, according to the WOS database

Countries/Regions	**Record Count**	**% of 3.127**
Peoples R China	1117	35.721
Usa	728	23.281
Italy	246	7.867
India	232	7.419
England	174	5.564
Spain	139	4.445
South Korea	138	4.413
Australia	131	4.189
Canada	114	3.646
Germany	104	3.326
Japan	95	3.038
Poland	89	2.846
Brazil	77	2.462
Iran	77	2.462
France	72	2.303
Netherlands	67	2.143
Switzerland	59	1.887
Sweden	53	1.695
Taiwan	45	1.439
Mexico	41	1.311
Ireland	39	1.247
Saudi Arabia	39	1.247

Belgium	35	1.119
Singapore	34	1.087
Denmark	30	0.959

Showing 25 out of 104 entries
3 record(s) (0.096%) do not contain data in the field being analyzed

Table 3. List of the top 25 institutions conducting research on the relationship between Alzheimer's disease and gut microbiota between 2009 and 2026, according to the WOS database

Affiliations	**Record Count**	**% of 3.127**
University Of California System	87	2.782
University Of Texas System	71	2.271
Harvard University	70	2.239
Chinese Academy of Sciences	64	2.047
University Of London	60	1.919
Harvard University Medical Affiliates	57	1.823
State University System of Florida	54	1.727
Harvard Medical School	53	1.695
Zhejiang University	50	1.599
Shanghai Jiao Tong University	46	1.471
Capital Medical University	44	1.407
Institut National De La Sante Et De La Recherche Medicale Inserm	44	1.407
Consiglio Nazionale Delle Ricerche Cnr	37	1.183
University System of Ohio	36	1.151
Duke University	35	1.119
Us Department of Veterans Affairs	35	1.119
University Of California San Diego	34	1.087
Veterans Health Administration Vha	34	1.087
University Of Texas Health Science Center Houston	33	1.055
Pennsylvania Commonwealth System of Higher Education Pcshe	32	1.023
Huazhong University of Science Technology	31	0.991
Jiangnan University	31	0.991
Central South University	29	0.927
Sichuan University	29	0.927
University of South Florida	29	0.927

Showing 25 out of 4.019 entries
4 record(s) (0.128%) do not contain data in the field being analyzed

Table 4. Web of Science category indexes used to identify publications related to Alzheimer's disease and gut microbiota in the WOS database between 2009 and 2026.

Web of Science Index	Record Count	% of 3.127
Science Citation Index Expanded (SCI-EXPANDED)	2921	93.412
Emerging Sources Citation Index (ESCI)	204	6.524
Social Sciences Citation Index (SSCI)	199	6.364

Table 5. Top 10 list of publishers that published articles related to Alzheimer's disease and gut microbiota in the WOS database between 2009 and 2026

Publishers	Record Count	% of 3.129
Elsevier	625	19.968
Mdpi	531	16.965
Springer Nature	406	12.971
Frontiers Media Sa	372	11.885
Wiley	210	6.709
Taylor & Francis	102	3.259
Sage	98	3.131
Nature Portfolıo	87	2.780
Bentham Science Publ Ltd	65	2.077
Oxford Univ Press	63	2.013

Showing 10 out of 143 entries

DISCUSSION

This bibliometric analysis reveals that scientific publications examining the relationship between Alzheimer's disease and gut microbiota have shown a significant upward trend in recent years. A total of 3,129 publications were identified between 2009 and 2026, indicating a growing trend in this field of research. In particular, the rise in publication and citation numbers since 2017 (Figure 2) reflects interest in alternative approaches to understanding the complex nature of Alzheimer's pathogenesis, which cannot be fully explained by the classic amyloid-β and tau hypotheses. Although we are still in the early stages of 2026, the fact that the number of publications indexed in WOS reached 26 as of January 21 indicates that the upward trend in this

field continues. An analysis of WOS data shows that the number of citations to publications in this field has been steadily increasing since 2017.

The most recent bibliometric studies in this field cover the period between 2012 and 2022, with publication numbers ranging between 500 and 600 (Li et al., 2022; Sun et al., 2022; Trejo-Castro et al., 2022). The trend of ongoing research in this field parallels the increasing number of studies suggesting that the gut-brain axis and microbiota pathophysiology play a central role in Alzheimer's disease (Vaziri et al., 2025).

When examining the distribution of publications by year, it is evident that the increase in literature reflects not only quantitative growth but also a methodological transformation. The widespread adoption of next-generation sequencing technologies and the use of metagenomic and multi-omic approaches have enabled a more in-depth investigation of the relationships between microbiota composition and neurodegenerative processes (Zhao et al., 2025). Thanks to these technologies, findings suggest that the composition of microbiota and metabolite profiles may influence both neuroinflammation and cognitive impairment are increasingly emerging (Fan et al., 2025).

Keyword analyses and network visualizations provide important insights into the conceptual framework of this research field. The studies do not focus solely on changes in the microbiota composition; they also address multidimensional mechanisms such as neuroinflammation, barrier dysfunction, microglial activation, and the effects of neuromodulatory metabolites (Lei et al., 2025). These findings suggest that the role of the microbiome in Alzheimer's pathogenesis may not only be an association but also a potential mechanistic pathway.

The author with the most publications is Rima Kaddurah-Daouk (24 publications-1531 citations), while the authors with the most citations are Sanjay Asana (8 publications-2654 citations) and Cynthia M. Carsson (5 publications-2601 citations). The data obtained highlights some countries that have made significant contributions to this field of research.

When examining the distribution by country and institution, it is evident that certain countries and research centers are leading in this field. According to WOS data, the country with the most publications is China (1117 publications-37403 citations), while the country with the most citations is the USA (728 publications-40296 citations). These countries are followed by the United Kingdom (174 publications-13,753 citations) and Italy (246 publications-15,748 citations) in terms of both citation and publication numbers. However, when examining citation impact beyond quantitative data, it was found that the USA is the world leader with a total of 40,296 citations and has the highest scientific impact in this field. This is supported by the priority given to publication numbers by leading American institutions such as the University of California System (2.78%), the University of Texas System (2.27%), and Harvard University (2.24%).

China, USA, the United Kingdom and Italy stand out as the most productive countries. According to data published at the end of 2022, the population aged 60 and over in China reached 280.04 million (19.8%), while the population aged 65 and overreached 209.78 million (14.9%). In parallel, cases of Alzheimer's disease and related dementia are rapidly increasing, with approximately 17 million cases reported in China in 2021; it has been reported that the prevalence of the disease increases significantly with age and poses a serious health burden, especially on the elderly population (Zhi et al., 2025). China's high publication count likely reflects strong government policies supporting Alzheimer's research and microbial studies, likely backed by the National Natural Science Foundation and the Healthy China Action Plan (Chen et al., 2019). The USA's clear leadership in citations in this field highlights the depth and global impact of research in the region. In addition to strategic funding from institutions such as the National Institutes of Health (NIH) and National Institute on Aging (NIA), the pioneering role of American universities in multi-omic technologies has played a critical role in this success. In particular, the ability to translate basic science findings into clinical applications (translational research) has enabled US-based studies to become mechanistic reference points in the microbiota-Alzheimer's axis. This observation is consistent with recent bibliometric analyses examining global research trends, which identify the US as the country with the highest citation impact (Total Citation) and central collaborative network (Yang et al., 2022).

In our study, Elsevier, MDPI, Springer Nature, Frontiers Media SA, and Wiley were identified as the most productive journals in this field. These five publishers accounted for approximately 68.5% of total publications. However, in the digital age, the accessibility and searchability of research content has increased, and researchers are not limited to specific journals when accessing information. Although the analysis of journal productivity provides a general overview of the leading journals in a field, it may not directly guide researchers' choice of sources.

The most cited articles are considered the most valuable and influential studies that impact subsequent research. In our analysis, the article titled “The Microbiota-Gut-Brain Axis,” published in Physiological Reviews in 2019, was identified as the most cited study, having received a total of 3,366 citations as of the review date. This article comprehensively addresses the multifaceted interaction of the gut microbiota with host physiology, summarizing its fundamental regulatory roles in metabolism, the immune system, and neurodevelopment. It also discusses at a mechanistic level how imbalances in microbiota (dysbiosis) contribute to the pathogenesis of metabolic, inflammatory, and neurological diseases and highlights their therapeutic target potential (Cryan et al., 2019). The second most cited article, published in Microorganisms, is titled "What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment,

Diet, and Diseases," published in MDPI, emphasizes that a healthy microbiota balance is critical for host metabolism, immune function, and disease prevention, while addressing the relationship between dysbiosis and both intestinal and extraintestinal diseases (Rinninella et al., 2019). The third most cited study in our analysis is a noteworthy article published in the journal Scientific Reports. Titled ‘Gut microbiome alterations in Alzheimer’s disease’, this study is one of the first large-scale investigations to clinically demonstrate reduced microbial diversity in stool samples from Alzheimer's patients compared to a control group, along with changes in specific bacterial phyla (such as an increase in Bacteroidetes and a decrease in Firmicutes). With 1,521 citations, this study is one of the most influential papers in the field and has served as a cornerstone in establishing the microbiota-Alzheimer's relationship as a clinical reality beyond a theoretical assumption (Vogt et al., 2017).

Within this bibliometric structure, our analysis shows that the concepts of oxidative stress, inflammation, cellular aging, and neuroinflammation frequently appear in the literature examining the relationship between gut microbiota and Alzheimer's disease. Although the frequency of keywords does not definitively establish a causal relationship or biological significance, it suggests that these areas are active research topics.

The bibliometric findings suggest that the growing interest in the relationship between Alzheimer's disease and gut microbiota has also brought with it avenues that could lead to potential clinical applications. It is thought that the microbiome could be used as a biomarker in the early stages of the disease and that therapeutic interventions targeting the microbiome (e.g., fecal microbiota transplantation, probiotic/prebiotic treatments, dietary modifications) could slow the progression of Alzheimer's disease (Ren et al., 2025)

Considering these findings, certain limitations of the study should also be considered. Ideally, a single database should be used in bibliometric analyses (Donthu et al., 2021). This study utilized only the WOS database, and analyses were performed using the VOSviewer software. The use of VOSviewer software may have caused limited deviations in the data matching and visualization processes; however, this does not significantly affect the interpretation of general research trends regarding the relationship between Alzheimer's disease and gut microbiota. While bibliometric analyses are effective in revealing publication volume and research trends, they do not evaluate the scientific quality of individual studies. Therefore, the findings reflect quantitative trends in studies on the relationship between Alzheimer's disease and gut microbiota and should not be interpreted as definitive indicators of biomedical priorities.

CONCLUSION

This bibliometric analysis reveals that the relationship between Alzheimer's disease and gut microbiota is a rapidly expanding, interdisciplinary research field that has gained significant momentum over the past fifteen years. The increase in publication and citation trends indicates that studies focusing on mechanistic themes, particularly neuroinflammation, oxidative stress, immune regulation, and the gut-brain axis, are becoming increasingly important. Country, institution, and keyword analyses indicate that this field is evolving beyond basic science toward translational and clinical research. The findings suggest that gut microbiota may be considered a potential biomarker and therapeutic target in Alzheimer's pathogenesis. However, since the current literature is largely based on observational and experimental studies, well-designed mechanistic studies and randomized controlled clinical trials are needed to establish the causal role of microbiota in Alzheimer's disease. Such studies are expected to contribute significantly to the translation of microbiota-based diagnostic and therapeutic approaches into clinical practice.

REFERENCES

2024 Alzheimer's disease facts and figures. (2024). *Alzheimers Dement*, *20*(5), 3708-3821. https://doi.org/10.1002/alz.13809

Chen, P., Li, F., & Harmer, P. (2019). Healthy China 2030: moving from blueprint to action with a new focus on public health. *Lancet Public Health*, *4*(9), e447. https://doi.org/10.1016/s2468-2667(19)30160-4

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci*, *13*(10), 701-712. https://doi.org/10.1038/nrn3346

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., . . . Dinan, T. G. (2019). The Microbiota-Gut-Brain Axis. *Physiol Rev*, *99*(4), 1877-2013. https://doi.org/10.1152/physrev.00018.2018

Dias, D., & Socodato, R. (2025). Beyond Amyloid and Tau: The Critical Role of Microglia in Alzheimer's Disease Therapeutics. *Biomedicines*, *13*(2). https://doi.org/10.3390/biomedicines13020279

Donthu, N., Kumar, S., Mukherjee, D., Pandey, N., & Lim, W. M. (2021). How to conduct a bibliometric analysis: An overview and guidelines. *Journal of Business Research*, *133*, 285-296. https://doi.org/https://doi.org/10.1016/j.jbusres.2021.04.070

Fan, K. C., Lin, C. C., Chiu, Y. L., Koh, S. H., Liu, Y. C., & Chuang, Y. F. (2025). Compositional and functional gut microbiota alterations in mild cognitive impairment: links to Alzheimer's disease pathology. *Alzheimers Res Ther*, *17*(1), 122. https://doi.org/10.1186/s13195-025-01769-9

Fung, T. C., Olson, C. A., & Hsiao, E. Y. (2017). Interactions between the microbiota, immune and nervous systems in health and disease. *Nat Neurosci*, *20*(2), 145-155. https://doi.org/10.1038/nn.4476

Kowalski, K., & Mulak, A. (2019). Brain-Gut-Microbiota Axis in Alzheimer's Disease. *J Neurogastroenterol Motil*, *25*(1), 48-60. https://doi.org/10.5056/jnm18087

Lei, W., Cheng, Y., Liu, X., Gao, J., Zhu, Z., Ding, W., Xu, X., Li, Y., Ling, Z., Jiang, R., & Chen, X. (2025). Gut microbiota-driven neuroinflammation in Alzheimer's disease: from mechanisms to therapeutic opportunities. *Front Immunol*, *16*, 1582119. https://doi.org/10.3389/fimmu.2025.1582119

Li, Z.-L., Ma, H.-T., Wang, M., & Qian, Y.-H. (2022). Research trend of microbiota-gut-brain axis in Alzheimer's disease based on citespace (2012–2021): a bibliometrics analysis of 608 articles. *Frontiers in aging neuroscience*, *14*, 1036120.

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., . . . Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj*, *372*, n71. https://doi.org/10.1136/bmj.n71

Ren, J., Wang, Q., Hong, H., & Tang, C. (2025). Fecal Microbiota Transplantation in Alzheimer's Disease: Mechanistic Insights Through the Microbiota-Gut-Brain Axis and Therapeutic Prospects. *Microorganisms*, *13*(8). https://doi.org/10.3390/microorganisms13081956

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., & Mele, M. C. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms*, *7*(1). https://doi.org/10.3390/microorganisms7010014

Sokol, H. (2019). [Definition and roles of the gut microbiota]. *Rev Prat*, *69*(7), 776-782. (Définition et rôles du microbiote intestinal.)

Sun, H.-L., Feng, Y., Zhang, Q., Li, J.-X., Wang, Y.-Y., Su, Z., Cheung, T., Jackson, T., Sha, S., & Xiang, Y.-T. (2022). The microbiome–gut–brain axis and dementia: a bibliometric analysis. *International journal of environmental research and public health*, *19*(24), 16549.

Tenchov, R., Sasso, J. M., & Zhou, Q. A. (2024). Alzheimer's Disease: Exploring the Landscape of Cognitive Decline. *ACS Chem Neurosci*, *15*(21), 3800-3827. https://doi.org/10.1021/acschemneuro.4c00339

Trejo-Castro, A. I., Carrion-Alvarez, D., Martinez-Torteya, A., & Rangel-Escareño, C. (2022). A Bibliometric Review on Gut Microbiome and Alzheimer's Disease Between 2012 and 2021. *Front Aging Neurosci*, *14*, 804177. https://doi.org/10.3389/fnagi.2022.804177

van Eck, N. J., & Waltman, L. (2010). Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics*, *84*(2), 523-538. https://doi.org/10.1007/s11192-009-0146-3

Vaziri, Y., Olia, J. B. H., Avci, C. B., & Nourazarian, A. (2025). Molecular mechanisms of gut microbiota dysbiosis and metabolites in Alzheimer's disease pathogenesis: implications for precision therapeutics. *Mol Brain*, *18*(1), 92. https://doi.org/10.1186/s13041-025-01263-1

Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., Carlsson, C. M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B. B., & Rey, F. E. (2017). Gut microbiome alterations in Alzheimer's disease. *Sci Rep*, *7*(1), 13537. https://doi.org/10.1038/s41598-017-13601-y

Yang, J., Deng, Y., Cai, Y., Liu, Y., Peng, L., Luo, Z., & Li, D. (2022). Mapping trends and hotspot regarding gastrointestinal microbiome and neuroscience: A bibliometric analysis of global research (2002-2022). *Front Neurosci*, *16*, 1048565. https://doi.org/10.3389/fnins.2022.1048565

Yildiz, R., Afşar, M., & Elasan, S. (2024). Global Trends in Studies on the Presence of Demodex spp. in Patients Diagnosed with Blepharitis. *J Arthropod Borne Dis*, *18*(2), 84-93. https://doi.org/10.18502/jad.v18i2.17531

Zhao, H., Zhou, X., Song, Y., Zhao, W., Sun, Z., Zhu, J., & Yu, Y. (2025). Multi-omics analyses identify gut microbiota-fecal metabolites-brain-cognition pathways in the Alzheimer's disease continuum. *Alzheimers Res Ther*, *17*(1), 36. https://doi.org/10.1186/s13195-025-01683-0

Zhi, N., Ren, R., Qi, J., Liu, X., Yun, Z., Lin, S., Hu, Y., Li, H., Xie, X., Wang, J., Li, J., Zhu, Y., Gao, M., Yang, J., Wang, Y., Jing, Y., Geng, J., Cao, W., Xu, Q., . . . Wang, G. (2025). The China Alzheimer Report 2025. *Gen Psychiatr*, *38*(4), e102020. https://doi.org/10.1136/gpsych-2024-102020

Artificial Intelligence and Smart Physical Education: Technologies, Pedagogy and Challenges

Halil TANIR [1]

1- Associate Professor.; Uşak University, Faculty of Sport Sciences, Department of Coaching Education. halil.tanir@usak.edu.tr ORCID No: 0000-0001-5626-8362

ABSTRACT

Artificial intelligence (AI) and digital-intellectual technologies are increasingly transforming educational environments by enabling adaptive, data-driven and personalised learning processes. Within this transformation, physical education and sports education have emerged as important fields for the implementation of intelligent technologies such as machine learning, deep learning, wearable systems, motion analysis and virtual reality applications. This chapter examines the role of artificial intelligence in physical education and sports education through a comprehensive review of the existing literature. The chapter focuses on the technological foundations of AI, major application areas, pedagogical impacts and emerging ethical and structural challenges. The findings indicate that AI technologies contribute to personalised instruction, motor skill development, real-time performance assessment and increased student motivation. In particular, convolutional neural network (CNN)-based systems are widely used in motion analysis and technique evaluation processes. However, issues such as data privacy, algorithmic limitations and digital inequality continue to present significant barriers to sustainable implementation. The chapter further emphasises that AI technologies should be approached from a human-centred and interdisciplinary perspective in order to ensure pedagogically meaningful and ethically responsible integration into physical education environments. Overall, AI-supported systems have the potential to reshape physical education by creating more interactive, measurable and adaptive learning environments.

Keywords – artificial intelligence, physical education, sports education, digital-intellectual technologies, machine learning, smart education

INTRODUCTION

The rapid development of artificial intelligence (AI) technologies has created substantial transformations in educational systems worldwide. Initially limited to computer science and engineering fields, AI applications are now widely integrated into educational contexts through technologies such as machine learning, deep learning, natural language processing (NLP), learning analytics and intelligent tutoring systems. These technologies enable educational systems to become more adaptive, data-driven and personalised (Luan et al., 2020).

As education systems evolve, traditional teacher-centred instructional models are increasingly being replaced by student-centred and technology-supported learning environments (Bozkurt et al., 2021). In this transformation process, AI technologies provide opportunities for analysing student data, automating repetitive tasks and generating personalised

learning experiences. Furthermore, tools such as intelligent learning management systems, chatbots and speech recognition technologies contribute to improving learning processes and monitoring academic performance (Grassini, 2023).

Physical education and sports education have also been influenced by this broader digital transformation. Unlike many academic disciplines, physical education simultaneously involves cognitive, affective and psychomotor learning domains. Consequently, the integration of AI technologies into physical education requires not only technological adaptation but also pedagogical restructuring (White et al., 2021).

Recent technological developments such as wearable devices, motion tracking systems, virtual reality (VR), augmented reality (AR) and Internet of Things (IoT)-based applications have enabled physical education environments to become more measurable and interactive (Koekoek et al., 2018; Marttinen et al., 2020). Through these technologies, students' physical performance can be monitored in real time, personalised exercise programmes can be developed and motor skill development can be evaluated more effectively.

Artificial intelligence-supported physical education applications offer important opportunities for both teachers and students. AI systems support lesson planning, performance analysis, feedback generation and personalised instruction (Lian, 2022). In addition, AI-based systems contribute to increased student engagement and motivation through interactive and gamified learning environments.

However, despite these advantages, the integration of AI into physical education also raises significant concerns regarding ethics, data privacy, digital inequality and algorithmic limitations. In particular, the collection of biometric and behavioural data requires careful ethical governance and secure data management procedures.

Although the literature on AI in education has expanded rapidly in recent years, research specifically examining AI applications in physical education remains relatively limited. Existing studies frequently focus on technological functionality while giving insufficient attention to pedagogical and human-centred dimensions. Therefore, there is a need for a more comprehensive and interdisciplinary evaluation of AI applications in physical education and sports education.

This chapter aims to examine the technological foundations, application areas, pedagogical effects and challenges associated with AI-supported physical education and sports education systems.

2. Digital Transformation in Education

The digital transformation of education has accelerated significantly in recent decades due to advancements in information and communication technologies. Educational environments are no longer confined to traditional

classroom settings; instead, digital platforms, mobile technologies and intelligent systems increasingly shape learning processes (Zhang, 2025).

Artificial intelligence has emerged as one of the most influential drivers of this transformation. AI technologies support educational systems by analysing learner data, automating administrative tasks and generating adaptive instructional processes. Intelligent learning systems can monitor student performance continuously and personalise educational content according to learners' individual needs (Grassini, 2023).

This transformation has contributed to the development of student-centred educational models. Rather than applying identical teaching methods to all learners, AI-supported systems can adapt learning environments according to students' learning pace, motivation and performance levels (Ding et al., 2025).

Furthermore, technologies such as big data analytics, cloud computing and mobile learning platforms have increased opportunities for flexible and lifelong learning. Following the COVID-19 pandemic, hybrid and online learning environments became increasingly common, accelerating the adoption of AI-supported educational systems globally (Sousa et al., 2025)

Within this broader transformation, physical education has also begun integrating digital technologies into teaching and assessment processes. Wearable technologies, smart sensors and AI-supported systems are increasingly used for monitoring physical activity, analysing movement patterns and evaluating performance (Li et al., 2024).

3. Artificial Intelligence Technologies in Physical Education

3.1 Machine Learning

The use of machine learning techniques in the field of physical education has gained increasing importance in recent years alongside the rapid development of digital technologies. These technologies provide innovative opportunities in various areas such as evaluating students' physical performance, monitoring physical activity levels, personalizing instructional processes, and improving educational management systems. In particular, artificial intelligence-based systems and machine learning algorithms contribute to the development of more objective, data-driven, and individualized approaches in physical education classes.

Considering individual differences among students is one of the important factors that increase the efficiency of teaching processes in physical education. In this context, machine learning algorithms enable the personalization of teaching methods by analyzing students' performance data. In the study conducted by Jiang (2022), Support Vector Machines (SVM) were used to analyze deficiencies in students' sports skills and to provide recommendations for improving instructional processes. The findings revealed that students did not reach the expected competency level

in sports skills and that existing educational programs should be restructured. This demonstrates the importance of data-driven evaluation systems in physical education.

Machine learning methods are also widely used in the measurement and evaluation of physical activity. In the study by Liu and Zhuang (2022), the Random Forest algorithm was used to evaluate exercise effectiveness in physical education classes. The results indicated that the Random Forest model produced more successful outcomes than Support Vector Machines and fuzzy clustering-based methods. Through such algorithms, students' physical activity levels can be quantitatively analyzed, and the effectiveness of teaching processes can be evaluated more accurately.

The objective measurement of physical activity is an essential requirement for assessing the quality of physical education classes. In a study conducted by Scruggs (2007), middle school students' physical activity levels during physical education classes were measured using pedometers, and the number of steps per minute was found to reliably reflect physical activity duration. Integrating such data collection tools with machine learning algorithms enables more comprehensive analyses of students' physical activity patterns.

Artificial intelligence-supported physical education management systems have also become notable applications in recent years. The AI-based physical education management system developed by Zhang (2024) combines motion recognition, character analysis, and exercise evaluation algorithms to analyze students' movement techniques. The system demonstrated that the proportion of moderate-intensity physical activity performed by students during lessons met the exercise intensity criteria recommended by the World Health Organization. Such systems help teachers plan lessons more effectively and monitor students' physical activities in a more systematic manner.

The contribution of physical education classes to students' daily physical activity levels is another important research topic. In the study conducted by Kriemler et al. (2011), approximately one-third of elementary school students' time during physical education classes was spent in moderate-to-vigorous physical activity. Furthermore, students' overall daily physical activity levels increased on days when physical education classes were held, and this effect was also observed among overweight children. These findings indicate that physical education plays an important role in helping children develop healthy lifestyle habits.

Machine learning techniques are used not only in evaluating physical performance but also in analyzing students' psychological conditions. Ding et al. (2025) examined the effects of lifestyle variables and socioeconomic factors on predicting depressive symptoms among Chinese adolescents and demonstrated that depressive symptoms could be predicted with high accuracy using the Boruta-RF algorithm and Random Forest model.

Similarly, Sousa et al. (2025) investigated the relationship between physical activity levels and anxiety and depression symptoms among physical education students and found that moderate-to-vigorous physical activity had a protective effect against depressive symptoms. These studies demonstrate that machine learning can be effectively utilized in physical education for monitoring both physical and psychological health.

Teachers' attitudes toward physical education and their self-efficacy perceptions are also important variables affecting students' participation in physical activity. In the study conducted by Alemdağ et al. (2014), it was determined that pre-service classroom teachers' attitudes toward physical education differed according to gender, grade level, and regular exercise habits. Additionally, positive attitudes toward physical education significantly influenced self-efficacy perceptions. Analyzing such data through machine learning techniques may contribute to the improvement of teacher education programs.

Examining students' attitudes toward physical education classes is another application area of digital analysis systems. In the study conducted by Çavdar et al. (2018) on obese students, students generally exhibited positive attitudes toward physical education classes; however, male students demonstrated more positive attitudes than female students. Through machine learning algorithms, such demographic differences can be analyzed to develop educational strategies tailored to specific student groups.

Machine learning applications are also utilized in rehabilitation and health-related fields. In the study conducted by Gundogdu (2021), the effects of exercise on balance in individuals with osteoporosis-related kyphosis were evaluated using artificial neural networks and other classification algorithms. The findings indicated that artificial neural networks achieved the highest performance with an accuracy rate of 91.4%. These results suggest that machine learning techniques can be effectively applied in rehabilitation processes.

In recent years, advanced artificial intelligence methods such as deep learning and artificial neural networks have increasingly been used in the field of physical education. The hybrid artificial intelligence model developed by Zang et al. (2024) examined the effects of exercise on cognitive performance through big data analytics. The findings indicated that physical activity positively affected cognitive processes. Such studies demonstrate that physical education contributes not only to physical development but also to cognitive and psychological development.

In conclusion, machine learning techniques have multidimensional application areas in physical education. Artificial intelligence-supported applications provide significant advantages in evaluating students' physical performance, monitoring physical activity levels, analyzing psychological health indicators, personalizing instructional processes, and improving educational management systems. With the continued development of digital

technologies, machine learning-based systems are expected to become more widespread in physical education, making educational processes more scientific, efficient, and individualized.

3.2 Deep Learning and Motion Analysis

Physical education is a critical field for developing individuals' motor skills, improving physical fitness, and promoting healthy lifestyle behaviors. In this context, motion analysis and performance assessment have become essential components for enhancing the effectiveness of physical education practices. In recent years, advances in artificial intelligence, particularly deep learning (DL) techniques, have significantly improved the accuracy and applicability of motion analysis in educational settings. Deep learning-based approaches can automatically identify complex movement patterns, thereby offering new opportunities for both individual and group-based performance evaluation.

Deep learning is a subfield of machine learning that utilizes multi-layered artificial neural networks to extract high-level features from data. Among these models, Convolutional Neural Networks (CNNs) are widely used in image processing and motion analysis. CNN-based systems developed for human movement tracking process video and image data to perform tasks such as movement recognition, classification, and standardization (Shi, 2023). These technologies enable the objective evaluation of students' motor skills in physical education and provide real-time feedback to both teachers and learners (Beng et al., 2023).

One of the most prominent applications of deep learning in motion analysis is monocular human pose estimation, which involves estimating human body posture from a single camera view. These methods allow both 2D and 3D pose estimation, enabling detailed analysis of human movement. In a comprehensive review, Chen et al. (2020) examined deep learning-based pose estimation methods developed since 2014, highlighting datasets, performance metrics, and key challenges in this field. Such studies provide a foundational framework for accurately modeling movement in physical education contexts.

However, one of the main challenges in motion analysis is the visibility of joint points. In single-camera systems, certain joints may be occluded or partially invisible, leading to estimation errors. Wade et al. (2023) found that occluded joints significantly increase angular measurement errors in 2D markerless motion capture systems. Nevertheless, joint angles derived from visible points show acceptable accuracy when compared to marker-based systems. These findings highlight the importance of anatomical accuracy and high-quality datasets in developing robust deep learning-based pose estimation models.

Another important application of deep learning in physical education is the improvement of instructional processes. Beng et al. (2023)

demonstrated that human pose estimation technologies can be integrated into teaching and learning environments by embedding criterion-based movements into deep learning models. Students' movements are evaluated through these models, generating quantitative performance indices. This approach enables students to receive instant and objective feedback, while teachers can collect class-wide performance data to develop more effective instructional strategies.

Wearable sensors and Internet of Things (IoT) infrastructures also play a significant role in physical activity monitoring and analysis. Yuan et al. (2026) proposed an IoT-supported Convolutional Recurrent Neural Network (CRNN) framework that integrates spatial and temporal features of multimodal wearable sensor data for high-accuracy activity recognition. By utilizing physiological signals such as heart rate variability and triaxial accelerometer data, this model enables detailed analysis of physical activities. Consequently, real-time monitoring and evaluation of students' physical activity in physical education settings becomes feasible.

Another important application of deep learning is sports motion recognition. Shi (2023) developed the CSTGAT model, which extracts spatial-temporal features of skeletal movements using convolutional neural networks and achieves high accuracy in online educational environments. Similarly, Wang et al. (2023) developed CNN-based models for volleyball movement recognition, enabling more precise analysis of sport-specific movements. These approaches are particularly valuable for standardizing sports techniques and objectively measuring performance.

Advances in hardware also contribute to the practical implementation of motion analysis systems. Lee et al. (2019) developed a CNN accelerator that reduces computational cost through model compression techniques, enabling efficient deployment of deep learning models on mobile devices. Such developments support the widespread use of real-time motion analysis in physical education environments.

Deep learning applications also extend to health-related fields closely linked to physical education. Kitaguchi et al. (2021) developed a 3D CNN model for surgical skill assessment, providing objective feedback in surgical training. Similar approaches can be adapted for evaluating skill acquisition and performance development in physical education settings.

Data sources used in motion analysis are highly diverse. Liao et al. (2023) proposed a sparse IMU-based 3D human pose estimation method that achieves high accuracy with a limited number of sensors, improving user comfort. Additionally, Zhang et al. (2020) introduced the AdaFuse method, which adaptively integrates multi-camera data to improve the estimation of occluded joints. The integration of such multi-source data significantly enhances the accuracy and comprehensiveness of motion analysis in physical education.

The pedagogical integration of deep learning is also an important dimension. Yau et al. (2026) found that the use of human pose estimation tools in university-level physical fitness programs improved students' diagnostic reasoning skills. AI-supported visual feedback not only enhanced students' conceptual understanding but also transformed teachers' pedagogical roles. This highlights that deep learning is not merely a technical tool but also a transformative educational instrument.

The effectiveness of deep learning-based motion analysis models largely depends on dataset quality. Quan and Zhao (2024) improved motion recognition accuracy by integrating biomechanical data into a DK-LSTM model. The model effectively classifies different physical activities with high accuracy and contributes to optimizing training programs through biomechanical interpretation. This approach enables both macro- and micro-level analysis of physical education processes, supporting the development of more effective instructional strategies.

In conclusion, deep learning techniques have led to revolutionary advancements in motion analysis within physical education. From human pose estimation and wearable sensor data processing to pedagogical applications and hardware acceleration, these studies highlight the highly interdisciplinary nature of the field. The automation, accuracy, and real-time capabilities of deep learning systems enable more objective performance evaluation and more interactive and efficient teaching processes. In light of these developments, the future of physical education is increasingly shaped by AI-driven technologies.

3.3 Natural Language Processing

Natural Language Processing (NLP) technologies have increasingly been used in recent years across health and education domains. In physical education, NLP offers innovative solutions for personalizing learning processes, improving instructional methods, and automating student feedback. From a multidisciplinary perspective, NLP applications in physical education represent a growing research area with significant potential for enhancing both pedagogical practices and learner outcomes.

One of the primary applications of NLP in physical education is the analysis of teacher-student interactions, classification of instructional behaviors, and evaluation of learning processes. For instance, Transformer-based language models have been used to automatically classify teachers' classroom behaviors in physical education settings. The BETO model, a BERT-based architecture, has demonstrated high accuracy and F1-scores in distinguishing teaching styles such as autonomy-supportive, structuring, controlling, and chaotic instructional behaviors (Martín-Hoz et al., 2025). These findings indicate that NLP can contribute to more objective and scalable evaluation of pedagogical practices while providing meaningful feedback to teachers.

Beyond teacher behavior analysis, NLP is also widely used for processing learner feedback. In professional development contexts, studies comparing different NLP approaches have shown that large language model (LLM)-based clustering methods are effective in organizing short and less diverse open-ended responses into meaningful thematic groups (Maslej et al., 2025). This demonstrates that NLP can provide deeper insights into learners' experiences and perceptions in physical education environments.

