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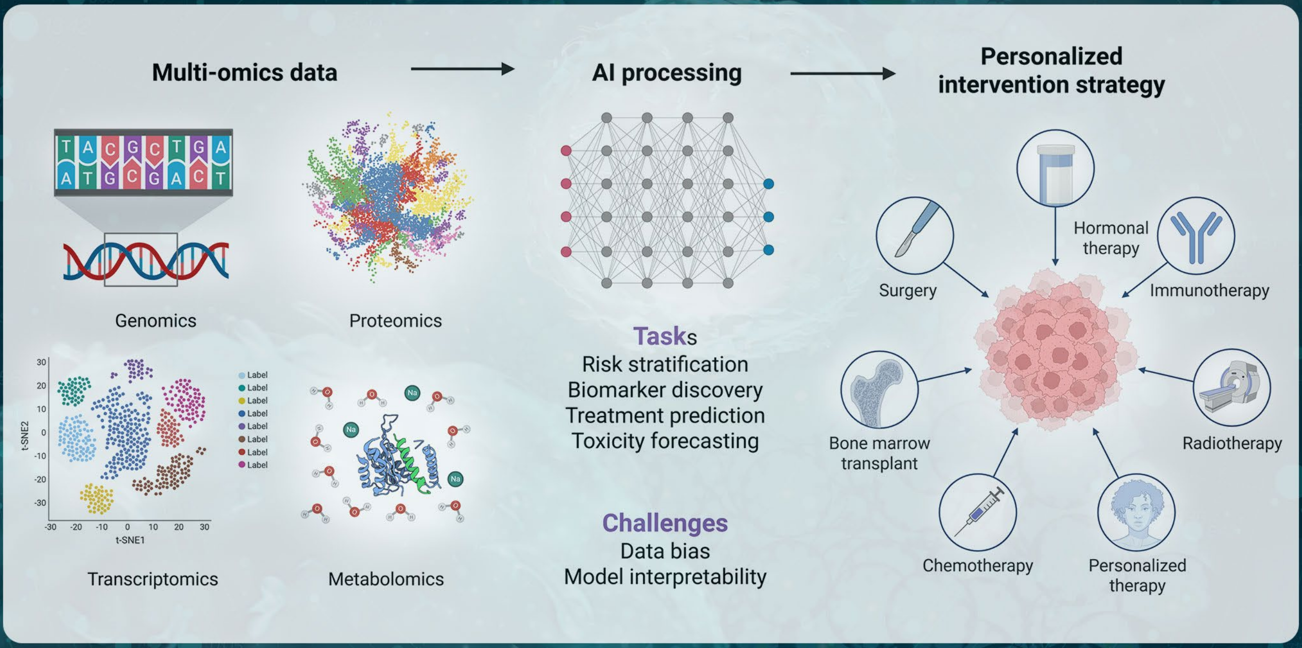

A conceptual framework for AI and multi-omics in survivorship care

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REVIEW ARTICLE

The sinonasal microbiome, host defense peptides, and cancer risk: Decoding the immune system's dilemma in chronic rhinosinusitis

Ghazaleh Omidvar[1†], **Shirin Esmaili Dolabinezhad**[1†], **Marjan Sistani**[1†], and **Saeid Besharati**[1,2*]

[1]Nursing Research Center of Respiratory Diseases, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medicine Sciences, Tehran, Iran

[2]Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Chronic rhinosinusitis (CRS) is a prevalent and debilitating condition characterized by persistent inflammation of the sinonasal mucosa. Despite advances in understanding its pathophysiology, the role of microbial communities and their interactions with the host immune system remains poorly understood. This review aims to elucidate the complex interplay between microbial dysbiosis, host immune responses, the dysregulated, cancer-like behavior of inflamed sinus tissue, and the production of antimicrobial peptides (AMPs) and lipids in CRS. A systematic literature review was conducted across databases such as PubMed, Scopus, and Web of Science. Keywords included "chronic rhinosinusitis," "microbial interactions," "antimicrobial peptides," "antimicrobial lipids," and "immune system." Studies published between 2020 and 2025 were included, focusing on microbial–immune interactions, AMPs, and lipids in CRS. Data were synthesized to identify key mechanisms and therapeutic implications. Microbial dysbiosis in CRS is associated with altered immune responses and impaired production of AMPs and lipids. Studies demonstrate that microbial interactions can either exacerbate or mitigate inflammation, depending on the balance between pathogenic and commensal species. Immune system dysregulation, particularly in T-cell responses and cytokine production, further exacerbates chronic inflammation. Emerging therapies, such as probiotics and AMP-based treatments, show promise in restoring microbial-immune balance. Future research should focus on developing targeted therapies that restore microbial balance and enhance innate immune defenses. Understanding the role of microbial interactions in CRS offers new avenues for personalized treatment strategies, potentially improving outcomes for patients with this challenging condition.

Keywords: Chronic rhinosinusitis; Microbial interactions; Antimicrobial peptides; Antimicrobial lipids; Immune system

1. Introduction

Chronic rhinosinusitis (CRS) is a prevalent and debilitating inflammatory disease of the paranasal sinuses, affecting a significant portion of the global population and imposing a substantial burden on healthcare systems and patient quality of life. Clinically characterized by symptoms such as nasal obstruction, rhinorrhea, facial

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[†] *These authors contributed equally to this work.*

***Corresponding author:**
Saeid Besharati
(saeid.besharati@sbmu.ac.ir)

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pain, and loss of smell persisting for more than 12 weeks, CRS is a heterogeneous disorder with diverse underlying pathophysiologies. While historically viewed as a simple consequence of bacterial infection or anatomical obstruction, our understanding of CRS has evolved to recognize it as a complex, multifactorial disease driven by a dysfunctional interplay between environmental factors, the sinus microbiome, and the host's immune response. Despite advances in medical and surgical management, a considerable number of patients remain refractory to treatment, underscoring the critical need to dissect the intricate molecular and cellular mechanisms that perpetuate chronic inflammation.[1]

The immune response in CRS is often persistent and self-perpetuating, sharing surprising similarities with the immune evasion strategies observed in cancer.[2] In this review, we explore the paradigm shift from infection to dysbiosis, examining how an imbalance in the sinus microbial community, rather than a single pathogen, contributes to a pro-inflammatory environment and disrupts mucosal homeostasis. The role of the host immune system is paramount. This review examines dysregulated immune responses in CRS, focusing on the aberrant activation of innate and adaptive immunity, the role of specific T-helper (Th) cell polarizations (e.g., Th1, Th2, and Th17), and the resulting cytokine milieu that defines various CRS endotypes.

A compelling and emerging concept in CRS is the observation of cancer-like characteristics in the chronically inflamed sinus tissue. We examine cancer-like features, including tissue remodeling, epithelial-to-mesenchymal transition, enhanced angiogenesis, and resistance to apoptosis, which collectively contribute to the recalcitrant, hyperplastic nature of the disease. The sinus epithelium is not a passive barrier but an active defender. We analyze the synthesis and function of key host-derived defense molecules, including antimicrobial peptides (AMPs; e.g., defensins and cathelicidins) and lipid mediators (e.g., eicosanoids and specialized pro-resolving mediators). The imbalance between protective and inflammatory mediators in these classes is a critical factor in determining disease chronicity and resolution.

By integrating these facets, this review seeks to offer a holistic and mechanistic perspective on CRS.[3] The epidemiology of CRS underscores its profound public health impact. It is estimated to affect 5–12% of the general population in developed countries, leading to millions of physician visits and a vast number of prescribed medications annually. Beyond direct healthcare costs, indirect costs from absenteeism, presenteeism, and reduced productivity are immense. However, the actual burden of CRS is perhaps most acutely felt in its devastating effect on patients' quality of life. Studies consistently show that the impact of CRS on physical functions, social functioning, and mental health is comparable to, or even exceeds, that of other chronic conditions, such as chronic obstructive pulmonary disease, congestive heart failure, and chronic back pain. The persistent nature of symptoms, particularly the loss of smell and chronic fatigue, leads to social isolation, nutritional issues, and a significant increase in the prevalence of depression and anxiety, creating a heavy personal and societal toll.[4-6]

The pathophysiology of CRS is complex and multifactorial, traditionally conceptualized as an interplay between host and environmental factors. The "unified airway" model links CRS closely with other inflammatory conditions, such as asthma and allergic rhinitis, suggesting shared pathogenic mechanisms. Central to the development of CRS is the disruption of the sinonasal homeostatic ecosystem. This involves a dysfunctional epithelial barrier, an aberrant immune response to both inhaled pathogens and environmental agents, and dysregulation of the local inflammatory milieu. Historically, research focused on the role of bacterial infection; however, the contemporary understanding has evolved to recognize that persistent inflammation, rather than active infection, is the cornerstone of the disease. This inflammation is sustained by a complex network of innate and adaptive immune cells, culminating in the characteristic tissue remodeling—including edema, fibrosis, polyp formation, and basement membrane thickening—observed in CRS patients.[7-10]

Current concepts posit that disruption of the homeostatic balance between the host and its microbial residents, known as dysbiosis, is a critical driver of the persistent inflammation observed in CRS. This dysbiosis is characterized not necessarily by the presence of a single pathogenic species, but by a shift in the overall microbial community structure, often with a loss of beneficial commensals and an expansion of pro-inflammatory pathobionts.[11] Concurrently, the host's immune response in CRS is marked by distinct endotypes, most notably the Th2-skewed inflammation seen in patients with comorbid asthma and nasal polyps and the more Th1-/Th17-dominated profile in those without polyps.[12] The central, yet incompletely resolved, question is how these specific immune responses and the sinus microbial ecosystem interact reciprocally to initiate and perpetuate the disease cycle.

Nasal secretions, often collected through lavage, swabbing, or an absorbent matrix, provide a unique and non-invasive window into the host–microbe interface. These secretions are a rich source of both microbial DNA, enabling profiling of the entire microbial community through 16S ribosomal RNA (rRNA) gene sequencing, and a plethora of host-derived immune factors, including cytokines, immunoglobulins, AMPs, and proteases.[13] The simultaneous analysis of these two components from the same sample offers an unprecedented opportunity to move beyond correlation toward a mechanistic understanding of CRS pathogenesis. Therefore, the primary objective of this review is to synthesize and critically evaluate the existing body of literature concerning the collection and examination of nasal secretions from CRS patients.[14,15]

In CRS, however, these defense mechanisms are often compromised. Alterations in the expression and function of AMPs and antimicrobial lipids have been observed, leading to impaired microbial clearance and chronic inflammation. In addition, the interplay between the microbiota and the immune system is disrupted, creating a vicious cycle of microbial dysbiosis, immune dysregulation, and tissue damage. Understanding these interactions is crucial for developing targeted therapies that restore microbial balance and enhance host defense mechanisms.[16-19] This review delves into the intricate molecular and cellular mechanisms that underpin the inflammatory state in CRS. We aim to move beyond a superficial description and provide detailed insights into several pertinent and interconnected aspects of the disease's pathogenesis. Specifically, we explore the critical role of microbial dysbiosis, where an imbalance in the sinonasal microbiome between beneficial and pathobiont organisms disrupts immune homeostasis and fuels inflammation. We then examine the consequent host immune reactivity, dissecting the dominant endotypes—namely, the type 2 inflammatory response, often associated with nasal polyps and eosinophilia, and the non-type 2 pathways. Furthermore, we discuss the emerging concept of cancer-like behavior in inflamed tissue, where processes such as epithelial-to-mesenchymal transition, sustained proliferation, and resistance to apoptosis contribute to polyp growth and recalcitrant disease. Finally, we synthesize the current understanding of the synthesis and function of AMPs and lipids in the sinonasal mucosa, highlighting how their deficiency or dysfunction impairs the first line of defense and perpetuates the cycle of inflammation and dysbiosis. By integrating these key facets, this review seeks to clarify the pathogenic landscape of CRS and illuminate potential avenues for novel, targeted therapeutic strategies.

2. Literature review methodology

A comprehensive literature search was performed across electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar. The search terms included combinations of the following keywords: "antimicrobial peptides," "immune system dynamics," "chronic rhinosinusitis," "microbial interactions," "host-pathogen interactions," "biofilms," "innate immunity," and "therapeutic applications." Articles published in English from 2020 to 2025 were included. Studies focusing on the role of AMPs in CRS, their interaction with microbial communities, and their impact on immune system dynamics were prioritized. Both *in vitro* and *in vivo* studies, as well as clinical trials, were considered. Duplicate articles were removed, and the remaining studies were screened based on titles and abstracts. Full-text articles were reviewed for relevance, and references from selected articles were cross-checked to identify additional studies (Table 1).

3. Results

3.1. Understanding CRS

CRS is broadly categorized into two subtypes: CRS with nasal polyps and CRS without nasal polyps. Symptoms include nasal congestion, facial pain, hyposmia (reduced sense of smell), and purulent nasal discharge. Traditional treatment approaches involve corticosteroids, saline irrigation, and antibiotics targeting common pathogens, such as *Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis*. However, a subset of patients fails to respond to conventional therapies, prompting clinicians to explore alternative etiologies, including atypical bacterial infections.[36,37]

3.2. The sinus microbiome in health and disease

The sinonasal cavity harbors a diverse community of microorganisms, collectively known as the sinus microbiome. In healthy individuals, this microbiome is relatively stable and consists of commensal bacteria that coexist harmoniously with the host, contributing to immune regulation and protection against pathogens. However, in CRS, this microbial balance is disrupted, leading to dysbiosis, a state of microbial imbalance that promotes inflammation and disease.[14] Studies using advanced sequencing technologies, such as 16S rRNA gene sequencing, have revealed distinct differences in the sinus microbiome of CRS patients compared to healthy controls. CRS is often associated with a reduction in microbial diversity and an overgrowth of pathogenic or opportunistic bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *H. influenzae*. These pathogens can form biofilms, which are structured communities of bacteria encased in a protective extracellular matrix. Biofilms are highly resistant to antibiotics and immune clearance, contributing to the chronic and recurrent nature of CRS.[38,39]

Table 1. Integration of AMPs and immune system dynamics in CRS, with a focus on microbial interactions

Categories	Hypothesis	Results	Example quotes	References
Immune modulation by AMPs	AMPs play a critical role in modulating immune responses in CRS.	AMPs such as defensins and cathelicidins regulate immune cell activity.	"Human neutrophil α-defensin HNP1 interacts with bacterial cell wall components to promote biofilm formation by *S. aureus*."[22(p1)]	20-22
Microbial dysbiosis and AMPs	Dysbiosis in CRS correlates with AMP dysregulation.	Imbalance in microbial communities reduces AMP efficacy.	"*S. aureus* biofilms suppress AMP production in CRS patients."[7(p4)]	7,23,24
Advances in AMP research	Advances in AMP research improve therapeutic strategies.	Advanced techniques in AMP synthesis and delivery.	"Advanced in peptide engineering enhances AMP stability and bioavailability."[26(p1)]	7,25
Immune system dynamics	Immune system dynamics are altered by microbial–AMP interactions.	AMPs influence cytokine profiles and T-cell responses.	"Cytokine family members have diverse biological functions, promoting protective immunity against many pathogens but also driving inflammatory pathology."[7(p4)]	7,26
Therapeutic applications of AMPs	AMP-based therapies show promise in CRS treatment.	Clinical trials on synthetic AMPs for CRS.	"Synthetic AMPs reduce bacterial load and inflammation in CRS patients."[30(p13)]	27,28
Microbial resistance mechanisms	Microbial resistance to AMPs is a growing concern.	Bacteria evolve resistance to AMPs through genetic mutations.	"*Pseudomonas aeruginosa* develops resistance by altering membrane composition."[30(p3)]	28-30
Host–microbiota interactions	AMPs interact with host microbiota to maintain homeostasis.	AMPs shape microbial communities in the sinonasal cavity.	"AMPs promote beneficial commensals while suppressing pathogens in CRS."[31(p3)]	30,31
Pathogen immune evasion	Immune evasion by pathogens reduces AMP efficacy.	Pathogens secrete proteases to degrade AMPs.	"*S. aureus* secretes proteases that cleave human β-defensins, evading immune attack."[35(p1)]	32-35

Abbreviations: AMPs: Antimicrobial peptides; CRS: Chronic rhinosinusitis; *S. aureus*: *Staphylococcus aureus*.