Another important application area of NLP in physical education is the development of intelligent tutoring systems and virtual simulation environments. During the COVID-19 pandemic, the Hepius virtual patient simulator integrated NLP algorithms to support learners' clinical reasoning and diagnostic skills. The system provided real-time feedback, helping students identify knowledge gaps and significantly improving short-term learning outcomes (Furlan et al., 2020; Furlan et al., 2021). These findings highlight the role of NLP in supporting both theoretical knowledge acquisition and practical skill development in physical education and related health education contexts.

However, the use of large language models in physical education also presents certain challenges. One key issue is the risk of "hallucination," where models generate incorrect or misleading feedback. In a badminton learning experiment, students using LLM-based applications showed lower performance in specific skill areas compared to the control group, suggesting that hallucinated outputs may negatively affect learning processes (Qiu, 2024). This emphasizes the need for careful validation and supervision of NLP-generated content in physical education settings.

NLP applications also contribute to the improvement of instructional strategies through multimodal learning analytics (MMLA). In a study on Baduanjin exercise instruction in China, multimodal data-including kinematic movement data, speech fluency, and facial expressions—were integrated to objectively evaluate teaching performance. The findings revealed that high-performing teachers provided fewer but more effective feedback instances, while excessive corrective feedback negatively affected students' skill acquisition (Guan et al., 2026). This demonstrates how NLP combined with multimodal analytics can support the optimization of pedagogical strategies in physical education.

The role of NLP in physical education also extends to health-oriented chatbots and AI-based personalized exercise recommendation systems. Large language models such as ChatGPT can be used to provide individualized physical activity guidance. However, critical issues such as accuracy, safety, privacy, and theoretical grounding must be carefully addressed in these systems (O'malley et al., 2026). Systematic reviews further show that LLM-based exercise recommendation tools can improve efficiency, enhance patient engagement, and increase accessibility, particularly in remote areas (Lai et al., 2025).

In parallel, NLP is also being applied in mental health education and personalized learning systems. For example, a knowledge graph and NLP-based personalized learning pathway system in mental health education has been shown to improve learning efficiency by adapting content to learners' knowledge levels (Huang & Zhan, 2025). Such interdisciplinary approaches highlight the intersection between physical education and health education in the context of NLP technologies.

NLP techniques have also been widely used in social media analysis related to health and physical activity. During the COVID-19 pandemic, sentiment analysis and topic modeling of Twitter data provided valuable insights into public responses to health policies (Jang et al., 2020; Jang et al., 2021). Similarly, studies analyzing geographic sentiment dynamics toward COVID-19 vaccination using BERT-based models demonstrated the potential of NLP for real-time public opinion analysis (Ye et al., 2022). These findings suggest that NLP can support health communication strategies and behavior change interventions relevant to physical education contexts.

From a pedagogical perspective, NLP has been used in curriculum analysis and discourse studies. Critical discourse analysis of curriculum materials has revealed complex relationships between social justice expectations and learning outcomes (Rossi et al., 2009). Furthermore, studies on how external providers interpret and translate physical education curricula into commercial products highlight the importance of preserving teachers' pedagogical expertise (Sperka et al., 2017; Enright et al., 2018). In this context, NLP provides powerful tools for analyzing educational discourse and supporting curriculum development processes.

In conclusion, NLP technologies offer a wide range of applications in physical education, including the analysis of teacher behavior, evaluation of student feedback, development of virtual simulators, optimization of instructional strategies through multimodal learning analytics, and provision of personalized exercise recommendations. However, issues related to reliability, accuracy, ethics, and responsible use remain critical. Therefore, continued interdisciplinary research is essential to fully realize the potential of NLP in physical education (Jiang et al., 2021; Maslej et al., 2025; Furlan et al., 2020; Martín-hoz et al., 2025; O'malley et al., 2026; Guan et al., 2026; Qiu, 2024).

3.4 Virtual Reality and Smart Technologies

The rapid advancement of technology in education has necessitated the adoption of innovative approaches in traditional disciplines such as physical education. In particular, virtual reality (VR) and smart technologies have increasingly been used to enrich learning experiences, enhance motor skill acquisition, and improve physical performance. Within this context, VR and smart technology applications in physical education offer significant

opportunities for supporting students' physical health, increasing motivation, and facilitating more effective learning processes.

Virtual reality is a technology that immerses users in a fully computer-generated three-dimensional environment, isolating them from the real world while enabling interactive experiences. In educational settings, VR is particularly effective in transforming abstract concepts into concrete experiences and supporting the learning of complex motor skills (Kamińska et al., 2019). In physical education, VR enables real-time monitoring of movements, immediate correction of errors, and personalized feedback, thereby making the learning process more effective and sustainable.

VR applications in physical education are particularly valuable for motor skill acquisition and the promotion of physical activity. For example, VR-based training programs designed to support motor development in children can be implemented at earlier developmental stages compared to traditional methods and can be adapted to individual learning needs (Kiefer et al., 2018). These programs are designed in accordance with children's cognitive and perceptual-motor development levels and support natural progression in motor skill acquisition. As a result, VR-based interventions increase children's engagement in physical activity and contribute to the long-term development of healthy lifestyle habits.

Smart technologies in physical education refer to sensor-based systems used for monitoring movement, analyzing performance, and optimizing physical activity. Wearable devices and motion capture technologies provide real-time biomechanical data, enabling both self-assessment by students and targeted instructional interventions by teachers. However, it is also noted that these technologies are still evolving and may present challenges in terms of reliability and accuracy (Shan, 2020).

The integration of VR and smart technologies in physical education not only enhances individual performance but also increases motivation and participation levels. Exergaming and interactive exercise-based games, for instance, reduce the monotony of traditional exercises while increasing students' engagement in physical activity (Klein & Simmers, 2009). These systems allow learners to simultaneously develop physical skills and enjoy the learning process. Moreover, VR-based exercise programs have been shown to have positive effects on cognitive functions; dual-task VR activities performed during walking, for example, can improve cognitive flexibility (Jung et al., 2021).

The use of VR in physical education is not limited to children. It is also widely applied in older adults and rehabilitation contexts. AR/VR applications have shown promising effects on physical and mental health in elderly populations (Carroll et al., 2021). In rehabilitation settings, VR-based exercise games have been found to enhance motivation and improve physical performance following upper limb injuries (Elor et al., 2022). Similarly, the combination of mixed VR exercises and occupational therapy

has been reported to significantly improve hand function (Roman et al., 2023). These applications not only enhance rehabilitation effectiveness but also improve patient adherence to treatment programs.

Another important contribution of VR and smart technologies in physical education is the personalization of instruction and the development of real-time feedback systems. For example, VR simulation platforms supported by biomechanical analysis help students learn correct posture and reduce the risk of injury (Chang, 2025). In such systems, deep learning algorithms are used to estimate joint angles and force metrics, thereby improving training quality. Additionally, augmented reality systems integrated with motion capture technologies are used in biomechanical evaluations, enabling users to optimize their performance based on motion data.

VR-based instruction in physical education also offers advantages in terms of social interaction and motivation. In activities such as dance, VR platforms allow users to engage with the system for different motivational purposes, including entertainment, fitness, and social interaction (Sarupuri et al., 2023). This not only increases participation in physical activity but also strengthens social connections among learners. Furthermore, VR-based games have been associated with positive mental health outcomes, and such applications may have therapeutic potential in conditions such as depression (Fleming et al., 2020).

The application of VR and smart technologies in physical education is also expanding in clinical contexts. For instance, VR technologies show promise in the assessment and rehabilitation of gait and balance disorders in Parkinson's disease patients (Canning et al., 2020). These systems enable a better understanding of motor-cognitive functions and support the development of personalized rehabilitation programs. In addition, VR interventions in patients with diabetes have been shown to improve balance ability and reduce fear of falling, although no significant effect on blood glucose control has been observed (Yim & Hur, 2023).

Wearable devices, which are considered part of smart technologies, are widely used for monitoring various physiological parameters, particularly respiratory health. These devices enable continuous monitoring of individuals in real-life environments, thereby supporting personalized healthcare delivery (Aliverti, 2017). In the context of physical education, wearable technologies can track parameters such as heart rate and energy expenditure during physical activity, allowing for more accurate evaluation of training effectiveness.

Despite their advantages, the widespread implementation of VR in physical education also presents several challenges. These include hardware costs, user adaptation difficulties, and concerns regarding safety and privacy (Farrell & Macdougall, 2023). Furthermore, differences in interaction patterns have been observed among therapists with limited VR rehabilitation

experience, indicating the need for structured training programs (Christensen & Holte, 2018). The use of extended reality (XR) technologies in remote research and education also presents technical limitations and user experience challenges, although it is considered a promising area for future development (Ratcliffe et al., 2021).

In conclusion, the integration of virtual reality and smart technologies into physical education offers a multifaceted approach that enhances learning processes, increases motivation, and supports physical performance. These technologies enable more interactive, personalized, and measurable learning experiences for students while also contributing significantly to rehabilitation processes. However, broader implementation requires improvements in technical infrastructure, user training, and careful consideration of ethical issues.

Table 1. Overview of Artificial Intelligence Technologies in Physical Education

AI Technology	Application Area	Educational Contribution
Machine Learning	Performance monitoring	Personalised instruction
Deep Learning (CNN)	Motion analysis	Technique evaluation
Natural Language Processing	Intelligent feedback systems	Interactive learning
Virtual Reality (VR)	Skill simulation	Increased engagement
IoT and Wearables	Real-time activity tracking	Data-driven assessment

4. Applications of Artificial Intelligence in Physical Education

Artificial intelligence (AI) has emerged as one of the most transformative technological developments in education in recent years. In physical education (PE), AI extends beyond traditional instructional approaches by enabling more effective, personalized, and data-driven learning environments. As a result, the integration of AI into physical education has become a complex and multidisciplinary field that requires consideration from pedagogical, technological, and ethical perspectives.

Physical education aims to develop motor skills, improve physical health, and promote lifelong physical activity habits. Traditionally, PE practices have been constrained by limited resources, non-individualized instruction, and manual assessment processes. However, the integration of AI technologies has significantly transformed these limitations by creating more dynamic and interactive learning environments (Lee & Lee, 2021). According to Lee and Lee (2021), AI supports personalized instruction, automated assessment, information delivery, and feedback systems in physical education, while also requiring teachers to adapt to new technological competencies.

At the university level, AI applications in physical education have demonstrated significant potential in enhancing performance analysis through motion capture systems and machine learning techniques. Zhang (2025) found that real-time biomechanical feedback provided via web-based

platforms improves skill acquisition and optimizes physiological load management. Similarly, Zhou and Wu (2025) developed systems that integrate wearable sensors and video-based motion capture data to achieve high-accuracy movement recognition, enabling adaptive and personalized learning environments. These technologies contribute to data-centric and adaptive physical education systems that enhance both student performance and teacher effectiveness.

The integration of Internet of Things (IoT) technologies into physical education represents another important advancement. Li et al. (2021) demonstrated that IoT-based physical activity monitoring devices enable real-time tracking of student behavior, with data processed in virtual environments to optimize individualized exercise intensity. These systems monitor physiological indicators such as heart rate and body temperature, thereby improving both physical and psychological outcomes. Compared to traditional observation methods, IoT systems provide higher accuracy and interactivity.

The complexity of motor learning processes further highlights the importance of AI applications in physical education. Schöllhorn et al. (2022) emphasize that motor learning is nonlinear, unpredictable, and highly context-dependent. Therefore, AI-based systems are particularly valuable in capturing individual differences and supporting adaptive learning processes. Personalized feedback is especially critical in dynamic environments where motor skill acquisition requires continuous adjustment.

Motion capture technologies also play a key role in operationalizing AI in physical education. Ozkaya et al. (2018) demonstrated that 3D motion capture data collected during repetitive upper limb movements can provide detailed insights into biomechanical performance. These datasets are essential for training advanced AI algorithms and improving the accuracy of movement analysis systems.

AI-supported peer-assisted learning (PAL) systems have also gained attention in recent research. Hsia et al. (2025) found that AI-enhanced PAL models improve students' skill performance, learning motivation, and sense of autonomy. These systems facilitate peer-to-peer feedback while reducing teachers' workload, thereby strengthening both academic and social learning outcomes.

Comparative studies have also shown the effectiveness of technology-enhanced feedback systems. Amara et al. (2015) demonstrated that video-based motion analysis and modeling techniques are more effective than verbal feedback in improving motor skill learning. This highlights the importance of visual and technological tools in physical education instruction.

Despite these advantages, the widespread adoption of AI in physical education raises important ethical concerns. Jovanovic and Kay (2023) highlight issues such as data ownership, privacy, and unintended

consequences in the use of wearable technologies in K-12 education. The ethical management of student-generated biometric data remains a critical issue in AI-enhanced physical education systems.

AI applications are also increasingly used in sport-specific training environments. Wu (2023) found that virtual training tools and online coaching platforms improve performance in martial arts training while increasing accessibility and student motivation. Similarly, Bačić and Hume (2018) developed a prototype system for evaluating tennis swing performance using 3D motion data, enabling personalized feedback and technical improvement in athletes.

Wearable technologies further expand the scope of AI applications in physical activity monitoring. Namba (2023) discusses the growing mobile health market and highlights the role of machine learning and deep learning in risk prediction and activity tracking using smartphones and wearable devices. These systems contribute to continuous monitoring and personalized health recommendations.

Tian (2024) provides a comprehensive review of AI-based physical education systems in higher education, emphasizing personalized instruction, real-time feedback, and multi-dimensional assessment approaches. However, challenges such as technological reliability, privacy concerns, and the need for teacher support are also identified.

In biomechanics and sports performance analysis, Ang and Kong (2023) demonstrate that wearable sensors combined with video analysis can effectively evaluate Olympic weightlifting movements. This confirms the applicability of hybrid AI systems in field-based performance assessment.

Deep learning, a core component of AI, has significantly advanced computer vision applications in physical education. Sindhwani et al. (2021) report that deep learning models achieve superior performance in image classification and segmentation tasks using frameworks such as TensorFlow, MXNet, and CNTK. These advancements improve the accuracy of movement recognition systems in PE contexts.

In human-computer interaction, Lv et al. (2022) show that deep learning-based gesture and speech recognition enables more natural and interactive virtual environments. In physical education, such technologies can enhance student engagement and motivation, particularly in immersive learning settings.

From a broader educational perspective, AI also supports teacher decision-making and student-centered learning. Istenič Starčič (2019) emphasizes that AI systems can monitor cognitive, social-emotional, and psychomotor domains while providing data-driven feedback to support individualized learning pathways.

The integration of machine learning education at K-12 levels is also gaining importance. Tedre et al. (2021) highlight challenges in integrating machine learning into curricula, emphasizing the need for new cognitive

paradigms beyond traditional programming approaches. This suggests that future physical education programs will increasingly incorporate AI-driven pedagogical models.

In sustainable education contexts, Lin et al. (2023) highlight that AI systems enable personalized learning experiences by monitoring student performance, emotional states, and engagement levels. However, issues such as data privacy, security, and algorithmic bias must be carefully addressed.

Finally, Deng et al. (2022) propose a big data-driven intelligent management system for monitoring and improving students' physical health. These systems enable personalized exercise prescriptions and support the integration of in-school and out-of-school physical education activities.

In conclusion, artificial intelligence applications in physical education encompass a wide range of technologies, including wearable devices, motion capture systems, deep learning models, and intelligent tutoring systems. These technologies are combined with pedagogical approaches such as personalized learning and peer-assisted learning, forming a highly multidisciplinary field. While AI offers significant opportunities for enhancing learning, performance analysis, and instructional design, it also raises important ethical, technical, and pedagogical challenges that must be carefully addressed in future research and practice.

Table 2. Overview of Artificial Intelligence Technologies in Physical Education

Dimension	Benefits	Challenges
Learning	Adaptive instruction	Technology dependence
Assessment	Continuous evaluation	Algorithmic bias
Motivation	Gamification	Reduced human interaction
Accessibility	Flexible learning	Digital inequality
Data Use	Learning analytics	Privacy concerns

5. Pedagogical Impacts of AI-Supported Physical Education

Artificial intelligence (AI) has led to significant pedagogical transformations in physical education (PE), reshaping traditional instructional models into more adaptive, personalized, and data-driven learning environments. As a discipline focused on motor skill development, physical health, and lifelong activity habits, PE has historically relied on standardized instruction and manual assessment. However, AI integration has introduced new possibilities for individualized learning pathways, real-time feedback, and enhanced teacher decision-making processes.

AI technologies in physical education enable adaptive learning experiences tailored to individual student needs. For example, AI-based platforms can dynamically adjust training programs based on students' physical conditions and learning progress while incorporating interactive game-based elements to increase engagement (Geng et al., 2026). Similarly, AI-supported motion analysis systems and virtual coaching tools provide real-time feedback, improving technical accuracy and optimizing learning

outcomes (Rodriguez & Cerezo, 2025). These innovations shift physical education from a uniform instructional model toward a more personalized and data-informed structure.

Beyond student performance, AI also significantly transforms the pedagogical role of teachers. Teacher competence in AI pedagogy has become a key factor in the effective implementation of these technologies. In Finland, systematic integration of AI-supported physical education pedagogy into teacher education programs has enhanced teachers' digital competencies and facilitated the adoption of innovative instructional models (Geng et al., 2026). This development enables teachers to provide more accurate and individualized feedback, while AI-based assessment systems support objective, data-driven evaluation of student progress (Wen, 2025).

Another major pedagogical impact of AI in physical education is the enhancement of student engagement and motivation. Traditional PE environments often struggle to maintain consistent student participation; however, AI-based gamified applications and interactive platforms have introduced new motivational mechanisms. These systems encourage active participation and make learning more enjoyable (Rodriguez & Cerezo, 2025). Additionally, adaptive content delivery allows students to progress at their own pace and interact with learning materials aligned with their interests (Zharmukhanbetov & Singh, 2023). This is particularly beneficial for students with diverse learning styles and abilities.

Teacher roles remain central to sustaining student motivation and engagement. In virtual learning environments, autonomy-supportive teaching behaviors have been shown to significantly increase student participation (Salami & Althaqafi, 2023). Furthermore, effective feedback mechanisms provided by teachers contribute to improved learning outcomes and deeper understanding of motor skill development. AI-supported feedback systems further strengthen this process by offering continuous, real-time, and personalized performance evaluations, thereby enhancing both teaching quality and learning experiences (Nyberg et al., 2025).

Learning analytics (LA) represents another important dimension of AI-supported physical education. LA tools collect and analyze student data to support evidence-based decision-making in teaching processes. These systems help identify students' strengths and weaknesses while fostering self-regulated learning skills (García-senín et al., 2022). Moreover, the integration of learning analytics into instructional design improves lesson planning and contributes to higher student achievement (Law & Liang, 2020). Although LA applications in physical education are still emerging, they are expected to play a growing role in future pedagogical frameworks.

The technical infrastructure supporting AI applications also plays a critical role in shaping pedagogical outcomes. Wearable sensor technologies and multimodal data fusion systems enable objective assessment of student performance by capturing both physical movements and contextual

behavioral data (Wen, 2025). These technologies allow teachers and students to optimize learning processes through real-time data feedback. However, effective implementation requires improvements in digital infrastructure and enhanced digital literacy among teachers (Mian et al., 2024).

The widespread adoption of remote and hybrid learning models, particularly after the pandemic, has further accelerated the need for new pedagogical approaches in physical education. Research on modular teaching methods indicates that while teachers generally demonstrate strong digital competencies, challenges such as technological infrastructure limitations and module distribution issues remain significant barriers (Mian et al., 2024). This highlights the importance of strengthening both technical and pedagogical support systems to ensure the sustainability of AI-supported physical education practices.

Disciplinary integration is another key aspect of AI-driven pedagogical transformation. Studies in Indonesia emphasize the importance of integrating physical education with other subjects as an innovative teaching approach, while also highlighting the need to improve teachers' content knowledge in this area (Budiman et al., 2024). AI technologies can support such interdisciplinary approaches by offering personalized learning pathways and facilitating flexible instructional design. Additionally, AI tools may accelerate pedagogical transformation in areas such as movement composition and creative learning within Physical Education Teacher Education (PETE) programs (Tolgfors et al., 2024).

At the policy level, AI-supported physical education also influences system-wide educational strategies. For example, coordinated school health programs in Tennessee emphasize the importance of physical activity policies and recommend increased use of technology in implementation processes. Such initiatives highlight the role of technological infrastructure in supporting the broader adoption of AI-based physical education systems (Nyberg et al., 2025).

Ethical considerations remain central to the pedagogical discourse on AI in physical education. Issues such as data privacy, algorithmic bias, and digital inequality require careful attention from educators and policymakers (Zharmukhanbetov & Singh, 2023; Rodriguez & Cerezo, 2025). Ensuring responsible AI integration necessitates the development of ethical guidelines and targeted teacher training programs, along with efforts to reduce digital divides in educational contexts (Rodriguez & Cerezo, 2025; Geng et al., 2026).

In conclusion, AI-supported physical education produces multidimensional pedagogical impacts by enhancing student engagement, improving assessment processes, and enabling personalized learning experiences. However, realizing its full potential requires strengthened technical infrastructure, updated teacher education programs, and robust ethical frameworks. Existing research clearly indicates that AI plays a

central role in the pedagogical transformation of physical education, and future studies will continue to shape and expand this rapidly evolving field.

Table 3: Selected Studies on AI and Digital-Intellectual Technologies in Physical Education

Author(s)	Year	Focus Area	Technology
Koekoek et al.	2018	Digital video technology	Educational technology
Lian	2022	Badminton instruction	Deep learning
Marttinen et al.	2020	Wearable technologies	IoT
Zhong et al.	2025	Smart physical education	Digital-intellectual technologies
Bozkurt et al.	2021	AI in education	Artificial intelligence

6. Ethical and Structural Challenges

Despite the growing potential of AI-supported physical education, several important limitations remain.

6.1 Data Privacy and Security

AI-supported systems frequently collect sensitive biometric and behavioural data. Wearable technologies, movement tracking systems and performance analytics platforms generate substantial amounts of personal information. Consequently, ethical governance and secure data management procedures are essential.

6.2 Algorithmic Limitations

Human movement is highly dynamic and context-dependent. Existing AI systems may struggle to interpret complex physical behaviours accurately. Errors in movement recognition systems may negatively affect performance evaluation processes.

6.3 Digital Inequality

Digital inequality remains one of the most significant barriers to AI integration in education. Schools with limited technological infrastructure may lack access to advanced AI-supported systems and wearable technologies. This situation may increase educational disparities between institutions and regions.

7. Future Perspectives and Human-Centred AI

Future developments in physical education are expected to involve increasingly intelligent and interconnected systems. Smart gyms, AI-supported coaching systems, immersive VR environments and wearable ecosystems may become more widespread in educational settings.

However, future developments should prioritise human-centred approaches. Educational technologies should support holistic student development rather than focusing solely on data collection and performance optimisation.

Interdisciplinary collaboration among educators, computer scientists, psychologists and health professionals will be essential for developing sustainable and ethically responsible AI-supported educational systems.

Future research should particularly focus on:

- inclusive AI systems,
- teacher AI literacy,
- ethical governance,
- long-term pedagogical impacts,
- and sustainable smart education models.

8. Conclusion

Artificial intelligence and digital-intellectual technologies are transforming physical education and sports education by creating more adaptive, personalised and data-driven learning environments. AI-supported systems contribute to performance assessment, movement analysis, personalised instruction and increased student motivation.

At the same time, important challenges related to data privacy, algorithmic limitations and digital inequality continue to require careful consideration. Therefore, the successful integration of AI into physical education depends not only on technological innovation but also on ethical governance, pedagogical balance and equitable access.

Overall, AI-supported physical education systems have substantial potential to reshape educational practices and improve learning experiences. However, future developments should adopt interdisciplinary, ethical and human-centred approaches to ensure sustainable and meaningful implementation.

REFERENCES

Alemdağ, S., Öncü, E., ve Sakallıoğlu, F. (2014). Sınıf öğretmeni adaylarının beden eğitimi dersine yönelik tutum ve öz-yeterlikleri. Abant İzzet Baysal Üniversitesi Eğitim Fakültesi Dergisi, 14(2). https://doi.org/10.17240/aibuefd.2014.14.2-5000091527

Aliverti, A. (2017). Wearable technology: role in respiratory health and disease. Breathe, 13(2), e27–e36. https://doi.org/10.1183/20734735.008417

Amara, S., Mkaouer, B., Nassib, S. H., Chaaben, H., Hachana, Y., ve Salah, F. Z. B. (2015). Effect of video modeling process on teaching/learning hurdle clearance situations on physical education students. Advances in Physical Education, 05(04), 225–233. https://doi.org/10.4236/ape.2015.54027

Ang, C. L., ve Kong, P. W. (2023). Field-based biomechanical assessment of the snatch in Olympic weightlifting using wearable in-shoe sensors and videos—A preliminary report. Sensors, 23(3), 1171. https://doi.org/10.3390/s23031171

Bačić, B., ve Hume, P. A. (2018). Computational intelligence for qualitative coaching diagnostics: Automated assessment of tennis swings to improve performance and safety. Big Data, 6(4), 291–304. https://doi.org/10.1089/big.2018.0062

Beng, T. H., San, N. S. K., Meng, T. S., Peng, T. W., ve Komar, T. J. (2023). The use of human pose estimation to enhance teaching & learning in physical education. Proceedings of the 8th ACPES International Conference. https://doi.org/10.4108/eai.28-10-2022.2327418

Bozkurt, A., Karadeniz, A., Baneres, D., Guerrero-Roldán, A. E., ve Rodríguez, M. E. (2021). Artificial intelligence and reflections from educational landscape: A review of AI studies in half a century. Sustainability, 13(2), 800. https://doi.org/10.3390/su13020800

Budiman, A., Nopembri, S., Supriadi, D., Sinulingga, A., ve Andika, R. (2024). Interdisciplinary physical education: implementation and insights of Indonesian PE teachers. Retos, 57, 607–615. https://doi.org/10.47197/retos.v57.105910

Canning, C. G., Allen, N. E., Nackaerts, E., Paul, S. S., Nieuwboer, A., ve Gilat, M. (2020). Virtual reality in research and rehabilitation of gait and balance in Parkinson disease. Nature Reviews Neurology, 16(8), 409–425. https://doi.org/10.1038/s41582-020-0370-2

Carroll, J., Hopper, L., Farrelly, A. M., Lombard-Vance, R., Bamidis, P. D., ve Konstantinidis, E. I. (2021). A scoping review of augmented/virtual reality health and wellbeing interventions for older adults: Redefining immersive virtual reality. Frontiers in Virtual Reality, 2. https://doi.org/10.3389/frvir.2021.655338

Chang, Z. (2025). Development of a virtual simulation training platform for physical education teaching posture integrating biomechanics. Molecular & Cellular Biomechanics, 22(1), 915. https://doi.org/10.62617/mcb915

Chen, Y., Tian, Y., ve He, M. (2020). Monocular human pose estimation: A survey of deep learning-based methods. Computer Vision and Image Understanding, 192, 102897. https://doi.org/10.1016/j.cviu.2019.102897

Christensen, D. J. R., ve Holte, M. B. (2018). The impact of virtual reality training on patient-therapist interaction. Interactivity, Game Creation, Design, Learning, and Innovation, 127–138. https://doi.org/10.1007/978-3-319-76908-0_13

Çavdar, E., İlhan, E. L., ve Esentürk, O. K. (2018). An analysis of students' attitudes towards physical education. Journal of Human Sciences, 15(4), 2493. https://doi.org/10.14687/jhs.v15i4.5597

Ding, L., Wu, Z., Wu, Q., ve Li, E. (2025). Machine learning-based predictive modeling of depressive symptoms in Chinese adolescents. Journal of Affective Disorders, 385, 119399. https://doi.org/10.1016/j.jad.2025.119399

Elor, A., Powell, M., Mahmoodi, E., Teodorescu, M., ve Kurniawan, S. (2022). Gaming beyond the novelty effect of immersive virtual reality for physical rehabilitation. IEEE Transactions on Games, 14(1), 107–115. https://doi.org/10.1109/tg.2021.3069445

Enright, E., Williams, B., Sperka, L., ve Peucker, K. (2018). Pedagogic rights and the acoustics of health and physical education teacher education: Whose voices are heard? Physical Education and Sport Pedagogy, 23(5), 524–535. https://doi.org/10.1080/17408989.2018.1470616

Farrell, K., ve MacDougall, D. (2023). An overview of clinical applications of virtual and augmented reality. Canadian Journal of Health Technologies, 3(3). https://doi.org/10.51731/cjht.2023.600

Fleming, T., Bavin, L., Stasiak, K., Hermansson-Webb, E., Merry, S., Cheek, C., Lucassen, M., Lau, H., Pollmuller, B., ve Hetrick, S. (2020). Serious games and gamification for mental health: Current status and promising directions. https://doi.org/10.26686/wgtn.12331271.v2

Furlan, R., Gatti, M., Menè, R., Shiffer, D., Marchiori, C., Giaj Levra, A., Saturnino, V., Brunetta, E., ve Dipaola, F. (2021). A natural language processing–based virtual patient simulator and intelligent tutoring system for the clinical diagnostic process: Simulator development and case study. JMIR Medical Informatics, 9(4), e24073. https://doi.org/10.2196/24073

García-Senín, S., Arguedas, M., ve Daradoumis, T. (2022). Using learning analytics to support STEAM students' academic achievement and self-regulated learning. Research on Education and Media, 14(1), 36–45. https://doi.org/10.2478/rem-2022-0005

Grassini, S. (2023). Shaping the future of education: Exploring the potential and consequences of AI and ChatGPT in educational settings. Education Sciences, 13(7), 692. https://doi.org/10.3390/educsci13070692

Guan, T., Chew, R. S. Y., Wen, X., ve Huan, B. (2026). Using multimodal learning analytics as a formative assessment tool in AI-assisted physical education: A case study of Baduanjin teaching. Frontiers in Education, 11. https://doi.org/10.3389/feduc.2026.1744594

Gündoğdu, S. (2021). Comparison of artificial neural networks with other classification algorithms using data on balance changes caused by exercises applied to patients with kyphosis due to osteoporosis. Journal of the Institute of Science and Technology (Iğdır University), 11(4), 2660–2672. https://doi.org/10.21597/jist.743051

Hsia, L.-H., Lin, Y.-N., Lin, C.-H., ve Hwang, G.-J. (2025). Prompting somatic practice performance with AI-facilitated peer-assisted learning: A self-determination theory perspective. Education and Information Technologies, 30(16), 23331–23364. https://doi.org/10.1007/s10639-025-13681-8

Huang, C., ve Zhan, L. (2025). Personalized learning paths for mental health education using knowledge graph and NLP. Schizophrenia Bulletin, 51(Supplement_4), S368–S368. https://doi.org/10.1093/schbul/sbaf199.075

Istenič Starčič, A. (2019). Human learning and learning analytics in the age of artificial intelligence. British Journal of Educational Technology, 50(6), 2974–2976. https://doi.org/10.1111/bjet.12879

Jang, H., Rempel, E., Roth, D., Carenini, G., ve Janjua, N. Z. (2021). Tracking COVID-19 discourse on Twitter in North America: Infodemiology study using topic modeling and aspect-based sentiment analysis. Journal of Medical Internet Research, 23(2), e25431. https://doi.org/10.2196/25431

Jiang, Z. (2022). The teaching of sports physical education skills under exercise physiology based on support vector machine. Discrete Dynamics in Nature and Society, 2022. https://doi.org/10.1155/2022/4416617

Jovanovic, P., ve Kay, R. (2023). Exploring ethical issues with using wearable technology in K-12 classrooms: A review of the literature. Journal of Digital Life and Learning, 2(2), 3–11. https://doi.org/10.51357/jdll.v2i2.203

Kamińska, D., Sapiński, T., Wiak, S., Tikk, T., Haamer, R., Avots, E., Helmi, A., Ozcinar, C., ve Anbarjafari, G. (2019). Virtual reality and its applications in education: Survey. Information, 10(10), 318. https://doi.org/10.3390/info10100318

Kiefer, A. W., Pincus, D., Richardson, M. J., ve Myer, G. D. (2017). Virtual reality as a training tool to treat physical inactivity in children. Frontiers in Public Health, 5. https://doi.org/10.3389/fpubh.2017.00349

Kitaguchi, D., Takeshita, N., Matsuzaki, H., Igaki, T., Hasegawa, H., ve Ito, M. (2021). Development and validation of a 3-dimensional convolutional neural network for automatic surgical skill assessment based on spatiotemporal video analysis. JAMA Network Open, 4(8), e2120786. https://doi.org/10.1001/jamanetworkopen.2021.20786

Klein, M. J., ve Simmers, C. S. (2009). Exergaming: virtual inspiration, real perspiration. Young Consumers, 10(1), 35–45. https://doi.org/10.1108/17473610910940774

Koekoek, J., van der Mars, H., van der Kamp, J., Walinga, W., ve van Hilvoorde, I. (2018). Aligning digital video technology with game pedagogy in physical education. Journal of Physical Education, Recreation & Dance, 89(1), 12–22. https://doi.org/10.1080/07303084.2017.1390504

Kriemler, S., Meyer, U., Roth, R., Zahner, L., Gerber, M., Puder, J. J., ve Hebestreit, H. (2011). Physical activity levels during physical education lessons and their contribution to total physical activity. Medicine & Science in Sports & Exercise, 43(5), 342. https://doi.org/10.1249/01.mss.0000400941.52087.54

Lai, X., Chen, J., Lai, Y., Huang, S., Cai, Y., Sun, Z., Wang, X., Pan, K., Gao, Q., ve Huang, C. (2025). Using large language models to enhance exercise recommendations and physical activity in clinical and healthy populations: Scoping review. JMIR Medical Informatics, 13, e59309. https://doi.org/10.2196/59309

Lian, S. (2022). Experimental research on badminton teaching of physical education major based on deep learning in the multimedia environment. Computational Intelligence and Neuroscience, 2022, 9249635. https://doi.org/10.1155/2022/9249635

Liao, X., Dong, J., Song, K., ve Xiao, J. (2023). Three-dimensional human pose estimation from sparse IMUs through temporal encoder and regression decoder. Sensors, 23(7), 3547. https://doi.org/10.3390/s23073547

Lin, C.-C., Huang, A. Y. Q., ve Lu, O. H. T. (2023). Artificial intelligence in intelligent tutoring systems toward sustainable education: A systematic review. Smart Learning Environments, 10(1). https://doi.org/10.1186/s40561-023-00260-y

Liu, G., ve Zhuang, H. (2022). Evaluation model of multimedia-aided teaching effect of physical education course based on random forest algorithm. Journal of Intelligent Systems, 31(1), 555–567. https://doi.org/10.1515/jisys-2022-0041

Luan, H., Geczy, P., Lai, H., Gobert, J., Yang, S. J. H., Ogata, H., Baltes, J., Guerra, R., Li, P., ve Tsai, C.-C. (2020). Challenges and future directions of big data and artificial intelligence in education. Frontiers in Psychology, 11. https://doi.org/10.3389/fpsyg.2020.580820

Lv, Z., Poiesi, F., Dong, Q., Lloret, J., ve Song, H. (2022). Deep learning for intelligent human–computer interaction. Applied Sciences, 12(22), 11457. https://doi.org/10.3390/app122211457

Martín-Hoz, L., Yanes-Luis, S., Huerta Cejudo, J., Gutiérrez-Reina, D., ve Franco Álvarez, E. (2025). A comparative study of BERT-based models for teacher classification in physical education. Electronics, 14(19), 3849. https://doi.org/10.3390/electronics14193849

Marttinen, R., Landi, D., Fredrick, R. N., ve Silverman, S. (2020). Wearable digital technology in PE: Advantages, barriers, and teachers' ideologies. Journal of Teaching in Physical Education, 39(2), 227–235. https://doi.org/10.1123/jtpe.2018-0240

Maslej, M. M., Donner, K., Thakur, A., Islam, F., Costa-Dookhan, K. A., ve Sockalingam, S. (2025). Deriving insights from open-ended learner feedback: An exploration of natural language processing approaches. Journal of Continuing Education in the Health Professions, 45(3), 203–209. https://doi.org/10.1097/ceh.0000000000000597

Namba, H. (2023). ICT and physical activity. Advanced Virtual Assistants - A Window to the Virtual Future [Working Title]. https://doi.org/10.5772/intechopen.1001933

Nyberg, G., Quennerstedt, M., Tolgfors, B., ve Backman, E. (2025). Physical education teachers' experiences of the meaning of feedback in PE. European Physical Education Review, 32(2), 239–256. https://doi.org/10.1177/1356336x251340248

O'Malley, L., Halley, A., Willms, A., Edwards, I., ve Liu, S. (2026). Creating customized chatbots with ChatGPT to promote physical activity: A mini review. Frontiers in Digital Health, 8. https://doi.org/10.3389/fdgth.2026.1761193

Ozkaya, G., Jung, H. R., Jeong, I. S., Choi, M. R., Shin, M. Y., Lin, X., Heo, W. S., Kim, M. S., Kim, E., ve Lee, K.-K. (2018). Three-dimensional motion capture data during repetitive overarm throwing practice. Scientific Data, 5(1). https://doi.org/10.1038/sdata.2018.272

Qiu, Y. (2024). The impact of LLM hallucinations on motor skill learning: A case study in badminton. IEEE Access, 12, 139669–139682. https://doi.org/10.1109/access.2024.3444783

Quan, Z., ve Zhao, Y. (2024). Optimization strategies for physical education teaching movements using biomechanical analysis and deep learning techniques. Molecular & Cellular Biomechanics, 21(4), 465. https://doi.org/10.62617/mcb465

Ratcliffe, J., Soave, F., Bryan-Kinns, N., Tokarchuk, L., ve Farkhatdinov, I. (2021). Extended reality (XR) remote research: A survey of drawbacks and opportunities. Proceedings of the 2021 CHI Conference on Human Factors in Computing Systems, 1–13. https://doi.org/10.1145/3411764.3445170

Rodriguez, A. M., ve Cerezo, R. M. (2025). The integration of artificial intelligence in physical education, innovation, and sustainability in learning performance. In Innovation and Sustainability in Electric and Autonomous Mobility, 53–78. https://doi.org/10.4018/979-8-3373-2802-7.ch003

Roman, N., Baseanu, C., Tuchel, V. I., Nicolau, C., Repanovici, A., Manaila, A., Minzatanu, D., ve Miclaus, R. S. (2023). The benefits of combining mixed virtual reality exergaming with occupational therapy for upper extremity dexterity. Electronics, 12(6), 1431. https://doi.org/10.3390/electronics12061431

Rossi, T., Tinning, R., McCuaig, L., Sirna, K., ve Hunter, L. (2009). With the best of intentions: A critical discourse analysis of physical education curriculum materials. Journal of Teaching in Physical Education, 28(1), 75–89. https://doi.org/10.1123/jtpe.28.1.75

Salami, F. A., ve Althaqafi, A. S. (2023). The effectiveness of teacher autonomy supportive style on enhancing student engagement in EFL virtual classrooms. English Language and Literature Studies, 13(1), 44. https://doi.org/10.5539/ells.v13n1p44

Sarupuri, B., Kulpa, R., Aristidou, A., ve Multon, F. (2023). Dancing in virtual reality as an inclusive platform for social and physical fitness activities: A survey. The Visual Computer, 40(6), 4055–4070. https://doi.org/10.1007/s00371-023-03068-6

Schöllhorn, W. I., Rizzi, N., Slapšinskaitė-Dackevičienė, A., ve Leite, N. (2022). Always pay attention to which model of motor learning you are using. International Journal of Environmental Research and Public Health, 19(2), 711. https://doi.org/10.3390/ijerph19020711

Scruggs, P. W. (2007). Middle school physical education physical activity quantification. Research Quarterly for Exercise and Sport, 78(4), 284–292. https://doi.org/10.1080/02701367.2007.10599426

Shan, G. (2020). Challenges and future of wearable technology in human motor-skill learning and optimization. Sports Science and Human Health - Different Approaches. https://doi.org/10.5772/intechopen.91356

Shi, Y. (2023). Image recognition of skeletal action for online physical education class based on convolutional neural network. IEIE Transactions on Smart Processing & Computing, 12(1), 55–63. https://doi.org/10.5573/ieiespc.2023.12.1.55

Sindhwani, N., Anand, R., S., M., Shukla, R., Yadav, M., ve Yadav, V. (2021). Performance analysis of deep neural networks using computer vision. EAI Endorsed Transactions on Industrial Networks and Intelligent Systems, 8(29), 171318. https://doi.org/10.4108/eai.13-10-2021.171318

Sousa, I., Sobreira, S., ve Pedretti, A. (2025). Nível de atividade física, depressão e ansiedade em estudantes de Educação Física. Revista Andaluza de Medicina del Deporte, 18(1). https://doi.org/10.33155/ramd.v18i1.1222

Sperka, L., Enright, E., ve McCuaig, L. (2017). Brokering and bridging knowledge in health and physical education: A critical discourse analysis of one external provider's curriculum. Physical Education and Sport Pedagogy, 23(3), 328–343. https://doi.org/10.1080/17408989.2017.1406465

Tedre, M., Toivonen, T., Kahila, J., Vartiainen, H., Valtonen, T., Jormanainen, I., ve Pears, A. (2021). Teaching machine learning in K–12 classroom: Pedagogical and technological trajectories for artificial intelligence education. IEEE Access, 9, 110558–110572. https://doi.org/10.1109/access.2021.3097962

Tian, Y. (2024). Empowering college physical education: AI-driven training, teaching, and intelligent information processing. Molecular & Cellular Biomechanics, 21(1), 327. https://doi.org/10.62617/mcb.v21i1.327

Tolgfors, B., Backman, E., Nyberg, G., ve Quennerstedt, M. (2022). Exploring movement composition in the transition from physical education teacher education to school PE. Physical Education and Sport Pedagogy, 29(6), 670–684. https://doi.org/10.1080/17408989.2022.2153818

Wade, L., Needham, L., Evans, M., McGuigan, P., Colyer, S., Cosker, D., ve Bilzon, J. (2023). Examination of 2D frontal and sagittal markerless motion capture: Implications for 2D and 3D markerless applications. https://doi.org/10.1101/2023.01.17.523930

Wang, H., Jin, X., Zhang, T., ve Wang, J. (2023). Convolutional neural network-based recognition method for volleyball movements. Heliyon, 9(8), e18124. https://doi.org/10.1016/j.heliyon.2023.e18124

Wen, B. (2025). Wearable multimodal data fusion methods for intelligent assessment in physical education. Internet Technology Letters, 9(1). https://doi.org/10.1002/itl2.70134

White, R. L., Bennie, A., Vasconcellos, D., Cinelli, R., Hilland, T., Owen, K. B., ve Lonsdale, C. (2021). Self-determination theory in physical education: A systematic review of qualitative studies. Teaching and Teacher Education, 99, 103247. https://doi.org/10.1016/j.tate.2020.103247

Wu, M. (2023). Technological training tools in teaching physical education among martial arts athletes. International Journal of Social Science and Human Research, 06(03). https://doi.org/10.47191/ijsshr/v6-i3-39

Yau, Y., Shen, Y. C., ve Hooi, L. B. (2026). Leveraging human pose estimation for diagnostic feedback: Action research on instructional mediation and sustainable learning in coach education. Acta Psychologica, 263, 106128. https://doi.org/10.1016/j.actpsy.2025.106128

Ye, J., Hai, J., Wang, Z., Wei, C., ve Song, J. (2023). Leveraging natural language processing and geospatial time series model to analyze COVID-19 vaccination sentiment dynamics on Tweets. JAMIA Open, 6(2). https://doi.org/10.1093/jamiaopen/ooad023

Yim, Y.-R., ve Hur, M.-H. (2023). Effects of virtual reality program on glycated hemoglobin, static and dynamic balancing ability, and falls efficacy for diabetic patients: A systematic review and meta-analysis. Journal of Korean Academy of Fundamentals of Nursing, 30(2), 155–167. https://doi.org/10.7739/jkafn.2022.30.2.155

Yuan, J., Zhang, Y., ve Chen, B. (2026). An IoT-enabled CRNN framework for secure wearable sensor-based activity recognition in physical education. Scientific Reports, 16(1). https://doi.org/10.1038/s41598-026-42082-1

Zhang, F. (2023). Research on physical education management system in higher education institutions in the context of deep learning. Applied Mathematics and Nonlinear Sciences, 9(1). https://doi.org/10.2478/amns.2023.2.01612

Zhang, J. (2025). Leveraging artificial intelligence for enhanced physical education in universities. International Journal of Web-Based Learning and Teaching Technologies, 20(1), 1–15. https://doi.org/10.4018/ijwltt.393622

Zhang, R., Qi, Y., Kong, S., Wang, X., ve Li, M. (2024). A hybrid artificial intelligence algorithm for fault diagnosis of hot rolled strip crown imbalance. Engineering Applications of Artificial Intelligence, 130, 107763. https://doi.org/10.1016/j.engappai.2023.107763

Zhang, Z., Wang, C., Qiu, W., Qin, W., ve Zeng, W. (2020). AdaFuse: Adaptive multiview fusion for accurate human pose estimation in the wild. International Journal of Computer Vision, 129(3), 703–718. https://doi.org/10.1007/s11263-020-01398-9

Zharmukhanbetov, S., ve Singh, C. P. (2023). Enhancing flipped classroom engagement and personalized learning through AI-powered adaptive content delivery. 2023 3rd International Conference on Technological Advancements in Computational Sciences (ICTACS), 1411–1416. https://doi.org/10.1109/ictacs59847.2023.10389841

Zhou, J., ve Wu, W. (2025). Reforming university physical education with artificial intelligence for enhanced teaching and learning. Discover Artificial Intelligence, 6(1). https://doi.org/10.1007/s44163-025-00531-2

Global Research Trends on Artificial Intelligence in Pressure Injury Research: A Bibliometric Analysis for Nursing Practice

Ümmühan YİĞİT*

[1]Nursing/Faculty of Health Science, Bolu Abant Izzet Baysal University, Turkiye
*(ummuhanyigit@ibu.edu.tr) Email of the corresponding author

ABSTRACT

Objective: The objective of this study is to review the existing scientific literature on artificial intelligence applications in the prevention, prediction, and treatment of pressure ulcers in intensive care units using bibliometric methods and to reveal research trends, collaboration networks, and development dynamics in this field.
Method: A comprehensive search was conducted on March 11, 2026, using the Web of Science database with the keywords “pressure ulcer,” “pressure injury,” “machine learning,” “artificial intelligence,” and “deep learning.” The retrieved publications were analyzed using the Vosviewer program to determine the distribution of publications by year, the most productive authors, institutions, and countries, citation networks, and keyword co-occurrence maps. In addition, publications were classified into categories of artificial intelligence applications in the field of pressure ulcers through thematic analysis.
Results: Following the keyword search, 68,675 records related to pressure ulcers [pressure ulcer or pressure injury or ulcer] were identified in the Web of Science (WoS) database. Of these, 259 articles containing the terms “machine learning,” “artificial intelligence,” or “deep learning” in their titles were included in the analysis.
Conclusion: This bibliometric study indicate that artificial intelligence research on pressure ulcers holds significant potential for preventive care and risk assessment in nursing; however, due to a lack of multidisciplinary collaboration and clinical integration, future efforts should focus on developing clinically valid and practice-integrated systems.

Keywords – Bibliometrics, Nursing, Research Trends, Pressure Injury, Vosviewer

I. INTRODUCTION

Pressure injuries are defined by the National Pressure Ulcer Advisory Panel (NPUAP) as “localized damage to the skin and/or underlying soft tissue, typically over a bony prominence or in association with a medical device.” The injury may present as intact skin or an open wound and may be painful. The damage results from prolonged pressure or pressure combined with friction. The soft tissue’s tolerance to pressure can be influenced by factors such as nutrition, perfusion, and the individual’s overall health status. [1].

Pressure injuries are recognized as one of the quality indicators in the healthcare system, and their prevention and treatment require a comprehensive care approach. Pressure injuries, a preventable problem, negatively impact the

patient's quality of life, prolong hospital stays, increase dependence on family members and healthcare staff by causing a loss of independence, lead to physical and emotional problems in the patient, lead to social isolation, increase morbidity and mortality rates, heighten susceptibility to healthcare-associated infections, result in unnecessary medication use, and, in this context, increase the cost of healthcare services. It has been reported that 90% of pressure ulcers can be prevented through the identification of at-risk individuals and the provision of appropriate care. [1,2].

In units where high-risk patient groups are present, preventive measures against pressure injuries must be considered. Pressure injuries are a critical issue that must be addressed through a multidisciplinary approach, encompassing the patient's caloric intake, monitoring of hemodynamic parameters, tissue perfusion, laboratory findings, vital signs, infection control, wound care, positioning, skin care, and maintenance of skin integrity. [3–6].

It is important to enhance the competencies of healthcare professionals by adopting a multidisciplinary approach to the prevention, care, and treatment of pressure injuries based on current evidence. To mitigate the physical, psychosocial, and economic impacts of pressure injuries and reduce the burden they place on the healthcare system, it is essential to prevent pressure injuries before they occur. [7]. However, the limitations of traditional methods are increasing the need for more advanced and data-driven approaches.

In recent years, advancements in Artificial Intelligence and Machine Learning technologies have led to the emergence of innovative applications in the healthcare sector, particularly those supporting clinical decision-making processes. In the field of pressure injuries, AI applications have emerged as effective tools for risk prediction, early diagnosis, staging, and the development of prevention strategies. Indeed, it has been demonstrated that machine learning algorithms can perform risk prediction with high accuracy using electronic health records and clinical data and can be integrated into clinical decision support systems. [8,9].

However, the integration of AI-based approaches into nursing practice offers significant opportunities for improving the quality of care and ensuring patient safety. These technologies, which reduce the decision-making burden on nurses and contribute to the development of personalized care plans—particularly in intensive care, surgical, and chronic care settings—are increasingly being adopted in clinical practice. However, there is a need for a

systematic evaluation of the scope, trends, and collaborative networks within this field.

In this context, bibliometric analyses enable the quantitative examination of the scientific literature in a specific research field, the identification of research trends, and the determination of future research directions [10]. This study aims to examine the global scientific output on artificial intelligence applications in pressure injury research using bibliometric methods, to identify research trends, collaboration networks, and development dynamics in the field, and to contribute to nursing practice based on the findings.

II. METHODS

Study Design

This study aims to examine the existing scientific literature on artificial intelligence applications in pressure injuries through bibliometric methods and to identify research trends, collaboration networks, and the developmental dynamics of this field. Data has been collected for each category on publication years, countries where publications were made, countries with the most publications receiving citations, keywords most frequently used in publications, and journals with the highest number of publications in the field.

Research Method and Data Collection Process

This study was conducted using bibliometric analysis methods. The data for the research were obtained using Web of Science (WoS) on March 11, 2026. The data analysis was evaluated using VOSviewer (1.6.20, CWTS, Leiden, Netherlands). A separate search command was written for each category evaluated on WoS. The keywords “pressure ulcer, pressure injury, ulcer” were used for pressure sores, and “machine learning, artificial intelligence, deep learning” were used for artificial intelligence.

Initially, 68,675 records related to pressure ulcers [pressure ulcer OR pressure injury OR ulcer] were identified in the Web of Science (WoS) database. Among these, 259 articles containing the terms “machine learning,” “artificial intelligence,” or “deep learning” in their titles were included in the analysis. These publications have received a total of 1,708 citations.

Boolean query: TS=["pressure ulcer" OR "pressure injury" OR ulcer*

AND ["machine learning" OR "artificial intelligence" OR "deep learning" OR "neural networks"]

Inclusion Criteria

Studies indexed in the Web of Science (WoS) database.

Publications related to pressure injuries, containing at least one of the following terms in the title, abstract, or keywords: “pressure ulcer,” “pressure injury,” or “decubitus ulcer.”

Studies related to artificial intelligence applications, including terms such as “artificial intelligence,” “machine learning,” or “deep learning” in the title, abstract, or keywords.

Exclusion Criteria

Studies not directly related to pressure injuries or artificial intelligence.

III. RESULTS

The general characteristics of the publications included in the study, categorized by document type, Web of Science [WOS] research areas, geographic distribution, and funding agencies, are summarized in Table 1.

A total of 259 publications were analyzed. The majority of these documents were original Articles (n = 176, 67.95\%), followed by meeting abstracts (11.20\%] and review articles (10.81\%). Other document types, such as proceeding papers and early access articles, accounted for a smaller portion of the total output. In terms of WOS research areas, surgery was the most prominent field, representing 13.90\% of the total publications (n = 36). This was followed by dermatology (11.20\%) and medicine general internal (11.20\%), suggesting that the research topic is highly relevant to clinical and surgical disciplines. The nursing discipline ranked fourth, with 28 publications (10.81%), following these fields.

The analysis of country-level productivity revealed that the People's Republic of China was the leading contributor to the field, accounting for 29.34% (n = 76) of the analyzed literature. The USA followed with 18.92\% [n = 49], and India ranked third with 9.65\% (n = 25). Other significant contributors included England, Japan, and Portugal. These findings indicate a strong research focus in East Asia and North America. Although Turkey is not

among the top 10 countries in the publication ranking, it ranks 13th with 7 publications (2.703%).

Regarding the funding landscape, the National Natural Science Foundation of China (NSFC) emerged as the most frequent sponsor, supporting 8.88\% (n = 23) of the studies. US-based organizations, specifically the National Institutes of Health (NIH) and the Department of Health and Human Services, also provided significant support, each contributing to 3.09\% of the publications. The diversity of funding agencies across different regions highlights the global interest and investment in this research area.

Table 1. Distribution of the Included Publications by Document Type, WoS Research Areas, Geographic Distribution, and Funding Agencies

Document Type	**n**	**%**	**WoS Research Areas**	**n**	**%**
Article	176	67.954	Surgery	36	13.900
Meeting Abstract	29	11.197	Dermatology	29	11.197
Review Article	28	10.811	Medicine General Internal	29	11.197
Proceeding Paper	16	6.178	Nursing	28	10.811
Early Access	10	3.861	Medical Informatics	25	9.653
Letter	5	1.931	Gastroenterology Hepatology	21	8.108
Correction	2	0.772	Endocrinology Metabolism	20	7.722
Editorial Material	2	0.772	Computer Science Information Systems	19	7.336
Retracted Publication	1	0.386	Engineering Biomedical	19	7.336
Retraction	1	0.386	Health Care Sciences Services	19	7.336
			Endocrinology Metabolism	20	7.722
Geographic Distribution			Funding Agencies		
Peoples R Chına	76	29.344	Natıonal Natural Scıence Foundatıon Of Chına NSFC	23	8.880
USA	49	18.919	Natıonal Instıtutes Of Health Nıh Usa	8	3.089
Indıa	25	9.653	Unıted States Department of Health Human Servıces	8	3.089
England	16	6.178	Fundacao Para A Cıencıa EA Tecnologıa FCT	4	1.544

Japan	11	4.247	Natıonal Key Research Development Program Of Chına	4	1.544
Portugal	10	3.861	European Unıon EU	3	1.158
South Korea	10	3.861	Fundamental Research Funds For The Central Unıversıtıes	3	1.158
Taıwan	9	3.475	Mınıstry Of Educatıon Culture Sports Scıence And Technology Japan Mext	3	1.158
Saudı Arabıa	8	3.089	Natıonal Research Foundatıon of Korea	3	1.158
Australıa	7	2.703	Natıonal Scıence Foundatıon NSF	3	1.158

Citation information for articles

The chronological distribution of research productivity and its impact on the scientific community is illustrated in Figure 1. The data reveals three distinct phases in the evolution of this research field: a period of dormancy, a period of emergence, and a current phase of rapid expansion.

From 1993 to approximately 2018, the research area remained in a prolonged state of dormancy, with sporadic publication activity (averaging fewer than 2 papers per year) and negligible citation counts. A notable shift began in 2019, marking the transition to an emergence phase.

The most significant surge in academic interest occurred after 2020. The number of annual publications increased dramatically, rising from 12 papers in 2020 to a peak of 64 papers in 2025. This upward trajectory in output is closely mirrored by the citation trends, which exhibit an exponential increase. Total citations reached their zenith in 2025, exceeding 1,100 citations in a single year.

The data for 2026 shows a partial decrease; however, this is likely attributed to the incomplete data collection period for the current year rather than a decline in research interest. The high correlation between the volume of publications (bars) and the frequency of citations (line) suggests that the field is not only expanding in quantity but is also achieving substantial scholarly impact and recognition within the global scientific community.

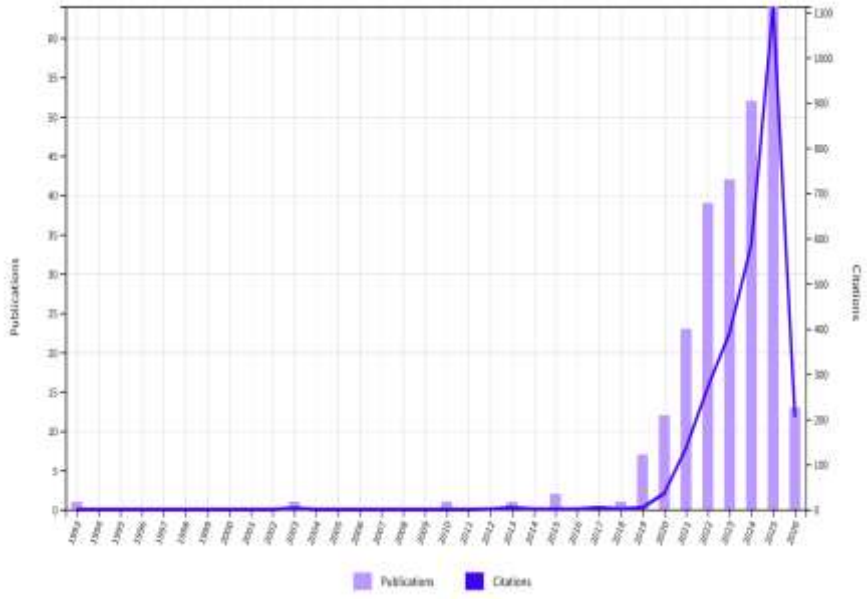


Figure 1 The chronological distribution of research productivity and its impact on the scientific community

Institutional collaborations

For institutional analysis, 124 items and 13 clusters were identified in the Vosviewer program, with the condition that there be at least 1 publication and at least 1 collaboration from an institution. A total of 401 link stranges were identified among these clusters.

The collaborative relationships between the most productive institutions were analyzed using a network visualization map (see Figure 2). In this map, the size of the nodes represents the volume of publications, while the thickness of the connecting lines indicates the strength of the co-authorship links. The analysis reveals several key clusters of academic collaboration:

A significant portion of the network is occupied by Chinese universities, indicating a highly integrated domestic and international research infrastructure.

- Tongji University, Xiamen University, and Shanghai University of Traditional Chinese Medicine (Green Cluster) form a central hub of collaboration.

• Capital Medical University (Purple Cluster) appears as one of the largest nodes, suggesting it is a leading contributor in terms of total publication count and acts as a bridge between different research groups.

• The North American Hub: Harvard Medical School and the University of Southern California represent major Western pillars in the network. Harvard's position toward the periphery of certain clusters suggests it may lead a specific niche or collaborate with a distinct set of partners [Pink/Yellow nodes].

• The Oceanic and Middle Eastern Links: Monash University (Australia), Birmingham City University (UK), and Qatar University form a vertical axis in the map (Red and Cyan clusters), showing a collaborative bridge between Europe, Oceania, and the Middle East.

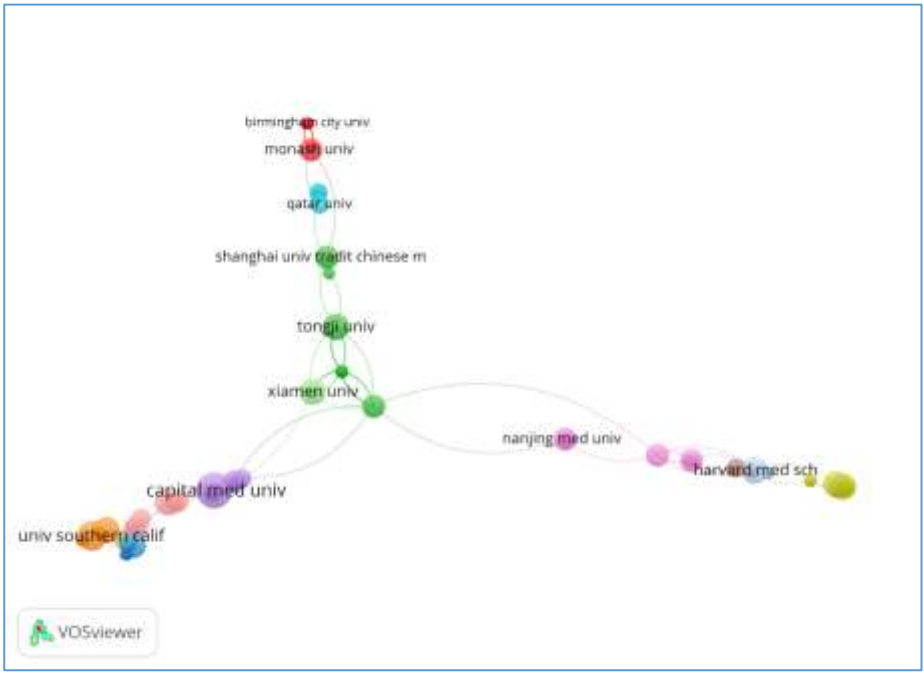


Figure 2. The collaborative relationships

Results: Author Co-authorship Network and Scholarly Collaboration

The intellectual structure of the field was further examined through a co-authorship network analysis (see Figure 3). This visualization identifies the core researchers, their productivity (node size), and the strength of their

collaborative ties (link thickness). The analysis revealed four primary research clusters:

1. The Central Academic Hub (Green Cluster)

The green cluster represents the most interconnected and central group within the network. Key figures such as Eliakim, Rami, Klang, Eyal, and Soffer, Shelly emerge as prominent nodes. Their central positioning and numerous connections suggest they are influential researchers who frequently lead multi-author studies and act as a bridge between different research subgroups.

2. High-Impact Collaborative Groups (Red and Blue Clusters)

- The Red Cluster: This group, featuring researchers like Shapira, Noam, Ben Horin, Shomron, and Ukashi, Offir, shows an extremely dense network of internal links. This density indicates a stable, long-term research team or laboratory that consistently publishes collaborative work.

- The Blue Cluster: Led by authors such as Yehuda, Reuma Margalit and Shanahan, Fergus, this cluster appears closely integrated with the central green hub, suggesting thematic or institutional overlap between these researchers.

3. Emerging or Specialized Groups (Yellow Cluster)

The yellow cluster, headed by Barash, Yiftach, is positioned toward the periphery of the main network. While these authors show strong internal collaboration (e.g., Lahat, Adi and Kiang, Eyal), their fewer links to the green and red clusters may indicate a specialized sub-focus or an emerging research group within the discipline.

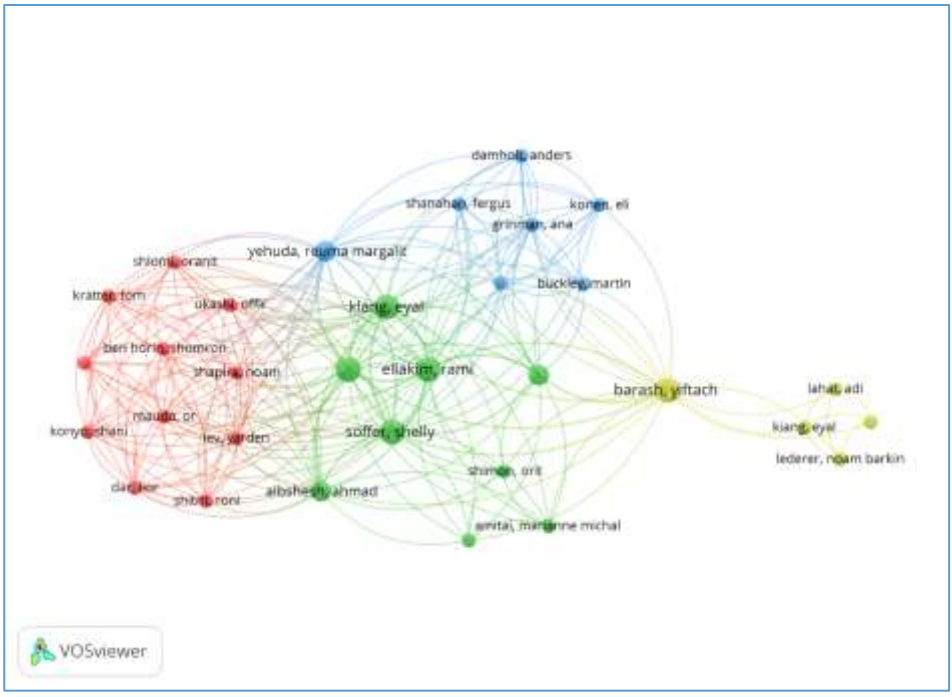


Figure 3. Autor collaborations

Autor collaborations

At least 1 publication and at least 1 citation were required, and 32 items and 4 clusters were identified. A total of 269 link strenges were identified among these clusters (Figure 3).

Key words collaborations

At least 1 publication and at least 1 citation were required, and 30 items and 5 clusters were identified. A total of 392 link strenges were identified among these clusters

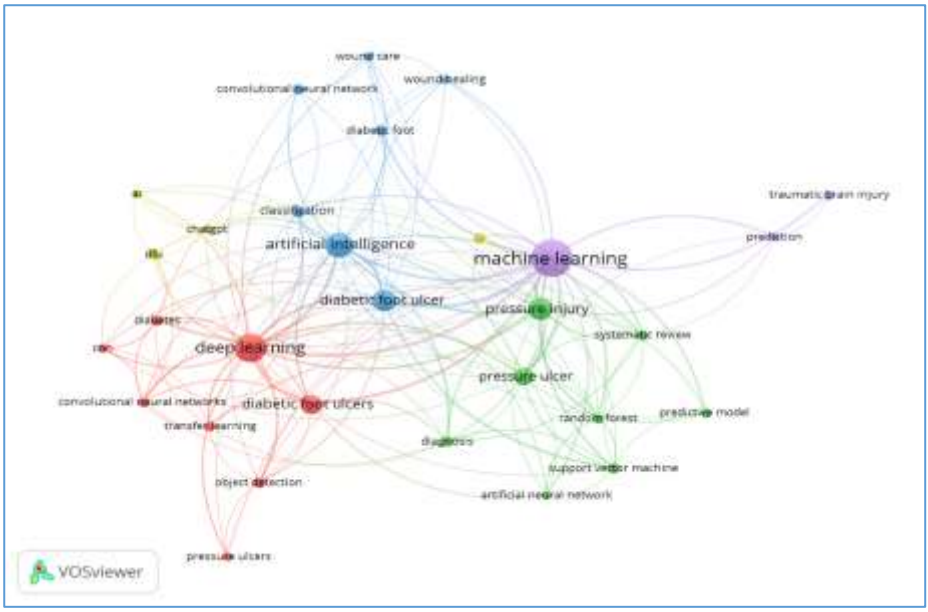


Figure 4. Key words collaborations

The main themes where the articles are concentrated

1. Deep Learning Models for Pressure Ulcer Classification: Several studies have developed convolutional neural networks (CNNs) and other deep learning models to classify and stage pressure ulcers with high accuracy, improving diagnostic precision in clinical settings [11,12].

2. Machine Learning for Diabetic Foot Ulcer Management: Research has focused on applying machine learning to identify, classify, and predict the healing of diabetic foot ulcers, which are critical complications of diabetes [13,14].

3. Integration of Explainability in AI Models: The incorporation of interpretability techniques such as SHAP and LIME in AI models to enhance transparency and trust in clinical decision-making [14,15].

4. Predictive Modeling for Pressure Ulcer Risk Assessment: Development of predictive models using electronic health records and machine learning to assess the risk of pressure ulcer development in hospital settings [16,17].