3.3. The role of microbial interactions in CRS

CRS is a persistent inflammatory condition of the paranasal sinuses and nasal mucosa, lasting for 12 weeks or longer. It is a common and debilitating condition that significantly impacts patients' quality of life, causing symptoms such as nasal congestion, facial pain, reduced sense of smell, and fatigue. While the exact etiology of CRS remains incompletely understood, emerging research highlights the critical role of microbial interactions in its pathogenesis. The complex interplay between bacteria, fungi, viruses, and the host immune system contributes to the chronicity and heterogeneity of CRS.[11,40]

3.4. The role of bacteria in CRS

Coneventionally, CRS has been associated with bacterial infections, particularly those caused by *S. aureus*, *P. aeruginosa*, and *H. influenzae*. However, the presence of these bacteria in sinus cultures does not always correlate with disease severity or treatment outcomes. This discrepancy has led researchers to explore alternative mechanisms, including the possibility that certain bacteria may evade the immune system and antibiotic therapy by residing inside host cells. Microbial interactions play a pivotal role in the development and persistence of CRS. These interactions occur not only between different microbial species but also between microbes and the host immune system.[41]

3.5. Bacterial involvement in CRS pathogenesis

In healthy individuals, the sinuses are sterile or colonized by a balanced microbiome that does not cause harm. However, in CRS, this balance is disrupted, leading to bacterial overgrowth or the presence of pathogenic species. Common bacterial pathogens associated with CRS include *S. aureus*, *P. aeruginosa*, *H. influenzae*, and *S. pneumonia*.[1,42]

3.6. Intracellular bacteria in patients with CRS: A hidden culprit?

CRS is a common and debilitating condition characterized by inflammation of the paranasal sinuses lasting for 12 weeks or longer. Despite its prevalence, the underlying causes of CRS remain poorly understood, with factors such as allergies, environmental triggers, and microbial infections playing complex roles. In recent years, emerging research has shed light on the potential involvement of intracellular bacteria in the pathogenesis of CRS, raising questions about their role in disease persistence and treatment resistance.[1,43]

3.7. Atypical bacterial infections in CRS

Atypical bacteria are microorganisms that do not respond to standard laboratory culturing techniques and often require specialized methods for detection. These pathogens are typically intracellular or have unique growth requirements, making them difficult to identify using traditional diagnostic approaches. In the context of CRS, the most commonly implicated atypical bacteria include *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *S. aureus* (small-colony variants). Atypical bacteria can induce a dysregulated immune response, leading to chronic inflammation. For example, C. pneumoniae and M. pneumoniae have been shown to stimulate the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which perpetuate sinus inflammation and tissue remodeling.[44,45]

3.7.1. Pathogen–pathogen interactions

In CRS, pathogenic bacteria often interact synergistically, enhancing each other's virulence. For example, *S. aureus* and *P. aeruginosa* are frequently co-isolated in CRS patients. These bacteria can engage in cross-communication through quorum sensing, a process that allows them to coordinate gene expression and biofilm formation. In addition, *S. aureus* can produce toxins that damage the epithelial barrier, facilitating the invasion of other pathogens such as *P. aeruginosa*.

3.7.2. Bacterial–fungal interactions

Fungi, particularly *Aspergillus* and *Candida* species, are also implicated in CRS, especially in cases of fungal ball or allergic fungal rhinosinusitis. Bacterial–fungal interactions can exacerbate inflammation and tissue damage. For example, *S. aureus* and *Candida albicans* can form mixed biofilms, which are more resistant to treatment than single-species biofilms. Furthermore, fungal proteases can degrade host tissues, creating a favorable environment for bacterial colonization.[46]

3.7.3. Viral–bacterial interactions

Viral infections, such as those caused by rhinoviruses or influenza viruses, can predispose individuals to bacterial superinfections in the sinuses. Viruses can impair mucociliary clearance, disrupt the epithelial barrier, and modulate the immune response, creating an environment conducive to bacterial colonization and biofilm formation. Viruses, particularly those responsible for upper respiratory tract infections, are increasingly recognized as key contributors to the initial inflammatory cascade that may lead to the development of CRS. Respiratory viruses, such as rhinovirus, influenza virus, respiratory syncytial virus, and adenovirus, are common culprits in acute viral rhinosinusitis. In most individuals, these infections are self-limiting and resolve without complications. However, in susceptible individuals, viral infections can trigger a dysregulated immune response, leading to persistent inflammation and mucosal damage. This aberrant response may be influenced by host factors, including genetic predisposition, environmental exposures, and underlying immune dysfunction.[47]

3.7.4. Microbe–host interactions

The host immune response to microbial colonization is a critical factor in CRS pathogenesis. In some patients, an exaggerated immune response to commensal or pathogenic microbes leads to persistent inflammation. For example, *S. aureus* produces superantigens that can trigger a robust T-cell response, contributing to tissue damage and chronic inflammation. In addition, dysbiosis can alter the production of AMPs and cytokines, further disrupting immune homeostasis.[48-50]

3.8. The overlooked virosphere: Viral influence on sinonasal immunity and microbial ecology

A central finding of this systematic review is the pronounced bias within the existing literature toward characterizing the bacterial component of the sinonasal microbiome. While these studies have been invaluable for establishing a baseline understanding of microbial communities in health and diseases such as CRS, the viral constituent of the virome remains largely unexplored. The majority of identified studies utilized 16S rRNA gene sequencing, a technique inherently blind to viral presence. Consequently, the role of viruses in shaping the sinonasal mucosal environment, modulating host defense mechanisms, and interacting with bacterial residents is critically under-represented. This creates a pro-inflammatory environment rich in damage-associated molecular patterns and depleted resources, which can paradoxically favor colonization by opportunistic pathogens such as *S. aureus* and *P. aeruginosa*.[51]

Beyond acute epithelial damage, viruses exert a more subtle and persistent influence on the production of key host-defense peptides (HDPs), a critical component of the innate immune system. AMPs such as β-defensins, S100 proteins (e.g., calprotectin), and cathelicidin (LL-37) are vital for maintaining microbial homeostasis on mucosal surfaces. Evidence suggests that viral infections can dysregulate the expression of these peptides. For example, rhinovirus infection has been shown to downregulate the expression of specific β-defensins in airway epithelial cells, potentially creating a permissive environment for secondary bacterial infection. Conversely, some viruses may induce certain AMPs as part of the generalized inflammatory response; however, the net effect often appears to be a failure of targeted, effective antimicrobial defense, leading to a state of immune dysregulation that benefits other members of the microbiome.[52]

Perhaps the most significant, yet least studied, aspect of sinonasal virology is the impact of bacteriophages—viruses that specifically infect bacteria. Bacteriophages are likely the most abundant biological entities in the sinonasal cavity and are pivotal players in bacterial ecology and evolution. Through predator–prey dynamics, they can directly modulate bacterial population densities and diversity. More importantly, phages drive bacterial evolution through lysogenic conversion, where the integration of phage DNA into the bacterial genome can confer new pathogenic traits. A canonical example is the conversion of a benign *S. aureus* strain into a toxigenic one through the acquisition of a phage encoding the Panton–Valentine leukocidin toxin.

In the context of CRS, phage-mediated transfer of genes encoding other virulence factors, biofilm-enhancing proteins, or antibiotic resistance determinants could fundamentally alter the disease's trajectory and chronicity. The interaction among viruses, HDPs, and bacteria is likely a complex, tripartite relationship. The host's production of AMPs in response to a viral threat may not only be ineffective against the virus but also apply selective pressure on the bacterial community, enriching for resistant species. Simultaneously, bacteria can modulate AMP expression, for example, *S. aureus* can upregulate its own biofilm formation in response to LL-37, enhancing its resilience. Bacteriophages, in turn, may exploit this stressed bacterial state for their own replication. Furthermore, some evidence suggests that certain HDPs can directly influence phage activity, either by neutralizing viral particles or by inducing the lytic cycle in lysogenic bacteria, effectively triggering the destruction of their bacterial host. This intricate web of interactions represents a significant gap in our current knowledge of sinonasal pathophysiology.

3.9. Implications for diagnosis and treatment

Understanding the role of microbial interactions in CRS has important implications for its diagnosis and treatment. Traditional culture-based methods often fail to capture the full complexity of the sinus microbiome, highlighting the need for molecular techniques such as metagenomic sequencing to characterize microbial communities and their functional potential. Personalized approaches that consider the unique microbial and immune profiles of individual patients may improve outcomes.[53-55] Current treatment strategies for CRS include antibiotics, corticosteroids, and surgery. However, these approaches often provide only temporary relief, as they do not address the underlying microbial dysbiosis and biofilm formation. Emerging therapies targeting microbial interactions, such as quorum-sensing inhibitors, probiotics, and bacteriophages, hold promise for restoring microbial balance and preventing recurrence. In addition, modulating the host immune response through biologics or immunotherapies may help break the cycle of chronic inflammation.[56,57]

3.10. The sinonasal microbiome: From initial homeostasis to pathologic dysregulation

A consistent theme emerging from the reviewed literature is the association between an altered sinonasal microbiome and a spectrum of chronic inflammatory diseases, most notably CRS. The included studies extensively cited the downstream consequences of microbial dysbiosis, effectively framing the microbiome as a central player in the effector phase of immune dysregulation. These downstream aspects include the breakdown of epithelial barrier integrity through the dysregulation of tight junction proteins; the sustained recruitment and activation of neutrophils, lymphocytes, and other innate immune cells; and the polarization of the adaptive immune response toward pathogenic Th2 or Th17 profiles, characterized by the production of cytokines such as IL-4, IL-5, IL-13, and IL-17.

Furthermore, several studies highlighted the role of dysbiotic communities in fostering a pro-inflammatory milieu through the production of specific virulence factors, toxins, and metabolites that directly damage host tissue and perpetuate a cycle of inflammation and microbial perturbation. While this body of evidence robustly describes the consequences of an established dysbiotic state, the early-stage triggering mechanisms that initiate the shift from a homeostatic to a dysbiotic microbiome remain a critical gap. To complete this model, we propose a framework that incorporates these initiating events, which can be broadly categorized into host-derived and environment-derived triggers.

3.11. Priming and disruption: The initiation of dysbiosis

The initial breach in sinonasal homeostasis potentially begins with an event that disrupts the delicate host–microbe interface. Key early triggering mechanisms include:

- Viral insult: A primary instigator is an acute viral upper respiratory infection (e.g., rhinovirus, influenza virus, and respiratory syncytial virus). These viruses do not merely cause transient inflammation; they can directly damage the ciliated respiratory epithelium, leading to sloughing of the protective mucus layer and exposing underlying receptors. This "hit-and-run" event creates a window of vulnerability by transiently suppressing innate immune responses and liberating host nutrients, thereby facilitating the initial colonization and overgrowth of opportunistic pathobionts residing within the community

- Failure of microbial competition: The early loss of key commensal species, particularly those producing bacteriocins or other antimicrobial compounds, can remove a critical ecological check on potential pathobionts such as *S. aureus* and *P. aeruginosa*. The depletion of these beneficial competitors, potentially through the indiscriminate use of antibiotics or the mechanisms above, creates a vacant niche that is rapidly filled by more inflammatory species.

3.12. Antibiotic resistance in CRS

The overuse and misuse of antibiotics in treating CRS have led to the emergence of antibiotic-resistant bacterial strains. *S. aureus*, particularly methicillin-resistant *S. aureus*, is a significant concern in CRS due to its resistance to multiple antibiotics. This resistance complicates treatment and often necessitates the use of more potent, more toxic antibiotics, which can have significant side effects.[49,58]

3.13. Biofilm formation and chronicity

One of the most significant challenges in treating CRS is the ability of bacteria to form biofilms. Biofilms are structured communities of bacteria encased in a self-produced extracellular matrix that adheres to the sinonasal mucosa. These biofilms are highly resistant to antibiotics and the host immune response, making them a key factor in the chronicity of CRS.[40,59,60]

4. Future directions and recommendations for empirical validation

The conceptual framework and literature synthesis presented in this paper provide a compelling model for understanding the complex interplay between microbial dysbiosis and immune dysregulation in CRS. The study has adeptly synthesized current literature to propose a plausible pathogenic cascade, identifying key microbial players and putative immune pathways. However, to transition from a compelling theoretical model to a foundational pillar of clinical understanding, the empirical rigor and persuasive capacity of this work must be bolstered through direct experimental or clinical validation.

A primary and highly feasible recommendation is to conduct controlled studies using well-characterized patient samples. While the review expertly extrapolates from existing studies, direct evidence generated from a dedicated cohort would powerfully substantiate its central thesis. A critical step in this direction would be the systematic collection and examination of nasal secretions or sinonasal tissue from CRS patients, stratified by phenotype (e.g., with or without nasal polyps) and endotype (e.g., Th2-high vs. Th2-low). The integration of these two datasets, microbiome and immunome, through advanced statistical modeling would be instrumental in verifying the theoretical analysis. For example, such an approach could directly test whether a high *S. aureus* burden is indeed correlated with elevated IL-5 and immunoglobulin E levels in the Th2-high subgroup, or whether a depletion of commensal *Corynebacterium* species is linked to a deficit in specific antimicrobial defenses. This methodology moves beyond correlation to provide a more mechanistic understanding of host–microbe interactions within the sinonasal niche.