5. Wound Segmentation and Classification Systems: Advances in using CNNs for the automatic segmentation and classification of chronic wounds, enhancing the precision and efficiency of wound care management [18,19].
6. Wearable Technology for Pressure Ulcer Prevention: Development of wearable devices that monitor pressure and tissue vitality to prevent the onset of pressure ulcers in high-risk patients [20,21].
7. AI in Wound Healing Prognosis: Use of AI to predict wound healing trajectories, providing valuable insights into the progression of wound healing and aiding in clinical decision-making [22,23].
8. Hybrid AI Models for Enhanced Wound Diagnosis: The combination of different AI approaches, such as CNNs and Bayesian networks, to improve the accuracy of wound diagnosis and classification [24].
9. AI-Driven Predictive Tools for Clinical Decision Support: Implementation of AI-based clinical decision support systems to enhance pressure ulcer prevention and management [15,25].
10. Multimodal Approaches to Ulcer Management: Combining machine learning with other medical technologies such as telemedicine and regenerative therapies to manage wound care effectively [26].

Journal collaborations

A minimum requirement of publishing at least one publication was set for journal searches. 139 items, 8 clusters, and a total of 8,674 links were reached.

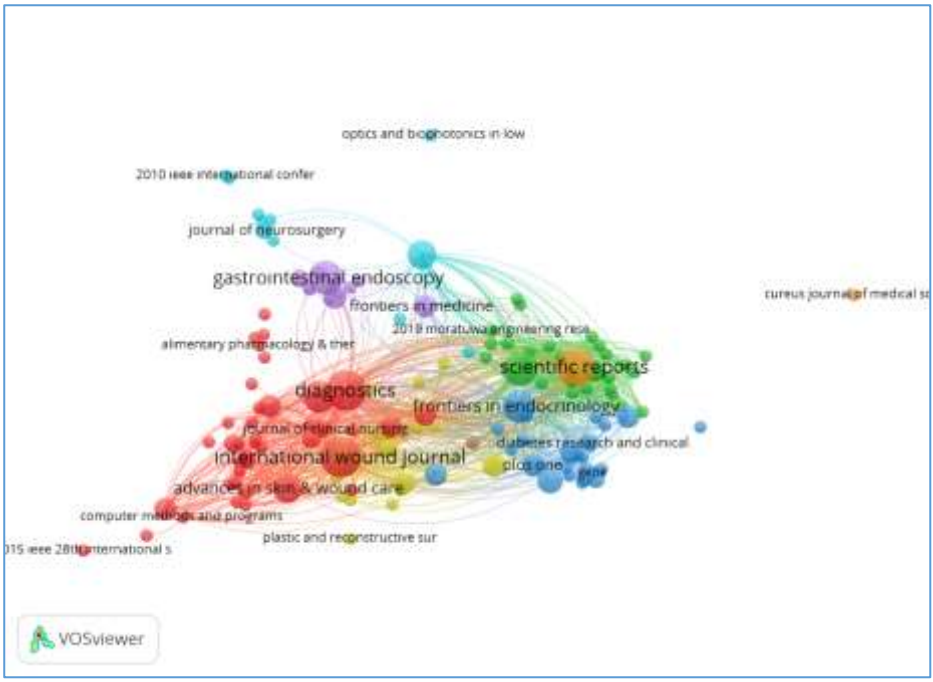


Figure 5. Journal collaborations

The intellectual structure and the interdisciplinary nature of the research field were visualized through a journal co-citation network (Figure 5). This analysis reveals how different scientific domains interact and which journals serve as the fundamental knowledge base for the topic. The network is organized into several distinct thematic clusters:

The red cluster represents the clinical core of the research, with a heavy emphasis on patient care and specialized treatments.

- International Wound Journal and Advances in Skin & Wound Care appear as the most significant nodes, indicating their role as the primary references for clinical practice.

- The inclusion of the Journal of Clinical Nursing within this cluster highlights the vital role of nursing sciences in the practical application of these research findings.

Table 2. The 10 Most Highly Cited Publications in Nursing Artificial İntelligence Research in Nursing For Pressure Ulcers

Authors	Years	Publication Title	Journal	NCite
Nakagami, G.; Yokota, s; Kitamura, A; Takahashi, T;Morita, K; Noguchi, H; Ohe, K; Sanada,H	2021	Supervised machine learning-based prediction for in-hospital pressure injury development using electronic health records: A retrospective observational cohort study in a university hospital in Japan	International Journal of Nursing Studies	35
Xu, J; Chen, T; Fang, X; Xia, L; Pan, X	2024	Prediction model of pressure injury occurrence in diabetic patients during ICU hospitalization--XGBoost machine learning model can be interpreted based on SHAP	Intensive and Critical Care Nursing	33
Ya-Han,H; Yi-Len,L; Ming-Feng, K; Pei-Ju, L	2020	Constructing Inpatient Pressure Injury Prediction Models Using Machine Learning Techniques	CIN: Computers, Informatics, Nursing	32
Ji-Yu, C; Man-Li, Z; Yi-Ping, S; Hong-Lin, C	2023	Predicting the Development of Surgery-Related Pressure Injury Using a Machine Learning Algorithm Model	Journal of Nursing Research	25
Seo, S; Kang, J; Eom,H; Song, H; Park, J H; Lee, Y; Lee, H	2023	Visual classification of pressure injury stages for nurses: A deep learning model applying modern convolutional neural networks	Journal of Advanced Nursing	15
Chun, X; Pan, LY; Lin, Y; Ye, LY; Liang, HY; Tao, JP; Luo, Y	2021	A model for predicting 7-day pressure injury outcomes in paediatric patients: A machine learning approach	Journal of Advanced Nursing	11
Mousa, KM; Mousa, FA; Mohamed, HS; Elsawy, MM	2023	Prediction of Foot Ulcers Using Artificial Intelligence for Diabetic Patients at Cairo University Hospital, Egypt	Sage Open Nursing	10
Myoung, K; Jung Mi; Choi,R; Kwan, B	2023	Development and Effectiveness of a Clinical Decision Support System for Pressure Ulcer Prevention	CIN: Computers, Informatics, Nursing	9

		Care Using Machine Learning A Quasi-experimental Study		
Alderden,J; Johnny,J; Brooks, K, R; Wilson,A; Yap, T, L; Zhao, Y; Laan, M; Kennerly, S	2024	Explainable Artificial Intelligence For Early Prediction Of Pressure Injury Risk	Amerıcan Journal Of Critical Care	6
Ikuta, K; Fukuoka; K, Kimura, Y, Nakagaki, M;, Ohga, M;, Suyama, Y;, Morita , M; Umeda,R;, Konishi, M; Nishikawa,H; Yagi, S.	2024	An ingenious deep learning approach for pressure injury depth evaluation with limited data	Journal of Tissue Viability	5

When studies on artificial intelligence in the field of pressure injuries within nursing are examined, it is evident that research has focused on several key clinical applications. These include the staging and visual classification of pressure injuries, the development of explainable artificial intelligence approaches for early prediction of pressure injury risk, and the creation of clinical decision support systems aimed at preventing pressure injuries. In addition, numerous studies have developed machine learning–based prediction models to estimate the likelihood of pressure injury development. Deep learning models utilizing image-based feature variables for the intelligent prediction of pressure injuries and artificial intelligence models designed to determine pressure injury depth have also gained increasing attention. Furthermore, research has explored patient-centered artificial intelligence models and interpretable machine learning approaches based on traditional risk assessment tools such as the Braden Scale to predict both pressure injury risk and mortality. Other studies have focused on predicting surgery-related pressure injuries, as well as forecasting hospital-acquired pressure injuries using supervised machine learning models based on electronic health records. Conducted across various clinical settings—including intensive care units, emergency departments, pediatric populations, and surgical intensive care units—these studies demonstrate that artificial

intelligence and machine learning approaches offer significant potential to support nursing care in the early detection, risk assessment, and prevention of pressure injuries (Table 2) [11,15,35–44,27–34].

In light of these findings, it can be said that artificial intelligence research on pressure injuries holds significant potential in the nursing discipline in terms of preventive care, personalized risk assessment, and improving the quality of care. However, the limited presence of multidisciplinary collaborations in the literature and the insufficient evaluation of the integration of certain models into clinical practice point to significant gaps for future research. Therefore, it is recommended that future studies focus on developing artificial intelligence systems that incorporate greater clinical validation, are integrable into nursing practice, and are user-friendly.

REFERENCES

1. National Pressure Injury Advisory Panel [NPIAP]. Prevention and Treatment of Pressure Ulcers/Injuries: Clinical Practice Guideline. 2019;

2. Visconti AJ, Sola OI, Raghavan P V. Pressure Injuries: Prevention, Evaluation, and Management. American Family Physician. 2023;108[2]:166–74.

3. García-Fernández FP, Agreda JJS, Verdú J, Pancorbo-Hidalgo PL. A new theoretical model for the development of pressure ulcers and other dependence-related lesions. Journal of Nursing Scholarship. 2014;46[1]:28–38.

4. de Sousa Júnior BS, da Silva Duarte FH, Pereira Rodrigues AN, Oliveira de Mendonça AE, de Vasconcelos Torres G, Vieira Dantas D, et al. Preventive Actions In Pressure Ulcer Development In Intensive Care Units. International Archives of Medicine. 2016;1–9.

5. Arai K, Yamamoto K hei, Suzuki T, Mitsukawa N, Ishii I. Risk factors affecting pressure ulcer healing: Impact of prescription medications. Wound Repair and Regeneration. 2020;28[3]:409–15.

6. Degerli MS. Pressure Ulcers in Palliative Care Unit Patients. Experimed. 2023;13[2]:133–41.

7. Mohamed SA, Weheida SM. Effects of implementing educational program about pressure ulcer control on nurses' knowledge and safety of immobilized patients. Journal of Nursing Education and Practice. 2014;5[3].

8. Topol E. Deep Medicine:How Artificial Intelligence Can Make Healthcare Human Again. Basic Books.; 2019.

9. Esteva A, Robicquet A, Ramsundar B, Kuleshov V, DePristo M, Chou K, et al. A guide to deep learning in healthcare. Nature Medicine [Internet].

2019;25[1]:24–9. Available from: http://dx.doi.org/10.1038/s41591-018-0316-z

10. Donthu N, Kumar S, Mukherjee D, Pandey N, Lim WM. How to conduct a bibliometric analysis: An overview and guidelines. Journal of Business Research [Internet]. 2021;133[April]:285–96. Available from: https://doi.org/10.1016/j.jbusres.2021.04.070

11. Seo S, Kang J, Eom IH, Song H, Park JH, Lee Y, et al. Visual classification of pressure injury stages for nurses: A deep learning model applying modern convolutional neural networks. Journal of Advanced Nursing. 2023;79[8]:3047–56.

12. Zalluhoğlu C, Akdoğan D, Karakaya D, Güzel MS, Ülgü MM, Ardalı K, et al. Region-Based Semi-Two-Stream Convolutional Neural Networks for Pressure Ulcer Recognition. Journal of Imaging Informatics in Medicine [Internet]. 2024;37[2]:801–13. Available from: https://doi.org/10.1007/s10278-023-00960-4

13. Spinazzola E, Picaud G, Becchi S, Pittarello M, Ricci E, Chaumont M, et al. Chronic Ulcers Healing Prediction through Machine Learning Approaches: Preliminary Results on Diabetic Foot Ulcers Case Study. Journal of Clinical Medicine. 2025;14[9]:1–16.

14. Rathore PS, Kumar A, Nandal A, Dhaka A, Sharma AK. A feature explainability-based deep learning technique for diabetic foot ulcer identification. Scientific Reports. 2025;15[1]:1–22.

15. Kim MS, Ryu JM, Choi BK. Development and Effectiveness of a Clinical Decision Support System for Pressure Ulcer Prevention Care Using Machine Learning: A Quasi-experimental Study. CIN - Computers Informatics Nursing. 2023;41[4]:236–45.

16. Song J, Gao Y, Yin P, Li Y, Li Y, Zhang J, et al. The random forest model has the best accuracy among the four pressure ulcer prediction models using machine learning algorithms. Risk Management and Healthcare Policy. 2021;14:1175–87.

17. Padula W V., Armstrong DG, Pronovost PJ, Saria S. Predicting pressure injury risk in hospitalised patients using machine learning with electronic health records: a US multilevel cohort study. BMJ Open. 2024;14[4].

18. Wang C, Anisuzzaman DM, Williamson V, Dhar MK, Rostami B, Niezgoda J, et al. Fully automatic wound segmentation with deep convolutional neural networks. Scientific Reports [Internet]. 2020;10[1]:1–9. Available from: https://doi.org/10.1038/s41598-020-78799-w

19. García-Zapirain B, Elmogy M, El-Baz A, Elmaghraby AS. Classification of pressure ulcer tissues with 3D convolutional neural network. Medical and Biological Engineering and Computing. 2018;56[12]:2245–58.

20. Gruenerbel L, Heinrich F, Böhlhoff-Martin J, Röper L, Machens HG,

Gruenerbel A, et al. Wearable Prophylaxis Tool for AI-Driven Identification of Early Warning Patterns of Pressure Ulcers. Bioengineering. 2023;10[10]:1–33.

21. Cicceri G, De Vita F, Bruneo D, Merlino G, Puliafito A. A deep learning approach for pressure ulcer prevention using wearable computing. Human-centric Computing and Information Sciences [Internet]. 2020;10[1]. Available from: https://doi.org/10.1186/s13673-020-0211-8

22. Gupta R, Goldstone L, Eisen S, Ramachandram D, Cassata A, Fraser RDJ, et al. Towards an AI-Based Objective Prognostic Model for Quantifying Wound Healing. IEEE Journal of Biomedical and Health Informatics. 2024;28[2]:666–77.

23. Berezo M, Budman J, Deutscher D, Hess CT, Smith K, Hayes D. Predicting Chronic Wound Healing Time Using Machine Learning. Advances in Wound Care. 2022;11[6]:281–96.

24. Veredas FJ, Mesa H, Morente L. A hybrid learning approach to tissue recognition in wound images. International Journal of Intelligent Computing and Cybernetics. 2009;2[2]:327–47.

25. Kim J, Lee C, Choi S, Sung DI, Seo J, Na Lee Y, et al. Augmented Decision-Making in wound Care: Evaluating the clinical utility of a Deep-Learning model for pressure injury staging. International Journal of Medical Informatics [Internet]. 2023;180[October]:105266. Available from: https://doi.org/10.1016/j.ijmedinf.2023.105266

26. Liao H, Liao T, Wu Y. Integrating Regenerative Therapies and Artificial Intelligence for Precision Management of Diabetic Foot Ulcers. 2026;00[00]:1–26.

27. Alderden J, Johnny J, Brooks KR, Wilson A, Yap TL, Zhao Y, et al. Explainable Artificial Intelligence for Early Prediction of Pressure Injury Risk. Am J Crit Care. 2024;33[5]:373–81.

28. Wei L, Lv H, Yue C, Yao Y, Gao N, Chai Q, et al. A machine learning algorithm-based predictive model for pressure injury risk in emergency patients: A prospective cohort study. International Emergency Nursing [Internet]. 2024;74[February]:101419. Available from: https://doi.org/10.1016/j.ienj.2024.101419

29. Ikuta K, Fukuoka K, Kimura Y, Nakagaki M, Ohga M, Suyama Y, et al. An ingenious deep learning approach for pressure injury depth evaluation with limited data. Journal of Tissue Viability [Internet]. 2024;33[3]:387–92. Available from: https://doi.org/10.1016/j.jtv.2024.05.009

30. Puttur N, Manoj R, Bhosale K, Malik N, Patil P, Niezgoda J, et al. A Within-Person Randomized Controlled Pilot Study to Evaluate the Ability of a Point-of-Care Artificial Intelligence-Enabled Multispectral Imaging Device to Manage Leg Ulcers in Leprosy. Advances in skin & wound care.

2025;38[9]:471–9.

31. Demir Üçtepe AS, Battal AE, Güleç C, Ergün E, Bakcaci A, Karadağ A, et al. Artificial Intelligence-Enabled Staging Classification of Pressure Injuries. Advances in skin & wound care. 2025;38[9]:480–6.

32. Kirkland-Kyhn H, Sengul T, Karadag A, Yilmaz Akyaz D, Cevizci T, Teleten O. Exploring Nurses' Acceptability and Readiness for Patient-centered Artificial Intelligence Systems in Pressure Injury Prevention. Advances in Skin and Wound Care. 2025;38[9]:488–95.

33. Morriss J, Edsberg LE, Cox J, Capasso V. Using Explainable Artificial Intelligence in a Systematic Literature Review of Pressure Injury Prevention: Lessons Learned. Journal of wound, ostomy, and continence nursing : official publication of The Wound, Ostomy and Continence Nurses Society. 2026;53[2]:94–101.

34. Mousa KM, Mousa FA, Mohamed HS, Elsawy MM. Prediction of Foot Ulcers Using Artificial Intelligence for Diabetic Patients at Cairo University Hospital, Egypt. SAGE Open Nursing. 2023;9.

35. Ganesan O, Morris MX, Guo L, Orgill D. A review of artificial intelligence in wound care. Artificial Intelligence Surgery. 2024;4[4]:364–75.

36. Chen B, Zhou J, Chen S, Wang F, Liu P, Xu Y, et al. Interpretable Machine Learning Prediction Model for Predicting Mortality Risk of ICU Patients With Pressure Ulcers Based on the Braden Scale: A Clinical Study Based on MIMIC-IV. Journal of Clinical Nursing. 2025;34[8]:3353–69.

37. Jeon JH, Chung J, Lim NK. A Longitudinal Investigation of Stage 2 Pressure Injury Outcomes With Machine Learning Technique to Identify Relevant Factors. Advances in skin & wound care. 2025;38[9]:E81–9.

38. Babaheidari TB, Tajvidi M, Bakhtiyari M, Kabir K, Baradaran Bagheri A, Aghajanian S. Comparative Evaluation of Pressure Injury Risk Assessment Tools and Machine Learning Models in Postoperative Surgical ICU Patients. Nursing in Critical Care. 2026;31[1]:1–9.

39. Liu X, Tang C, Guo L, Shao J, Wu G, Qi Y. Intelligent Prediction of Pressure Injury by Image-Based Feature Variable With Machine Learning. Nursing Research. 2025;74[6]:442–8.

40. Nakagami G, Yokota S, Kitamura A, Takahashi T, Morita K, Noguchi H, et al. Supervised machine learning-based prediction for in-hospital pressure injury development using electronic health records: A retrospective observational cohort study in a university hospital in Japan. International Journal of Nursing Studies [Internet]. 2021;119:103932. Available from: https://doi.org/10.1016/j.ijnurstu.2021.103932

41. Hu YH, Lee YL, Kang MF, Lee PJ. Constructing Inpatient Pressure Injury Prediction Models Using Machine Learning Techniques. CIN - Computers Informatics Nursing. 2020;38[8]:415–23.

42. Chun X, Pan L, Lin Y, Ye L, Liang H, Tao J, et al. A model for predicting 7-day pressure injury outcomes in paediatric patients: A machine learning approach. Journal of Advanced Nursing. 2021;77[3]:1304–14.

43. Xu J, Chen T, Fang X, Xia L, Pan X. Prediction model of pressure injury occurrence in diabetic patients during ICU hospitalization——XGBoost machine learning model can be interpreted based on SHAP. Intensive and Critical Care Nursing [Internet]. 2024;83[April]:103715. Available from: https://doi.org/10.1016/j.iccn.2024.103715

44. Cai JY, Zha ML, Song YP, Chen HL. Predicting the Development of Surgery-Related Pressure Injury Using a Machine Learning Algorithm Model. Journal of Nursing Research. 2021;29[1].

Healing Not Only the Patient But the Planet: Sustainability in Green Operating Room Processes and The Role of Nurses

Ümmühan Yiğit*

[1]Nursing/Faculty of Health Science, Bolu Abant Izzet Baysal University, Turkiye
*(ummuhanyigit@ibu.edu.tr] Email of the corresponding author

ABSTRACT

The healthcare sector is a significant source of global carbon emissions; operating rooms, as the units within hospitals that generate the most waste and consume the most energy, are at the center of this environmental burden. With the vision of "Healing Not Just the Patient, but the World," there is a strategic role for nurses in implementing sustainability practices within operating room processes and driving this transformation. Florence Nightingale's "Environmental Theory" and the "do no harm" principle—the cornerstone of nursing—are being redefined today as an ecological responsibility. In the transition from the traditional linear economic model to a circular economy, waste management, control of anesthetic gases, energy efficiency, and green procurement strategies form the core focus areas. The operating room nurse, through their roles as case coordinator, educator, and supervisor, stands at the center of these sustainable interventions, serving as a key actor in safeguarding both clinical excellence and ecosystem health.

The aim of this study is to examine sustainability practices in green operating room processes, which aim to develop climate-sensitive applications, and to highlight the critical roles assumed by the operating room nurse in these processes in light of the academic literature**.**

Keywords – Operating Room, Sustainability, Green Operating Room

I. INTRODUCTION

With advances in medical technology, modern medical practices have made greater contributions to human health and recovery. While this improves patients' quality of life, it ironically places a heavy burden on the ecosystem.

Sustainability is defined as "meeting the needs of the present without compromising the ability of future generations to meet their own needs." [1]. It is defined as a comprehensive approach that encompasses maintaining the quality of care, ensuring the effective use of resources, and guaranteeing the long-term sustainability of healthcare services while addressing existing health issues. [2]. In this context, sustainability in health systems is addressed from environmental, economic, and social perspectives [3].

The flawed waste sorting process in operating rooms results in non-medical waste being unnecessarily disposed of as medical waste. The toxic gases and particulate matter released into the atmosphere during this process trigger respiratory diseases—a long-term public health issue—thereby violating the principle of "do no harm," which is the cornerstone of nursing. [4]. It is very clear that sustainability is an ethical imperative in nursing care.

Sustainable operating room practices encompass multifaceted strategies such as waste management, energy efficiency, water usage, sustainable procurement, and the optimization of anesthesia practices [5]. In this process,

healthcare professionals—particularly operating room nurses—play a critical role in the planning, implementation, and evaluation of sustainable practices [Alshqaqeeq et al., 2020]. Nurses' active roles in waste management, the proper use of resources, and fostering team awareness directly influence the success of sustainability initiatives [6].

1. Key Areas of Focus for Sustainability in the Healthcare System

1.1. Environmental Dimension

The environmental dimension of sustainability aims to minimize the negative impacts of healthcare services on ecosystems and to manage natural resources in a way that does not jeopardize the health needs of future generations [7].

Efficient resource use in operating rooms relies on waste reduction, reusable systems, smart anaesthesia, and energy optimisation without compromising care quality [8].

Waste Management and Reduction: Waste is any material that cannot be reused after use or that poses a hazard to the environment. Operating rooms are the units in hospitals that generate the most waste. Operating rooms account for 30–70% of hospital waste; up to 74% of this waste consists of preoperative waste that can be recycled. [6,9,10].

Strategic Sustainability Practices:

- In operating rooms, streamlining surgical sets according to the specific procedure and transitioning to a lean tray system containing only essential instruments—thereby eliminating unnecessary tools—is an effective solution that both reduces sterilization costs and lightens the nurses' workload. → This results in a 13% reduction in CO_2 emissions per case and a corresponding decrease in costs. [11,12].
- Proper medical waste segregation [separating non-hazardous waste from infectious/contaminated waste] increases the volume of recyclable waste while significantly reducing disposal costs [9,13].
- Reuse and disposible materials benefits the environment and society [9].

Sustainable Management of Energy and Water Resources: Although operating rooms occupy a small area within hospitals, they account for approximately 40% of the facility's total energy consumption. This high energy consumption is primarily due to high-efficiency heating, ventilation, and air conditioning (HVAC) systems that operate continuously 24 hours a day, as well as surgical robots and advanced imaging equipment. [14].

Reducing the air exchange rate in operating rooms to meet standards when the rooms are not in use [at night or between procedures] can reduce energy consumption by up to 50%. Turning off medical devices [monitors,

cauterizers, etc.] completely rather than leaving them in standby mode between procedures can also save energy. [15].

Water consumption in operating rooms is high due to factors such as surgical hand washing, the sterilization of surgical instruments and drapes, and HVAC systems. To improve water efficiency, using sensor-activated systems instead of manual faucets prevents waste by ensuring water flows only when needed [10].

Parienti et al. (2002) reported that using alcohol-based surgical scrub solutions instead of traditional handwashing with soap and water could reduce water consumption without increasing the risk of surgical site infections [16].

Anesthetic Gases: Desflurane and N_2O have the highest global warming and ozone-depleting impact and can account for %50 of an OR's carbon footprint and up to %5 of hospital CO_2 emissions in high-income settings [5,17,18] .

Sustainability for anesthetic gases centers on avoiding desflurane and N_2O, using the lowest safe fresh gas flows, favoring regional when clinically suitable, and, where available, capturing waste gases. Department-wide education, policy changes, and decision-support can deliver rapid, large emission cuts without sacrificing patient safety [19].

1.2. Economic Dimension

The circular economy model has emerged as a sustainable development paradigm in opposition to the linear economic model—commonly characterized as "take–make–dispose"—which originated with the Industrial Revolution, the advent of mass production, and the assumption of inexhaustible natural resources. The circular economy aims to maximize the lifespan of resources, prevent waste generation at its source, and ensure the continuous reintegration of materials into the system through recycling processes. [20].

By reducing the use of single-use products and opting for reusable medical devices, and by returning surgical waste to the economy as raw material, we can both prevent environmental harm and generate economic benefits [14].

The circular economy is a sustainable development model that aims to reduce the negative environmental impacts of economic activities by minimizing waste generation in production and consumption processes through the reuse and recycling of resources [20]. The principles of the circular economy, initially known as the 3Rs—Reduce, Reuse, and Recycle—have evolved over time to include additional principles, resulting in the 10Rs. [Figure 1] [20]. The principles of "reduce, reuse, repair, refuse, rethink, renew, recycle, remanufacture, repurpose, and recover" outlined in the 10R Model serve as an effective guide for ensuring surgical sustainability [21]. The Society of American Gastrointestinal and Endoscopic Surgeons [SAGES] and the European Association for Endoscopic Surgery (EAES) report that the 10R model used in the circular economy is an effective approach to sustainability in surgical practice [21].

Figure 1. 10R model of surgical sustainability

1.3. The Social Dimension

Social sustainability in operating rooms is an ethical responsibility that protects the health of staff, prioritizes patient safety, and safeguards the public's future right to health through the equitable use of resources. In this context, nurses are not merely technical practitioners but also advocates for equity in healthcare and safe working environments.

3. The Role of Operating Room Nurses in Sustainability

Hospitals are at the center of the climate crisis from an ecological perspective, due to their high resource consumption, production of 30-70% of all medical waste, and high energy requirements [22,23]. Operating rooms are the hospital departments that contribute most significantly to a hospital's carbon footprint, as they require substantial amounts of energy, advanced technology, and single-use equipment and supplies. In addition, anesthetic gases are potent greenhouse gases that are released directly into the environment and contribute to global warming [24,25]. Healthcare is not only about healing the individual, but also about protecting the world in which that individual lives and breathes [21].

Sustainability is no choice for operating room nursing; it has become a professional responsibility. As the coordinator of the surgical team, the operating room nurse is the primary power behind the “green” transformation at every step, from waste management to the materials used.

Improving nurses' knowledge and awareness is critical to the success of sustainable practices in operating rooms [26].

The origins of sustainable nursing practices are based on Florence Nightingale’s “Environmental Theory.” Nightingale defined the nurse’s fundamental responsibility as manipulating environmental factors—such as ventilation, cleanliness, waste management, and lighting—to support the patient’s recovery process [27]. Today’s principles of sustainability [conservation of resources, prevention of pollution] are, in fact, a modern version of this 150-year-old theory. The focus of nursing care extends to the patient’s environment. Since Florence Nightingale’s “Environmental Theory,” the role of a healthy environment in the healing process has been well understood. Today, because the harm caused by medical practices to the environment [carbon footprint, plastic pollution, etc.] indirectly threats public health, nursing’s “do no harm” principle has evolved into an “ecological do no harm” responsibility that includes minimizing harm to the ecosystem during the delivery of healthcare services [Anåker ve ark., 2018].

Operating room and surgical nurses can “green” operating room by correct segregation, minimizing waste generation, preferring reusables, and promoting recycling systems [28–30]. Nurses can encourage team members to turn off equipment and lights in the operating room to save energy [24].

The transformation of operating rooms into “green operating rooms”—where climate-sensitive practices are adopted to protect and improve global health, ensure the sustainable use of natural resources, and reduce environmental impact—is considered a critical strategy. In this context, it is recommended to prioritize reusable products over single-use materials to reduce emissions from operating rooms, optimize the use of medical and surgical gases, and select appropriate anesthesia and sterilization methods. Additionally, shortening hospital stays, reducing waste volume and energy consumption, and transitioning documentation and communication processes to digital platforms are among the other key practices contributing to the reduction of carbon emissions. [31].

REFERENCES

1. Bruntdland GH. Our common future, report of the world commission on environment and development. World Commission on Environment and Development. 1987;42–427.
2. Kaplan S, Sadler B, Little K, Franz C, Orris P. Can Sustainable Hospitals Help. The Commonwealth Fund. 2012;29[1641].
3. Rafaih A Bin, Ari K. Sustainability in Surgery: Problems, Solutions, and Recommendations. Cureus. 2025;17[10].
4. Monroe O. The Role of Nurses in Environmental Sustainability [Internet]. Undergraduate Research and Creative Practice. 2024. Available from: https://scholarworks.gvsu.edu/honorsprojects
5. Sherman JD, Thiel C, MacNeill A, Eckelman MJ, Dubrow R, Hopf H, et al. The Green Print: Advancement of Environmental Sustainability in Healthcare. Resources, Conservation and Recycling [Internet]. 2020;161[July 2020]:104882. Available from: https://doi.org/10.1016/j.resconrec.2020.104882
6. Azouz S, Boyll P, Swanson M, Castel N, Maffi T, Rebecca AM. Managing barriers to recycling in the operating room. American Journal of Surgery. 2019;217[4]:634–8.
7. Heinzova R, Hoke E. Sustainability and Green Management in Hospitals. Entrepreneurship and Sustainability Issues. 2025;13[2]:313–25.
8. Nieuwenhuizen KE Van, Michels CTJ, Both IGIA, Guinée JB. Evidence-based Integration of Environmental Sustainability into Clinical Guidelines for Operating Rooms A Systematic Review. Ann Surg. 2026;283[4]:593–607.
9. Mastronardi M, Fracon S, Ahuja M, Qi V, Ngo J, Westwood E, et al. Review Greening the Operating Room : A Narrative Review of Global. 2025;1167–79.
10. Wu S, Cerceo E. Sustainability Initiatives in the Operating Room. Joint Commission Journal on Quality and Patient Safety [Internet]. 2021;47[10]:663–72. Available from: https://doi.org/10.1016/j.jcjq.2021.06.010
11. Lam K, Gadi N, Acharya A, Beatty JW, Darzi A, Purkayastha S. Interventions for sustainable surgery : a systematic review. 2022;19.
12. Vu C, Ibarra-vega A, Yang CD, Manzanarez-felix K, Ting CL. Interventions to Reduce Surgical Waste Burden : A Systematic Review. :1–8.
13. Wu S, Cerceo E. Sustainability Initiatives in the Operating Room. The Joint Commission Journal on Quality and Patient Safety [Internet]. 2026;47[10]:663–72. Available from: https://doi.org/10.1016/j.jcjq.2021.06.010
14. MacNeill AJ, Lillywhite R, Brown CJ. The impact of surgery on global climate: a carbon footprinting study of operating theatres in three health systems. The Lancet Planetary Health [Internet]. 2017;1[9]:e360–7. Available from: http://dx.doi.org/10.1016/S2542-5196[17]30162-6
15. Cacabelos-Reyes A, Loper-Gonzales J, Gonzalez-Gil A, Febrero-garrido L. Assessing the Energy Demand Reduction in a Surgical Suite by Optimizing the HVAC Operation During O ff -Use Periods. Applied Sciences. 2020;10.
16. Parienti J, Thibon P, Heller R. Hand-Rubbing With an Aqueous Alcoholic Solution vs Traditional Surgical Hand-Scrubbing and 30-Day Surgical Site

Infection Rates. JAMA. 2002;288[6]:722–7.
17. Mcgain F, Harris RD, Sherman JD. Action guidance for addressing pollution from inhalational anaesthetics. Anaesthesia. 2022;1023–9.
18. Ramaswamy P, Shah A, Kothari R, Schloemerkemper N. An Accessible Clinical Decision Support System to Curtail Anesthetic Greenhouse Gases in a Large Health Network : Implementation Study Corresponding Author : 2022;5:1–13.
19. Hammer S, Eichlseder M, Klivinyi C, Lang-illievich K, Moser A, Pichler A, et al. Effects of departmental green anaesthesia interventions on carbon dioxide equivalent emissions : a systematic review. British Journal of Anaesthesia [Internet]. 2025;135[1]:79–88. Available from: https://doi.org/10.1016/j.bja.2025.03.038
20. Kara HT, Babaoğlu C. Financing The Circular Economy Approach Its Applicability in Local Governments in Türkiye. Journal of Environment, Urban Planning, and Climate. 2025;4[7]:48–67.
21. Johnson SM, Marconi S, Sanchez-Casalongue M, Francis N, Huo B, Alseidi A, et al. Sustainability in surgical practice: a collaborative call toward environmental sustainability in operating rooms. Surgical Endoscopy. 2024;38[8]:4127–37.
22. Dalkılıç M, Bilik Ö, Vural F. Why Is Medical Waste Segregation Important In The Operating Room? Journal of Health Sciences and Clinical Research. 2025;4[2]:160–75.
23. Balch JA, Krebs JR, Filiberto AC, Montgomery WG, Berkow LC, Upchurch GR, et al. Methods and evaluation metrics for reducing material waste in the operating room: a scoping review. Surgery [United States] [Internet]. 2023;174[2]:252–8. Available from: https://doi.org/10.1016/j.surg.2023.04.051
24. Flynn FM, Martinsen SS, Øiseth F, Flo J, Leonardsen ACL. Sustainability in the operating room: a cross-sectional survey of nurse anaesthetists' and operating room nurses' views and practice. BMC Nursing. 2025;24[1].
25. Kagoma Y, Stall N, Rubinstein E, Naudie D. People, planet and profits: The case for greening operating rooms. CMAJ Canadian Medical Association Journal. 2012;184[17]:1905–11.
26. Kırtıl İ, Çağan Kişin Ş, Akyüz N. Nurses' Perceptions and Experiences Regarding Environmental Sustainability in the Operating Room: A Phenomenological Study. Nursing and Health Sciences. 2025;27[3].
27. Nightingale F. Notes on Nursing: What it is, and what it is not. Dover Publications.; 1969.
28. Hassan ET, Mahdy NE, Ahmed NG. Barriers to Green Practice regarding Waste Management among Operating Room Nurses What are the barriers to green practice regarding waste management among operating room nurses ? 2024;15[2].
29. Sürme Y. Recycling , responsible consumption and nursing : A qualitative study of surgical nurses ' recycling and medical waste management. 2022;[October]:4514–22.
30. Moya E, Bryant SA, Horneck N, Taylor V, Alayleh A, Alawa J, et al. The American Journal of Surgery Advancing sustainability in healthcare : A scoping review of global recycling practices in operating rooms. The

American Journal of Surgery [Internet]. 2025;241[December 2024]:116178. Available from: https://doi.org/10.1016/j.amjsurg.2024.116178
31. Kara Ö, Seyhan Ak E. Carbon Footprint and Sustainability in The Operating Room: What to Know on the Road to Becoming a Green Operating Room. Lokman Hekim Journal. 2025;15[2]:389–99.

Proline as a Quality and Authenticity Marker in Albanian Honey

Xhulieta Hamiti*

Aleksia Zaimi[1]

Dorina Shëngjergji[2]

[1]Department of Chemistry/University of Tirana, Albania
[2]Department of Nursing and Physiotherapy/Europian University of Tirana, Albania
*(xhulieta.hamiti@fshn.edu.al) Email of the corresponding author

ABSTRACT

Proline, the predominant amino acid in honey, is considered one of the most reliable indicators of honey ripeness and authenticity. In the present study, the proline content of 44 Albanian honey samples collected from different geographical regions and floral origins was evaluated using LC-MS/MS amino acid profiling. The investigated samples included both monofloral and polyfloral honeys originating from Castanea sativa, Erica spp., Arbutus unedo, and other botanical sources. The results demonstrated remarkably high levels of proline in Albanian honey, with values ranging from 242.83 to 1113.68 mg/kg and an average concentration of 621.23 ± 232.18 mg/kg. Monofloral honeys, particularly Castanea sativa samples, showed significantly higher proline concentrations compared to several polyfloral samples. The obtained values were substantially higher than the minimum international quality threshold generally accepted for authentic honey. The findings suggest that Albanian honey possesses excellent maturity and authenticity characteristics and may reflect the unique botanical diversity and environmental conditions of Albania. Furthermore, the elevated proline content may contribute to the biological and antioxidant potential of these honeys. The present study highlights the usefulness of proline as a biochemical marker for evaluating honey quality and authenticity and provides new scientific data regarding the amino acid composition of Albanian honey.

Keywords – Albanian honey; proline; amino acids; authenticity; LC-MS/MS.

I. INTRODUCTION

Honey is a natural sweet substance produced by honeybees from nectar or honeydew through enzymatic transformation, dehydration, and maturation processes within the hive. Due to its nutritional composition and biological activities, honey has been extensively studied for its antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties. The chemical composition of honey is highly complex and depends on several factors, including floral origin, geographical location, climate, soil conditions, storage conditions, and processing methods [1].

In addition to sugars, honey contains a wide range of minor bioactive compounds such as phenolic acids, flavonoids, organic acids, vitamins, enzymes, minerals, proteins, and amino acids. Although amino acids are present in relatively low concentrations compared to carbohydrates, they play an important role in the evaluation of honey quality and authenticity.

Among all amino acids identified in honey, proline is generally recognized as the predominant amino acid, representing approximately 50–85% of the total amino acid fraction in many honey types. Proline originates mainly from bee salivary secretions during nectar transformation and therefore serves as an indicator of honey maturity and bee activity. For this reason, proline concentration is commonly used as a criterion for determining honey authenticity and detecting adulteration or immature honey [2], [3].

According to international recommendations, authentic mature honey should generally contain more than 180 mg/kg proline. Lower concentrations may indicate adulteration, artificial feeding of bees, excessive processing, or premature harvesting. Several studies have demonstrated that proline levels vary according to botanical origin and environmental conditions. Chestnut, heather, and honeydew honeys are often characterized by particularly high proline concentrations.

Albania possesses exceptional botanical biodiversity and favorable climatic conditions for beekeeping. However, despite the increasing scientific interest in Albanian honey, information regarding its amino acid composition, especially proline content, remains limited [4], [5]. Most studies on Albanian honey have focused primarily on physicochemical parameters, antioxidant activity, phenolic compounds, and pollen analysis [6].

Therefore, the aim of the present study was to evaluate the proline content of 44 Albanian honey samples collected from different geographical regions and botanical origins and to investigate the role of proline as a quality and authenticity marker. Additionally, the study aimed to compare proline levels among different honey types and discuss the relationship between elevated proline concentrations and the overall quality characteristics of Albanian honey [7], [8].

II. Materials and Method

A. Honey samples

A total of 44 honey samples from different Albanian regions were included in the study. The samples represented several botanical origins, including *Castanea sativa*, *Arbutus unedo*, *Erica arborea*, *Trifolium pratense*, *Salvia officinalis L.*, *Nigella sativa*, *Citrus aurantium*, *Staehelina uniflosculosa*, and polyfloral honey. The dataset included 22 polyfloral samples, 11 *Castanea sativa* samples, and smaller groups or individual samples from other botanical origins [9].

B. Determination of amino-acids with LC-MS/MS equipment

The amino acid profile of the honey samples was determined using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Prior to analysis, honey samples were homogenized and diluted appropriately with ultrapure water. Sample preparation included filtration through membrane filters before chromatographic analysis.

The LC-MS/MS analysis was performed using an Agilent chromatographic system coupled with a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive ionization mode. Multiple reaction monitoring (MRM) transitions were used for amino acid identification and quantification.

Calibration curves were prepared using analytical amino acid standards. Quantification of proline and other amino acids was carried out using external standard calibration. The results were expressed as mg/kg honey.

Amino acid concentrations were expressed in mg/kg. The analyzed amino acids included tryptophan, taurine, phenylalanine, tyrosine, leucine, isoleucine, methionine, gamma-aminobutyric acid, valine, 2-aminoadipic acid, glutamic acid, aspartic acid, threonine, serine, alanine, glycine, hydroxyproline, asparagine, proline, sarcosine, glutamine, citrulline, histidine, arginine, argininosuccinic acid, and lysine. Total amino acids were calculated as the sum of quantified amino acids.

C. Statistical Analysis

Descriptive statistics were calculated for each amino acid, including mean, standard deviation, minimum, median, maximum, and coefficient of variation. PCA was performed using standardized amino acid variables, excluding total amino acids to avoid redundancy. The association between botanical origin and amino acid composition was evaluated using the correlation ratio eta squared (η^2), which estimates the proportion of variability in each amino acid explained by categorical botanical origin. One-way ANOVA was also applied for botanical groups with at least two samples, while interpretation of groups with one sample was considered descriptive only.

III. RESULTS

A. *Descriptive statistics of aminoacid composition*

The amino acid profile showed substantial variability among the 44 Albanian honey samples. Total amino acids ranged from 400.44 to 1349.72 mg/kg, with a mean value of 835.55 mg/kg and a median of 798.25 mg/kg. The coefficient of variation for total amino acids was 31.44%, indicating moderate variability among samples.

Proline was the dominant amino acid in the dataset. Its concentration ranged from 242.83 to 1113.68 mg/kg, with a mean value of 621.23 mg/kg and a median of 547.02 mg/kg. The coefficient of variation for proline was 37.37%, showing considerable natural variability among honey samples. The predominance of proline confirms its importance as the main amino acid marker in Albanian honey.

After proline, the most abundant amino acids were phenylalanine, glutamic acid, asparagine, tyrosine, threonine, aspartic acid, sarcosine, and alanine. Phenylalanine presented a mean value of 58.52 mg/kg, ranging from 5.35 to 199.47 mg/kg. Glutamic acid showed a mean concentration of 17.36 mg/kg, while tyrosine averaged 13.10 mg/kg. The high coefficient of variation observed for phenylalanine and tyrosine indicates that these amino acids may contribute to differentiation among botanical origins.

B. *Proline ranking among honey samples*

The highest proline concentration was observed in sample HPE, classified as *Staehelina uniflosculosa*, with 1113.68 mg/kg. Other samples with high proline values included HSA6 (*Trifolium pratense*), HDI2 (*Castanea sativa*), HKO1 (polyfloral), HSH3 (*Castanea sativa*), HTR3 (*Nigella sativa*), HBU2 (polyfloral), HSA3 (*Arbutus unedo*), HDI1 (*Castanea sativa*), and HGJ2 (polyfloral). These samples also generally showed high total amino acid content, suggesting a strong contribution of proline to the total amino acid pool.

The lowest proline concentrations were observed in some polyfloral and citrus samples, including HRR2, HBU3, HSA5, HSA4, and HBU1. However, even the lowest proline values remained within a range that supports the natural amino acid richness of the samples.

C. *Aminoacid composition by botanical origin*

Botanical groups showed different mean proline and total amino acid values. *Trifolium pratense* samples presented the highest mean proline

concentration among groups with at least two samples, with an average of 822.57 mg/kg and mean total amino acids of 1090.62 mg/kg. *Arbutus unedo* samples showed a mean proline value of 660.34 mg/kg and mean total amino acids of 882.93 mg/kg. *Castanea sativa* samples had a mean proline concentration of 632.13 mg/kg and mean total amino acids of 827.43 mg/kg. Polyfloral samples showed a mean proline concentration of 575.03 mg/kg and mean total amino acids of 781.51 mg/kg.

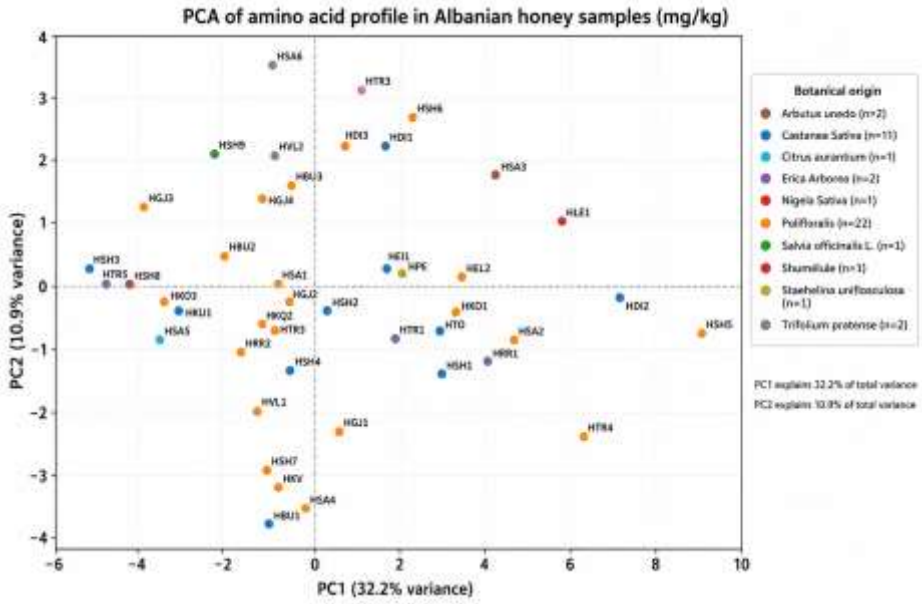
These results suggest that botanical origin influences amino acid composition, although interpretation must consider the unequal number of samples among botanical groups. The larger polyfloral and *Castanea sativa* groups allow more reliable comparison, while botanical types represented by one or two samples should be interpreted as preliminary observations.

D. *Principal component analisis*

PCA was applied to standardized amino acid variables to evaluate the overall structure of the dataset. The first principal component explained 32.17% of the total variance, while the second principal component explained 10.86%. Together, PC1 and PC2 accounted for 43.04% of the total variability.

PC1 was mainly associated with amino acids such as 2-aminoadipic acid, valine, gamma-aminobutyric acid, glutamine, alanine, aspartic acid, glycine, and glutamic acid. This component appears to represent a general amino acid enrichment axis. PC2 was more strongly influenced by phenylalanine, tyrosine, methionine, taurine, threonine, hydroxyproline, arginine, and argininosuccinic acid. Therefore, PC2 may reflect differences related to aromatic amino acids and specific nitrogen-containing compounds.

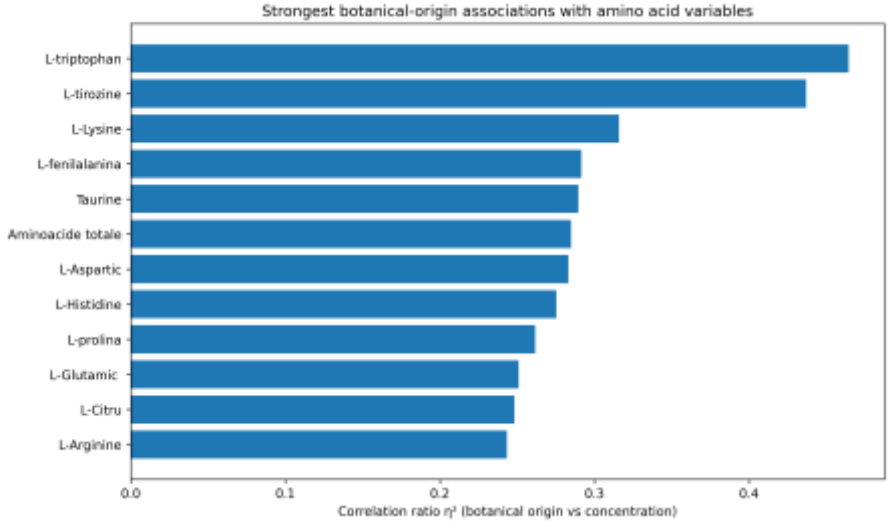
The PCA score plot showed partial grouping of samples according to botanical origin, especially for the larger polyfloral and *Castanea sativa* groups. However, overlap among groups was observed, indicating that amino acid composition alone may not completely separate all botanical origins. This is expected because honey composition is influenced by multiple factors beyond floral source, including region, climate, harvest period, and bee metabolism.



E. *Association between Botanical Origin and Amino Acid Variables*

The botanical-origin association analysis using eta squared showed that several amino acid variables were moderately associated with botanical origin. The strongest associations were observed for tryptophan ($\eta^2 = 0.464$), tyrosine ($\eta^2 = 0.437$), lysine ($\eta^2 = 0.316$), phenylalanine ($\eta^2 = 0.292$), taurine ($\eta^2 = 0.290$), total amino acids ($\eta^2 = 0.285$), aspartic acid ($\eta^2 = 0.283$), histidine ($\eta^2 = 0.275$), and proline ($\eta^2 = 0.262$).

These results indicate that botanical origin explains part of the variability in amino acid composition. Proline showed a moderate association with botanical origin, but it was not the only discriminating amino acid. Tryptophan, tyrosine, lysine, phenylalanine, and taurine appeared to contribute more strongly to botanical differentiation. Therefore, while proline is the most important quality and maturity marker, a broader amino acid profile is more informative for botanical classification.



IV. DISCUSSION

The results confirm that proline is the predominant amino acid in Albanian honey. This finding is consistent with previous studies reporting proline as the major free amino acid in honey and a useful marker of honey maturity and authenticity ([10], [11]). The high mean proline concentration observed in the present dataset supports the maturity and natural biochemical quality of the analyzed Albanian honey samples.

Proline is mainly introduced into honey through bee salivary secretions during nectar processing. Therefore, its concentration reflects the degree of nectar transformation and enzymatic activity. Honey harvested too early or adulterated with sugar syrups may contain lower proline concentrations because the natural maturation process is incomplete [12], [13].

The mean proline value obtained in this study was 621.23 mg/kg, which is comparable with or higher than values reported for several international honey types. The dominance of proline in the total amino acid fraction confirms that it can be used as a central biochemical marker for evaluating Albanian honey quality. However, proline should not be interpreted alone. The PCA and botanical-origin association results showed that other amino acids, particularly tryptophan, tyrosine, lysine, phenylalanine, and taurine, also contribute to sample differentiation.

The PCA results suggest that the amino acid profile provides useful information for honey characterization but does not fully separate all botanical origins. Similar findings have been reported by Hermosín [2], who

demonstrated that free amino acid composition is useful for botanical differentiation but may show overlap among honey types. Iglesias [3] also emphasized that amino acid profiles can help discriminate honeydew and floral honeys, especially when combined with chemometric methods.

In the present study, the strongest botanical-origin associations were not limited to proline. Tryptophan and tyrosine showed the highest eta squared values, suggesting that aromatic amino acids may play an important role in differentiating honey types. Phenylalanine also showed high variability among samples and contributed strongly to PC2. This supports the idea that a complete amino acid profile may provide better botanical discrimination than a single marker.

The higher mean proline and total amino acid values observed in *Trifolium pratense*, *Arbutus unedo*, and *Castanea sativa* honeys may reflect differences in nectar composition and plant-specific nitrogen compounds. However, because some botanical groups were represented by only one or two samples, these findings should be interpreted cautiously. Larger sample numbers for each botanical origin would be necessary to confirm botanical-specific amino acid fingerprints.

Overall, the results indicate that proline is a strong quality and authenticity marker, while the full amino acid profile offers additional value for botanical classification. Combining amino acid data with melissopalynological analysis, physicochemical parameters, phenolic profiles, and antioxidant activity would provide a more complete characterization of Albanian honey.

V. CONCLUSIONS

This study demonstrated that proline is the dominant amino acid in Albanian honey and represents a useful marker for honey quality, maturity, and authenticity. The analyzed samples showed a mean proline concentration of 621.23 mg/kg and a mean total amino acid content of 835.55 mg/kg. These values indicate a rich amino acid profile and support the natural biochemical quality of the studied samples.

PCA revealed that amino acid composition contributes to the differentiation of honey samples, although botanical groups showed partial overlap. The first two principal components explained 43.04% of the total variance. Botanical-origin association analysis showed that tryptophan, tyrosine, lysine, phenylalanine, taurine, total amino acids, and proline were among the most informative variables.

The findings confirm that proline is a reliable quality marker, but botanical classification benefits from the evaluation of the complete amino acid profile. Future research should include a larger number of samples per botanical origin and integrate amino acid profiling with pollen analysis, phenolic composition, antioxidant activity, and physicochemical quality parameters.

REFERENCES

[1] Anklam, E. (1998). *A review of the analytical methods to determine the geographical and botanical origin of honey*. Food Chemistry, 63, 549–562.

[2] Hermosín, I., Chicón, R. M., & Cabezudo, M. D. (2003). *Free amino acid composition and botanical origin of honey*. Food Chemistry, 83, 263–268.

[3] Iglesias, M. T., Martín-Álvarez, P. J., Polo, M. C., de Lorenzo, C., & Pueyo, E. (2004). *Usefulness of amino acid composition to discriminate between honeydew and floral honeys*. Journal of Agricultural and Food Chemistry, 52, 84–89.

[4] Hoxha, F., Lamçe, F., Beqo, M., Kongoli, R., Malollari, I., & Kyçyk, O. (2019). *Quality Evaluation of Commercialised Honey in Tirana Using Physicochemical Analysis*. Albanian Journal of Agricultural Sciences, 18(1), 26–31.

[5] Shahu, E., Shehu, S., & Mara, V. (2019). *Determination of honey quality of the south region of Albania based on physico-chemical parameters*. European Academic Research, VII (8).

[6] Hamiti, X., Shallari, G., Pupuleku, B., & Lazo, P. (2025). *The total phenolic content and antioxidant activity of Albanian honey samples*. International Journal of Ecosystems and Ecology Science (IJEES).

[7] International Honey Commission. (2009). *Harmonised methods of the International Honey Commission*.

[8] Thrasyvoulou, A., Tananaki, C., Giraudel, J. L., Manikis, I., & Kontominas, M. (2018). *Legislation of honey criteria and standards*. Journal of Apicultural Research, 57, 88–96.

[9] Von der Ohe, W., Persano Oddo, L., Piana, M. L., Morlot, M., & Martin, P. (2004). *Harmonized methods of melissopalynology*. Apidologie, 35, S18–S25.

[10] White, J. W., & Doner, L. W. (1980). *Honey composition and properties*. In *Beekeeping in the United States, Agriculture Handbook No. 335*, 82–91.

[11] Bogdanov, S. (2008). *Honey composition*. In *The Honey Book*. Bee Product Science.

[12] Bogdanov, S., Ruoff, K., & Oddo, L. P. (2004). *Physico-chemical methods for the characterization of unifloral honeys: A review*. Apidologie, 35, S4–S17

[13] da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). *Honey: Chemical composition, stability and authenticity*. Food Chemistry, 196, 309–323.

The Role of Nitrosamine Formation and The Nitrite-Nitrate Cycle in Cancer Development: A Biochemical Approach

Tugba AGBEKTAS[1]
Cemile ZONTUL[2]

Sivas Cumhuriyet University, Yıldızeli Vocational School, Department of Food Processing, Food Technology Program, Sivas, Türkiye. tubaagbektas@cumhuriyet.edu.tr
Orcid ID: 0009-0004-4533-0019
Sivas Cumhuriyet University, Yıldızeli Vocational School, Department of Chemistry and Chemical Processing Technologies, Biochemistry Program, Sivas, Türkiye. cemilezontul@cumhuriyet.edu.tr, Orcid ID: 0000-0002-1436-5145

ABSTRACT

Nitrosamines are among the most significant chemical carcinogens due to their potent mutagenic and carcinogenic properties. This chapter aims to provide a comprehensive and integrative overview of the biochemical mechanisms underlying nitrosamine formation, nitrite–nitrate metabolism, and their roles in cancer development. The chapter is based on a critical evaluation of current experimental, epidemiological, and molecular studies focusing on endogenous nitrosation reactions, metabolic activation pathways, oxidative stress, inflammation, DNA damage, epigenetic alterations, and environmental risk factors associated with nitrosamine exposure.

Current evidence demonstrates that nitrosamines can be formed both exogenously through dietary and environmental exposure and endogenously through nitrosation reactions occurring in the gastrointestinal system. Following metabolic activation primarily mediated by cytochrome P450 enzymes, particularly CYP2E1, nitrosamines generate reactive electrophilic intermediates capable of inducing DNA alkylation, oxidative and nitrosative stress, mitochondrial dysfunction, chronic inflammation, and epigenetic dysregulation. The formation of DNA adducts such as O^6-methylguanine, activation of inflammatory signaling pathways including NF-κB and STAT3, and disruption of cellular redox balance are recognized as major contributors to multistage carcinogenesis. In addition, emerging evidence highlights the important roles of the gut microbiota, dietary composition, antioxidant status, and environmental exposures such as tobacco use in modulating nitrosamine formation and toxicity.

Recent advances in food safety regulations, pharmaceutical quality control, antioxidant-based preventive approaches, and microbiota-targeted interventions further support the growing importance of nitrosamine-related research in public health and cancer prevention strategies. In conclusion, this chapter emphasizes that nitrosamine-associated carcinogenesis is a multifactorial biochemical process involving complex interactions among metabolism, inflammation, oxidative stress, epigenetic regulation, nutrition, and environmental factors. A deeper understanding of these molecular mechanisms may contribute to the development of novel preventive, diagnostic, and therapeutic approaches for cancer management.

Keywords – Nitrosamines, Nitrite–nitrate cycle, Carcinogenesis, Oxidative stress, DNA damage, Epigenetic alterations, Inflammation, Microbiota, CYP2E1, Reactive nitrogen species

1. INTRODUCTION

Cancer comprises a heterogeneous group of diseases that arise through complex interactions between genetic alterations and environmental exposures. Among chemical carcinogens, N-nitrosamines have long attracted scientific attention because of their potent mutagenic and carcinogenic properties. These compounds may occur in processed foods, tobacco products, contaminated drinking water, cosmetic materials, and certain pharmaceutical formulations. In recent years, the detection of nitrosamine contamination in pharmaceutical products has further increased toxicological and public health concerns regarding these compounds (Li & He, 2023).

The formation of nitrosamines is closely associated with nitrite and nitrate metabolism. Nitrate (NO_3^-) and nitrite (NO_2^-) are natural intermediates of the physiological nitric oxide pathway; however, under favorable conditions, they can generate nitrosating agents capable of reacting with secondary amines. The resulting N-nitrosamines contribute to carcinogenesis through multiple mechanisms, including DNA alkylation, oxidative stress induction, chronic inflammation, and epigenetic modifications (Snodin et al., 2024).

Epidemiological evidence has demonstrated significant associations between nitrosamine exposure and various gastrointestinal malignancies. Meta-analytical studies indicate that elevated nitrite intake may increase the risk of gastric and esophageal cancers (Seyyedsalehi et al., 2023; Ghasemi et al., 2024). Nevertheless, nitrate and nitrite metabolism presents a paradoxical nature. While dietary nitrates derived from vegetables are generally associated with beneficial cardiovascular effects, nitrites commonly present in processed meat products have been linked to enhanced nitrosamine formation. This phenomenon is often referred to as the “nitrate paradox.” Factors such as the food matrix, antioxidant composition, and cooking methods are considered critical determinants of these differing biological outcomes.

This chapter aims to comprehensively examine the biochemical basis of the nitrite–nitrate cycle and the mechanisms underlying nitrosamine formation. In addition, metabolic activation pathways, DNA damage, oxidative stress, inflammatory responses, nutritional factors, microbiota interactions, environmental exposures, and endogenous protective mechanisms will be discussed in light of current scientific literature.

2. Biochemical Bases of Nitrate and Nitrite Metabolism

Nitrate (NO_3^-) and nitrite (NO_2^-) are fundamental components of nitrogen metabolism and were historically regarded primarily as toxic or potentially carcinogenic compounds. However, advances in molecular biology, biochemistry, and physiology have demonstrated that these molecules are not merely harmful metabolites but also physiological precursors of nitric

oxide (NO). Today, the nitrate–nitrite–nitric oxide pathway is recognized as an important regulatory system involved in vascular homeostasis, immune modulation, neurotransmission, mitochondrial energy metabolism, and intracellular signaling pathways (Barbosa et al., 2024).

Nitric oxide is a short-lived free radical molecule with potent biological activity in mammalian systems. Initially identified as an endothelium-derived relaxing factor, NO promotes vasodilation through activation of guanylate cyclase in vascular smooth muscle cells, inhibits platelet aggregation, and contributes to the maintenance of endothelial integrity. In addition, it plays essential roles in immune regulation, neurotransmitter release, and cellular proliferation processes (Chen, 2024).

Under physiological conditions, nitric oxide is mainly synthesized from L-arginine through nitric oxide synthase (NOS) enzymes. During this oxidative reaction, L-arginine is converted into citrulline and nitric oxide. Three major NOS isoforms have been identified: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). While eNOS and nNOS generate relatively low levels of NO under normal physiological conditions, iNOS can produce large amounts of nitric oxide during inflammatory responses. Excessive NO production, particularly in chronic inflammatory states, has been associated with the generation of reactive nitrogen species and the promotion of tumor development (Chen, 2024).

In recent years, an alternative biochemical pathway in addition to the classical L-arginine–NOS pathway has been identified. This pathway, known as the "nitrate–nitrite–NO pathway," becomes especially active under hypoxic or low-oxygen conditions. Dietary nitrate is rapidly absorbed from the small intestine into the systemic circulation. Approximately 25% of circulating nitrate is actively concentrated in the salivary glands and secreted into the oral cavity, where it is reduced to nitrite by oral microbiota. This process is referred to as the enterosalivary nitrate cycle (Barbosa et al., 2024).

Anaerobic bacteria located primarily on the dorsal surface of the tongue convert nitrate into nitrite through nitrate reductase activity. A portion of the generated nitrite enters the circulation, whereas the remainder reaches the stomach. Under acidic gastric conditions, nitrite is protonated to form nitrous acid, an unstable intermediate capable of generating nitrosyl cations (NO^+), diazonium ions, and other reactive nitrogen species. These reactive compounds can interact with biological amines, leading to the formation of N-nitrosamines. Acidic gastric pH, elevated nitrite concentrations, and protein-rich diets are considered important factors that accelerate nitrosation reactions (Ghasemi et al., 2024).

The major sources of nitrate and nitrite are dietary intake and endogenous metabolic processes. Most dietary nitrate is obtained from vegetables such as beetroot, arugula, lettuce, celery, and spinach. According to data from the World Health Organization, approximately 70–80% of daily nitrate intake

originates from vegetables. In contrast, nitrite exposure is largely associated with processed meat products. Nitrite is commonly added to sausages, salami, ham, bacon, and similar products to inhibit microbial growth, prolong shelf life, and stabilize color (WHO, 2016).

The concept of the "nitrate paradox" has gained increasing attention in recent literature. While vegetable-derived nitrate has been associated with protective cardiovascular effects, nitrite and nitrate from processed meats have been linked to increased cancer risk. This difference appears to depend largely on the food matrix and accompanying antioxidant compounds. Vegetables are rich in ascorbic acid, polyphenols, and flavonoids, which suppress nitrosation reactions. In contrast, processed meats contain heme iron and are frequently subjected to high-temperature processing, both of which facilitate nitrosamine formation (Frontiers in Nutrition, 2024).

The biological effects of nitrate and nitrite metabolism extend beyond nitric oxide generation. These compounds also play important roles in oxidative stress mechanisms. The reaction between nitric oxide and superoxide radicals leads to the formation of peroxynitrite ($ONOO^-$), a highly reactive oxidizing molecule. Peroxynitrite contributes to protein nitration, lipid peroxidation, mitochondrial dysfunction, and oxidative DNA damage. Disruption of the balance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) impairs cellular redox homeostasis and contributes to the initiation of multiple pathological processes, including carcinogenesis (Wu et al., 2024; Glorieux et al., 2024).

The intestinal microbiota also plays a significant role in nitrate and nitrite metabolism. Recent studies indicate that both oral and gut microbiota act as major regulators of nitrate reduction. Dysbiosis may enhance nitrosation reactions, intensify inflammatory responses, and increase the risk of gastrointestinal cancers. In particular, Helicobacter pylori infection has been shown to promote gastric inflammation and facilitate nitrosamine formation.

Current epidemiological evidence suggests that the health effects of nitrate metabolism are source-dependent. Plant-derived nitrate intake has been associated with lower risks of cardiovascular disease, dementia, and metabolic disorders, whereas nitrate and nitrite exposure from processed meats and contaminated drinking water has been linked to gastrointestinal cancers and liver diseases. Findings from the Danish Diet and Cancer Cohort demonstrated that plant-based nitrate consumption was associated with healthier lifestyle patterns, whereas animal-derived nitrite intake correlated with higher inflammatory risk (Ghasemi et al., 2024).

2.1. Dietary Sources of Nitrate and Nitrite

Nitrate (NO_3^-) and nitrite (NO_2^-) are nitrogen-containing compounds widely present in the human diet and are mainly obtained through food consumption. Vegetables represent the principal dietary source of nitrate,

whereas processed meat products and foods containing preservative additives constitute the major sources of nitrite. The biological effects of nitrate and nitrite intake depend not only on their concentration but also on their origin, food composition, preparation methods, and the presence of accompanying antioxidant compounds (Lundberg, Carlström & Weitzberg, 2018). Leafy green vegetables are particularly rich in naturally occurring nitrates. Vegetables such as arugula, lettuce, beetroot, celery, spinach, and chard contain especially high nitrate levels. Plants absorb nitrate from the soil and utilize it during amino acid and protein synthesis. Several factors, including fertilization practices, light exposure, irrigation conditions, and storage duration, can significantly influence nitrate accumulation in vegetables. According to the World Health Organization, nearly 70–80% of total daily nitrate intake originates from vegetable consumption (WHO, 2016). The health effects of vegetable-derived nitrate have been reconsidered in recent years. Although nitrate was previously regarded mainly as a potentially harmful compound, current evidence suggests that it may exert beneficial cardiovascular effects through enhancement of nitric oxide bioavailability. In particular, plant-derived nitrate has been associated with vasodilation, preservation of endothelial function, and blood pressure regulation (Kapil et al., 2020). One of the primary reasons for these beneficial effects is that vegetables are rich not only in nitrate but also in antioxidant molecules such as ascorbic acid, polyphenols, flavonoids, and carotenoids. These antioxidant compounds can inhibit nitrosation reactions and thereby reduce nitrosamine formation. The conversion of nitrate into nitrite occurs mainly through the activity of the oral microbiota. After ingestion, dietary nitrate is absorbed in the small intestine, enters the systemic circulation, and is subsequently concentrated in the salivary glands before being secreted into the oral cavity. Anaerobic bacteria present in the oral flora reduce nitrate to nitrite via nitrate reductase enzymes. This enterosalivary circulation pathway is considered one of the key physiological mechanisms contributing to endogenous nitric oxide production (Lundberg & Weitzberg, 2022). Processed meat products are recognized as the primary dietary sources of nitrite. Nitrite is widely used as a preservative additive in sausages, salami, frankfurters, ham, bacon, and smoked meat products. In the food industry, nitrite is primarily added to inhibit microbial growth, extend shelf life, stabilize product color, and enhance flavor characteristics. Nitrite has long been utilized as an important food additive because of its antimicrobial properties, particularly its ability to inhibit the growth of Clostridium botulinum infection and reduce the risk of botulism. In meat products, nitrite reacts with myoglobin to form nitrosomyoglobin, which is responsible for the characteristic pink-red color of cured meats (Honikel, 2008). Despite these technological advantages, the use of nitrite in processed meat products has raised significant concerns regarding nitrosamine formation. Cooking methods involving high temperatures, such as frying,

grilling, and smoking, as well as prolonged storage conditions, may markedly increase nitrosamine synthesis. Under these conditions, nitrite can react with amines derived from proteins to generate highly carcinogenic N-nitroso compounds, including N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPYR), and N-nitrosodiethylamine (NDEA) (EFSA, 2023). The formation of nitrosamines is particularly enhanced in high-fat and protein-rich products such as bacon when exposed to elevated cooking temperatures. According to the 2023 evaluation published by the European Food Safety Authority, NDMA concentrations detected in fried bacon and smoked meat products were higher than those observed in many other processed meat products (EFSA, 2023). In addition, pyrolysis products generated during smoking processes may further promote nitrosation reactions. Nitrate and nitrite exposure does not originate solely from dietary products but may also occur through drinking water. Elevated nitrate levels in groundwater and surface water are frequently associated with intensive agricultural fertilizer application. Consumption of nitrate-contaminated water is recognized as a major risk factor for methemoglobinemia, commonly referred to as "blue baby syndrome," particularly in infants. Moreover, chronic exposure to high nitrate concentrations has been suggested to contribute to the development of gastrointestinal malignancies (Ward et al., 2018). In recent years, the concept known as the "nitrate paradox" has attracted increasing scientific attention. Nitrates naturally present in vegetables are generally associated with cardioprotective and beneficial physiological effects, whereas nitrite and nitrate derived from processed meat products are linked to increased cancer risk. This distinction appears to depend largely on the nutritional matrix and accompanying bioactive compounds. Vegetables contain substantial amounts of antioxidants, including vitamin C, polyphenols, and flavonoids, which suppress nitrosation reactions. In contrast, processed meats contain heme iron, elevated protein levels, and compounds generated during high-temperature processing that facilitate nitrosamine formation (Habermeyer et al., 2015).