4.1. The interplay between antimicrobial agents and innate immunity in CRS

The relationship between antimicrobial agents and host innate immunity in CRS is complex and bidirectional. On one hand, antimicrobial agents can modulate the immune response by reducing microbial load and biofilm formation, thereby alleviating inflammation. On the other hand, the immune system influences the efficacy of antimicrobial therapies by shaping the microbial environment and determining the extent of inflammation. For example, the use of macrolides in CRS has been shown to downregulate pro-inflammatory cytokines and enhance epithelial repair, highlighting the immunomodulatory properties of specific antimicrobial agents. Conversely, the innate immune system's ability to recognize and respond to pathogens can be compromised in CRS, leading to recurrent infections and the need for prolonged antimicrobial therapy.[61-63]

4.2. Host innate immunity in CRS

The innate immune system serves as the first line of defense against microbial invaders and is intricately involved in the pathogenesis of CRS. The sinonasal epithelium is equipped with physical barriers, such as mucociliary clearance, and chemical defenses, including AMPs such as defensins and cathelicidins. These components work in concert to prevent microbial colonization and invasion. In CRS, however, dysregulation of innate immunity is frequently observed. Pattern recognition receptors, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs), detect pathogen-associated molecular patterns on microbes and initiate immune responses. In CRS patients, aberrant signaling through these receptors can lead to excessive production of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, contributing to chronic inflammation. In addition, defects in epithelial barrier function and impaired mucociliary clearance further exacerbate the disease by allowing persistent microbial colonization.[44,64]

4.3. AMPs in CRS

CRS is a complex inflammatory condition of the paranasal sinuses that persists for 12 weeks or longer, often leading to significant morbidity and reduced quality of life. The pathogenesis of CRS involves a dysregulated immune response, microbial colonization, and impaired mucosal defense mechanisms. Among the key players in the innate immune defense of the sinonasal mucosa are AMPs, which are small, cationic molecules that play a critical role in combating pathogens and modulating inflammation. AMPs are produced by epithelial cells and immune cells, and their expression is often altered in CRS, contributing to the disease's persistence and severity.[65-68]

4.4. Defenses in CRS

The sinonasal epithelium serves as the first line of defense against inhaled pathogens, allergens, and environmental irritants. In CRS, this barrier function is often compromised, leading to increased susceptibility to infections and chronic inflammation. AMPs are a crucial component of this defense system, providing rapid, non-specific immunity against bacteria, viruses, and fungi.[65]

4.5. Cathelicidins in CRS

In CRS, the expression of LL-37 is often altered, with studies showing both increased and decreased levels depending on the subtype of CRS (e.g., with or without nasal polyps). In CRS with nasal polyps, LL-37 levels are typically reduced, which may contribute to the impaired antimicrobial defense and increased bacterial colonization observed in these patients. Conversely, in CRS without nasal polyps, LL-37 expression may be upregulated as part of the inflammatory response. The dysregulation of cathelicidins in CRS highlights their potential role in the disease's pathogenesis and their potential as therapeutic targets.[69,70]

4.6. Deleted in malignant brain tumors 1 (DMBT-1) in CRS

In CRS, the expression of DMBT-1 is often downregulated, particularly in patients with nasal polyps. This reduction in DMBT-1 levels may impair the sinonasal epithelium's ability to clear pathogens and maintain mucosal homeostasis, contributing to the chronic inflammation and microbial colonization seen in CRS. In addition, DMBT-1 has been shown to interact with other AMPs and immune mediators, suggesting that its dysregulation may have broader implications for the immune response in CRS.[71,72]

4.7. S-100 proteins in CRS

In CRS, specific S-100 proteins, such as S100A7 (psoriasin) and S100A8/A9 (calprotectin), have been implicated in the disease's pathogenesis. S100A7 is an AMP that exhibits antimicrobial activity against bacteria and fungi, and its expression is often increased in CRS, particularly in patients with nasal polyps. The role of S-100 proteins in CRS underscores the complexity of the immune response in this condition and highlights their potential as biomarkers or therapeutic targets.[65]

4.8. Indolicidin in CRS

Indolicidin exhibits immunomodulatory properties by influencing cytokine production and promoting wound healing, which could aid in the repair of damaged sinus mucosa. However, its role in CRS remains under investigation, with studies exploring its potential as a therapeutic agent to combat persistent infections and inflammation.

4.9. CP10A in CRS

CP10A, a synthetic analog of the cathelicidin family of AMPs, has demonstrated significant antimicrobial and anti-inflammatory effects. In CRS, CP10A has been shown to inhibit biofilm formation, a key factor in the persistence of chronic infections. Its ability to modulate the immune response by reducing pro-inflammatory cytokines such as IL-6 and TNF-α makes it a promising candidate for managing CRS-related inflammation. Furthermore, CP10A's stability and low toxicity profile enhance its potential for clinical application in sinusitis treatment.[69]

4.10. Tachyplesin in CRS

In CRS, tachyplesin's ability to neutralize lipopolysaccharides and inhibit biofilm formation may help alleviate chronic infections. Its immunomodulatory effects, including the suppression of excessive inflammatory responses, further highlight its potential as a therapeutic agent in CRS management.

4.11. Microcins in CRS

In CRS, microcins may play a role in modulating the sinus microbiome by targeting pathogenic bacteria while sparing commensal species. This selective antimicrobial activity could help restore microbial balance in the sinuses, thereby reducing chronic inflammation. Research into the application of microcins in CRS is still in its early stages, but their potential for targeted therapy is promising.[73]

4.12. PR-39 in CRS

In CRS, PR-39 has been shown to promote tissue repair and reduce inflammation by modulating the activity of immune cells and inhibiting the production of reactive oxygen species. Its ability to enhance epithelial regeneration and restore mucosal integrity makes it a potential therapeutic candidate for managing the chronic inflammatory and tissue-damaging processes associated with CRS.[74,75]

4.13. Sinus mucus secretion composition to combat CRS

CRS is a complex inflammatory condition of the paranasal sinuses characterized by persistent symptoms and mucosal dysfunction. The sinus mucus, a critical component of the innate immune system, plays a pivotal role in defending against pathogens and maintaining sinus health. Understanding the specific components of sinus mucus and their roles in CRS pathogenesis is essential for developing targeted therapeutic strategies.[75-77] The composition of sinus mucus and its immune components plays a critical role in the pathogenesis of CRS. Understanding the roles of lysozyme, lactoferrin, secretory leukocyte proteinase inhibitor (SLPI), TLRs, NLRs, and other immune molecules provides valuable insights into the mechanisms underlying CRS and offers opportunities for developing targeted therapies to restore mucosal defense and reduce inflammation.

4.14. Lysozyme in CRS

In CRS, lysozyme levels are often dysregulated, with studies showing both increased and decreased expression depending on the disease phenotype. Reduced lysozyme activity may compromise the mucus barrier, allowing bacterial colonization and biofilm formation, which are common in CRS. Conversely, elevated lysozyme levels may contribute to tissue damage due to excessive inflammation. Restoring lysozyme homeostasis is a potential therapeutic target to enhance mucosal defense in CRS.[78]

4.15. Lactoferrin in CRS

In CRS, lactoferrin expression is often altered, with some studies reporting reduced levels in patients with severe disease. Lactoferrin's ability to modulate immune responses and protect against microbial invasion makes it a promising candidate for therapeutic interventions in CRS.[79]

4.16. SLPI in CRS

In CRS, SLPI levels are often diminished, particularly in patients with neutrophilic inflammation or recurrent infections. The loss of SLPI activity may exacerbate tissue damage and impair mucosal repair, highlighting its importance in maintaining sinus health. Augmenting SLPI levels could help mitigate inflammation and promote healing in CRS.[80,81]

4.17. Other immune system components to combat CRS

In addition to lysozyme, lactoferrin, and SLPI, sinus mucus contains other immune components such as defensins, cathelicidins, and immunoglobulins that contribute to mucosal defense. Defensins and cathelicidins are AMPs that directly kill pathogens and modulate immune responses. Immunoglobulins, particularly immunoglobulin A, play a crucial role in neutralizing pathogens and preventing their adherence to mucosal surfaces. Dysregulation of these components in CRS can impair the mucus barrier and exacerbate disease severity. Targeting these immune molecules may offer novel therapeutic avenues for CRS management.[4,82] In CRS, TLR expression and signaling are often dysregulated, contributing to aberrant immune activation and chronic inflammation. For example, TLR2 and TLR4 are implicated in recognizing bacterial components such as lipopeptides and lipopolysaccharides, respectively.[83] NLRs are intracellular sensors that detect microbial components and cellular damage, triggering inflammatory responses through the formation of inflammasomes. In CRS, NACHT, LRR, and PYD domains-containing protein 3 (NLRP3), inflammasome activation has been linked to the release of pro-inflammatory cytokines such as IL-1β and IL-18, which drive chronic inflammation.[76]

4.18. Future directions: Elucidating the pathogenesis of antimicrobial dysregulation in CRS

While the downregulation of key AMPs and lipids in the sinonasal epithelium of CRS patients is well-documented, the precise upstream mechanisms driving this critical immune defect remain inadequately defined. To further elucidate the pathogenesis of CRS, future research must delve beyond correlation and uncover the exact causal mechanisms responsible for this dysregulated expression. A primary area of investigation should focus on identifying modifications in the intracellular signaling pathways that govern AMP and lipid synthesis. For example, it is crucial to determine whether defects exist in the TLR, IL-related (e.g., IL-4/IL-13 through signal transducer and transcription activator 6), or epidermal growth factor receptor pathways, which directly modulate the transcription of molecules such as β-defensins and S100 proteins. Furthermore, the role of disruptions in intercellular communication warrants exploration; this includes examining how altered crosstalk between epithelial cells, immune cells (e.g., T-cells and macrophages), and the microbiome through cytokine networks or gap junctions can perpetuate a state of immune tolerance or suppression. In addition, the impact of elevations in metabolic stress within the inflammatory sinus environment should be considered. Hypoxia, nutrient deprivation, and oxidative stress can activate pathways such as hypoxia-inducible factor 1-alpha and 5'-AMP-activated protein kinase, which may inadvertently suppress energy-intensive host defense mechanisms. Systematic investigation into these areas—

through targeted in vitro models, animal studies, and analysis of human tissue samples—will not only provide a more coherent understanding of CRS pathogenesis but also reveal novel, druggable targets to restore the innate immune barrier and break the cycle of inflammation and microbial colonization that characterizes this debilitating disease.

4.19. Lifestyle in CRS susceptibility

While the primary triggers for cytokine release syndrome are often linked to specific immunotherapies (e.g., chimeric antigen receptor T-cell [CAR-T cell] therapy and bispecific antibodies) and underlying tumor burden, a comprehensive understanding of its incidence requires looking beyond these immediate factors. Subtle determinants such as host genetics, environmental exposures, and lifestyle factors may also influence the inflammatory milieu and immune reactivity in cytokine release syndrome. The interplay of host genetic predispositions, environmental exposures, and lifestyle choices can significantly modulate an individual's susceptibility to CRS, its severity, and clinical trajectory. Integrating these elements into the discussion provides a more holistic and personalized framework for risk stratification and management.

4.20. Host genetic predispositions

Individual genetic variation plays a fundamental role in determining the intensity of immune responses. Polymorphisms in genes encoding cytokines, their receptors, and key signaling molecules can create a pro-inflammatory "set-point" that predisposes patients to severe CRS. For example, single-nucleotide polymorphisms in the promoters of *IL-6* and *TNFA* can lead to heightened expression on immune activation. Similarly, variations in genes involved in TLR signaling or the NLRP3 inflammasome pathway may lower the threshold for uncontrolled macrophage and monocyte activation, a key driver of CRS. Future research, and indeed the discussion of CRS incidence, would be strengthened by considering how such germline genetics interact with therapeutic triggers to determine clinical outcomes.

4.21. Environmental influences and the microbiome

The patient's environment, particularly the composition of their gut microbiome, is emerging as a critical modulator of systemic immunity. A diverse, commensal-rich microbiome helps maintain immune homeostasis and regulates inflammatory pathways. In contrast, dysbiosis—often induced by prior broad-spectrum antibiotic use, dietary patterns, or other environmental factors—can skew the immune system toward a hyperinflammatory state. This "primed" immune background may exacerbate the cytokine storm following CAR-T-cell infusion or bispecific antibody engagement. Investigating and discussing the impact of microbiome status, along with other environmental exposures such as prior viral infections (e.g., cytomegalovirus and Epstein–Barr virus) that shape the T-cell repertoire, could provide valuable insights into inter-patient variability in CRS incidence.

4.22. The role of lifestyle and comorbidities

Lifestyle factors and pre-existing comorbidities represent another layer of complexity. Chronic conditions such as obesity, metabolic syndrome, and cardiovascular disease are associated with a state of low-grade, systemic inflammation, characterized by elevated baseline levels of C-reactive protein, IL-6, and other acute-phase reactants. This pre-activated inflammatory state may serve as a catalyst, amplifying the cytokine cascade initiated by immunotherapy. Furthermore, lifestyle choices such as smoking, chronic alcohol consumption, and nutritional status (e.g., Vitamin D deficiency) can impair immune regulation and endothelial integrity, potentially worsening CRS severity and contributing to related complications such as capillary leak syndrome.

4.23. Host genetic factors

Emerging evidence underscores the significant contribution of heritable factors to CRS susceptibility. Polymorphisms in genes governing the innate and adaptive immune response can profoundly alter an individual's reaction to sinonasal insults. For example, variations in genes encoding TLRs, which are crucial for pathogen recognition, may lead to a blunted or exaggerated inflammatory response to common allergens and microbes. Similarly, alterations in the cystic fibrosis transmembrane conductance regulator gene, even in subclinical, non-cystic fibrosis forms, can impair mucociliary clearance, creating a permissive environment for persistent inflammation. Furthermore, genes involved in epithelial barrier function, such as those encoding filaggrin, and in the regulation of Th cell differentiation (e.g., toward a Th2-dominant phenotype in CRS with nasal polyps) are areas of active investigation. A more comprehensive genetic characterization of patient cohorts could enable risk stratification and identify novel therapeutic targets for specific endotypes of CRS.

4.24. Environmental influences

The external environment serves as a constant source of challenge to the sinonasal epithelium. Chronic exposure to airborne pollutants, including particulate matter (PM2.5 and PM10), nitrogen dioxide, and ozone, has been correlated with an increased prevalence and severity of CRS. These pollutants are thought to induce oxidative stress, directly damage the ciliated respiratory epithelium, and act as adjuvants that potentiate allergic inflammation. Occupational exposures represent another critical dimension; individuals in professions involving dust (e.g., wood and flour), chemical fumes, and other irritants are demonstrably at higher risks. Moreover, the role of the indoor microbiome and early-life exposure to a diverse microbial environment (the "hygiene hypothesis") may be pivotal in shaping immune tolerance, with implications for the development of atopic conditions, including CRS.

4.25. Lifestyle and comorbid behaviors

Lifestyle choices and associated comorbidities are modifiable factors that can significantly impact CRS incidence and disease course. Smoking, both active and passive, is a well-established risk factor, as it paralyzes cilia, increases mucus viscosity, and promotes neutrophilic inflammation. Dietary patterns may also play a role; diets high in refined sugars and saturated fats may promote a pro-inflammatory state, while diets rich in antioxidants and omega-3 fatty acids could have a protective effect. The management of comorbid conditions is equally critical. Poorly controlled gastroesophageal reflux disease may contribute to CRS through the direct irritation of the posterior pharynx and sinonasal mucosa with refluxate. Furthermore, psychological stress, which can dysregulate immune function, and inadequate sleep are increasingly recognized as factors that may exacerbate symptom burden and hinder treatment response.

5. Conclusion

Microbial interactions are central to the pathogenesis of CRS, influencing disease onset, progression, and persistence. CRS can be reframed as a disease of failed host–microbe communication, driven by dysregulated immune responses with cancer-like features. This review highlights the role of microbial interactions in the pathogenesis of CRS, focusing on the interaction between the host immune system and microbial communities. We emphasize the importance of AMPs and antimicrobial lipids in maintaining sinus homeostasis and their potential disruption in CRS. This review synthesizes current knowledge on how microbial interactions influence immune responses, including the modulation of AMPs and lipids, and their contribution to chronic inflammation. We also highlight emerging therapeutic strategies targeting microbial–immune interactions to restore sinus health. By integrating findings from recent studies, this article provides a comprehensive overview of the molecular mechanisms underlying CRS and highlights the importance of microbial–immune interactions in disease progression and management.

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Author contributions

Conceptualization: Saeid Besharati
Writing–original draft: Ghazaleh Omidvar, Saeid Besharati, Shirin Esmaili Dolabinezhad
Writing–review & editing: Marjan Sistani, Saeid Besharati

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REVIEW ARTICLE

From cancer treatment strategies to survivorship care: Advances in integrating multi-omics and artificial intelligence

Ling Yin[1,2], Bao Chun Cheng[3], Xueyi Wang[3], Li Xia[3], Lei Ye[3], Xuefei Ji[3], Hui Wang[3], and Lu Gao[3]*

[1]Department of Medicine, Weill Cornell Medicine, Cornell University, New York, United States of America

[2]Department of Epidemiology and Biostatistics, College of Medicine, Nanjing Medical University, Nanjing, Jiangsu, China

[3]Department of Neurosurgery, First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China

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***Corresponding author:**
Lu Gao
(yfy1082035@fy.ahmu.edu.cn)

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Abstract

Advances in oncology are successfully shifting clinical focus beyond initial tumor control to address the complex, lifelong needs of a growing survivor population. This review examines the evolution of therapeutic paradigms, dissecting the mechanistic underpinnings and clinical impact of modern immunotherapies and gene-based modalities. It further addresses the multifaceted challenges in survivorship care, from managing persistent morbidity and psychological distress to overcoming systemic barriers in care coordination. Central to this discussion is the promise of multi-omics—which offers a high-resolution lens into tumor and host biology—to enable preemptive risk profiling. Concurrently, artificial intelligence (AI) emerges as the computational engine to distill these complex datasets into actionable intelligence for personalized surveillance. Furthermore, this review explores the translational hurdles facing AI, with a critical examination of inherent vulnerabilities, such as algorithmic bias, model opacity, and ethical pitfalls, in clinical deployment. By synthesizing these domains, this article provides a conceptual framework to steer the evolution of evidence-based, equitable, and patient-focused survivorship care.

Keywords: Cancer treatment strategy; Cancer survivorship care; Multi-omics; Artificial intelligence; Personalized medicine

1. Introduction

The management of cancer embodies a dynamic interplay between scientific innovation and evolving clinical imperatives. Grounded in the hallmarks of genomic instability and dysregulated cell proliferation, cancer continues to impose a significant global health burden, necessitating relentless refinement across the entire spectrum of oncological care, from prevention to long-term management.[1,2] The therapeutic arsenal has been fundamentally transformed over the past decades, moving decisively from broadly cytotoxic regimens toward a precision medicine paradigm defined by molecularly targeted strategies.[3,4]

This shift finds a powerful exemplar in the rise of cancer immunotherapy. Modalities such as immune checkpoint inhibitors (ICIs), which disrupt pivotal suppressive pathways like programmed cell death 1/programmed death ligand 1, and the engineering of chimeric antigen receptor (CAR) T-cells, are designed to overcome tumor-mediated immune suppression and reinvigorate endogenous antitumor responses, yielding profound and durable remissions across diverse cancer types.[5,6] The strategic incorporation of these novel agents with conventional treatments has substantially augmented survival rates, thereby propelling the oncology community into a critical new era: the transition from acute, tumor-focused care to long-term, patient-centered survivorship care.[7]

Consequently, the global cohort of cancer survivors is expanding, presenting healthcare systems with a complex and escalating challenge.[8] Contemporary survivorship care constitutes a comprehensive, longitudinal endeavor focused on mitigating the long-term and late-onset effects of both the malignancy and its often-toxic treatments. Its core mandate spans the prevention of disease recurrence and secondary neoplasms, vigilant surveillance for a spectrum of physical and psychological sequelae, active intervention for symptom control, and seamless coordination between oncology specialists and primary care providers.[9,10] Prevailing care models, however, frequently prove inadequate in addressing profound physical morbidities (e.g., persistent fatigue, neuropathies, pain syndromes), significant psychological distress (e.g., anxiety, depression, fear of recurrence), and the pervasive socioeconomic burdens, including financial toxicity and challenges in social reintegration.[11,12] The development of integrated, evidence-based, and personalized survivorship frameworks is therefore an urgent priority to optimize functional outcomes and quality of life for this burgeoning population.

Within this context, the convergence of two technological frontiers—multi-omics and artificial intelligence (AI)—holds exceptional potential to bridge the gap between therapeutic breakthroughs and comprehensive survivorship. Multi-omics profiling, through the concerted application of genomics, transcriptomics, proteomics, and metabolomics, furnishes an unprecedented, holistic vista of both tumor pathophysiology and the individual's host environment.[13] This integrative approach facilitates a deeper interrogation of resistance mechanisms, unveils biomarkers predictive of late effects, and informs the creation of dynamic, personalized surveillance strategies anchored in individual risk.[14] In parallel, AI and machine learning (ML) provide the indispensable computational power to decipher the immense complexity inherent in multi-omics data, electronic health records, and medical imaging.[15] These tools are being leveraged to augment diagnostic acuity, forecast individual therapeutic responses and toxicities, automate administrative workflows to enhance care coordination, and empower patients through intelligent digital tools.[16]

The translation of these sophisticated technologies into routine clinical practice, however, is fraught with formidable challenges. The multi-omics field must contend with issues of data standardization, integrative bioinformatics, and consequential biological interpretation.[16,17] Similarly, the widespread adoption of AI is tempered by valid concerns regarding algorithmic bias, model interpretability (the "black box" problem), data privacy, and the imperative for robust, prospective clinical validation.[17,18] A critical and discerning evaluation is thus paramount to translate their considerable promise into tangible clinical benefit.

The successful transition from acute cancer therapy to long-term survivorship care unveils a critical gap: The lack of a systematic framework that integrates technological advances with persistent clinical and ethical challenges. This review is motivated by the urgent need to synthesize these disparate domains and guide the evolution of next-generation survivorship models. To this end, it makes four primary contributions. First, it synthesizes the mechanistic evolution of cancer therapeutics and delineates the complex landscape of survivorship challenges they have engendered. Second, it provides a critical appraisal of the synergistic roles of multi-omics profiling and AI in enabling precision survivorship care. Third, it analyzes the formidable technical, translational, and ethical hurdles that currently impede the routine clinical adoption of these technologies. Finally, it proposes a forward-looking roadmap to navigate these barriers, with the ultimate goal of steering the field toward evidence-based, equitable, and patient-centered survivorship care ecosystems.

This review is crafted for clinical oncologists, translational researchers, and digital health innovators seeking a nuanced perspective on the integrated future of cancer care. This review critically synthesizes advances in cancer therapeutics, delineates the intricacies of modern survivorship, and places particular emphasis on the synergistic roles of multi-omics and AI. Distinct from a merely descriptive compilation, this work furnishes a balanced appraisal of both the capabilities and constraints of these technologies, with the ultimate objective of guiding the creation of effective, equitable, and patient-centered oncology care models.

2. The evolution of modern cancer treatment strategies

The landscape of cancer intervention has been radically reconfigured, evolving from an era dominated by empirical cytotoxics to a new age defined by mechanism-driven therapeutics.[18] This transition mirrors a deeper comprehension of oncogenic processes, enabling the design of interventions with enhanced specificity and a more favorable therapeutic index, thereby accelerating the adoption of precision oncology.[18,19] The subsequent sections provide a critical appraisal of the foundational modalities that constitute modern cancer care, with a dedicated focus on their operational mechanisms and clinical implementation—advances that, in their success, inherently demand the concurrent development of sophisticated survivorship frameworks.

2.1. The immunotherapy revolution

2.1.1. ICIs

ICIs constitute a landmark achievement in oncology, functioning through the targeted disruption of intrinsic immune regulatory circuits. These biologic agents, primarily monoclonal antibodies, selectively antagonize inhibitory receptors, such as programmed cell death-1 and cytotoxic T-lymphocyte-associated protein 4, and molecular pathways frequently co-opted by malignancies to induce T-cell exhaustion and evade immune destruction.[20,21] By impeding these negative signals, ICIs reinstate potent antitumor immunity and have demonstrated remarkable, durable clinical activity across a spectrum of cancers, including melanoma and non-small cell lung carcinoma.[22,23] Nonetheless, their application is circumscribed by heterogeneous response patterns and a unique spectrum of immune-related adverse events, underscoring the pressing need for reliable predictive biomarkers and refined patient selection algorithms.[24,25] While ICIs represent a paradigm shift, their clinical utility is constrained by unpredictable efficacy and toxicity. Future development hinges on the discovery of robust composite biomarkers that integrate tumor genomics with host immune contexture to guide personalized application.

2.1.2. Adoptive cell transfer

Adoptive cell transfer represents a pinnacle of personalized cancer immunotherapy, epitomized by CAR-T therapy. This sophisticated modality involves the genetic reprogramming of a patient's own T lymphocytes to express synthetic receptors that target specific tumor-associated antigens, such as CD19 in B-cell malignancies.[26,27] The process generates a potent, expanded population of cytotoxic T-cells capable of mediating antigen-directed tumor cell lysis. Successive iterations of CAR-T constructs have incorporated co-stimulatory domains (e.g., CD28, 4-1BB) to augment their *in vivo* persistence and cytotoxic efficacy.[28,29] In a complementary approach, T-cell receptor-engineered T-cell therapy leverages the specificity of natural T-cell receptors to recognize intracellular tumor antigens presented by major histocompatibility complexes, showing considerable promise for targeting solid tumors.[30,31]

2.2. Gene therapy: A vectorized approach

Recombinant adeno-associated virus has emerged as a leading vector platform for *in vivo* gene delivery in oncological applications, prized for its favorable safety profile and capacity for sustained transgene expression.[32,33] Engineered recombinant adeno-associated virus vectors can be armed with a diverse array of therapeutic transgenes—encoding entities such as tumor suppressors, anti-angiogenic factors, and immunomodulatory cytokines—for direct localized delivery to tumor sites.[34,35] This strategy of *in situ* gene transfer enables the targeted production of biologic agents within the tumor microenvironment, minimizing systemic exposure and potentially bypassing conventional drug resistance mechanisms. Contemporary research is concentrated on refining vector tropism to enhance tumor selectivity and overcoming the challenge of pre-existing neutralizing antibodies in patient populations.[36,37]

2.3. Molecular targets as therapeutic levers

The development of molecularly targeted agents represents a cornerstone of precision oncology, focusing on the specific pathways that fuel tumorigenesis.[19] The clinical deployment of these agents, however, reveals a complex interplay of opportunity and challenge. For instance, while dysregulation of the human epidermal growth factor receptor (HER) tyrosine kinase network provides a compelling therapeutic rationale in cancers such as HER 2-amplified breast and gastric carcinomas, the efficacy of targeted inhibitors is frequently constrained by adaptive pathway reactivation and acquired resistance mutations.[38,39] Similarly, targeting the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome presents a unique conundrum due to its context-dependent role in tumorigenesis—where it can promote progression in some malignancies while exerting antitumor effects in others, necessitating meticulous patient stratification.[40,41] This functional duality represents a significant unresolved debate, complicating the development of NOD-, LRR-, and pyrin domain-containing protein 3-targeted therapies and underscoring the need for precise biomarkers to stratify patients based on tumor context. Furthermore, cancer stem cells persist as a formidable therapeutic barrier, implicated in treatment resistance and disease relapse. These cells are maintained by evolutionarily conserved developmental pathways, such as Wnt/β-catenin and Hedgehog signaling, suggesting that their targeted disruption may be indispensable for achieving durable disease control.[42,43]

2.4. Synthesis and forward perspective

The collective progress in immunotherapy, gene therapy, and molecularly targeted agents has fundamentally recalibrated the management of cancer. The strategic deployment of these modalities, particularly within rational combination regimens, has markedly improved clinical outcomes across a diverse range of malignancies.[6,44] This very therapeutic success, however, has engendered a new set of challenges, giving rise to a growing and complex population of survivors with multifaceted long-term needs.[7]

3. The landscape and challenges of cancer survivorship care

The remarkable achievements in cancer therapeutics, while unequivocally improving survival trajectories, have concurrently unveiled a new dimension of clinical complexity: the long-term management of cancer survivorship. As this global population continues to expand, healthcare systems are confronted with the intricate task of addressing multifaceted needs that extend far beyond the conclusion of primary anticancer treatment.[7] Modern cancer survivorship care embodies a holistic, proactive approach dedicated to monitoring and alleviating the long-term and late-arising consequences of cancer and its treatments, promoting overall health, and optimizing quality of life across the entire survivorship continuum.[8]

The architecture of contemporary survivorship care is built upon four foundational pillars: first, the prevention of recurrent and second primary cancers; second, systematic surveillance for cancer recurrence and the detection of late-onset complications; third, active intervention for consequences related to cancer and its treatment; and fourth, coordinated care between oncology specialists and primary care services to ensure continuity and comprehensiveness.[9] This structured paradigm acknowledges survivorship as a distinct and prolonged phase of the cancer journey, demanding specialized knowledge, protocols, and resources to effectively address its unique challenges.

The clinical reality for survivors is often marked by a constellation of diverse and persistent issues. Physical sequelae represent a significant burden, with a high prevalence of cancer-related fatigue, chemotherapy-induced peripheral neuropathy, chronic pain conditions, and various organ-specific toxicities that can profoundly impair daily functioning and diminish quality of life.[10] In parallel, psychological distress manifests as clinically significant anxiety, depression, and particularly, the fear of cancer recurrence—a pervasive concern that can persist indefinitely after active treatment ends, casting a long shadow over a survivor's life.[11] These clinical challenges are frequently compounded by socioeconomic determinants, most notably financial toxicity stemming from treatment-related expenses, employment disruption, and ongoing healthcare costs, which collectively erect substantial barriers to accessing optimal survivorship care.[45]

Systemic and infrastructural barriers further impede the effective delivery of survivorship care. The transition from active treatment to long-term follow-up represents a critically vulnerable period, often characterized by fragmented communication and poorly defined care responsibilities between oncology specialists and primary care providers.[46] A scarcity of standardized, evidence-based protocols for monitoring and managing late effects, coupled with insufficient clinician training and education regarding survivorship care, frequently leads to the suboptimal recognition and management of treatment-related complications. Moreover, prevailing care models often fail to adequately conceptualize and address the intricate interplay between the physical, psychological, and social determinants that collectively define the survivor experience.[47] These multifaceted challenges underscore the critical imperative for innovative, scalable, and personalized approaches to survivorship care. The development of integrated, evidence-informed frameworks that strategically leverage emerging technologies represents an urgent and non-negotiable priority within modern oncology.