The gut microbiota also plays a crucial role in nitrate metabolism. Certain intestinal microorganisms can reduce nitrate to nitrite, whereas others may inhibit nitrosamine formation. Alterations in microbial composition, particularly during dysbiosis, have been associated with increased nitrosative stress, enhanced inflammatory responses, and a higher risk of gastrointestinal cancer development (Lundberg & Weitzberg, 2022).

2.2. Formation of Nitrous Acid and Nitrosating Species

A critical biochemical step in nitrosamine synthesis is the conversion of nitrite into nitrous acid (HNO_2) under acidic gastric conditions. The pH of human gastric fluid typically ranges between 1 and 3, creating an optimal

environment for this chemical transformation. Nitrite ions derived from dietary intake or transported to the stomach through saliva readily react with protons in the gastric lumen, leading to nitrous acid formation.

Nitrous acid is highly unstable and rapidly decomposes into several reactive nitrosating intermediates within the stomach. Among these, dinitrogen trioxide (N_2O_3), nitrosyl cation (NO^+), nitrosonium ions, and diazonium intermediates are considered the major contributors to nitrosation reactions. These reactive species interact with secondary amines, amides, and other nitrogen-containing organic compounds, ultimately resulting in the formation of N-nitrosamines (SKLM Commentary, 2024).

The generation of dinitrogen trioxide represents a particularly important step in the nitrosation pathway. Under acidic conditions, two molecules of nitrous acid undergo dehydration to produce N_2O_3, a potent nitrosating agent capable of reacting with biological amines and initiating nitrosamine synthesis. Kinetic studies have demonstrated that gastric pH plays a central role in regulating nitrosation efficiency. Nitrosation reactions are reported to occur most effectively at approximately pH 3–3.5. At lower pH levels, excessive protonation of amines suppresses the reaction rate, whereas at higher pH levels the formation of nitrous acid decreases significantly (Mirvish mechanism; SKLM Commentary, 2024).

In addition to gastric acidity, several other factors influence nitrosation reactions, including nitrite concentration, gastric emptying time, protein-rich dietary patterns, and the presence of catalytic compounds. Secondary amines and amides found in processed meat products provide favorable substrates for endogenous nitrosamine formation. Recent investigations have further shown that carbonyl compounds can markedly accelerate nitrosation kinetics. Compounds such as formaldehyde and acetaldehyde may react with amines to form intermediate iminium ions, thereby enhancing nitrosamine synthesis (Pan et al., 2024).

Nitrosation within the stomach is not solely governed by chemical reactions; microbial factors also contribute substantially to this process. In particular, Helicobacter pylori infection promotes chronic gastric inflammation and facilitates endogenous nitrosamine production. Gastric atrophy and hypochlorhydria associated with H. pylori infection increase gastric pH and stimulate bacterial nitrate reduction, leading to elevated nitrite concentrations within gastric fluid. Increased nitrite availability subsequently accelerates nitrosation reactions and enhances endogenous nitrosamine formation (Ghasemi et al., 2024).

During chronic gastritis and inflammatory conditions, activated immune cells release large amounts of nitric oxide. Enhanced nitric oxide production mediated by inducible nitric oxide synthase (iNOS) contributes to the formation of reactive nitrogen species and the development of nitrosative stress. Nitrosative stress is known to promote carcinogenesis through

multiple cellular damage pathways, including DNA base modifications, protein nitration, and lipid peroxidation (Snodin et al., 2024).

Recent evidence suggests that antioxidant compounds may significantly suppress nitrosation reactions in the gastric environment. Ascorbic acid (vitamin C) and polyphenolic compounds such as catechins, gallic acid, and proanthocyanidins exhibit nitrite-scavenging activity, thereby reducing the generation of reactive nitrosating species. Experimental studies using simulated gastric models have demonstrated that catechin and proanthocyanidin B2 effectively inhibit nitrosamine synthesis. Consequently, gastric nitrosation reactions are regulated by the complex interplay among gastric pH, nitrite concentration, dietary composition, microbiota balance, inflammatory status, and antioxidant capacity. A comprehensive understanding of these mechanisms is essential for reducing the risk of nitrosamine-associated carcinogenesis (Ren et al., 2024).

3. Nitrosamine Formation and Carcinogenic Mechanisms

N-nitrosamines are biologically active chemical compounds generated through the reaction of nitrosating agents with secondary or tertiary amines. These compounds have long attracted considerable attention in the fields of toxicology, biochemistry, and cancer research because of their potent mutagenic and carcinogenic properties. Experimental animal studies have consistently demonstrated that many nitrosamines possess strong cancer-inducing potential. Accordingly, numerous nitrosamine compounds have been classified as “probable” or “confirmed” human carcinogens by the International Agency for Research on Cancer (IARC, 2010).

One of the most important characteristics of nitrosamines is their ability to form not only through environmental exposure but also endogenously within the human body. This endogenous synthesis distinguishes nitrosamines from many other chemical carcinogens. Nitrosamine formation primarily occurs through nitrosation reactions. Under acidic conditions, nitrite ions are converted into nitrous acid, which subsequently decomposes into reactive nitrogen intermediates such as nitrosyl cation (NO^+), dinitrogen trioxide (N_2O_3), and related nitrosating species. These reactive compounds interact with biological amines and promote the synthesis of N-nitrosamines (Pan et al., 2024).

Nitrosation reactions occur more efficiently in strongly acidic environments, particularly within the stomach. In these reactions, secondary amines serve as substrates, while nitrosyl cations act as nitrosating agents, ultimately resulting in the formation of N-nitrosamine compounds. The efficiency of nitrosamine synthesis is influenced by multiple variables, including gastric pH, nitrite concentration, amine structure, temperature, and the presence of catalytic ions. Diets rich in protein and processed meat products are

considered especially favorable for endogenous nitrosamine formation because they provide abundant amine substrates (Seyyedsalehi et al., 2023).

Environmental exposure sources of nitrosamines are widespread. Processed meats, smoked foods, tobacco products, cosmetic formulations, pesticides, rubber and latex materials, and certain pharmaceutical agents are among the major contributors to nitrosamine exposure. In recent years, the detection of nitrosamine contamination in medications such as valsartan, ranitidine, and metformin has raised major concerns regarding pharmaceutical quality and drug safety (Li & He, 2023).

The ability of nitrosamines to form not only through external exposure but also endogenously within the gastric and intestinal environment substantially increases their potential risk to human health. Food processing and preparation methods are particularly important determinants of nitrosamine formation. Experimental and epidemiological studies have demonstrated that high-temperature cooking techniques, including frying, grilling, and smoking, markedly enhance nitrosamine synthesis. When nitrite-containing meat products are exposed to elevated temperatures, the formation of carcinogenic compounds such as N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPYR) is accelerated. Moreover, amines released during the thermal degradation of amino acids in protein-rich foods provide additional substrates for nitrosation reactions (EFSA, 2023).

The carcinogenicity of nitrosamines is primarily associated with their metabolic activation. Most nitrosamines are relatively unreactive in their native forms and therefore require bioactivation before interacting with cellular macromolecules such as DNA. This activation process mainly occurs through the cytochrome P450 enzyme system, particularly in the liver. Among these enzymes, CYP2E1 plays a central role in nitrosamine biotransformation (Snodin et al., 2024).

Metabolic activation generates highly reactive electrophilic intermediates capable of covalently binding to DNA bases, leading to the formation of DNA adducts. One of the most critical lesions associated with nitrosamine exposure is the formation of O^6-methylguanine. This altered DNA base disrupts normal base pairing during replication. Under physiological conditions, guanine pairs with cytosine; however, methylated guanine may incorrectly pair with thymine, resulting in G:C → A:T transition mutations. Such mutations can activate proto-oncogenes or inactivate tumor suppressor genes, thereby contributing to carcinogenesis (Snodin et al., 2024).

Nitrosamine-induced genetic damage is not restricted to direct DNA alkylation. Recent studies indicate that nitrosamines also influence epigenetic regulatory mechanisms. Alterations in DNA methylation profiles, histone modifications, and dysregulation of microRNA expression have all been associated with nitrosamine exposure. These epigenetic disturbances may promote uncontrolled cellular proliferation, suppress apoptosis, and

create a microenvironment favorable for tumor initiation and progression (Wu et al., 2024).

Oxidative and nitrosative stress mechanisms also contribute substantially to the carcinogenic effects of nitrosamines. During metabolic activation, increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) promotes lipid peroxidation, protein oxidation, mitochondrial dysfunction, and oxidative DNA damage. In particular, peroxynitrite ($ONOO^-$), generated through the interaction between nitric oxide and superoxide radicals, is a highly reactive oxidizing molecule capable of causing extensive cellular injury (Glorieux et al., 2024).

Chronic exposure to nitrosamines has additionally been linked to persistent inflammatory responses. Nitrosative stress activates several transcription factors, including NF-κB, STAT3, and AP-1, leading to elevated expression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. Sustained inflammation contributes to increased cellular proliferation, angiogenesis, and remodeling of the tumor microenvironment, thereby facilitating cancer development (Chen, 2024).

Epidemiological evidence strongly supports the association between nitrosamine exposure and gastrointestinal malignancies. Recent meta-analyses have reported that high nitrite intake is associated with increased risks of gastric, esophageal, and colorectal cancers (Ghasemi et al., 2024). Furthermore, tobacco-specific nitrosamines have been implicated in the pathogenesis of lung, pancreatic, and oral cavity cancers.

Among tobacco-specific nitrosamines, NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] and NNN (N′-nitrosonornicotine) are recognized as some of the most potent carcinogenic compounds derived from tobacco products (Peterson, Stanfill & Hecht, 2024). These compounds have been strongly associated with the development of multiple malignancies, particularly cancers of the lung, oral cavity, and pancreas.

Individual susceptibility also plays an important role in nitrosamine metabolism and carcinogenicity. Factors such as genetic polymorphisms, gut microbiota composition, antioxidant intake, smoking habits, alcohol consumption, and chronic inflammatory diseases may significantly influence endogenous nitrosamine formation. In particular, Helicobacter pylori infection has been shown to promote chronic inflammation in the gastric mucosa, thereby facilitating nitrosation reactions. Patients with chronic gastritis often exhibit elevated nitrite concentrations in gastric fluid, which may contribute to increased endogenous nitrosamine synthesis (Seyyedsalehi et al., 2023).

Conversely, several antioxidant compounds have been reported to suppress nitrosation reactions and reduce nitrosamine formation. Molecules such as ascorbic acid, tocopherols, and polyphenols can inhibit the generation of reactive nitrosating species. Vitamin C, in particular, reduces nitrosyl cations and interferes with nitrosamine synthesis pathways. For this reason, diets

rich in fruits and vegetables are considered potentially protective against nitrosamine-related carcinogenesis. Current evidence suggests that dietary patterns such as the Mediterranean diet may exert beneficial effects by reducing nitrosative stress and inflammation (Frontiers in Nutrition, 2024).

Overall, nitrosamines are highly potent chemical carcinogens that may originate from both environmental exposure and endogenous biochemical processes. Their carcinogenic effects involve multiple interconnected mechanisms, including DNA alkylation, oxidative and nitrosative stress, chronic inflammation, and epigenetic dysregulation. Consequently, minimizing nitrosamine exposure has become an important public health objective in the areas of food processing technologies, pharmaceutical manufacturing, environmental safety, and nutritional practices.

3.1. Nitrosation Reaction

Nitrosamine formation primarily occurs through nitrosation reactions, which represent one of the fundamental biochemical mechanisms underlying the synthesis of N-nitroso compounds. Nitrosation is a chemical process in which nitrosating agents interact with amine-containing molecules, leading to the production of N-nitroso derivatives. In particular, the reaction between secondary amines and nitrosyl cations (NO^+) results in the formation of N-nitrosamines, compounds widely recognized for their potent mutagenic and carcinogenic properties (EFSA, 2023).

The nitrosation pathway involves several sequential biochemical reactions. Initially, nitrite ions (NO_2^-) become protonated in acidic environments such as the stomach, producing nitrous acid (HNO_2). Because nitrous acid is chemically unstable, it rapidly decomposes into highly reactive nitrosating intermediates, including nitrosyl cations (NO^+), dinitrogen trioxide (N_2O_3), and nitrosonium ions (Pan et al., 2024). These reactive nitrogen species subsequently interact with secondary amines and initiate N-nitrosamine synthesis.

The efficiency of nitrosation reactions is strongly influenced by environmental pH. Acidic conditions promote the generation of nitrosyl cations and therefore accelerate nitrosamine formation. Since the physiological pH of human gastric fluid generally ranges from approximately 1 to 3, the stomach provides a highly favorable biochemical environment for endogenous nitrosation reactions (Mirvish, 1995).

Several additional factors regulate nitrosation within the gastric environment, including nitrite concentration, amine availability, gastric emptying time, temperature, and dietary composition. Protein-rich diets may increase the availability of secondary amines, thereby enhancing nitrosamine synthesis. Processed meat products are considered particularly important contributors because they contain substantial amounts of both nitrites and amine precursors (Habermeyer et al., 2015).

Recent research has further demonstrated that food preparation techniques significantly affect nitrosation dynamics. High-temperature cooking methods such as frying, grilling, and smoking are associated with increased nitrosamine production. During thermal processing, protein degradation generates additional amine substrates that participate in nitrosation reactions. Elevated concentrations of N-nitrosodimethylamine (NDMA) detected in bacon and smoked meat products support the importance of this mechanism (EFSA, 2023).

Nitrosation reactions are not restricted to the stomach but may also occur within the oral cavity and intestinal tract. The oral microbiota contributes to nitrate reduction by converting nitrate into nitrite through bacterial nitrate reductase activity. Anaerobic bacteria residing in the oral cavity therefore play a critical role in the enterosalivary nitrate cycle and in the endogenous generation of nitrosating species (Lundberg & Weitzberg, 2022).

The intestinal microbiota likewise has a substantial influence on nitrosation processes. Dysbiosis has been associated with increased nitrite production, enhanced nitrosative stress, and elevated gastrointestinal cancer risk. In particular, Helicobacter pylori infection promotes gastric inflammation and facilitates nitrosation reactions within the stomach. Studies involving individuals with chronic gastritis have demonstrated elevated gastric nitrite levels and enhanced endogenous nitrosamine formation (Seyyedsalehi et al., 2023).

Conversely, antioxidant compounds may inhibit nitrosation reactions and reduce nitrosamine synthesis. Molecules such as ascorbic acid (vitamin C), tocopherols, and polyphenols can neutralize reactive nitrosating species and suppress N-nitrosamine formation. Consequently, diets rich in fruits and vegetables are believed to exert protective effects against nitrosative stress. The Mediterranean dietary pattern, in particular, has been associated with reduced nitrosative damage and lower gastrointestinal cancer risk (Lundberg, Carlström & Weitzberg, 2018).

The biological consequences of nitrosation extend beyond nitrosamine production alone. Reactive nitrogen species generated during these reactions may induce DNA damage, protein nitration, lipid peroxidation, and mitochondrial dysfunction. Such alterations disrupt cellular redox homeostasis and contribute to mutagenesis and carcinogenesis through multiple interconnected molecular pathways (Wu et al., 2024).

3.2. Major Types of Nitrosamines

N-nitrosamines are classified into different subgroups according to their chemical structure, origin, and biological effects. While hundreds of different nitrosamine compounds have been identified to date, a significant portion have been shown to possess mutagenic and carcinogenic properties. In particular, some nitrosamine species can cause tumor development even at

very low doses in experimental animal models and are therefore considered potent chemical carcinogens. The International Agency for Cancer Research (IARC) has classified many nitrosamine compounds as "probable" or "definite" human carcinogens (IARC, 2010).
The most frequently studied nitrosamine compounds, and those of significant importance to human health, are as follows:

- ✓ N-nitrosodimethylamine (NDMA)
- ✓ N-nitrosodiethylamine (NDEA)
- ✓ N-nitrosopyrrolidine (NPYR)
- ✓ N-nitrosonornicotine (NNN)
- ✓ 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

These compounds are found in various environmental sources and can exhibit carcinogenic effects in different organ systems.

N-Nitrosodimethylamine (NDMA)

N-nitrosodimethylamine (NDMA) is one of the most extensively studied and highly carcinogenic nitrosamines. NDMA has been detected in a variety of environmental and dietary sources, including processed meat products, smoked foods, alcoholic beverages such as beer, fish products, and contaminated drinking water. In recent years, NDMA contamination identified in several pharmaceutical products has also become a major public health concern. Medications such as ranitidine, valsartan, and certain metformin formulations were recalled from the market because of unacceptable NDMA levels (Li & He, 2023). The toxic and carcinogenic effects of NDMA are primarily associated with its metabolic activation in the liver. This bioactivation process is mainly mediated by the CYP2E1 enzyme of the cytochrome P450 system. Following metabolic conversion, highly reactive methyldiazonium intermediates are generated, which induce DNA alkylation. One of the most critical consequences of this process is the formation of O^6-methylguanine DNA adducts, which contribute significantly to mutation development and genomic instability (Snodin et al., 2024). Experimental animal studies have demonstrated strong associations between NDMA exposure and the development of tumors in several organs, particularly the liver, stomach, pancreas, and colorectal tissues. Furthermore, chronic exposure to low concentrations of NDMA has been reported to promote oxidative stress, mitochondrial dysfunction, and inflammatory signaling pathways, all of which contribute to carcinogenesis and cellular damage (EFSA, 2023).

N-Nitrosodiethylamine (NDEA)

N-nitrosodiethylamine (NDEA) is an important nitrosamine commonly detected in processed meat products, alcoholic beverages, tobacco smoke,

and certain cosmetic formulations. Among nitrosamines, NDEA is particularly recognized for its potent hepatocarcinogenic activity. Experimental animal studies have demonstrated that chronic exposure to NDEA may induce progressive liver damage, including hepatic fibrosis, cirrhosis, and ultimately hepatocellular carcinoma (Li et al., 2024). The carcinogenic effects of NDEA are primarily associated with its metabolic activation. Following biotransformation, reactive ethyl intermediates are generated, which interact directly with DNA and induce ethylation of nucleic acid bases. These DNA modifications contribute to genomic instability, mutation accumulation, and enhanced cellular proliferation. In addition to its genotoxic effects, recent studies suggest that NDEA may also influence epigenetic regulatory pathways. Alterations in DNA methylation patterns, gene expression profiles, and epigenetic signaling mechanisms have all been associated with NDEA exposure, further contributing to carcinogenic progression (Wu et al., 2024).

N-Nitrosopyrrolidine (NPYR)

N-nitrosopyrrolidine (NPYR) is a nitrosamine commonly formed in fried bacon, smoked meat products, and protein-rich foods exposed to high-temperature processing. This compound is generated through the nitrosation of pyrrolidine-containing amines, and its formation is significantly enhanced during cooking methods such as frying, grilling, and smoking (Habermeyer et al., 2015).

Experimental studies have demonstrated that NPYR is associated with the development of liver, esophageal, and gastric cancers. Similar to other carcinogenic nitrosamines, NPYR requires metabolic activation before exerting its genotoxic effects. Following bioactivation, reactive intermediates form DNA adducts that contribute to mutation development and genomic instability.

In addition to direct DNA damage, NPYR has been reported to promote oxidative stress through mechanisms involving mitochondrial dysfunction and lipid peroxidation. Increased production of reactive oxygen species and disruption of cellular redox balance are considered important contributors to the carcinogenic effects of this compound (Glorieux et al., 2024).

Tobacco-Specific Nitrosamines: NNK and NNN

Tobacco-specific nitrosamines (TSNAs) are regarded as some of the most potent carcinogenic compounds within the nitrosamine group. Among these, NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] and NNN (N′-nitrosonornicotine) are considered the most biologically significant. These compounds are generated through the nitrosation of nicotine-derived

alkaloids during the curing, fermentation, aging, and combustion processes of tobacco leaves (Peterson, Stanfill & Hecht, 2024).

NNK is recognized as a highly potent pulmonary carcinogen and has been strongly implicated in lung cancer development. Molecular studies have demonstrated that NNK promotes cellular proliferation through activation of β-adrenergic receptors and nicotinic acetylcholine receptors. In addition, NNK stimulates several oncogenic signaling pathways, including PI3K/Akt, MAPK, and NF-κB, thereby enhancing tumor initiation, survival, and progression (Peterson et al., 2024).

NNN, on the other hand, has been particularly associated with cancers of the esophagus and oral cavity. Experimental investigations have shown that exposure to NNN induces extensive mutational damage within the esophageal epithelium and significantly increases the risk of squamous cell carcinoma formation (Hecht, 2023).

One of the most important characteristics of tobacco-specific nitrosamines is their widespread presence not only in cigarette smoke but also in smokeless tobacco products. Significant TSNA concentrations have been detected in electronic cigarette liquids, hookah products, chewing tobacco, and other alternative tobacco formulations. These findings indicate that the carcinogenic risks associated with tobacco use are not limited to combustion-derived toxicants alone but also involve substantial exposure to nitrosamine compounds (Peterson et al., 2024).

Organ-Specific Carcinogenicity

One of the most important characteristics of nitrosamines is their ability to exhibit organ-specific carcinogenic effects. Different nitrosamine compounds can promote tumor development in distinct tissues depending on their metabolic activation properties. This tissue specificity is largely associated with the expression patterns of cytochrome P450 enzymes in the affected organs (Snodin et al., 2024).

3.3. Metabolic Activation of Nitrosamines

Although nitrosamines possess strong carcinogenic potential, most of them are not directly reactive in their native form. To exert their biological and toxic effects, they must first undergo metabolic activation within the organism. The biotransformation of nitrosamines primarily occurs through microsomal enzyme systems located in the liver, although extrahepatic tissues such as the lungs, kidneys, pancreas, and gastrointestinal tract also contribute to this process. Among the metabolic enzymes involved, the cytochrome P450 (CYP450) family plays a central role. In particular, the CYP2E1 isoenzyme is regarded as one of the principal pathways responsible for nitrosamine activation (Snodin et al., 2024). During metabolic activation,

nitrosamines initially undergo α-hydroxylation reactions catalyzed mainly by CYP2E1. This reaction produces unstable α-hydroxynitrosamine intermediates, which rapidly decompose into highly reactive compounds, including diazonium ions, carbonium ions, and electrophilic alkylating species. These reactive metabolites subsequently interact with nucleophilic regions of cellular macromolecules, particularly DNA, resulting in covalent adduct formation and genetic damage (Hecht, 2023). One of the most significant DNA lesions induced by nitrosamine metabolism is the formation of O^6-methylguanine adducts. Such modifications interfere with normal DNA replication fidelity and promote mutagenesis. Under physiological conditions, guanine pairs specifically with cytosine; however, O^6-methylguanine has an increased tendency to pair incorrectly with thymine. This mismatch results in G:C → A:T transition mutations, which are strongly associated with proto-oncogene activation and tumor suppressor gene inactivation during carcinogenesis (Snodin et al., 2024; Hecht, 2023).
The mutagenic and carcinogenic potency of nitrosamines is closely related to their metabolic activation efficiency. According to Snodin et al. (2024), the biological toxicity of nitrosamines is directly associated with the amount of reactive electrophilic metabolites generated through CYP450-mediated metabolism. Nitrosamines containing hydrogen atoms at the α-carbon position are generally more susceptible to metabolic activation and therefore tend to exhibit stronger mutagenic activity. Consequently, the chemical structure of individual nitrosamines is a major determinant of their carcinogenic potential. The CYP2E1 enzyme is also involved in the metabolism of ethanol, acetaminophen, and various organic solvents. Chronic alcohol consumption has been shown to increase CYP2E1 expression and enzymatic activity. Enhanced CYP2E1 activity may accelerate nitrosamine biotransformation and increase the generation of reactive intermediates. For this reason, alcohol intake is believed to have a synergistic effect on nitrosamine-induced carcinogenesis and may further elevate cancer risk associated with nitrosamine exposure (Lu & Cederbaum, 2023).
Metabolic activation of nitrosamines is closely associated with their organ-specific carcinogenic effects. Since the expression levels of cytochrome P450 enzymes differ among tissues, the target organs affected by individual nitrosamines also vary. For example, N-nitrosodimethylamine (NDMA) primarily exerts hepatocarcinogenic effects because of its extensive metabolism in the liver, whereas the tobacco-specific nitrosamine NNK undergoes significant metabolic activation in lung tissue and is strongly linked to pulmonary tumor development (Peterson, Stanfill & Hecht, 2024).
Nitrosamine metabolism results not only in DNA alkylation but also in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), both of which contribute to oxidative and nitrosative stress. One of the most damaging reactive molecules formed during this process is

peroxynitrite ($ONOO^-$), which is generated through the interaction between nitric oxide and superoxide radicals. Peroxynitrite promotes protein nitration, lipid peroxidation, mitochondrial dysfunction, and oxidative DNA damage, thereby amplifying the carcinogenic potential of nitrosamines (Wu et al., 2024).

Recent evidence indicates that nitrosamines exert not only genotoxic but also epigenetic effects. Alterations in DNA methylation patterns, histone modifications, and dysregulation of microRNA expression have all been associated with nitrosamine exposure. These epigenetic abnormalities may contribute to abnormal gene expression, uncontrolled cellular proliferation, and tumor progression (Glorieux et al., 2024).

Interindividual genetic variability also influences nitrosamine metabolism and susceptibility to carcinogenesis. Polymorphisms within the CYP2E1 gene may increase the metabolic activation rate of nitrosamines in certain individuals, resulting in enhanced formation of reactive metabolites and greater mutagenic potential. Such genetic differences are considered important factors underlying variability in cancer susceptibility among exposed populations (Lu & Cederbaum, 2023).

Cellular antioxidant defense systems play a crucial role in limiting the harmful consequences of nitrosamine metabolism. Antioxidant molecules and enzymes, including glutathione, superoxide dismutase, catalase, and ascorbic acid, help neutralize reactive intermediates and reduce oxidative DNA damage. In particular, vitamin C has been shown to suppress nitrosamine synthesis by inhibiting nitrosation reactions, thereby providing a protective effect against nitrosamine-associated carcinogenesis (Habermeyer et al., 2015).

4. DNA Damage and Molecular Carcinogenesis

The carcinogenic effects of nitrosamines are primarily based on the interaction between reactive alkylating metabolites, generated following metabolic activation, and cellular DNA. Nitrosamines activated through the cytochrome P450 (CYP450) enzyme system produce highly reactive electrophilic intermediates capable of attacking various cellular macromolecules. Among the most critical targets of these reactive metabolites are the nucleophilic sites within DNA, particularly guanine bases (Hecht, 2023; Snodin et al., 2024).

4.1. Formation of DNA Adducts

Nitrosamine metabolites form various covalent binding products on DNA, known as DNA adducts. These adducts generally arise through the transfer of alkyl groups to DNA bases, thereby disrupting the accurate replication and transcription of genetic information. One of the most common and well-

characterized forms of nitrosamine-induced DNA damage is the formation of O^6-methylguanine. O^6-methylguanine is a highly mutagenic DNA lesion that promotes incorrect base pairing during DNA replication. Under normal physiological conditions, guanine pairs specifically with cytosine (C); however, methylated guanine exhibits an increased tendency to mispair with thymine (T). Over time, this abnormal pairing leads to the accumulation of G:C → A:T transition mutations. Such mutations may cause critical functional alterations in tumor suppressor genes and proto-oncogenes, thereby contributing to carcinogenic transformation (Snodin et al., 2024). The progressive accumulation of these genetic alterations creates a molecular environment favorable for uncontrolled cellular proliferation and the acquisition of a malignant phenotype (Hecht, 2023; Wu et al., 2024).

4.2. Epigenetic Alterations

Recent studies have demonstrated that nitrosamines contribute to carcinogenesis not only through direct genetic damage but also by inducing epigenetic alterations. Epigenetic modifications involve long-term changes in gene expression without alterations in the underlying DNA sequence. The major epigenetic disturbances associated with nitrosamine exposure include abnormal DNA methylation patterns, histone modifications such as altered acetylation and methylation, and dysregulation of microRNA (miRNA) expression. These epigenetic abnormalities may disrupt cellular homeostasis, silence tumor suppressor genes, and promote oncogene overexpression. In particular, DNA hypermethylation is regarded as a critical mechanism contributing to cancer development through transcriptional silencing of protective genes (Wu et al., 2024). One of the major consequences of nitrosamine-induced epigenetic dysregulation is the impairment of normal cell cycle control mechanisms. This disruption promotes uncontrolled cellular proliferation while simultaneously suppressing apoptotic pathways. In addition, activation of inflammatory signaling pathways, including NF-κB and STAT3, further supports the establishment and maintenance of a tumor-promoting microenvironment (Glorieux et al., 2024). The combined influence of DNA damage and epigenetic alterations is often interpreted within the framework of a multistage carcinogenesis model. According to this concept, nitrosamines contribute to both the initiation and promotion phases of tumor development. Chronic exposure to low concentrations of nitrosamines may progressively impair cellular DNA repair capacity, resulting in the gradual accumulation of irreversible genetic and epigenetic abnormalities. This mechanism is considered particularly important in explaining the long latency period observed in several cancers, especially gastrointestinal and lung malignancies (Hecht, 2023).

5. Oxidative Stress and Inflammation

One of the major mechanisms underlying the carcinogenic effects of nitrosamine exposure is the induction of oxidative and nitrosative stress at the cellular level. During nitrosamine metabolism, not only reactive alkylating intermediates but also reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated. These highly reactive molecules induce oxidative damage to lipids, proteins, and nucleic acids, thereby disrupting cellular integrity and redox homeostasis (Wu et al., 2024).
Oxidative stress fundamentally arises from an imbalance between pro-oxidant and antioxidant systems in favor of pro-oxidant activity. Increased ROS production during nitrosamine biotransformation adversely affects cellular metabolism, particularly by impairing mitochondrial electron transport chain function. Mitochondrial dysfunction subsequently enhances intracellular oxidative burden, promotes lipid peroxidation, and contributes to oxidative DNA damage (Glorieux et al., 2024).

5.1. Peroxynitrite Formation

One of the most important contributors to nitrosamine-induced oxidative and nitrosative stress is the formation of peroxynitrite ($ONOO^-$). Peroxynitrite is a highly potent oxidizing and nitrating molecule generated through the rapid interaction between nitric oxide (NO) and the superoxide radical (O_2^-). Due to its strong reactivity, peroxynitrite can induce extensive cellular and molecular damage in biological systems. It has been particularly associated with pathological processes such as nitration of tyrosine residues in proteins, lipid peroxidation of cellular membranes, mitochondrial DNA damage, impaired mitochondrial energy production, and disruption of enzymatic activities.
Peroxynitrite-mediated protein nitration may alter intracellular signaling pathways and contribute to the activation of apoptosis and necrotic cell death mechanisms (Glorieux et al., 2024).

5.2. Chronic Inflammation and the Tumor Microenvironment

Nitrosamine exposure is not limited to direct DNA damage and oxidative stress, but also supports tumor development through the activation of chronic inflammatory responses. Chronic inflammation plays a critical role in creating a "tumor-initiating and propagating microenvironment" in cancer development (Hanahan, 2022). Nitrosamines have been shown to activate inflammatory signaling pathways and are particularly effective through the following mechanisms:

- ✓ Activation of NF-κB (nuclear factor kappa B)
- ✓ Increased TNF-α (tumor necrosis factor-alpha) levels

- ✓ Release of pro-inflammatory cytokines such as IL-6 and IL-1β
- ✓ Upregulation of COX-2 (cyclooxygenase-2) expression

These inflammatory responses promote cellular proliferation while simultaneously suppressing apoptotic mechanisms, thereby facilitating the survival and progression of tumor cells (Wu et al., 2024). Persistent activation of the NF-κB signaling pathway is considered one of the key molecular links between chronic inflammation and carcinogenesis. Cytokines released through this pathway exert both autocrine and paracrine effects, contributing to the maintenance and stability of the tumor microenvironment. In particular, activation of the IL-6/STAT3 signaling axis enhances proliferative and anti-apoptotic signaling in tumor cells. Chronic inflammation also supports angiogenesis, an essential process for tumor growth and progression. Increased expression of VEGF (vascular endothelial growth factor) promotes neovascularization within tumor tissue, enabling enhanced nutrient and oxygen supply. This process accelerates tumor expansion and contributes to increased metastatic potential (Hanahan, 2022).

6. Nutrition, Microbiota, and Environmental Factors

The biological effects of nitrate and nitrite metabolism depend not only on the quantity consumed but also on their dietary source, the composition of the gut microbiota, and various environmental exposures. In this context, the concept known as the "nitrate paradox" has emerged as an important framework for explaining why vegetable-derived nitrates may exert beneficial cardiovascular effects, whereas nitrites derived from processed meat products are more frequently associated with carcinogenic processes. Although vegetables contain high concentrations of nitrate, they are simultaneously rich in antioxidant compounds such as ascorbic acid, polyphenols, and flavonoids. These bioactive molecules suppress nitrosation reactions and reduce nitrosamine formation. Consequently, vegetable-derived nitrate may contribute to beneficial physiological outcomes, including vasodilation, improved endothelial function, and regulation of blood pressure through enhanced nitric oxide bioavailability (Lundberg, Carlström & Weitzberg, 2018). In contrast, processed meat products provide a more favorable biochemical environment for endogenous nitrosamine synthesis. The interaction between nitrite and protein-derived amines, combined with the relatively low antioxidant content of these foods, facilitates nitrosation reactions and increases the formation of potentially carcinogenic N-nitroso compounds. Therefore, current evidence suggests that the biological effects of nitrate and nitrite should be evaluated not only according to dose but also within the framework of "source-dependent toxicity." The gut microbiota also functions as an important regulator of the nitrate–nitrite cycle. Both oral and intestinal microbial communities actively

participate in the reduction of nitrate to nitrite and consequently influence nitrosamine formation. Certain bacterial species increase nitric oxide bioavailability through nitrate reductase activity, whereas others may enhance nitrosative reactions and promote the formation of harmful N-nitroso compounds. In conditions of dysbiosis, where microbial balance is disrupted, nitrosation reactions may become more pronounced. Dysbiosis has also been associated with impaired intestinal barrier integrity, increased intestinal permeability ("leaky gut"), chronic inflammatory activation, and elevated risk of systemic endotoxemia (Lundberg & Weitzberg, 2022). Environmental factors, particularly tobacco exposure, also play a major role in nitrosamine-related carcinogenesis. Tobacco products are regarded as one of the most significant environmental sources of tobacco-specific nitrosamines (TSNAs). Among these compounds, NNK and NNN are recognized as highly potent organ-specific carcinogens strongly associated with cancers of the lung and upper aerodigestive tract. The carcinogenicity of tobacco-specific nitrosamines is primarily mediated through metabolic activation, subsequent DNA adduct formation, activation of oncogenic pathways such as KRAS, and induction of cellular transformation processes. NNK has been shown to stimulate proliferative signaling pathways including PI3K/Akt and MAPK in pulmonary tissue, thereby supporting tumor progression. Similarly, NNN contributes to genetic instability within the esophageal epithelium and increases the risk of squamous cell carcinoma development (Peterson, Stanfill & Hecht, 2024).