4. The integrative role of multi-omics in cancer survivorship care

The transformative success of contemporary cancer therapies has catalyzed the emergence of a growing survivor population confronting distinct long-term health challenges. While traditional surveillance paradigms remain heavily reliant on imaging and symptomatic presentation, multi-omics technologies are poised to redefine precision survivorship through comprehensive molecular phenotyping. This integrative methodology, which concurrently analyzes genomic, transcriptomic, proteomic, and metabolomic data, generates dynamic molecular signatures capable of guiding individualized survivorship management strategies.[12,13]

4.1. Molecular profiling for recurrence risk stratification

The assimilation of multi-omics data enables a sophisticated recalibration of risk assessment in cancer survivors through advanced molecular surveillance. The analysis of circulating tumor DNA (ctDNA) has emerged as a powerful tool for detecting minimal residual disease (MRD), with substantial evidence demonstrating its capacity to identify molecular recurrence with a significant lead time compared to conventional radiological modalities.[48] For instance, in early-stage non-small cell lung cancer, patient-specific phylogenetic ctDNA profiling after surgery detected MRD with a median lead time of 70 days before radiographic relapse. In this study, 93% (13/14) of patients who later experienced clinical recurrence had detectable ctDNA post-operatively, highlighting the high predictive value of ctDNA-based MRD assessment for impending relapse.[48] This utility is further evidenced by studies utilizing methylation signatures[49] and multi-analyte approaches[50] for sensitive cancer detection.

The predictive utility of ctDNA monitoring is further strengthened when integrated with other molecular analytes. Integrated interrogation of DNA methylation patterns with fragmentomic characteristics in cell-free DNA enhances sensitivity for early recurrence detection across multiple cancer types.[49] Furthermore, the incorporation of protein biomarker profiles alongside ctDNA quantification yields complementary information that refines risk stratification, proving particularly valuable in early-stage diseases where ctDNA abundance may be minimal.[50]

Beyond liquid biopsies, tissue-based multi-omics approaches furnish unique insights into recurrence mechanisms. Spatial transcriptomic profiling of histologically normal adjacent tissues reveals molecular field effects that may predispose to local recurrence,[51] while proteomic characterization of the tumor microenvironment uncovers immune contextures associated with durable treatment responses.[52] Single-cell RNA sequencing further delineates the cellular heterogeneity within residual disease populations, identifying transcriptional programs correlated with metastatic competence.[53]

The full potential of multi-omics is realized through the integration of these disparate data types via advanced computational methodologies. ML algorithms can synthesize genomic, transcriptomic, and proteomic features to generate composite recurrence risk scores that outperform predictions grounded solely in clinicopathological parameters.[54] Nevertheless, the clinical translation of these integrated models confronts substantial hurdles, including technical batch effects across omics platforms, the necessity for large, meticulously annotated cohorts for algorithm training, and the biological interpretation of complex, algorithmically-derived signatures. These sophisticated models facilitate genuinely personalized surveillance approaches, optimizing monitoring intensity and modality based on individual molecular risk profiles rather than population-derived guidelines.[55] The promise of multi-omics in recurrence risk stratification is clear, yet its clinical translation remains in early stages. Widespread adoption requires not only technical standardization and cost reduction but also prospective validation proving that risk-adapted surveillance improves survival or quality of life compared to standard follow-up.

4.2. Prediction and management of long-term treatment-related complications

Multi-omics approaches are revolutionizing the prediction and management of treatment-related sequelae through comprehensive molecular profiling of individual susceptibility and incipient pathological processes.[12] Pharmacogenomic investigations have identified numerous genetic variants that modulate vulnerability to specific treatment toxicities, enabling risk-adapted therapeutic selection and tailored surveillance strategies.[56] A prime example is a coding variant in the *RARG* gene (rs2229774), which has been robustly associated with a significantly increased risk of anthracycline-induced cardiotoxicity in childhood cancer survivors, illustrating how pre-emptive genotyping can guide personalized therapy and monitoring strategies.[56] Recent advances in genome-wide association studies have substantially expanded our understanding of the genetic architecture underlying complications such as chemotherapy-induced neurotoxicity and radiation-induced tissue damage.[57]

Metabolomic profiling delivers a dynamic readout of physiological perturbations following oncological treatments, offering distinctive insights into the mechanisms of long-term complications. Characteristic metabolic signatures have been discerned for diverse treatment-related toxicities.[58] Analogously, proteomic analyses reveal circulating biomarkers that reflect ongoing processes of tissue injury and repair, creating opportunities for pre-emptive detection and intervention.[59] In childhood cancer survivors, exercise intolerance—as measured by low peak oxygen uptake—is prevalent and is associated with all-cause mortality, with hazard ratios as high as 3.93 (95% confidence interval: 1.09–14.14). Impaired organ systems, including cardiac (e.g., abnormal global longitudinal strain), autonomic (chronotropic incompetence), pulmonary (forced expiratory volume in 1 s < 80%), and muscular (reduced quadriceps strength), contribute to this intolerance, underscoring the potential role of multimodal biomarker assessment in identifying high-risk survivors for targeted intervention.[59]

The convergence of multiple omics layers generates compelling insights for complex conditions, such as cancer therapy-related cardiac dysfunction.[60] Combined genomic, proteomic, and metabolomic profiling has delineated distinct molecular subtypes of cardiac injury, each possessing characteristic biomarker signatures and clinical trajectories.[60] These refined molecular classifications enable more targeted monitoring and preventive strategies predicated on the specific pathophysiological processes active in individual patients.[61]

The preceding analysis delineates the independent potential of genomics, transcriptomics, proteomics, and metabolomics in elucidating the molecular foundations of survivorship outcomes. However, the translation of this multidimensional data into clinically actionable insights necessitates a robust computational scaffold. As illustrated in Figure 1, the integration of these heterogeneous multi-omics data streams constitutes the foundational input for AI models, which function as the central analytical engine for prediction and inference.

4.3. Translational hurdles: Cost, feasibility, and accessibility

Despite its transformative potential, the widespread integration of multi-omics into routine survivorship care faces substantial practical barriers, with cost, feasibility, and accessibility constituting the most significant clinical hurdles.

Cost remains a primary obstacle. Comprehensive multi-omics profiling—encompassing whole-genome sequencing, transcriptomics, proteomics, and metabolomics—carries a high financial burden. While costs are decreasing, they are still prohibitive for most healthcare systems outside of research settings, and reimbursement mechanisms are underdeveloped. The economic case for widespread adoption relies on demonstrating not just clinical utility, but also long-term cost-effectiveness through prevented recurrences and reduced late-effect management.

Feasibility challenges span the entire workflow. Pre-analytical variables such as sample collection, processing, and storage require stringent standardization to ensure data quality and reproducibility across different centers. The analysis and interpretation of multi-omics data demand specialized bioinformatics expertise, which is scarce in many clinical environments. Integrating these complex data streams into existing electronic health records and clinical decision support systems presents further technical and operational complexities.

Accessibility concerns threaten to exacerbate existing health disparities. The infrastructure and expertise required for advanced omics profiling are concentrated in large, academic tertiary care centers. This creates an "omics divide," where survivors treated in community or rural

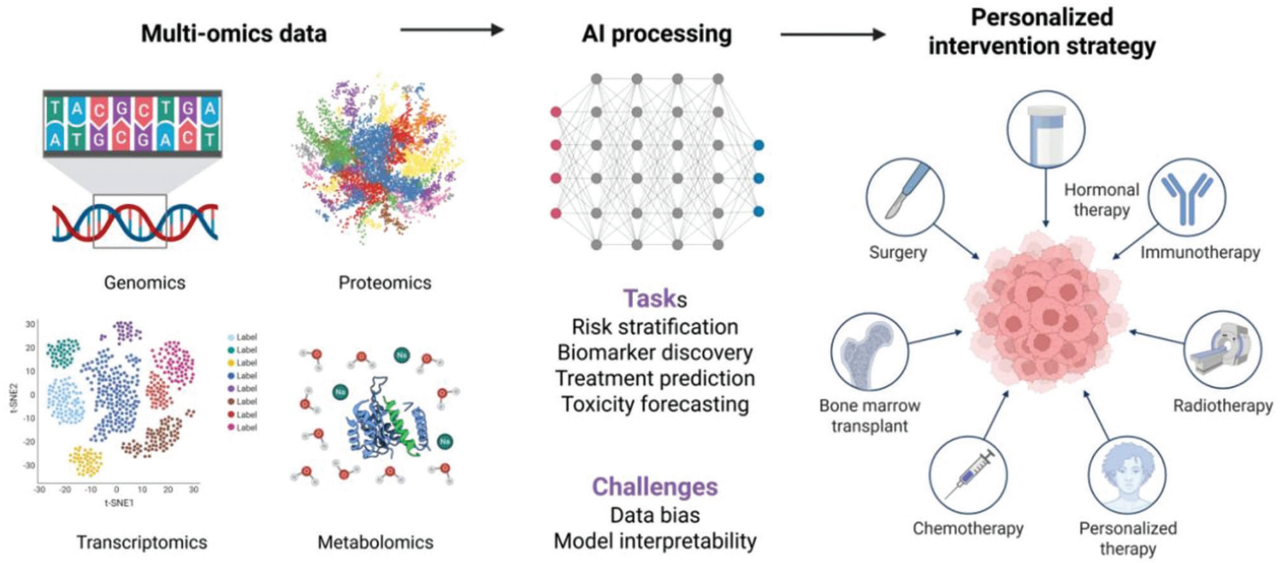

Figure 1. A conceptual framework for AI and multi-omics in survivorship care. This schematic outlines the pathway from multi-omics data acquisition to computational analysis and the subsequent delivery of personalized SCPs. The AI processing stage highlights central analytical tasks while acknowledging prevailing limitations. The final output enables precision interventions tailored to the individual survivor's dynamic molecular risk profile. Created with BioRender.com, Ling Yin (2026), https://app.biorender.com/illustrations/68e4bbdd87048e01114d2269.
Abbreviations: AI: Artificial intelligence; SCPs: Survivorship care plans.

settings, or in low- and middle-income countries, may not have access to these precision tools. Without deliberate policy interventions, equitable access frameworks, and the development of simplified, point-of-care omics technologies, the promise of precision survivorship risks becoming a privilege for the few, rather than a standard for all.

Addressing these translational hurdles is therefore not a secondary concern but a prerequisite for the ethical and sustainable implementation of multi-omics in cancer survivorship. Future efforts must prioritize the development of cost-effective assays, automated analysis pipelines, and scalable delivery models that can extend the benefits of molecular profiling to diverse and underserved survivor populations.

5. The integration of AI into cancer survivorship care

The inadequacy of conventional, generalized survivorship models necessitates a fundamental transition toward precision care. In this context, AI arises as a transformative enabler. Augmenting the foundational data derived from multi-omics, AI supplies the computational prowess to decode profound complexity, forecast individual risks, and automate care processes, thereby bridging the chasm between innovative therapy and comprehensive long-term wellness (Figure 1).

5.1. AI in multi-omics data integration and biomarker discovery

The molecular underpinnings of recurrence risk and late effects, as elucidated through multi-omics, yield datasets of immense intricacy. AI and ML are becoming indispensable for distilling this information into clinically actionable intelligence.

A primary application lies in the fusion of disparate omics layers—genomic, transcriptomic, proteomic, metabolomic—to construct unified predictive models. Deep learning architectures are capable of identifying non-linear interactions between diverse molecular features that remain opaque to conventional statistical methods. For instance, ML models can amalgamate somatic mutation profiles with plasma proteomic data to generate a composite risk score for recurrence that significantly surpasses the predictive performance of models based solely on clinical parameters.[13] Furthermore, AI demonstrates exceptional proficiency in *de novo* biomarker discovery. In the analysis of cell-free DNA, ML algorithms can not only detect the presence of ctDNA but also harness fragmentomic patterns and methylation signatures to predict MRD and imminent relapse with superior sensitivity and lead time compared to standard techniques.[48,49] AI can also be deployed to anticipate specific late effects; for example, by analyzing pre-treatment genomic data and on-treatment metabolomic shifts, models can stratify patients according to their risk for chemotherapy-induced cognitive impairment or cardiotoxicity, enabling preemptive interventions.[56,58] A critical limitation, however, is that many of these AI-discovered biomarkers and models remain associative rather than demonstrative of causation. Their clinical adoption, therefore, hinges on subsequent rigorous biological validation in independent cohorts and functional studies to elucidate the underlying mechanisms, ensuring they reliably guide clinical decision-making. This underscores a fundamental tension in AI-driven discovery: the trade-off between identifying powerful statistical associations and establishing biologically causal, mechanistically interpretable relationships—a debate central to the field's credibility and clinical translation. AI is pivotal for unlocking patterns within complex multi-omics data, moving beyond simple association toward predictive modeling. However, the field must evolve to develop more efficient, interpretable, and biologically grounded models to ensure robust clinical translation.

5.2. Automated monitoring of late effects in radiology and pathology

Many long-term consequences of cancer therapy manifest as structural alterations in organs, detectable through medical imaging and histopathological examination. AI-powered computer vision is redefining the surveillance of these sequelae.

In radiology, radiomics—the high-throughput extraction of quantitative features from medical images—facilitates the objective and sensitive monitoring of tissue damage. For instance, a deep learning model can be trained to automatically quantify the progression of radiation-induced pulmonary fibrosis on serial chest computed tomography scans, tracking subtle changes long before they become discernible to the human eye. Similarly, in cardio-oncology, AI algorithms applied to echocardiograms or cardiac magnetic resonance imaging can precisely measure myocardial strain, enabling the early identification of subclinical cardiac dysfunction in survivors exposed to cardiotoxic agents such as anthracyclines or HER 2-targeted therapies.[60] In digital pathology, AI models perform automated "digital biopsies." These systems can screen histopathological slides for specific treatment-related toxicities, such as identifying features of immunotherapy-related colitis in colon biopsies or drug-induced liver injury in liver biopsies, thereby offering a scalable tool for systematic tissue-level surveillance.[62] It is crucial to note, however, that the regulatory approval and routine clinical deployment of such AI tools significantly lag behind their technical development. A key barrier is the necessity for prospective, randomized trials to conclusively demonstrate that AI-driven monitoring leads to improve patient outcomes, such as reduced morbidity or enhanced quality of life, rather than merely increasing the detection of incidental findings. AI-driven image analysis offers a robust, objective method for tracking subclinical organ damage. Bridging the gap from technical promise to proven clinical benefit is the paramount challenge, demanding trials that measure impact on patient-centric outcomes, not just algorithmic performance.

5.3. AI-powered risk stratification and personalized care plan generation

The transition from active treatment to survivorship is a critical juncture that demands personalized planning. AI moves beyond static guidelines by generating dynamic, individual-specific SCPs.

The power of AI here lies in data fusion and dynamic updating. ML models can synthesize a patient's complete history—including tumor type and stage, treatment modalities and cumulative doses, genomic susceptibility variants, current comorbidities, and real-time patient-reported outcomes—to generate a dynamic risk profile. This profile estimates the personalized probability of recurrence, second primary cancers, and specific late effects (e.g., fatigue, anxiety, lymphedema). This computational risk assessment can then directly inform the SCP. For example, an AI system could automatically generate a tailored plan recommending: a specific imaging surveillance schedule based on recurrence risk; a customized exercise regimen to combat fatigue; and targeted screening for psychological distress based on the patient's profile.[63,64] Emerging applications of large language models can further translate these structured recommendations into an easy-to-understand, patient-facing SCP document, improving health literacy and adherence.[65,66]

5.4. AI tools for patient self-management and remote monitoring

Effective survivorship care extends beyond the clinic into the patient's daily life. AI-driven digital health tools are empowering survivors and creating a continuous feedback loop between patients and their care teams.[15]

Conversational AI agents and chatbots provide 24/7 access to vetted information and support. They can answer questions about medication side effects, diet, and managing common symptoms such as pain or nausea. More advanced systems can conduct proactive check-ins, using natural language processing to screen for worsening symptoms or psychological distress (e.g., fear of cancer recurrence) and escalating alerts to human providers when necessary.[67,68] When integrated with data from wearable devices (e.g., smartwatches), AI algorithms can monitor physiological parameters such as heart rate, sleep patterns, and activity levels. Deviations from an individual's baseline can serve as early warning signs for complications, such as arrhythmias, infections, or decompensating heart failure, facilitating timely intervention.[69]

The applications of AI and multi-omics discussed herein present a promising yet uneven landscape for addressing the diverse challenges in cancer survivorship. Table 1 provides a synthesized and critical overview, mapping specific survivorship challenges to their corresponding technological solutions, and crucially, evaluating the current level of evidence and primary limitations associated with each approach. This summary underscores that while some applications, such as ctDNA-based recurrence monitoring, are advancing toward clinical adoption, others remain primarily in the research or conceptual phase, facing significant technical and translational hurdles.

6. Challenges and future perspectives

Despite the compelling potential of integrated multi-omics and AI, their trajectory toward widespread clinical integration is fraught with formidable challenges that warrant acknowledgment. Transitioning from robust proof-of-concept to routine practice mandates overcoming a complex array of technical, translational, and ethical hurdles.

Notwithstanding these present impediments, the overarching ambition of this technological convergence is to inaugurate a fundamentally new paradigm for cancer survivor care. As conceptualized in Figure 2, we envisage a future centered on an AI-driven, dynamic closed-loop ecosystem for precision survivorship. This intelligent system transcends the limitations of a static, one-time plan by evolving into a self-refining learning entity that perpetually optimizes itself through continuous data assimilation, ensuring clinical decisions remain precisely synchronized with the survivor's evolving health status and individual needs.

This conceptual model illustrates the continuous cycle of data aggregation (Figure 2A), AI-powered analytics (Figure 2B), personalized intervention delivery (Figure 2C), and real-world monitoring with feedback (Figure 2D). Multi-omics and clinical data are integrated to enable risk prediction and personalized recommendations under human oversight and an ethical framework. The system dynamically refines care plans based on adherence tracking, symptom changes, and outcome measurements,

Table 1. Multi-omics and AI in cancer survivorship care: Applications and translational challenges

Survivorship care challenge	Multi-omics and data enablers	AI applications	Key limitations
Recurrence and second cancers	Circulating tumor DNA analysis; integrated genomic and epigenomic signatures; proteomic and metabolomic profiling	Machine learning models for dynamic risk stratification by integrating multi-omics and clinical data; deep learning for biomarker discovery from complex data	High cost and lack of standardized assays limit widespread adoption; prospective trials are needed to confirm that AI-driven interventions improve patient outcomes
Physical sequelae	Pharmacogenomic variants for toxicity susceptibility; metabolomic and proteomic profiles for early pathophysiological changes	Natural language processing for automated toxicity extraction and grading from electronic health records; computer vision for subclinical organ dysfunction detection in medical images	Predictive models are primarily associative and lack causal validation; performance and generalizability can be poor in underrepresented patient populations
Psychological distress	Polygenic risk scores for anxiety and depression; proteomic biomarkers of chronic stress response	Natural language processing for screening psychological distress from patient-reported outcomes and clinical notes; conversational AI agents for scalable support and triage of at-risk patients	Raises significant ethical and data privacy concerns; risk of algorithmic bias and requires rigorous human clinical oversight
Fragmented care coordination	Structured data from electronic health records on treatment history and comorbidities	Automated generation of personalized survivorship care plans using large language models; predictive analytics for care pathway optimization and resource allocation	Faces significant integration barriers with the existing healthcare information technology infrastructure; regulatory pathways for the clinical use of autonomous AI tools are unclear

Abbreviation: AI: Artificial intelligence.

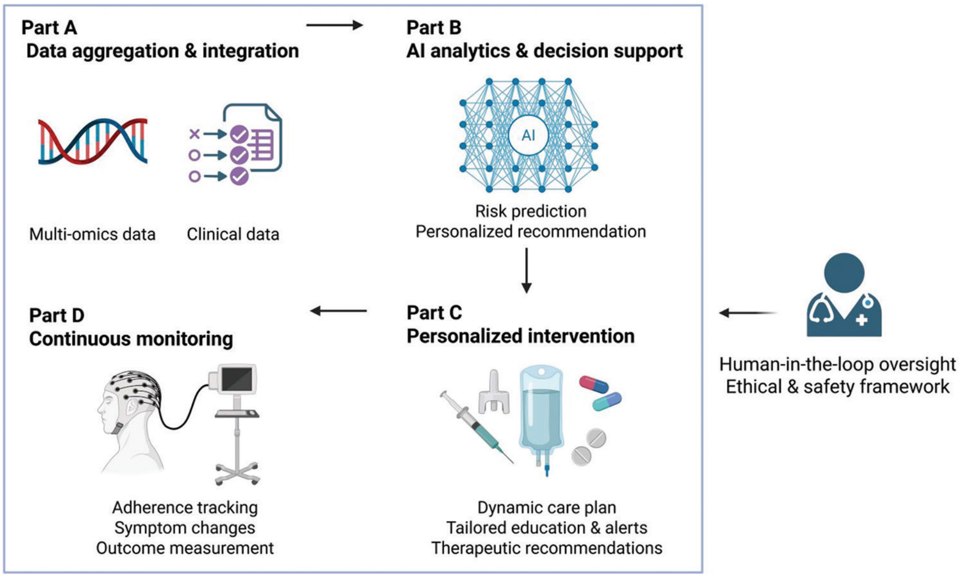

Figure 2. (A-D) A dynamic closed-loop framework for AI-driven precision survivorship care. Created with BioRender.com, Ling Yin (2026), https://app.biorender.com/illustrations/68e4ea8cea42bbf99d5bad06.
Abbreviation: AI: Artificial intelligence.

ensuring adaptive and patient-centered survivorship management.

6.1. Technical and analytical hurdles

The field of multi-omics must overcome issues of data standardization across different sequencing platforms and laboratories, and the computational complexity of integrative analysis.[16] For instance, harmonizing genomic variants called from different pipelines or integrating transcriptomic data from fresh-frozen versus formalin-fixed paraffin-embedded tissues remains a non-trivial challenge. For AI, the "black box" problem—where model decisions are not easily interpretable by clinicians—remains a significant barrier to trust and adoption.[17] This creates a core contradiction: the most complex, high-performing models (e.g., deep neural networks) are often the least interpretable, forcing a difficult choice between predictive accuracy and clinical transparency. Without insight into the reasoning behind an AI's prediction of high recurrence risk, a clinician may justifiably hesitate to act upon it. Furthermore, AI models are highly susceptible to data bias; if trained on non-representative populations (e.g., predominantly of European ancestry or from tertiary care centers), they can perpetuate and even exacerbate existing healthcare disparities when deployed in more general settings.[70]

6.2. Clinical translation and ethical implementation

The transition from research to clinical practice requires robust, prospective validation in diverse patient cohorts to prove efficacy and cost-effectiveness. A model that predicts cardiotoxicity in a retrospective dataset must be validated in a prospective trial to see if it truly enables preemptive interventions that preserve cardiac function. Key implementation barriers include inadequate digital infrastructure in community oncology practices, data privacy concerns governing the sharing of sensitive multi-omics and health data, and the lack of regulatory frameworks tailored to adaptive AI systems that learn and evolve. This highlights a critical, unresolved debate: balancing the imperative for large, diverse datasets to train robust and equitable AI models against the equally vital need to protect patient autonomy and confidentiality. Ethically, the deployment of these technologies must be guided by principles of equity and transparency, ensuring that they augment, rather than replace, the clinician–patient relationship. This includes establishing clear accountability for AI-assisted decisions and ensuring that these tools do not widen the gap in cancer outcomes between affluent and underserved populations.[71]

6.3. A roadmap for the future: From integration to implementation

The future of cancer survivorship care lies not only in the synergistic convergence of therapeutic advances, deep molecular profiling, and intelligent computation but also in creating a sustainable ecosystem for their implementation. To this end, we propose a focused roadmap. First, prioritizing the development of explainable AI—a dedicated subfield aimed at solving the "black box" problem—is crucial. Explainable AI systems are designed to provide clinically intuitive justifications for their outputs, such as highlighting the specific genomic variants or imaging features that most contributed to a risk prediction.[72] Second, actively fostering interdisciplinary collaboration through shared funding initiatives and the creation of "translational triads" that bring together oncologists, data scientists, and bioethicists at the project inception stage. Finally, shifting the research paradigm to prioritize large-scale, pragmatic clinical trials that measure the real-world impact of these integrated approaches on patient-centric outcomes, such as quality of life, functional status, and financial toxicity.[73] By adhering to this roadmap, the field can transform the current promise into a tangible reality: The establishment of evidence-based, equitable, and patient-centered survivorship models that are as sophisticated and precise as the cancer treatments that made them necessary.

7. Conclusion

The oncology paradigm is undergoing a fundamental reorientation, shifting from a narrow focus on tumor eradication to a comprehensive framework dedicated to long-term survivorship care. This review has charted this evolution, underscoring how mechanistic breakthroughs in immunotherapy, gene therapy, and targeted agents have successfully created a large and growing population of survivors, thereby unveiling a complex new landscape of long-term and late-onset sequelae. In confronting these challenges, the integration of multi-omics data and AI has emerged as a cornerstone for the next generation of precision medicine. Multi-omics profiling furnishes a high-resolution, dynamic molecular portrait of the individual, elucidating the biological underpinnings of recurrence risk and treatment-related complications. Concurrently, AI provides the essential computational framework to distill these complex datasets into clinically actionable intelligence, enabling dynamic risk stratification, personalized surveillance protocols, and preemptive management strategies.

The translation of this technological potential into routine clinical practice, however, is contingent upon overcoming significant translational barriers. Foremost among these are the imperatives of ensuring robust data standardization, mitigating algorithmic bias to promote health equity, resolving the "black box" problem through explainable AI, and securing rigorous prospective validation of these tools against meaningful patient-centric outcomes. The objective is not the supplanting of clinical judgment but the creation of a synergistic, human-in-the-loop paradigm wherein data-driven insights augment clinical expertise to facilitate more informed and personalized decision-making.

In perspective, the future of cancer survivorship lies in the construction of a learning, adaptive healthcare system. The convergent application of therapeutic innovation, deep molecular phenotyping, and advanced computation holds the promise of transitioning survivorship care from a one-size-fits-all model to a dynamic, lifelong state of personalized health management. Realizing this vision mandates a concerted, interdisciplinary endeavor that unites oncologists, computational biologists, clinical trial specialists, and bioethicists. The ultimate metric of success for this integrated approach will be its demonstrable capacity to optimize the quality of survival, ensuring that advancements in cancer therapy translate directly into sustained well-being for the global community of survivors.

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Author contributions

Conceptualization: Ling Yin, Lu Gao
Visualization: Ling Yin
Writing – original draft: Ling Yin, Bao Chun Cheng, Xueyi Wang
Writing – review & editing: Li Xia, Lei Ye, Xuefei Ji, Hui Wang, Lu Ga

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ACCSCIENCE PUBLISHING

Global Translational Medicine

ORIGINAL RESEARCH ARTICLE

Wound-healing efficacy of *Libidibia ferrea* fruit-derived topical formulations in Wistar rats

Ana Karina Lima Alves Cerdeira[1,2], Francineide Pereira da Silva Pena[3], Cecília Rafaela Salles Ferreira[3], Sérgio Gabriell Leite Brito[1,4], Luiza Pinon Nery de Oliveira[1,4], Helison de Oliveira Carvalho[1,2], Ricardo Luiz Cavalcanti de Albuquerque Jr[5], Erdi Can Aytar[4], and José Carlos Tavares Carvalho[1,2,6]*

[1]Department of Biological and Health Sciences, Laboratory of Drug Research, Faculty of Pharmacy, Federal University of Amapá, Macapá, Amapá, Brazil

[2]Department of Biological and Health Sciences, Innovation Pharmaceutical Program, Faculty of Pharmacy, Federal University of Amapá, Macapá, Amapá, Brazil

[3]Basic Health Unit, Department of Biological and Health Sciences, Faculty of Nursing, Federal University of Amapá, Macapá, Amapá, Brazil

[4]Department of Horticulture, Faculty of Agriculture, Usak University, Mende/Merkez/Uşak, Türkiye

[5]Department of Health Sciences, Postgraduate Program in Health and Environment, Faculty of Medicine, Tiradentes University, Aracaju, Sergipe, Brazil

[6]Clinical Research Center, University Hospital, Federal University of Amapá, Macapá, Amapá, Brazil

Abstract

Libidibia ferrea var. *ferrea* (Mart. ex Tul.) L.P. Queiroz (syn. *Caesalpinia ferrea*), popularly known as jucá, is widely distributed in the Amazon region and northeastern Brazil and is conventionally used to treat various diseases, including diabetes, infections, and inflammatory conditions. This study evaluated the healing effects of topical formulations derived from *L. ferrea* fruits on cutaneous wounds induced in Wistar rats. Three topical preparations were assessed: A glycolic extract of *L. ferrea*, a fruit infusion formulation (InLf), and a carbopol gel formulation (GLf). The phytochemical compounds present in the formulations, such as tannins and flavonoids, are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which are essential for the healing process. Histopathological methods were used to evaluate healing, including analysis of epithelialization, granulation tissue formation, and collagen deposition. The results demonstrated that InLf and GLf significantly accelerated healing, with greater type I collagen deposition in the groups treated with these formulations, suggesting a more advanced tissue repair process. Among the formulations, InLf demonstrated the most pronounced healing effects, possibly due to its higher flavonoid content. The *in silico* study showed that the main markers of this species—gallic acid and ethyl gallate—are involved in the observed pharmacological response, modulating vascular proliferation and fibroblast activity. Overall, these findings reinforce the therapeutic potential of *L. ferrea* in the development of natural healing agents.