7. Current Approaches and Preventive Strategies

Due to the potential carcinogenic effects of nitrosamines on human health, significant regulatory measures and risk-reduction strategies have been developed in recent years in both the food safety and pharmaceutical industries. In particular, the U.S. Food and Drug Administration and the European Medicines Agency have published comprehensive guidelines aimed at preventing nitrosamine contamination in pharmaceutical products. These guidelines require stricter control mechanisms during raw material selection, manufacturing processes, storage conditions, and quality assurance procedures. Such developments demonstrate that nitrosamine formation should be regarded not only as an environmental issue but also as a major industrial safety concern (FDA, 2023; EMA, 2023). Preventive strategies should be considered at both individual and public health levels. Modification of dietary habits represents one of the most important approaches. Reducing the consumption of processed meat products is regarded as a key strategy for decreasing nitrite and nitrosamine exposure. Conversely, adoption of a diet rich in antioxidant compounds may suppress nitrosation reactions and reduce endogenous nitrosamine formation.

Increased consumption of vegetables, fruits, and whole grains is therefore widely recommended (Lundberg, Carlström & Weitzberg, 2018).
Lifestyle-related factors also play an essential role in prevention. Smoking cessation significantly reduces exposure to tobacco-specific nitrosamines (TSNAs) and consequently lowers the risk of lung and upper aerodigestive tract cancers. In addition, optimization of nitrite concentrations used in food processing and the development of alternative preservation methods, such as low-temperature processing techniques and the use of natural preservatives, are considered important industrial strategies for minimizing nitrosamine formation (Hecht, 2023). In recent years, increasing attention has also been directed toward modulation of the gut microbiota through probiotic and prebiotic interventions. Maintenance of microbiota balance may help preserve nitrate–nitrite metabolism at more physiological levels and thereby reduce nitrosative stress. Furthermore, certain probiotic microorganisms have been reported to suppress nitrosative reactions and exert indirect anti-carcinogenic effects (Lundberg & Weitzberg, 2022).
The inhibitory effects of natural antioxidants on nitrosation reactions have likewise been demonstrated in numerous studies. Compounds such as ascorbic acid (vitamin C), tocopherols (vitamin E), and polyphenolic molecules can reduce or stabilize reactive nitrosating species, thereby decreasing N-nitrosamine formation. These findings support the concept that dietary antioxidants play a protective role against chemical carcinogenesis. In particular, the ability of vitamin C to reduce nitrosyl cations is considered one of the most important biochemical mechanisms involved in preventing endogenous nitrosamine synthesis (EFSA, 2023).

8. CONCLUSION

The nitrite–nitrate cycle is a natural and physiologically important metabolic pathway, particularly in the regulation of nitric oxide bioavailability in the human body. However, under certain biochemical and environmental conditions, this cycle may also trigger potentially carcinogenic processes through the formation of nitrosamines. Current evidence strongly demonstrates that, following metabolic activation, nitrosamines contribute to multistage carcinogenesis through mechanisms including DNA alkylation, induction of oxidative stress, chronic inflammation, and epigenetic dysregulation (Hecht, 2023; Snodin et al., 2024; Wu et al., 2024).
Recent epidemiological and mechanistic studies indicate that consumption of processed meat products and exposure to tobacco significantly increase nitrosamine formation and constitute major risk factors for cancers of the gastrointestinal tract, lungs, and upper aerodigestive system. In contrast, dietary nitrate derived from vegetables appears to exert different and generally protective biological effects because of its high antioxidant content and its role in enhancing nitric oxide bioavailability. These findings suggest

that the health effects of nitrate and nitrite are determined not only by dose but also by their source, food matrix, and accompanying biological factors (Lundberg, Carlström & Weitzberg, 2018).

Recent research has additionally highlighted the regulatory role of the gut microbiota in nitrate–nitrite metabolism and nitrosamine formation. The relationship between alterations in microbial composition and nitrosative stress has contributed to a better understanding of carcinogenic mechanisms. Moreover, the involvement of epigenetic alterations and inflammatory signaling pathways is increasingly recognized as a critical factor in nitrosamine-associated tumor development (Lundberg & Weitzberg, 2022; Wu et al., 2024).

Future advances in molecular biology, epigenetic analyses, and microbiota research are expected to provide a more comprehensive understanding of nitrosamine-related carcinogenic mechanisms and contribute to the development of personalized nutritional and preventive strategies. In this context, multidisciplinary approaches aimed at reducing nitrosamine exposure are considered important public health tools for cancer prevention.

REFERENCES

Barbosa, P. O., Tanus-Santos, J. E., Cavalli, R. C., Bengtsson, T., Montenegro, M. F., & Sandrim, V. C. (2024). The nitrate-nitrite-nitric oxide pathway: Potential role in mitigating oxidative stress in hypertensive disorders of pregnancy. Nutrients, 16(10), 1475.

Chen, T. (2024). Unveiling the significance of inducible nitric oxide synthase: Its impact on cancer progression and clinical implications. *Cancer Letters, 592*, 216931.

EFSA Panel on Contaminants in the Food Chain. (2023). Risk assessment of N-nitrosamines in food. *EFSA Journal, 21*(3), 7884.

EMA. (2023). *Information on nitrosamine impurities in human medicines*. European Medicines Agency.

Ghasemi, M., Koutenaei, M. B., Ghasemi, A., Alizadeh-Navaei, R., & Moosazadeh, M. (2024). A systematic review and dose-response meta-analysis of the association between nitrate and nitrite intake and gastroesophageal cancer risk. *Nitric Oxide, 153*, 61–71.

Glorieux, C., Liu, S., Trachootham, D., et al. (2024). Targeting ROS in cancer: Rationale and strategies. *Nature Reviews Drug Discovery, 23*, 583–606.

Habermeyer, M., Roth, A., Guth, S., Diel, P., Engel, K. H., Epe, B., Fürst, P., Heinz, V., Humpf, H. U., Joost, H. G., et al. (2015). Nitrate and nitrite in the diet:

How to assess their benefit and risk for human health. *Molecular Nutrition & Food Research, 59*(1), 106–128.

Hanahan, D. (2022). Hallmarks of cancer: New dimensions. *Cancer Discovery, 12*(1), 31–46.

Hecht, S. S. (2023). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Reviews Cancer, 23*(6), 377–395.

Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science, 78*(1–2), 68–76.

IARC. (2010). *Ingested nitrate and nitrite, and cyanobacterial peptide toxins*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 94.

Kapil, V., Khambata, R. S., Jones, D. A., Rathod, K., Primus, C., Massimo, G., Fukuto, J. M., Ahluwalia, A., & Webb, A. J. (2020). The noncanonical pathway for in vivo nitric oxide generation: The nitrate–nitrite–nitric oxide pathway. *Pharmacological Reviews, 72*(3), 692–766.

Li, X., & He, Y. (2023). Nitrosamine impurities in pharmaceuticals: Sources, analytical methods, and mitigation strategies. *Regulatory Toxicology and Pharmacology, 139*, 105365.

Li, X., Zhou, X., Guan, Y., Wang, Y. X. J., Scutt, D., & Gong, Q. Y. (2006). N-nitrosodiethylamine-induced pig liver hepatocellular carcinoma model: radiological and histopathological studies. *Cardiovascular and interventional radiology*, *29*(3), 420-428.

Lu, Y., & Cederbaum, A. I. (2023). CYP2E1 and oxidative liver injury by alcohol. *Free Radical Biology and Medicine, 198*, 163–176.

Lundberg, J. O., & Weitzberg, E. (2022). Nitric oxide signaling in health and disease. *Cell, 185*(16), 2853–2878.
Lundberg, J. O., & Weitzberg, E. (2022). Nitric oxide signaling in health and disease. *Cell, 185*(16), 2853–2878.

Lundberg, J. O., Carlström, M., & Weitzberg, E. (2018). Metabolic effects of dietary nitrate in health and disease. *Cell Metabolism, 28*(1), 9–22.

Mirvish, S. S. (1995). Role of N-nitroso compounds and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer. *Cancer Letters, 93*(1), 17–48.

Pan, Y., Breider, F., Barrios, B., Minakata, D., Deng, H., & von Gunten, U. (2024). Role of carbonyl compounds for N-nitrosamine formation during nitrosation: Kinetics and mechanisms. *Environmental Science & Technology, 58*(10), 4792–4801.

Peterson, L. A., Stanfill, S. B., & Hecht, S. S. (2024). An update on the formation in tobacco, toxicity and carcinogenicity of N′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Carcinogenesis, 45*(5), 275–287.

Peterson, L. A., Stanfill, S. B., & Hecht, S. S. (2024). An update on the formation in tobacco, toxicity and carcinogenicity of N′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Carcinogenesis, 45*(5), 275–287.

Peterson, L. A., Stanfill, S. B., & Hecht, S. S. (2024). An update on the formation in tobacco, toxicity and carcinogenicity of N′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Carcinogenesis, 45*(5), 275–287.

Ren, S., Hu, H., Zhu, X., Wang, S., Zhao, W., Xie, D., Xi, J., & Liu, K. (2024). Inhibitory effects and reactions of gallic acid, catechin, and procyanidin B2 with nitrosation under stomach simulating conditions. *Food & Function, 15*, 3130–3140.

Seyyedsalehi, M. S., Mohebbi, E., Tourang, F., Sasanfar, B., Boffetta, P., & Zendehdel, K. (2023). Association of dietary nitrate, nitrite, and N-nitroso compounds intake and gastrointestinal cancers: A systematic review and meta-analysis. *Toxics, 11*(2), 190. https://doi.org/10.3390/toxics11020190

SKLM Commentary. (2024). Commentary of the SKLM to the EFSA opinion on risk assessment of N-nitrosamines in food. *Archives of Toxicology*.

Snodin, D. J., Trejo-Martin, A., Ponting, D. J., Smith, G. F., Czich, A., Cross, K., & Dobo, K. L. (2024). Mechanisms of nitrosamine mutagenicity and their relationship to rodent carcinogenic potency. *Chemical Research in Toxicology, 37*(2), 181–198. https://doi.org/10.1021/acs.chemrestox.3c00327

Snodin, D. J., Trejo-Martin, A., Ponting, D. J., Smith, G. F., Czich, A., Cross, K., et al. (2024). Mechanisms of nitrosamine mutagenicity and their relationship to rodent carcinogenic potency. *Chemical Research in Toxicology, 37*(2), 181–198.

Snodin, D. J., Trejo-Martin, A., Ponting, D. J., Smith, G. F., Czich, A., Cross, K., et al. (2024). Mechanisms of nitrosamine mutagenicity and their relationship

to rodent carcinogenic potency. *Chemical Research in Toxicology, 37*(2), 181–198.

Stanfill, S. B., Hecht, S. S., Joerger, A. C., González, P. J., Maia, L. B., & Crack, J. C. (2023). From cultivation to cancer: Formation of N-nitrosamines and other carcinogens in smokeless tobacco and their mutagenic implications. *Critical Reviews in Toxicology, 53*(10), 1–32.

US Food and Drug Administration. (2023). Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs)-Guidance for Industry. US Food and Drug Administration: Silver Spring, MD, USA.

Ward, M. H., Jones, R. R., Brender, J. D., de Kok, T. M., Weyer, P. J., Nolan, B. T., Villanueva, C. M., & van Breda, S. G. (2018). Drinking water nitrate and human health: An updated review. *International Journal of Environmental Research and Public Health, 15*(7), 1557.

World Health Organization. (2016). *Nitrate and nitrite in drinking-water*. Geneva: WHO Press.

Wu, K., El Zowalaty, A. E., Sayin, V. I., & Papagiannakopoulos, T. (2024). The pleiotropic functions of reactive oxygen species in cancer. Nature Cancer, 5, 384–399.

Wu, K., El Zowalaty, A. E., Sayin, V. I., & Papagiannakopoulos, T. (2024). The pleiotropic functions of reactive oxygen species in cancer. *Nature Cancer, 5*, 384–399.

Bioactive Compounds as Modulators of Chemo Resistance in Gastric Cancer Therapy Current Evidence and Future Perspectives

Cagla TEKIN[1]

Melis ERCELIK[1*]

Tugse Nezihe YILDIZ[2]

Berrin TUNCA[1]

[1] Department of Medical Biology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey
[2] Acibadem Mehmet Ali Aydinlar University, Department of Medical Biotechnology, Graduate School of Health Sciences, Istanbul, Turkey
***Contact info:** Melis Erçelik, Ph.D., Bursa Uludag University Faculty of Medicine Medical Biology Department, Gorukle, Bursa, Turkey. Phone: +902242954168, melisercelik@uludag.edu.tr

ABSTRACT

Gastric cancer (GC) remains a malignancy with a poor prognosis, mainly due to the development of multidrug resistance (MDR), which limits the efficacy of chemotherapy. Despite advances in surgical resection and systemic treatments, the 5-year survival rate is relatively low. MDR in GC results from the effects of multiple mechanisms, including increased drug efflux, decreased drug uptake, enhanced DNA damage repair, apoptosis resistance, epithelial-mesenchymal transition. Current chemotherapy mainly involves platinum derivatives, 5-fluorouracil (5-FU), pyrimidine analogs which are used alone or in combination. Despite tolerable toxicity, combination therapies for GC reduce quality of life and are recommended as second- or third-line treatments in advanced cases. Bioactive compounds derived from natural sources have gained attention as promising agents to enhance chemotherapy efficacy and overcome MDR in GC. These compounds induce apoptosis, inhibit tumor metastasis, and modulate the tumor microenvironment, improving treatment response. Combining traditional chemotherapy with bioactive compounds has emerged as an effective strategy to counteract chemotherapy resistance, a critical factor in enhancing GC prognosis. Despite promising preclinical data, clinical translation remains limited, and further well-designed trials are essential before their integration into standard GC therapies. This review highlights the importance of bioactive molecules in overcoming drug resistance, summarizing their roles in improving chemotherapy effectiveness and offering insights into recent studies focused on their therapeutic benefits in GC.

Keywords: Gastric Cancer, Drug Resistance, Bioactive Compounds

1. Introduction

Gastric cancer is a poor prognosis disease originating from the epithelium of the gastric mucosa and is the third leading cause of cancer-related deaths [1,2]. More than 1 million new cases of GC are identified each year worldwide, and approximately more than 700,000 deaths occur each year [3]. Most patients with GC receive their diagnosis at late stages, leading to a median survival of only 12-15 months [2]. Current approaches to GC treatment include surgical resection, chemotherapy, radiotherapy, immunotherapy and targeted therapy [4]. Early-stage GC is treated with surgical resection, while chemotherapy is usually the first-line approach for advanced-stage GC [5]. Current chemotherapeutic approaches mainly include platinum derivatives, 5-FU and other pyrimidine analogs, and anthracyclines such as doxorubicin and epirubicin [5]. These chemotherapy drugs are usually administered in monotherapy or combination [6].

The current chemotherapy approach prevents GC recurrence and metastasis, promotes increased survival, reduces symptoms, and improves quality of life

[5]. However, the effectiveness of chemotherapy is limited, and the 5-year overall survival rate of GC remains below 40%, indicating that the disease still has a poor prognosis [7]. The main reason for this is the development of the multidrug resistance (MDR) mechanism in GC cells, which causes less sensitivity to chemotherapy and thus limits its effectiveness [8]. MDR results from the combined effects of multiple factors and pathways, including increased drug efflux, decreased drug uptake, drug inactivation, increased DNA damage repair, reduced apoptosis, modification of drug-active target proteins, alterations in signaling pathways, and the presence of tumor stem cells (Figure 1) [1,5]. Overcoming drug resistance to chemotherapy is a critical factor in improving the prognosis of GC. Combining traditional chemotherapy agents with bioactive compounds has become a popular approach to overcoming drug resistance to chemotherapy.

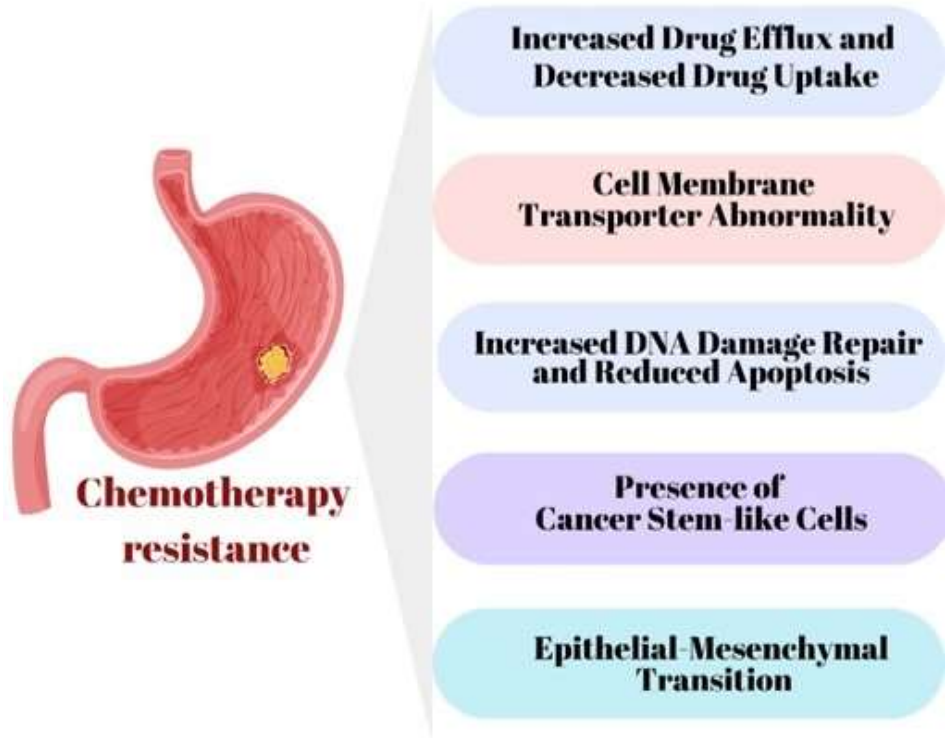


Fig. 1. Main mechanisms of chemotherapy resistance development in GC.

GC is a malignancy with high incidence and mortality worldwide and is driven by multifactorial etiological contributors, including *Helicobacter pylori* infection, Epstein–Barr virus (EBV) association, genetic predispositions, dietary carcinogens, smoking, and alcohol consumption [9,10,11]. In this context, the present review aims to comprehensively summarize the major factors underlying chemotherapy resistance in GC patients and to discuss the potential roles of bioactive molecules as promising therapeutic strategies to overcome this resistance. These factors not only initiate carcinogenesis but also drive disease progression through chronic inflammation, epigenetic modifications, and genomic instability [12,13]. Molecular alterations including mutations in tumor suppressor genes (e.g., *TP53*), activation of oncogenes (e.g., *KRAS*, *HER2*), and dysregulation of cell cycle regulators contribute significantly to gastric tumorigenesis and are often linked with poor therapeutic [14,15,16]. A central challenge in GC management is the development of MDR, which limits the long-term efficacy of standard chemotherapies [17]. MDR is a multifaceted phenomenon, commonly associated with the overexpression of ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp/ABCB1), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP/ABCG2), which actively efflux chemotherapeutic agents from tumor cells [18]. Furthermore, enhanced DNA damage repair mechanisms, alterations in apoptosis-related proteins (such as p53 and Bcl-2 family members), modifications of drug-target molecules, and activation of pro-survival signaling pathways including PI3K/AKT, MAPK, and NF-κB further reinforce resistance [19,20]. The presence of cancer stem cell subpopulations within gastric tumors adds another layer of complexity, as these cells exhibit inherent chemoresistance and contribute to relapse and metastasis [21]. In recent years, there has been growing interest in combining conventional chemotherapy with naturally occurring bioactive compounds, including polyphenols (curcumin, resveratrol, epigallocatechin gallate), flavonoids, terpenoids, and alkaloids [22,23]. These molecules exhibit diverse pharmacological activities, such as modulating drug efflux transporters, enhancing apoptosis, generating reactive oxygen species (ROS), and suppressing oncogenic signaling cascades [24,25,26]. Importantly, bioactive compounds have shown synergistic effects with chemotherapeutic agents, restoring drug sensitivity and reducing systemic toxicity [27]. Given the limitations of existing chemotherapy and the multifactorial nature of MDR, there is an unmet clinical need for safe adjunctive strategies. Bioactive compounds may fulfill this role by modulating resistance pathways.

2. Mechanisms of Drug Resistance in Gastric Cancer

Drug resistance in GC arises through multiple cellular and molecular mechanisms that collectively reduce the efficacy of chemotherapeutic agents

and limit treatment success (Figure 2). Among these mechanisms, ATP-binding cassette (ABC) transporter proteins play a critical role by increasing drug excretion out of the cell and reducing intracellular drug concentrations through active removal of drugs from their sites of action [28]. ABCB1 transporter is overexpressed in GC cells, potentially reducing chemotherapy sensitivity. While its association with poor prognosis remains debated, most studies indicate that chemotherapy induces ABCB1 expression, increasing drug resistance [28]. Blocking ABCB1 may enhance prognosis by reversing resistance. Similarly, other ABC transporters, such as ABCC1, contribute to drug resistance in GC [29]. DNA damage repair is one of the mechanisms of drug resistance, as most chemotherapeutics induce apoptosis by damaging DNA [1]. In GC, resistance to platinum compounds involves XRCC1 (BER-pathway) and BRCA1 (HR-pathway). Additionally, apoptosis resistance contributes to MDR, with factors such as p53, Bcl-2, NF-κB, Caspase-3, and TNF-α playing crucial roles [1,5]. Cancer stem-like cells resist chemotherapy by remaining in the G0-phase and cause tumor recurrence (Huang et al., 2020; Liu et al., 2024). Epithelial-mesenchymal transition (EMT) is associated with tumor metastasis and promotes drug resistance [1,5]. E-cadherin expression decreases in drug-resistant cells, while increased Vimentin expression promotes metastasis and MDR development in GC [1,5].

In addition to the overexpression of ABC transporters, GC cells exploit several complementary mechanisms to resist the cytotoxic effects of chemotherapy. One important mechanism is the alteration of drug uptake channels. Reduced expression or structural modifications in solute carrier (SLC) family transporters, such as copper transporter 1 (CTR1), directly decrease the intracellular accumulation of platinum-based drugs, limiting their efficacy [30]. Furthermore, tumor cells may upregulate drug-metabolizing enzymes such as glutathione S-transferases (GSTs), which detoxify chemotherapeutic compounds and contribute to MDR [31].

Epigenetic reprogramming also plays a pivotal role in the development of resistance. Aberrant DNA methylation, histone modifications, and dysregulation of non-coding RNAs can silence tumor suppressor genes or activate resistance-related genes, thereby promoting drug tolerance [32]. For example, hypermethylation of DNA repair genes has been linked with resistance to platinum derivatives, while microRNAs such as miR-21 and miR-200 regulate pathways associated with apoptosis and EMT [33].

The tumor microenvironment (TME) further complicates therapeutic outcomes. Hypoxia-inducible factor-1α (HIF-1α) signaling, enhanced angiogenesis, and stromal interactions all contribute to MDR by promoting cell survival and limiting drug penetration [34]. Hypoxia-induced EMT, characterized by decreased E-cadherin and increased Vimentin expression, not only drives metastasis but also enhances chemoresistance [35]. In addition,

immune cells within the TME, such as tumor-associated macrophages (TAMs), secrete cytokines including IL-6 and TNF-α, which activate pro-survival signaling pathways and reinforce drug resistance [36].

Finally, emerging evidence highlights the role of autophagy in GC chemoresistance. While autophagy is a survival mechanism under stress conditions, its upregulation in cancer cells enables them to withstand chemotherapy-induced stress and apoptosis [37]. Inhibition of autophagy has therefore been proposed as a strategy to resensitize resistant GC cells to chemotherapeutic agents, suggesting a potential therapeutic target for combination therapy [38].

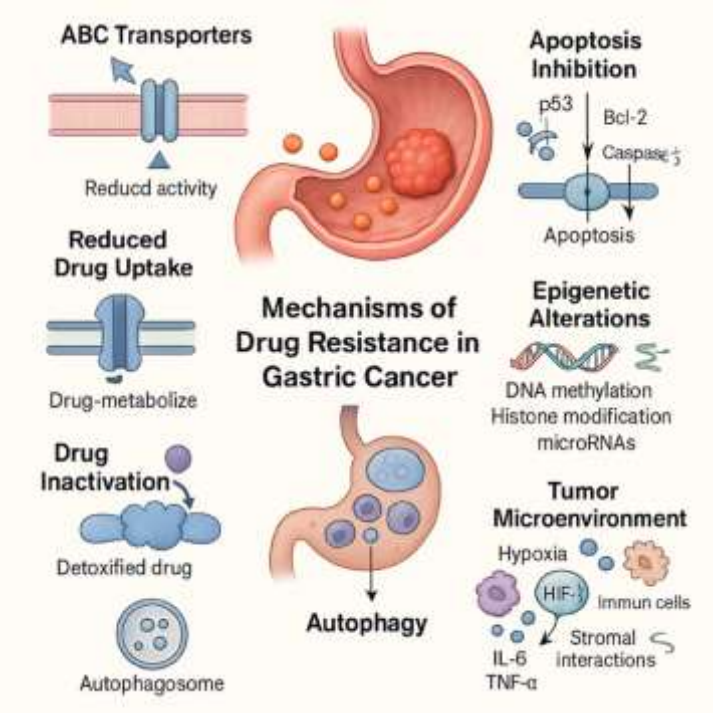


Fig. 2. A diagram summarizing the main mechanisms leading to drug resistance GC in - including the increased drug efflux by ABC transporters, reduced drug uptake, drug inactivation, suppression of apoptosis, occurrence of epigenetic alterations, resistance development by the tumor microenvironment, and protection of cells through autophagy.

Overall, these mechanisms act in concert, highlighting the need for multi-targeted approaches such as combination therapies with bioactive compounds.

Table 1. Key Mechanisms of Drug Resistance in Gastric Cancer and Potential Modulation by Bioactive Compounds

Mechanism of Resistance	Key Molecular Players	Functional Effect	Example Bioactive Compounds (modulators)	Ref
ABC Transporters	MDR1 (P-gp), MRP1, BCRP	Increased drug efflux → reduced intracellular drug accumulation	Paeoniflorin, Tetrandrine, Curcumin	[5,39,40]
DNA Damage Repair	ERCC1, PARP, BRCA1/2	Enhanced repair of chemotherapy-induced DNA damage	Quercetin, Liquiritin	[41,42]
Apoptosis Inhibition	Bcl-2, Bax, Caspases	Blockade of apoptosis, cell survival despite chemotherapy	Curcumin, Bufalin, Oleanolic acid	[40,43]
Epithelial–Mesenchymal Transition (EMT)	E-cadherin ↓, N-cadherin ↑, β-catenin, Snail	Increased invasiveness, resistance to apoptosis and chemotherapy	Curcumin, Apigenin	[5,44]
Tumor Microenvironment (TME)	VEGF, HIF-1α, IL-6, CAFs	Angiogenesis, hypoxia, cytokine signaling supporting drug resistance	Quercetin, Apigenin, Resveratrol	[5,44]
Autophagy	Beclin-1, LC3-II, mTOR	Protective mechanism under stress → drug tolerance	Apigenin, Genistein	[44]
Epigenetic Alterations	HDACs, DNMTs, miRNAs	Aberrant gene expression promoting MDR	EGCG (catechin), Curcumin, Genistein	[5,40]

3. Combination Drug Use to Overcome Drug Resistance

Tumor cells genetic and molecular heterogeneity limits the effectiveness of single anticancer drugs, potentially leading to drug resistance [45]. Therefore, combining two chemotherapy drugs that work with different mechanisms

increases treatment effectiveness by reducing the development of resistance. The mechanisms of action of chemotherapy drugs are different. For example, 5-FU causes cytotoxicity by inhibiting thymidylate synthase, disrupting basic biosynthetic processes, or incorrectly incorporating its metabolites into RNA and DNA [5]. Leucovorin enhances 5-FU's activity by binding thymidylate synthase, significantly improving therapeutic efficacy in combination therapy [5]. Combining 5-FU and leucovorin was reported to increase 5-FU-induced cytotoxicity in 5-FU-resistant GC cells [46]. On the other hand, platinum-based compounds such as cisplatin prevent DNA synthesis by forming cross-links within DNA and between strands [5]. Cisplatin is often used in combination with fluoropyrimidines, especially 5-FU or capecitabine [47]. A sub analysis of 501 patients showed that adding anthracycline to Cisplatin+5-FU significantly improved overall survival [48]. Similar to these combination drug applications, there are examples of different combinations of different chemotherapy drugs. Although combination drug therapies for GC have a relatively tolerable toxicity profile, long-term use reduces the patient's quality of life, limiting their therapeutic efficacy. Therefore, they are recommended as second or third-line therapy in advanced GC cases [49].

Together with traditional doublet regimens, triplet combinations have also been investigated to improve outcomes in GC. For instance, epirubicin, cisplatin, and 5-FU (ECF regimen) have been evaluated in several clinical trials, demonstrating enhanced response rates compared to doublet therapy, although at the cost of increased toxicity [50]. The REAL-2 trial further validated the substitution of cisplatin with oxaliplatin and 5-FU with capecitabine, which maintained efficacy while improving tolerability [51]. These studies indicate that rationally designed triplet regimens may provide superior benefits in selected patients.

Targeted agents combined with chemotherapy have also shown promise in overcoming resistance. The addition of trastuzumab, an anti-HER2 monoclonal antibody, to cisplatin and fluoropyrimidine chemotherapy significantly improved overall survival in HER2-positive GC patients in the pivotal ToGA trial [52]. Similarly, combining immune checkpoint inhibitors such as nivolumab with chemotherapy has demonstrated encouraging efficacy in advanced GC, highlighting the potential of chemo-immunotherapy combinations [53].

Furthermore, nanoparticle-based drug delivery systems are emerging as a novel strategy to enhance combination therapy efficacy. Nanocarriers allow for the co-delivery of two or more chemotherapeutic agents with improved pharmacokinetics, reduced systemic toxicity, and enhanced tumor penetration [54]. Preclinical studies have demonstrated that nanoformulations of cisplatin and 5-FU, when delivered simultaneously, can synergistically inhibit tumor growth while minimizing side effects [55].

Despite these advances, the balance between efficacy and toxicity remains a major challenge for combination drug use in GC. While novel regimens and drug delivery approaches aim to overcome resistance and improve outcomes, careful patient selection and biomarker-driven strategies will be essential to optimize therapeutic benefit and minimize adverse effects [56]. While combination chemotherapy has improved outcomes modestly, toxicity and acquired resistance remain major barriers, which natural bioactive compounds may help to mitigate.

4. The Importance of Bioactive Compounds in Overcoming Drug Resistance

In recent years, bioactive compounds have become increasingly crucial for overcoming resistance to traditional chemotherapy in treating GC [5,40,41]. Bioactive compounds are naturally found in fruits, vegetables, and medicinal plants. Compared to traditional chemotherapy drugs, bioactive compounds offer significant advantages such as low toxicity, minimal side effects, and wide availability [5,40].Numerous studies have shown that these bioactive compounds can enhance the efficacy of chemotherapeutic agents by exhibiting various biological effects, such as inducing apoptosis in GC cells, suppressing migration, inhibiting MDR mechanisms, and modulating the tumor microenvironment (Figure 3) [1,5,41].

Increasing research has demonstrated that bioactive compounds can be combined with the primary drug to overcome drug resistance and strengthen its effectiveness [41]. Bioactive compounds, particularly plant polyphenols, flavonoids, coumarins, and alkaloids, have fewer side effects than conventional treatments and have gained prominence recently due to their potential to suppress cancer cell aggression [40]. In a study by Tekin et al. [42], the authors demonstrated that olive leaf extract (OLE) can enhance the efficacy of conventional chemotherapeutic agents by regulating EMT in GC cells. Specifically, combining OLE with 5-fluorouracil or cisplatin reduced EMT marker expression (e.g., E-cadherin, N-cadherin, vimentin) and increased apoptosis, sensitizing tumor cells to chemotherapy [57]. These results support the idea that bioactive natural extracts may help overcome drug resistance by targeting non-canonical pathways, such as EMT suppression, when applied with standard chemotherapy.