Keywords: Wound healing; *Libidibia ferrea*; Tannins; Flavonoids; Collagen; Skin wounds; Wistar rats

***Corresponding author:**
José Carlos Tavares Carvalho
(farmacos@unifap.br)

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1. Introduction

Wound healing is a dynamic and complex biological process that restores tissue integrity after injury. This process involves several phases: inflammation, cell proliferation, granulation tissue formation, and remodeling.[1,2] The development of new healing agents, particularly those derived from natural sources, has recently intensified, with a focus on medicinal plants that have a long tradition of widespread use.[3,4]

Libidibia ferrea var. *ferrea* (Mart. ex Tul.) LP Queiroz (synonym of *Caesalpinia ferrea*), popularly known as jucá, is a plant widely distributed in the Amazon region and northeastern Brazil, and is traditionally used to treat various diseases, including diabetes, infections, and inflammatory conditions[5,6] Scientific studies have demonstrated that the fruits, bark, and leaves of this plant possess a wide range of pharmacological properties, including anti-inflammatory, antimicrobial, and antioxidant activities, which are essential for healing.[7,8]

Several bioactive compounds have been isolated from *L. ferrea*, including gallic acid and methyl gallate, which have demonstrated antitumor potential in *in vitro* and *in vivo* assays, inhibiting the early activation of Epstein–Barr virus antigens and other models of carcinogenesis.[9,10] Furthermore, the aqueous extract of *L. ferrea* has demonstrated beneficial effects, including protection against gastric ulcers in experimental models, reduced gastric secretion volume, and promotion of tissue regeneration.[11,12]

The wound-healing activity of medicinal plants is of particular interest for the development of topical pharmaceutical formulations, such as gels and creams, that can accelerate wound healing and reduce the risk of complications, including infections.[3,13] In previous studies, the crude extract of *L. ferrea* demonstrated potent anti-inflammatory activity in carrageenan-induced edema models and analgesic properties, suggesting its use in managing pain associated with the inflammatory process.[7] In addition to its antimicrobial activity against oral pathogens, such as *Candida albicans* and *Streptococcus mutans*, these effects indicate that *L. ferrea* may effectively prevent infections during healing.[14] A literature review on the *L. ferrea* highlighted the need for studies investigating its healing activity.[15]

Furthermore, *L. ferrea* has demonstrated positive effects on immune regulation, particularly by stimulating myelopoiesis in infection and tumor models, which may enhance the immune response during wound healing.[16] This is particularly relevant for the clinical use of the plant, as an adequate immune response is crucial for tissue regeneration and the prevention of complications during the healing process.[1]

The present study aims to evaluate the wound-healing effects of two topical preparations of *L. ferrea* fruits, namely an Infusion formulation (InLf) and a Gel formulation (GLf) containing glycolic extract of *L. ferrea* (GcLf), in cutaneous wounds induced in Wistar rats. Previous studies indicate that the plant has great potential for wound healing due to its anti-inflammatory, antimicrobial, and immunomodulatory properties.[7,8,15] The evaluation of these pharmaceutical formulations aims to explore the therapeutic potential of *L. ferrea* for wound treatment and to contribute to the development of new natural healing agents.

2. Materials and methods

2.1. Plant material

The fruits of *L. ferrea* were collected from the Medicinal Plant Garden of the Institute of Studies and Research of the State of Amapá, from a specimen previously identified and cataloged in the Herbarium of the Agronomic Institute of the North (IAN185211, Fabaceae: *L. ferrea* var. *ferrea* [Mart. ex Tul.)] LP Queiroz) (Figure 1).

The *L. ferrea* species was used to extract GcLf for the preparation of InLf and GLf. The methods employed followed those described in the Phytotherapeutic Formulary of the Brazilian Pharmacopeia.[17] Tannins and flavonoids in the preparations were quantified using the method described by Gonzalez.[18]

2.2. Preparation of topical formulations

The GLf were prepared using a Carbopol base. GcLf of *L. ferrea* was incorporated into the base at a concentration of 5% (w/w). The choice of this concentration was based on

Figure 1. *Libidibia ferrea* collected from the Medicinal Plant Garden of the Institute of Studies and Research of the State of Amapá. The specimen was previously identified and cataloged in the Herbarium of the Agronomic Institute of the North (IAN185211, Fabaceae: *Libidibia ferrea* var. *ferrea* [Mart. ex Tul.] LP Queiroz).

previous pilot studies that demonstrated optimal stability and efficacy without causing skin irritation.[15] Total tannin and flavonoid contents were determined using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively, as described by Gonzalez.[18]

2.3. Animals used in the study

The Research Ethics Committee of the Federal University of Amapá approved the study under protocol no. 008/2017 of 08/23/2017. A total of 20 male Wistar rats, albino variety, weighing 300 ± 40 g, were obtained from the Laboratory Animal Science at the Multidisciplinary Center for Biological Research, University of Campinas. The rats were 21 days old and acclimated in polyethylene cages under controlled temperature conditions (22 ± 3°C), with free access to food and water ad libitum, under a 12-h light/12-h dark cycle. After the acclimation period, the animals were individually placed in metabolic cages.

Each experimental group consisted of five animals, housed in polyethylene cages in an air-conditioned room at 25 ± 2°C. The animals were deprived of food for 24 h before the experiment, but had access to water ad libitum. They received standardized food (Labina®) and water throughout the experiment, with a 12-h light/dark cycle maintained for 7 days.

2.4. Experimental groups for healing activity

Animals were allocated to four groups (n = 5 per group), balanced for body weight, as follows:

(i) The first group was treated with GLf at a concentration of 5% topically, with a dose of 188 mg/kg.

(ii) The second group (positive control) was treated topically with fibrinolysin (Fbn; Cristália Chemical Pharmaceutical Products Ltd., Brazil), with a dose of 31 mg/kg.

(iii) The third group was treated with InLf at a concentration of 5%, at a dose of 182 mg/kg.

(iv) The fourth group, defined as the negative control (Con), was treated with 0.5 mL of a 0.9% saline solution (sodium chloride).

Different treatments were applied to the wounds immediately after the surgical procedure and administered daily, topically, using a disposable spatula, at the same time each day.

2.5. Surgical procedure and morphometric evaluation

All surgical procedures were performed under general anesthesia, using 45 mg/kg of sodium thiopental (Cristália, Brazil) administered intraperitoneally, as described by Alves Barros *et al.*[19]

After shaving the dorsocervical region, a rectangular area of approximately 3 cm × 5 cm was outlined using an electric clipper. The skin was disinfected with iodized alcohol. A 6-mm full-thickness excisional wound was created on the cervical region of the animal's back using a skin biopsy punch. The tissue was removed with scissors and forceps until the dorsal muscle fascia was exposed. Immediately after hemostasis, treatments began, administered once daily at standardized times (12:00–13:00) for 7 days, with the wounds remaining without dressings throughout the experiment.

The wounds were measured in a standardized manner in the craniocaudal direction, from the 1st day (immediately after surgery) to the seventh postoperative day, using a digital caliper. The rate of wound healing was analyzed. Based on the measured values, wound contraction was calculated using the following formula:

$$\text{Wound contraction percentage (\%)} = \frac{\text{Initial area} - \text{final area}}{\text{Initial area}} \times 100 \quad (1)$$

Digital images of the wounds were obtained in a standardized manner immediately after the surgical procedure (day 1) and continued until the seventh postoperative day. For this purpose, a camera (Sonic 7.0, Brazil) was used, attached to a support fixed perpendicular to the horizontal plane and kept at a distance of 8 cm from this plane, providing adequate wound focusing. The digitized images were stored in Windows Bitmap (BMP) format and analyzed in ImageLab Software Version 6.1 (Bio-Rad Laboratories Inc., Brazil).

On the 7th day after the surgical procedure, the animals were anesthetized, placed in a carbon dioxide chamber, and euthanized. Following sacrifice, the wound was excised with a 0.5-mm margin of intact skin around the lesion, extending down to the muscle fascia, under aseptic conditions, making a median cut, and the tissue was fixed in 10% formalin.

2.6. Histopathological evaluation of wounds

The histopathological evaluation was performed according to the method described by Alves Barros *et al.*[19] The skin fragments fixed in 10% formalin were dehydrated and embedded in paraffin. They were then cut at 5 µm and stained with hematoxylin-eosin (HE) and picrosirius. The quantitative and qualitative analyses of the effects of both GLf and InLf included counting fibroblasts and inflammatory cells and assessing angiogenesis (blood vessels) on histopathological slides at ×400 magnification.

To determine epithelialization rates (ER), histological sections stained with HE were used to evaluate the extent of epithelialization in wounds by measuring epidermal migration from the average wound margin to the point where progression stopped. ER was calculated as the percentage of wound length covered by newly formed epithelium, determined in four high-power fields (×400) on each slide using morphometry software (Imagetool®, ImageTool 3.0 - Informer Technologies, Inc., USA), according to the equation as follows:

$$\mathrm{ER}(\%)=\left(1-\frac{\mathrm{W_A}\text{-}\mathrm{E_A}}{\mathrm{E_A}}\right)\times 100\begin{cases}\mathrm{ER}(\%)-\text{Epithelialization rates}\\ \mathrm{W_A}-\text{Wound surface area}\\ \mathrm{E_A}-\text{Epithelialized area}\end{cases} \tag{2}$$

For the assessment of collagen deposition, histological sections stained with picrosirius and analyzed under polarized light were used for descriptive evaluation. Collagen fibers were analyzed according to their birefringence pattern (greenish/yellow-greenish or orange, orange reddish), morphological appearance (wavy or stretched, thin or thick, short or long), and disposition (parallel-arranged or interlaced).

2.7. Statistical analysis

All experimental quantitative results were expressed as mean ± standard deviation, and one-way analysis of variance was used, followed by Tukey's test for multiple comparisons. Results with significance levels of $p<0.05$ were considered statistically significant. Statistical analyses were conducted using GraphPad Prism (version 5.03, GraphPad Software, Inc., San Diego, California, USA).

2.8. Molecular docking

The three-dimensional (3D) structure of the vascular endothelial growth factor receptor (VEGFR)-2/vascular endothelial growth factor (VEGF)-A complex (PDB ID: 3V2A) was retrieved from the Protein Data Bank (https://www.rcsb.org/). Initially, water molecules and cofactors were excluded, and hydrogen atoms were added using AutoDockTools 4.0 (Molecular Graphics Lab, The Scripps Research Institute, La Jolla, Califórnia, USA). The 3D models of natural product compounds were downloaded in SDF format from PubChem and converted to PDB format using Discovery Studio Visualizer (Dassault Systèmes BIOVIA, Massachusetts, USA) for subsequent docking simulations.

Before performing docking, the grid center for each protein target was determined using the AutoGrid program in AutoDockTools. The grid size was set to 40 points per dimension, with a spacing of 0.375 Å across all simulations. Molecular docking was conducted using AutoDock Vina 1.2.5 (The Scripps Research Institute, La Jolla, California, USA)[20] to calculate the binding affinities of each ligand to the target protein. The number of output poses (modes) was set to 10, and the energy range was adjusted to 9 kcal/mol. To improve docking accuracy, the exhaustiveness parameter was set to 1,000. Two-dimensional and 3D visuals were generated using BIOVIA Discovery Studio Visualizer software (Dassault Systèmes BIOVIA, Massachusetts, USA).[21]

2.9. Density functional theory computational analysis

Density functional theory calculations were performed using the B3LYP functional with the 6-311G(d,p) basis set. This approach was employed to evaluate the properties of gallic acid and ethyl gallate. The B3LYP method is widely recognized for its cost-effectiveness and reliability in predicting the geometries of both natural and synthetic molecules in quantum chemistry. All computations were conducted using the GAUSSIAN 09W software package (Gaussian, Inc., Wallingford, Connecticut, USA), and the optimized molecular structures were visualized with GaussView 6.0 (Semichem Inc., Kansas, USA).[22]

3. Results

3.1. Phytochemical assay and evaluation of wound contraction

Phytochemical analyses (Table 1) indicated that the GcLf of the fruits of *L. ferrea* presented a tannin content of 23% and flavonoids of 0.25%, while InLf had 21% tannins and 0.32% flavonoids. GLf demonstrated the lowest contents, with 18% tannins and 0.14% flavonoids.

The results of topical treatments on wound healing (Figure 2) showed that both InLf and GLf from *L. ferrea* significantly reduced the wound area at all measured time points ($p<0.001$), indicating that the treatments accelerated tissue repair. The area under the curve data showed that the treatments differed significantly from the control group (Con). The difference was observed mainly in the later days of treatment, where the difference from the control group was more pronounced.

Table 1. Tannin and flavonoid content in glycolic extract, infusion, and gel of *Libidibia ferrea*

Product	Tannin (%)	Flavonoid (%)
GcLf	23	0.25
InLf	21	0.32
GLf	18	0.14

Abbreviations: GcLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion formulation.

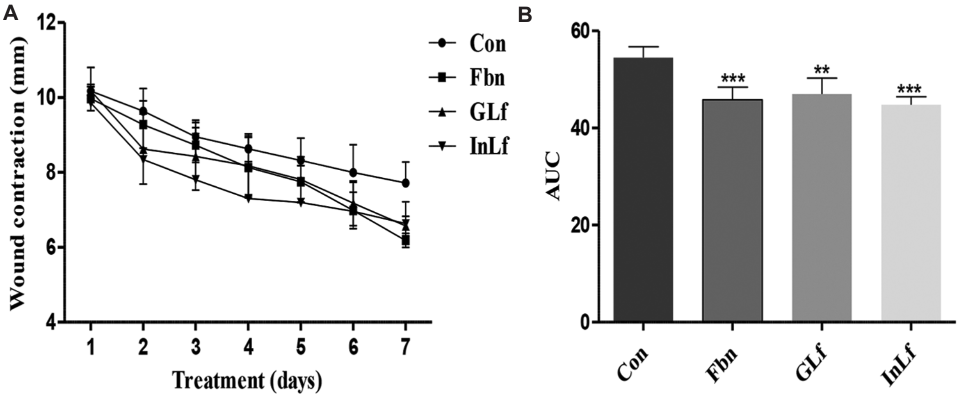

Figure 2. Effect of topical administration of GLf (188 mg/kg), InLf (182 mg/kg), and Fbn (31 mg/kg) on cutaneous wound healing. (A) Wound contraction throughout treatment. (B) AUC for each group.

Notes: Data are presented as mean±standard deviation. $^{**}p<0.01$ and $^{***}p<0.001$ versus Con group, according to Tukey's *post hoc* test following one-way analysis of variance.

Abbreviations: AUC: Area under the curve; Con: Control; Fbn: Fibrinolysin; GcLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion.

3.2. Histopathological evaluation of wounds

The findings revealed that the GLf group (Figure 3) showed a moderate inflammatory infiltrate in the marginal portion of the wound, predominantly lymphocytic, as well as intense proliferation of plump fibroblasts organized in parallel, associated with the deposition of rectilinear, delicate bundles of collagen exhibiting varying thickness. The vascular component was represented by capillary vessels, some of which were congested. The surface of the wound was covered with acanthotic orthokeratinized squamous epithelium. The most central portion of the wound exhibited a superficial third of the wound bed, representing a prominent, highly vascular granulation reaction. The deepest third of the wound bed exhibits a mature fibrocellular granulation reaction, characterized by moderate lymphocytic infiltration, fibroblastic proliferation, and collagen deposition, similar to the pattern observed at the margins.