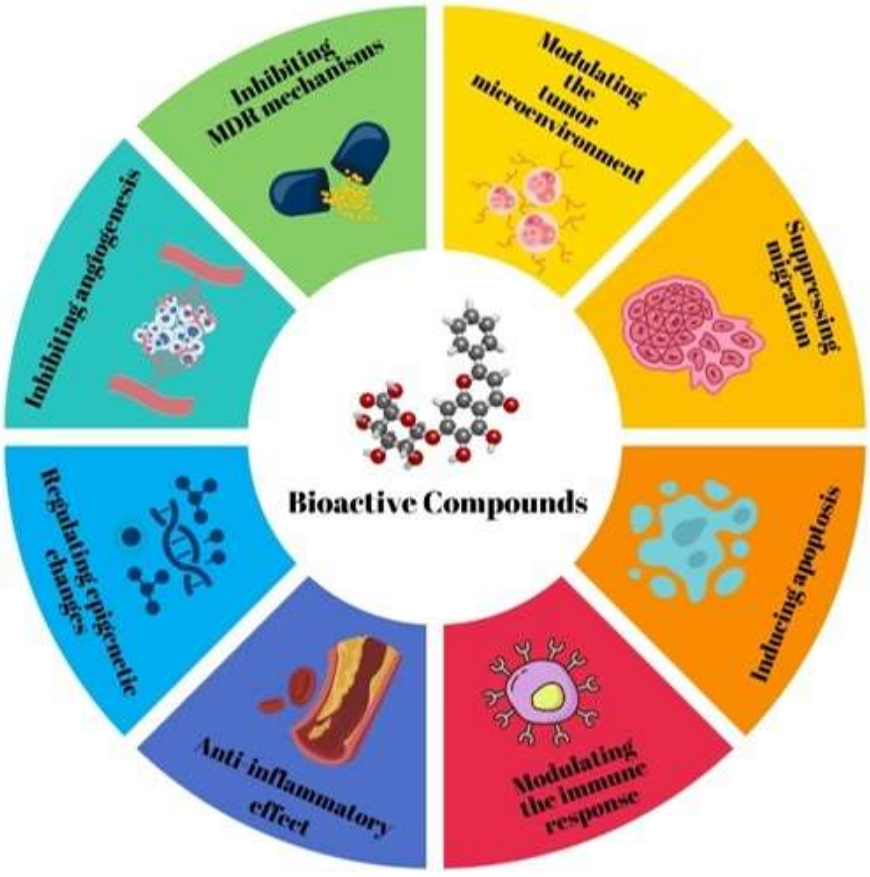


Fig. 3. Potential roles of bioactive compounds for GC therapy include a regulatory role in regulating various GC phenotypes, including tumor growth, proliferation, invasion, metastasis, angiogenesis and cell death.

Curcumin is a natural phenolic compound found in various Curcuma species and exhibits broad-spectrum anticancer effects. Curcumin reverses the MDR mechanism by directly inhibiting drug resistance in GC cells [1]. Several studies have determined that the mechanism of reversing MDR in GC cells by curcumin is associated with the inhibition of the NF-κB pathway and the induction of apoptosis [1]. Kang et al. found that curcumin inhibited cell proliferation by down-regulating the NF-κB signaling pathway in GC cells, overcoming resistance to 5-FU and doxorubicin [1,58]. Moreover, researchers (Ham et al., 2022) reported that curcumin reduced chemotherapy resistance by inhibiting the JAK2/STAT3 pathway and exhibited a synergistic effect with 5-FU in treating xenograft GC tumors. Quercetin is a natural flavonoid found

in abundance in many fruits, vegetables, and plants and has antioxidant, antitumor, and anti-inflammatory properties [59]. Several studies have shown that quercetin suppresses the proliferation of GC cells, induces apoptosis, and prevents metastasis [60]. Researchers [61] reported that quercetin increased the efficacy of chemotherapy by regulating metastasis-associated factors in the combined treatment of AGS cells with irinotecan and its metabolite SN-38, a DNA topoisomerase I inhibitor. Oleuropein is a polyphenolic compound with powerful antioxidant, anti-inflammatory and anticancer properties in olive leaves and olive oil [57]. Researchers [62] demonstrated the therapeutic effects of oleuropein by reducing the proliferation of GC cells and regulating the expression of apoptotic, metastatic genes and microRNAs from non-coding RNAs, one of the epigenetic mechanisms. Catechin, an antioxidant flavonoid, increased the sensitivity of SNU620 GC cells to 5-FU, indicating that combining it with 5-FU enhanced the cytotoxic effect on GC cells [63]. Several studies have indicated that Liquiritin, a major component of licorice, inhibits cisplatin-resistant GC cell proliferation and migration [64,65]. Furthermore, Liquiritin has been shown to suppress cell proliferation by downregulating cyclin D1, cyclin A, and CDK4 and increasing p53 and p21 in cisplatin combination therapy [5]. Studies have shown that Tetrandrine, an alkaloid isolated from the roots of Stephaniae Tetrandrae Radix, has the potential to overcome drug resistance by down-regulating the expression levels of genes such as MDR1 and MRP1 in resistant GC cells [1,5].

Collectively, bioactive compounds have become the focus of numerous studies due to their significant advantages over traditional chemotherapy drugs in the treatment of GC. Moreover, it has been shown in numerous studies that it overcomes chemotherapy resistance and increases chemotherapy effectiveness. Table 1 in this review collects bioactive compounds with the potential to overcome drug resistance in GC.

Table 2. Role of Bioactive Compounds in Gastric Cancer Treatment: Mechanisms, Sources, and Combination Effects

Bioactive Compound	**Natural Source**	**Chemotherapeutic Agent (Combination)**	**Mechanism / Target**	**Pathway**	**Observed Effect (Synergy / Outcome)**	**Ref**
Paeoniflorin	*Paeonia lactiflora* (peony root)	Vincristine	MDR1, Bcl-xL, Bcl-2 inhibition	NF-κB	Reversed MDR, enhanced apoptosis	[1]
Bufalin	*Bufo bufo gargarizans* (toad venom)	Cisplatin	Bax/Bcl-2 modulation	Akt/mTOR	Increased chemosensitivity, apoptosis	[1]
Oleanolic acid	Olive leaves, medicinal plants	Cisplatin	Bax, Bcl-2, Caspase-3	–	Enhanced cisplatin-induced apoptosis	[1]
Tetrandrine	*Stephania tetrandra*	–	MDR1, MRP1 inhibition	–	Reduced efflux-mediated resistance	[1]
Curcumin	*Curcuma longa* (turmeric)	– / 5-FU	β-catenin, LRP6 inhibition	Wnt/β-catenin, JAK/STAT3	Reduced EMT, restored drug sensitivity	[5]
Quercetin	Fruits, vegetables, onions	Irinotecan	Bcl-2, Bax, VEGF regulation	PI3K-Akt	Suppressed angiogenesis, improved apoptosis	[5,44]

Liquiritin	*Glycyrrhiza uralensis* (licorice)	Cisplatin	p53, p21, PARP activation	–	Restored cisplatin sensitivity	[5]
Genistein	Soy isoflavones	–	Gli1, CD44 inhibition	Hedgehog	Reduced stemness, decreased resistance	[5]
Apigenin	Parsley, celery, chamomile	–	p-mTOR, p62, HIF-1α, Ezh2 inhibition	mTOR	Suppressed glycolysis, inhibited EMT	[44]
Luteolin	Celery, green pepper, carrots	–	Bax, Caspase-3, Cyt C, Bcl-2	–	Promoted apoptosis, overcame MDR	[44]
Catechin (EGCG)	Green tea (*Camellia sinensis*)	5-FU	Caspase-3, Bax, Bcl-2	–	Synergistic cytotoxicity with 5-FU	[44]
Myricetin	Berries, tea, vegetables	–	PARP, Bax, Bcl-2	PI3K/Akt/mTOR	Enhanced apoptosis, reduced proliferation	[44]
Rutin	Citrus fruits, buckwheat	Cisplatin, Oxaliplatin	p38/Caspase activation	–	Synergistic apoptosis, restored sensitivity	[44]

Beyond their direct cytotoxic effects, bioactive compounds have been recognized for their ability to modulate the TME, which plays a crucial role in chemotherapy resistance. Natural agents such as resveratrol and EGCG suppress pro-tumorigenic cytokines, reduce angiogenesis, and inhibit cancer-associated fibroblast activation, thereby enhancing the sensitivity of GC cells to standard treatments [66]. These immunomodulatory and anti-inflammatory properties highlight the potential of bioactive compounds to act not only as chemosensitizers but also as regulators of the complex interactions within the TME.

Another important consideration for the therapeutic use of bioactive compounds is their pharmacokinetic limitations. Poor water solubility, low bioavailability, and rapid metabolism have restricted their translation from bench to bedside [67]. To overcome these barriers, advanced drug delivery systems such as liposomes, polymeric nanoparticles, phytosomes, and micelles have been developed. For instance, nanoparticle-encapsulated curcumin has shown improved stability and accumulation in GC tissues, enhancing its efficacy in combination with 5-FU or cisplatin in preclinical models [68]. These approaches demonstrate that formulation innovations are essential for realizing the full therapeutic potential of bioactive compounds.

Looking ahead, future research will require well-designed biomarker-driven clinical trials to validate the safety and synergistic efficacy of bioactive compounds in GC patients. While preclinical studies have provided compelling evidence of their anti-tumor properties, clinical validation remains limited. Integrating omics-based technologies and personalized medicine strategies may help identify patient subgroups most likely to benefit from bioactive compound-based therapies [69]. Such precision approaches will be pivotal in translating bioactive compounds from promising adjuncts into integral components of GC treatment regimens.

5. Nano-Encapsulation Strategies for Bioactive Compounds to Overcome Chemoresistance in Gastric Cancer

Nano-encapsulation strategies have been extensively explored to improve the delivery and chemosensitizing effects of natural bioactive compounds in GC, particularly against drug resistance. Curcumin-loaded PLGA nanoparticles significantly enhanced cytotoxicity and apoptosis in GC cells compared with free curcumin, while also exerting anti-Helicobacter pylori activity, suggesting dual benefits in both carcinogenesis prevention and tumor control [70]. Similarly, resveratrol-loaded mesoporous silica nanoparticles (MSN-Res) and metal–organic framework carriers such as Res@ZIF-90 increased resveratrol stability, tumor accumulation and mitochondrial targeting, leading to stronger inhibition of proliferation, migration and

invasion, and greater induction of apoptosis in GC xenograft models than free resveratrol [71,72]. Co-delivery systems that combine phytochemicals with chemotherapeutics further address multidrug resistance: EGCG–doxorubicin nanoparticles targeting CD44/P-selectin–positive GC achieved superior antitumor efficacy and reduced off-target toxicity compared with the free drug combination, in part by enhancing intracellular drug retention and apoptosis [73] while injectable cellulose-based microgels co-loading 5-FU and curcumin produced sustained release, increased ROS-mediated apoptosis, and effectively overcame 5-FU resistance in vitro and in vivo [74]. Across these platforms, nano-delivery enhances solubility, stability, tumor-specific accumulation and controlled release of bioactive compounds, enabling more effective modulation of resistance pathways and the tumor microenvironment and supporting their use as nano-chemosensitizers in GC therapy [75,76,77,78].

6. Limitations and Future Perspectives of Bioactive Compounds in GC Therapies

Although chemotherapy has a significant effect on improving the course of the disease in advanced GC treatment, the development of drug resistance is an important factor limiting the success of treatment. To overcome this problem, natural bioactive compounds that can increase the effectiveness of chemotherapy by minimizing its side effects and, at the same time, directly suppress the aggressiveness of cancer cells have been gaining increasing attention in recent years. These bioactive compounds have anti-tumor properties and increase the effectiveness of treatment by showing synergistic effects in combination treatments with chemotherapy. However, these compounds limit the effectiveness of treatment due to factors such as low solubility, short biological half-life and limited bioavailability when used in free form. Therefore, appropriate formulation strategies are needed to increase the clinical effectiveness of these bioactive compounds. In recent years, the number of studies on nano formulations of bioactive compounds has significantly increased. Nano formulations offer the potential to increase the bioavailability of these compounds, overcome solubility problems and provide more specific targeting to cells. Furthermore, an in-depth study of these compounds' pharmacokinetic and pharmacodynamic properties and further evaluation of their preclinical analyses are critical to optimize future treatment strategies.

Despite compelling preclinical activity, the clinical translation of bioactive compounds remains constrained by formulation and quality-control issues. Plant-derived preparations often vary in composition across batches, which complicates dose standardization and reproducibility in trials. In parallel, herb–drug interactions and safety signals—such as catechin-related hepatotoxicity at high supplemental doses—necessitate rigorous toxicity

monitoring and pharmacovigilance when these agents are combined with cytotoxic chemotherapy [69,79]. Establishing Good Manufacturing Practice (GMP)–grade materials with analytically verified content and impurities is therefore a prerequisite for robust clinical evaluation.

To address poor solubility, rapid metabolism, and limited bioavailability, advanced delivery platforms (liposomes, polymeric nanoparticles, solid-lipid nanoparticles, phytosomes, and micelles) are increasingly used to co-deliver bioactive molecules with standard drugs. Such systems can improve pharmacokinetics, enhance tumor accumulation, and enable schedule-controlled co-exposure—features that have yielded stronger chemosensitization in GC models (e.g., nano-curcumin with 5-FU or cisplatin) while mitigating systemic toxicity [67,68]. Moving forward, head-to-head comparisons of delivery technologies and prespecified pharmacokinetic/pharmacodynamic (PK/PD) endpoints will be essential to select clinically translatable formulations.

Future progress will hinge on biomarker-driven development. Because GC is molecularly heterogeneous, response to bioactive compounds likely depends on tumor subtype and context (e.g., EMT status, ABC-transporter expression, TME cytokine signatures). Patient-derived organoids and ex vivo pharmacotyping can prospectively match combinations to sensitive phenotypes and accelerate trial design, while umbrella/enrichment trials can validate synergy in molecularly selected cohorts [69,80,81]. Together, these strategies can bridge the bench-to-bedside gap and clarify where bioactive compounds add the greatest value in GC therapy.

7. Conclusion

GC is still one of the leading global health issues owing to its incidence rate, prognosis, and potential development of drug resistance, making it difficult to achieve long-term chemotherapeutic responses [1,5,7]. Natural sources have emerged as a promising lead in trying to complement existing therapies, and their mechanisms of action include inhibition of drug efflux transporters, induction of apoptosis, reversal of the epithelial-to-mesenchymal transition, and TME regulation [16,17,18,22,43]. Evidence in the literature shows that these compounds have the potential to make GC cells sensitive to chemotherapeutic agents with reduced toxicity [17,18,24].

Nonetheless, the use of these bioactive molecules in a clinical setting is a nascent area. Lack of solubility, low bioavailability, lack of pharmacokinetic uniformity, and limited data from adequately powered human clinical studies continue to be significant hurdles for their use in mainstream management [5,16]. New developments in formulations, especially nanoparticle formulations, along with the use of patient selection based on biomarkers, can potentially address these hurdles [5,16,44].

Individually, the available data suggests that bioactive compounds represent an emerging therapeutic concept rather than an accepted standard of care. Additional mechanistic studies, rigorous validation in the preclinical setting, and properly designed Phase I to III clinical studies would be required to unlock their full promise. If these hurdles can be overcome, bioactive compounds could become crucial parts of precision therapy approaches for overcoming drug resistance in GC [2,5,17].

Acknowledgment

We acknowledge the Scientific and Technological Research Council of Türkiye (TÜBİTAK) for supporting the author, Cagla Tekin, within the scope of the 2211-A doctoral program scholarship.

References

[1]. Huang WJ, Ruan S, Wen F, Lu XN, Gu SP, Chen XX, et al. Multidrug Resistance of Gastric Cancer: The Mechanisms and Chinese Medicine Reversal Agents. Cancer Manag Res. 2020:12, 12385–12394. https://doi.org/10.2147/CMAR.S274599

[2]. Che G, Yin J, Wang W, Luo Y, Chen Y, Yu X, et al. Circumventing drug resistance in gastric cancer: A spatial multi-omics exploration of chemo and immuno-therapeutic response dynamics. Drug Resist Updat. 2024:74, 101080. https://doi.org/10.1016/j.drup.2024.101080

[3]. Lin JL, Lin JX, Lin GT, Huang CM, Zheng CH, Xie JW, et al. Global incidence and mortality trends of gastric cancer and predicted mortality of gastric cancer by 2035. BMC public health. 2024:24(1), 1763. https://doi.org/10.1186/s12889-024-19104-6

[4]. Joshi SS, Badgwell BD. Current treatment and recent progress in gastric cancer. CA Cancer J Clin. 2021:71(3), 264-279.

[5]. Liu J, Yuan Q, Guo H, Guan H, Hong Z, Shang D. Deciphering drug resistance in gastric cancer: Potential mechanisms and future perspectives. Biomed Pharmacother. 2024:173, 116310. https://doi.org/10.1016/j.biopha.2024.116310

[6]. Zhang SX, Liu W, Ai B, Sun LL, Chen ZS, Lin LZ. Current advances and outlook in gastric cancer chemoresistance: a review. Recent patents on anti-cancer drug discovery. 2022: 17(1), 26-41. doi: 10.2174/1574892816666210929165729

[7]. Li Y, Feng A, Zheng S, Chen C, Lyu J. Recent Estimates and Predictions of 5-Year Survival in Patients with Gastric Cancer: A Model-Based Period Analysis. Cancer Control. 2022:29, 10732748221099227. https://doi.org/10.1177/10732748221099227

[8]. Marin JJG, Al-Abdulla R, Lozano E, Briz O, Bujanda LM, Banales J, et al. Mechanisms of resistance to chemotherapy in gastric cancer. Anticancer Agents Med Chem. 2016:16(3), 318-334.

[9]. Rawla P, Barsouk A. Epidemiology of gastric cancer: Global trends, risk factors and prevention. *Prz Gastroenterol.* 2019;14(1):26–38.

[10]. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of gastric cancer attributable to *Helicobacter pylori*. *Int J Cancer.* 2015;136(2):487–490.

[11]. Camargo MC, Kim WH, Chiaravalli AM, et al. Improved survival of gastric cancer with EBV association: A multi-national pooled analysis. *Gut.* 2014;63(2):236–243.

[12]. Correa P, Piazuelo MB. The gastric precancerous cascade. *J Dig Dis.* 2012;13(1):2–9.
Cunningham D, Okines AF, Ashley S. (2010). *Capecitabine and oxaliplatin in advanced oesophagogastric cancer: REAL-2 trial update.* Lancet Oncol, 11(5): 465–473.

[13]. Song H, Held M, Sandin S, et al. Risk of gastric cancer among men and women with changes in smoking, drinking and obesity: A population-based cohort study. *Int J Cancer.* 2019;144(5):1105–1112.

[14]. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature.* 2014;513(7517):202–209.

[15]. Kim JW, Nam KH, Ahn SH, et al. Prognostic implications of genetic alterations in gastric cancer. *Clin Cancer Res.* 2014;20(5):1253–1262.

[16]. Gravalos C, Jimeno A. HER2 in gastric cancer: A new prognostic factor and a novel therapeutic target. *Ann Oncol.* 2008;19(9):1523–1529.

[17]. Chen T, Li Q, Liu Z, et al. Multidrug resistance in gastric cancer: Mechanisms and potential therapeutic strategies. *Cancer Commun.* 2021;41(5):389–418.

[18]. Robey RW, Pluchino KM, Hall MD, et al. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer.* 2018;18(7):452–464.

[19]. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: An evolving paradigm. *Nat Rev Cancer.* 2013;13(10):714–726.

[20]. Galluzzi L, Senovilla L, Vitale I, et al. Molecular mechanisms of cisplatin resistance. *Oncogene.* 2012;31(15):1869–1883.

[21]. Ishimoto T, Nagano O, Yae T, et al. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc− and thereby promotes tumor growth. *Cancer Cell.* 2011;19(3):387–400.

[22]. Kunnumakkara AB, Bordoloi D, Harsha C, et al. Curcumin mediates anticancer effects by modulating multiple cell signaling pathways. *Clin Sci.* 2017;131(15):1781–1799.

[23]. Weng CJ, Yen GC. Flavonoids, a ubiquitous dietary phenolic subclass, exert extensive in vitro anti-invasive and in vivo anti-metastatic activities. *Cancer Metastasis Rev.* 2012;31(1–2):323–351.

[24]. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: Preclinical and clinical studies. *Anticancer Res.* 2003;23(1A):363–398.

[25]. Yang CS, Wang H, Sheridan ZP. Studies on prevention of obesity, metabolic syndrome, diabetes, cardiovascular diseases and cancer by tea. *J Food Drug Anal.* 2018;26(1):1–13.

[26]. Gupta SC, Tyagi AK, Deshmukh-Taskar P, Hinojosa M, Prasad S, Aggarwal BB. Downregulation of tumor necrosis factor and other proinflammatory biomarkers by polyphenols. *Arch Biochem Biophys.* 2014;559:91–99.

[27]. Prasad S, Gupta SC, Tyagi AK, Aggarwal BB. Curcumin, a component of golden spice: From bedside to bench and back. *Biotechnol Adv.* 2014;32(6):1053–1064.

[28]. Shi WJ, Gao JB. Molecular mechanisms of chemoresistance in gastric cancer. World J Gastrointest Oncol. 2016: 8(9), 673–681. https://doi.org/10.4251/wjgo.v8.i9.673

[29]. Yu P, Du Y, Cheng X, Yu Q, Huang L, Dong R. Expression of multidrug resistance-associated proteins and their relation to postoperative individualized chemotherapy in gastric cancer. World J Surg Oncol. 2014:12, 307. https://doi.org/10.1186/1477-7819-12-307

[30]. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. (2013). *Cancer drug resistance: An evolving paradigm.* Nat Rev Cancer, 13(10), 714–726.

[31]. Zhang Y, Yang J, Li H, Wu Y, Zhang H. (2020). *GSTP1 and chemotherapy resistance in gastric cancer.* Oncol Lett, 19(1), 1–10.

[32]. Qi L, Ding Y. (2022). *Epigenetic regulation in gastric cancer drug resistance.* Front Oncol, 12, 868403.

[33]. Ueda T, Volinia S, Okumura H, et al. (2010). *Relation between microRNA expression and progression and prognosis of gastric cancer: A microRNA expression analysis.* Lancet Oncol, 11(2), 136–146.

[34]. Xue L, Ren C, Yang T, et al. (2021). *The role of tumor microenvironment in gastric cancer chemoresistance.* Front Oncol, 11, 768305.

[35]. Nieto MA, Huang RY, Jackson RA, Thiery JP. (2016). *EMT: 2016.* Cell, 166(1), 21–45.

[36]. Shibuya K, Fujimoto H, Nomura S, et al. (2020). *IL-6-mediated signaling in the tumor microenvironment promotes gastric cancer chemoresistance.* Cancer Sci, 111(4), 1256–1268.

[37]. Amaravadi RK, Kimmelman AC, Debnath J. (2019). *Targeting autophagy in cancer: Recent advances and future directions.* Cancer Discov, 9(9), 1167–1181.

[38]. Xie C, Jin J, Bao X, et al. (2022). *Autophagy inhibition potentiates chemotherapy in gastric cancer by inducing apoptosis.* J Exp Clin Cancer Res, 41, 51.

[39]. Huang, W., Lai, C., Peng, S., Hsu, C., Hsu, P., Chu, P., Feng, C., & Lin, Y. (2021). Targeting Tumor Cells with Nanoparticles for Enhanced Co-Drug Delivery in Cancer Treatment. Pharmaceutics, 13. https://doi.org/10.3390/pharmaceutics13091327

[40]. Zou JY, Chen QL, Luo XC, Damdinjav D, Abdelmohsen UR, Li HY, et al. Natural products reverse cancer multidrug resistance. Front Pharmacol. 2024:15, 1348076. https://doi.org/10.3389/fphar.2024.1348076

[41]. SY, Hwang D, Shin S, Park J, Kim M, Rahman MDH, et al. Potential of Bioactive Food Components against Gastric Cancer: Insights into Molecular Mechanism and Therapeutic Targets. Cancers (Basel). 2021:13(18), 4502. https://doi.org/10.3390/cancers13184502.

[42]. Tekin C, Ercelik M, Dunaev P, Galembikova A, Tezcan G, Aksoy SA, et al. Leaf Extract from European Olive (Olea europaea L.) Post-Transcriptionally Suppresses the Epithelial-Mesenchymal Transition and Sensitizes Gastric Cancer Cells to Chemotherapy. Biochemistry (Mosc). 2024:89(1), 97–115. https://doi.org/10.1134/S0006297924010061.

[43]. Galluzzi L, Senovilla L, Vitale I, et al. Molecular mechanisms of cisplatin resistance. *Oncogene.* 2012;31(15):1869–1883.

[44]. Cai J, Tan X, Hu Q, Pan H, Zhao M, Guo C, et al. Flavonoids and Gastric Cancer Therapy: From Signaling Pathway to Therapeutic Significance. Drug Des Devel Ther. 2024 25;18:3233-3253. https://doi.org/10.2147/DDDT.S466470.

[45]. Sethy C, Kundu CN. 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: Implication of DNA repair inhibition. Biomed Pharmacother. 2021:137, 111285. https://doi.org/10.1016/j.biopha.2021.111285

[46]. Nakamura A, Nakajima G, Okuyama R, Kuramochi H, Kondoh Y, Kanemura T, et al. Enhancement of 5-fluorouracil-induced cytotoxicity by leucovorin in 5-fluorouracil-resistant gastric cancer cells with upregulated expression of thymidylate synthase. Gastric Cancer. 2014:17(1), 188–195. https://doi.org/10.1007/s10120-013-0249-7

[47]. Kang YK, WK, Shin DB, Chen J, Xiong J, Wang J, et al. Capecitabine/cisplatin versus 5-fluorouracil/cisplatin as first-line therapy in patients with advanced gastric cancer: a randomised phase III noninferiority trial. Ann Oncol. 2009: 20(4), 666–673. https://doi.org/10.1093/annonc/mdn717

[48]. Rivera F, Vega-Villegas ME, López-Brea MF. Chemotherapy of advanced gastric cancer. Cancer Treat Rev. 2007:33(4), 315-324.

[49]. Yamaguchi K, Nakagawa S, Yabusaki H, Nashimoto A. Combination chemotherapy with 5-fluorouracil, cisplatin and paclitaxel for pretreated patients with advanced gastric cancer. Anticancer Res. 2007: 27(5B), 3535–3539.

[50]. Cunningham D, Starling N, Rao S, et al. (2008). *Capecitabine and oxaliplatin for advanced esophagogastric cancer.* N Engl J Med, 358(1):36–46.

[51]. Cunningham D, Okines AF, Ashley S. (2010). *Capecitabine and oxaliplatin in advanced oesophagogastric cancer: REAL-2 trial update.* Lancet Oncol, 11(5): 465–473.

[52]. Bang YJ, Van Cutsem E, Feyereislova A, et al. (2010). *Trastuzumab in combination with chemotherapy versus chemotherapy alone for HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3 trial.* Lancet, 376(9742): 687–697.

[53]. Janjigian YY, Shitara K, Moehler M, et al. (2021). *First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a phase 3 trial.* Lancet, 398(10294): 27–40.

[54]. Xu X, Ho W, Zhang X, Bertrand N, Farokhzad O. (2015). *Cancer nanomedicine: from targeted delivery to combination therapy.* Trends Mol Med, 21(4):223–232.

[55]. Wang Y, Wu H, Dong L, et al. (2020). *Co-delivery of cisplatin and 5-fluorouracil via nanoparticles enhances chemosensitivity of gastric cancer cells.* Int J Nanomedicine, 15: 7733–7745.

[56]. Lordick F, Shitara K, Janjigian YY. (2022). *New agents on the horizon in gastric cancer.* Ann Oncol, 33(10):1001–1015.

[57]. Barbaro B, Toietta G, Maggio R, Arciello M, Tarocchi M, Galli A, et al. Effects of the olive-derived polyphenol oleuropein on human health. Int J Mol Sci. 2014:15(10), 18508–18524. https://doi.org/10.3390/ijms151018508

[58]. Kang Y, Hu W, Bai E, Zheng H, Liu Z, Wu J, et al. Curcumin sensitizes human gastric cancer cells to 5-fluorouracil through inhibition of the NFκB survival-signaling pathway. Onco Targets Ther. 2016:9,7373–7384. https://doi.org/10.2147/OTT.S118272

[59]. Ham IH, Wang L, Lee D, Woo J, Kim TH, Jeong HY, et al. Curcumin inhibits the cancer-associated fibroblast-derived chemoresistance of gastric cancer through the suppression of the JAK/STAT3 signaling pathway. Int J Oncol.2022:61(1), 85.

[60]. Yang L, Hu Z, Zhu J, Liang Q, Zhou H, Li J, et al. Systematic Elucidation of the Mechanism of Quercetin against Gastric Cancer via Network Pharmacology Approach. Biomed Res Int. 2020:3860213. https://doi.org/10.1155/2020/3860213

[61]. Lei CS, Hou YC, Pai MH, Lin MT, Yeh SL. Effects of quercetin combined with anticancer drugs on metastasis-associated factors of gastric cancer cells: in vitro and in vivo studies. J Nutr Biochem. 2018:51, 105–113. https://doi.org/10.1016/j.jnutbio.2017.09.011

[62]. Asghariazar V, Makaremi S, Zare E, Danesh H, Matin S, Fouladi N, et al. Oleuropein induces apoptosis in gastric cancer cell lines by regulating mir-34a, mir-21, and related genes: An experimental and bioinformatic study. *Int J Biol Macromol*. 2024;265(Pt 1):130527. https://doi.org/10.1016/j.ijbiomac.2024.130527

[63]. Han, J. H., Kim, M., Kim, H. J., Jang, S. B., Bae, S. J., Lee, I. K., ... & Ha, K. T. (2021). Targeting lactate dehydrogenase A with catechin resensitizes SNU620/5FU gastric cancer cells to 5-fluorouracil. *International journal of molecular sciences*, *22*(10), 5406.

[64]. Wei, F., Jiang, X., Gao, H. Y., & Gao, S. H. (2017). Liquiritin induces apoptosis and autophagy in cisplatin (DDP)-resistant gastric cancer cells in vitro and xenograft nude mice in vivo. *International Journal of Oncology*, *51*(5), 1383-1394.

[65]. Bhat, A. A., Moglad, E., Afzal, M., Agrawal, N., Thapa, R., Almalki, W. H., ... & Gupta, G. (2025). The anticancer journey of liquiritin: insights into its mechanisms and therapeutic prospects. *Current Medicinal Chemistry*, *32*(28), 6026-6041.

[66]. Pan MH, Chiou YS, Chen WJ, et al. (2019). *Biological activities of bioactive compounds and their roles in modulating the tumor microenvironment.* Biomed Pharmacother, 113:108750.

[67]. Yallapu MM, Jaggi M, Chauhan SC. (2019). *Pharmacokinetics and bioavailability of curcumin in cancer therapy.* Cancer Lett, 465:1–7.

[68]. Wang J, Fang D, Zhang S, et al. (2021). *Nanoparticle-based curcumin delivery enhances chemosensitivity of gastric cancer cells to 5-FU and cisplatin.* Front Pharmacol, 12:642852.

[69]. Shitara K, Van Cutsem E, Bang YJ, et al. (2023). *Future perspectives on biomarker-driven therapy in gastric cancer.* Nat Rev Clin Oncol, 20(6): 382–400.

[70]. Alam, J., Dilnawaz, F., Sahoo, S., Singh, D., Mukhopadhyay, A., Hussain, T., & Pati, S. (2022). Curcumin Encapsulated into Biocompatible Co-Polymer PLGA Nanoparticle Enhanced Anti-Gastric Cancer and Anti-Helicobacter Pylori Effect. Asian Pacific Journal of Cancer Prevention: APJCP, 23, 61- 70. https://doi.org/10.31557/apjcp.2022.23.1.61

[71]. Lin, M., Yao, W., Xiao, Y., Dong, Z., Huang, W., Zhang, F., Zhou, X., & Liang, M. (2021). Resveratrol-modified mesoporous silica nanoparticle for tumor-targeted therapy of gastric cancer. Bioengineered, 12, 6343- 6353. https://doi.org/10.1080/21655979.2021.1971507

[72]. Qiu, G., Cai, L., Li, G., Ren, Y., Li, E., Deng, K., Zhu, M., Han, S., Che, X., Li, X., & Fan, L. (2024). Res@ZIF-90 suppress gastric cancer progression by disturbing mitochondrial homeostasis. Translational Oncology, 51. https://doi.org/10.1016/j.tranon.2024.102179

[73]. Huang, W., Lai, C., Peng, S., Hsu, C., Hsu, P., Chu, P., Feng, C., & Lin, Y. (2021). Targeting Tumor Cells with Nanoparticles for Enhanced Co-Drug Delivery in Cancer Treatment. Pharmaceutics, 13. https://doi.org/10.3390/pharmaceutics13091327

[74]. Zhou, J., Zhang, Z., Zhang, Z., Wu, T., Li, H., Hao, X., Liu, X., Gong, T., Liu, D., & Wei, S. (2025). A facile dual-drug delivery system using cellulose-based microgel/hydrogel for enhanced gastric cancer therapy.. Colloids and surfaces. B, Biointerfaces, 254, 114813 . https://doi.org/10.1016/j.colsurfb.2025.114813

[75]. Rai, A., Kaur, S., Rawat, S., & Singh, I. (2023). Herbal bioactive encapsulated nano-formulations for the treatment of gastric cancer: a concise review. Nano Futures, 7. https://doi.org/10.1088/2399-1984/acf822
[76]. Chavda, V., Patel, A., Mistry, K., Suthar, S., Wu, Z., Chen, Z., & Hou, K. (2022). Nano-Drug Delivery Systems Entrapping Natural Bioactive Compounds for Cancer: Recent Progress and Future Challenges. Frontiers in Oncology, 12. https://doi.org/10.3389/fonc.2022.867655
[77]. Gupta, J., Ahmed, A., Tayyib, N., Zabibah, R., Shomurodov, Q., Kadheim, M., Alsaikhan, F., Ramaiah, P., Chinnasamy, L., & Samarghandian, S. (2023). A state-of-art of underlying molecular mechanisms and pharmacological interventions/nanotherapeutics for cisplatin resistance in gastric cancer.. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 166, 115337 https://doi.org/10.1016/j.biopha.2023.115337.

[78]. Nejati, K., Rastegar, M., Fathi, F., Dadashpour, M., & Arabzadeh, A. (2022). Nanoparticle-based drug delivery systems to overcome gastric cancer drug resistance. Journal of Drug Delivery Science and Technology. https://doi.org/10.1016/j.jddst.2022.103231

[79]. Nelson KM, Dahlin JL, Bisson J, et al. The essential medicinal chemistry of curcumin. *J Med Chem.* 2017;60(5):1620–1637.

[80]. Nanki K, Toshimitsu K, Takano A, et al. A patient-derived organoid biobank of gastric cancer for therapeutic screening. *Cell Stem Cell.* 2018;23(6):882–897.e11.

[81]. Vlachogiannis G, Hedayat S, Vatsiou A, et al. Patient-derived organoids model treatment response of metastatic GI cancers. *Science.* 2018;359(6378):920–926.