In the marginal portion, Figure 4 shows that the Fbn group exhibited moderate inflammatory infiltration and a granulation reaction, with a less significant vascular-capillary component. The connective tissue appeared denser, consisting of thin, short fibrils, accompanied by intense fibroblast proliferation. In addition, clear epithelialization at the wound edges was observed. In the central region, a fibrovascular granulation reaction was observed. The connective tissue consisted of a highly loose extracellular matrix composed of thin, delicate, and very short collagen fibrils. Peripheral epithelialization was also visible.

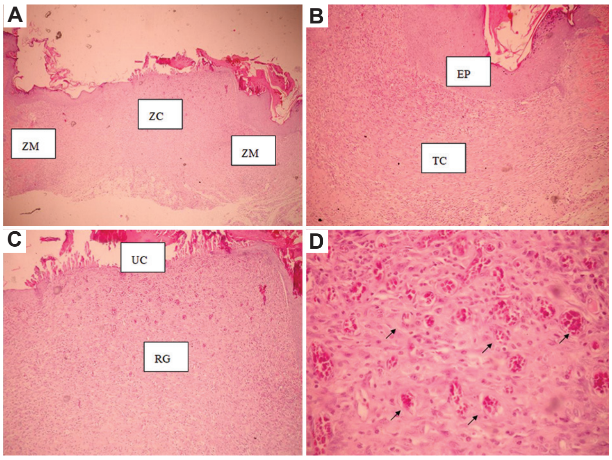

Figure 3. Histopathological evaluation of wounds treated with the gel formulation (188 mg/kg). (A) Overall view of the wound, highlighting the ZM and ZC zones (scale bar: 250 µm; magnification: ×40). (B) ZM zone, highlighting an area of EP and underlying inflamed TC (scale bar: 100 µm; magnification: ×100). (C) ZC zone, highlighting the RG and UC zone without epithelial lining (scale bar: 50 µm; magnification: ×100). (D) Vascular components (denoted with arrows) in the superficial region (scale bar: 25 µm; magnification: ×400).

Abbreviations: EP: Epithelialization; RG: Granulation reaction; TC: Connective tissue; UC: Ulcerated zone; ZC: Central zone; ZM: Marginal zone.

Figure 5 shows that the InLf group exhibited a moderate lymphohistiocytic inflammatory infiltrate, along with intense proliferation of plump fibroblasts arranged parallel to the wound surface. These features are associated with the deposition of straight and delicate collagen bundles of varying thickness. Well-defined marginal epithelialization was present, accompanied by evident acanthosis of the newly formed epithelium. In the central portion of the wound, an immature, predominantly fibrovascular granulation reaction extended from the surface toward the deeper layers.

Figure 6 shows that, in the Con group, the marginal portion exhibited a lymphohistiocytic inflammatory

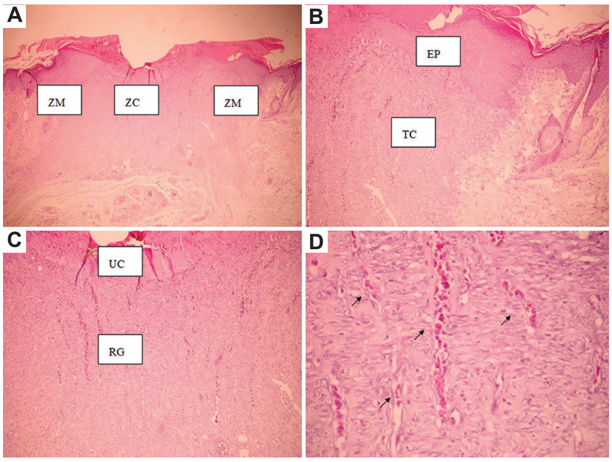

Figure 4. Histopathological evaluation of wounds treated with fibrinolysin (31 mg/kg). (A) Overall view of the wound, highlighting the ZM and ZC zones (scale bar: 250 µm; magnification: ×40). (B) ZM zone, highlighting an area of EP and underlying inflamed TC (scale bar: 100 µm; magnification: ×100). (C) ZC zone, highlighting the RG and UC zone without epithelial lining (scale bar: 50 µm; magnification: ×100). (D) Vascular components (denoted with arrows) in the superficial region (scale bar: 25 µm; magnification: 400×).
Abbreviations: EP: Epithelialization; RG: Granulation reaction; TC: Connective tissue; UC: Ulcerated zone; ZC: Central zone; ZM: Marginal zone.

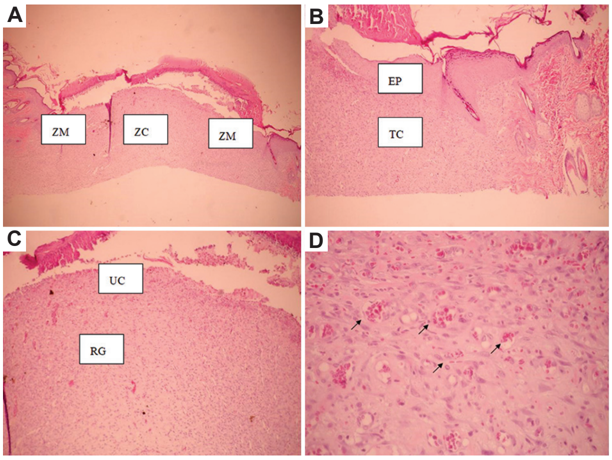

Figure 5. Histopathological evaluation of wounds treated with the infusion formulation (182 mg/kg). (A) Overall view of the wound, highlighting the ZM and ZC zones (scale bar: 250 µm; magnification: ×40). (B) ZM zone, highlighting an area of EP and underlying inflamed TC (scale bar: 100 µm; magnification: ×100). (C) ZC zone, highlighting the RG and UC zone without epithelial lining (scale bar: 50 µm; magnification: ×100). (D) Vascular components (denoted with arrows) in the superficial region (scale bar: 25 µm; magnification: ×400).
Abbreviations: EP: Epithelialization; RG: Granulation reaction; TC: Connective tissue; UC: Ulcerated zone; ZC: Central zone; ZM: Marginal zone.

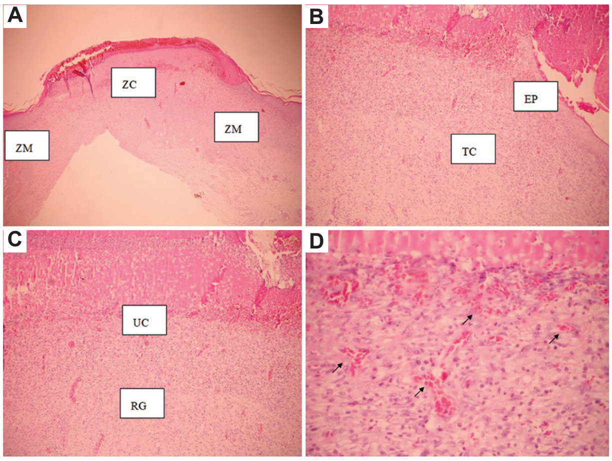

Figure 6. Histopathological evaluation of untreated (control) wounds. (A) Overall view of the wound, highlighting the ZM and ZC zones (scale bar: 250 µm; magnification: ×40). (B) ZM zone, highlighting an area of EP and underlying inflamed TC (scale bar: 100 µm; magnification: ×100). (C) ZC zone, highlighting the RG and UC zone without epithelial lining (scale bar: 50 µm; magnification: ×100). (D) Vascular components (denoted with arrows) in the superficial region (scale bar: 25 µm; magnification: ×400).
Abbreviations: EP: Epithelialization; RG: Granulation reaction; TC: Connective tissue; UC: Ulcerated zone; ZC: Central zone; ZM: Marginal zone.

reaction with a prominent vascular component of the granulation tissue. However, the vasculature consisted of small, poorly formed capillaries with irregular morphology (“slit-like” vessels), accompanied by moderate fibroblast proliferation and deposition of short, delicate fibrillar collagen, arranged in widely spaced parallel distribution, typical of highly immature connective tissue.

In the central portion, a richly vascularized granulation reaction extended from the surface to the deeper layers, along with moderate interstitial edema. Persistent superficial neutrophilic infiltration and moderate interstitial edema were observed in all specimens.

3.3. Assessment of epithelialization, angiogenesis, and inflammatory cells

The ER (Figure 7A) in the GLf- and Fbn-treated groups was significantly higher ($p<0.001$) than in the Con group. Moreover, the InLf-treated group also showed a significant difference ($p<0.05$) compared to the Con group.

In the microscopic analysis at ×400 magnification, the presence of inflammatory cells (Figure 7B) showed that the mean cell count in the Con group was significantly higher than in the other groups. In the groups treated with GLf, InLf, and Fbn, significantly lower values were observed ($p<0.001$), with means of 9.1 ± 4.48, 11 ± 3.22, and 11.2 ± 3.28, respectively.

Regarding angiogenesis (Figure 7C), a highly significant difference was observed in the mean values of the three groups compared to the Con group. For example, in the GLf-treated group, a significant increase ($p<0.001$) in

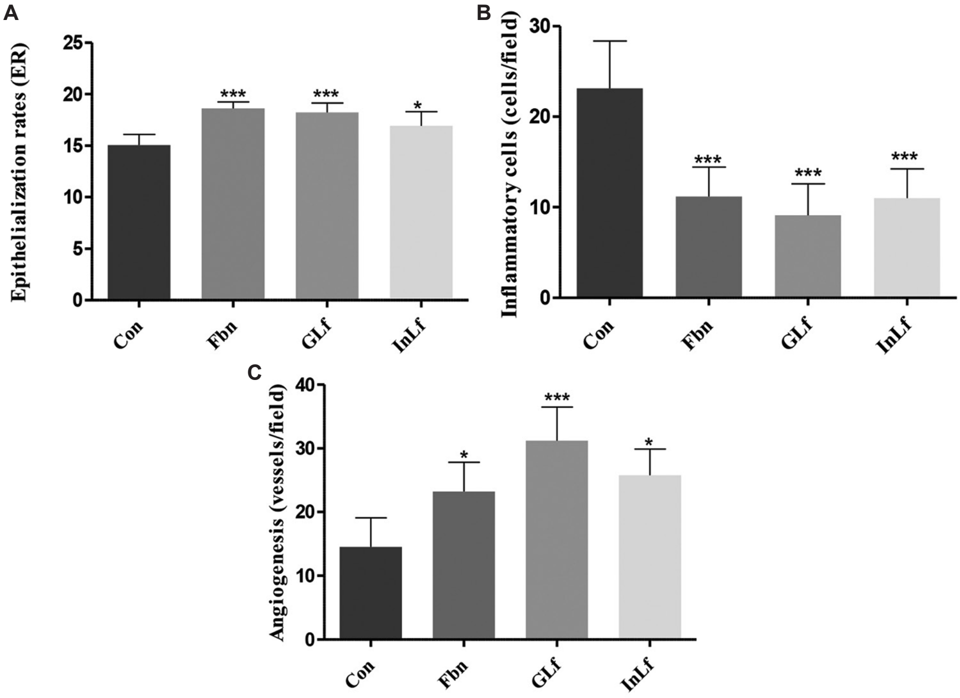

Figure 7. Results of histological assessments. (A) Epithelialization rate of the GLf (188 mg/kg), InLf (182 mg/kg), and Fbn (31 mg/kg) groups. (B) Number of inflammatory cells (cells/field). (C) Angiogenesis values (vessels/field) of the treated groups.
Notes: $*p<0.05$ and $***p<0.001$ versus Con group, according to Tukey's *post hoc* test following one-way analysis of variance.
Abbreviations: Con: Control; Fbn: Fibrinolysin; GcLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion.

the mean number of vessels per field was observed (31.2 ± 5.27). In groups treated with Fbn and InLf, significant differences ($p<0.05$) were observed when compared to the Con group, with mean values of 23.2 ± 4.62 and 25.8 ± 4.08, respectively.

3.4. Quantification of fibroblasts and collagen deposition

Fibroblast quantification in histopathological slides (Figure 8) revealed a significant increase ($p<0.01$) was observed in the average number of new fibroblasts in the GLf-treated group, with a value of 27.88 ± 5.24 cells/field. In the groups treated with InLF and Fbn, significant differences ($p<0.05$) were also observed compared to the Con group. The Con group exhibited significantly reduced fibroblast proliferation, with an average value of 13.2 ± 4.6 cells/field.

The analysis of collagen deposition in histopathological sections stained with picrosirius and examined under polarized light microscopy (Figure 9) revealed that, in the GLf and Fbn groups, short, thin, and delicate fibrous bundles with a slightly wavy appearance, irregular arrangement, and greenish to yellow-green birefringence were observed, consistent with deposition of reticular type III collagen. The bundles were more abundant in deeper regions and at the wound edges, and fibrils with yellow-orange birefringence, compatible with type I collagen, were also observed. Interfibrillar spaces were plentiful. The pattern observed in the InLf group was similar, but type I collagen deposition was minimal; even in the marginal zones, this collagen was

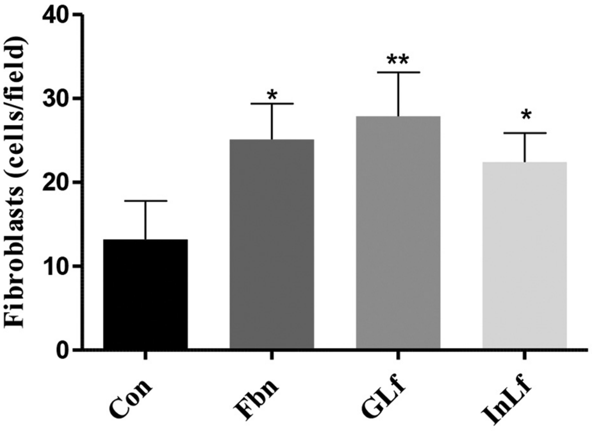

Figure 8. Fibroblast proliferation (cells/field) in groups treated with GLf (188 mg/kg), InLf (182 mg/kg), and Fbn (31 mg/kg).
Notes: $*p<0.05$ and $**p<0.01$ versus Con group, according to Tukey's *post hoc* test following one-way analysis of variance
Abbreviations: Con: Control; Fbn: Fibrinolysin; GcLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion.

scarce. In contrast, in the Con group, only type III collagen was observed along the entire wound, with the bundles remaining relatively thin and arranged in a reticular pattern.

3.5. Molecular docking

The binding interactions of gallic acid and ethyl gallate with the target protein 3V2A were analyzed, revealing significant differences in their binding energy, ligand efficiency, and estimated inhibition constants (Table 2). Gallic acid exhibited a binding energy of −5.5 kcal/mol, ligand efficiency of −0.460, and an inhibition constant (Ki) of 92.7 µM. In contrast, ethyl gallate demonstrated slightly higher binding affinity, with a binding energy of −5.6 kcal/mol, a ligand efficiency of −0.400, and a Ki of 83.9 µM. These results indicate that ethyl gallate may form more stable interactions with the target protein than gallic acid. However, the differences in ligand efficiency suggest that gallic acid achieves more effective binding per heavy atom, despite ethyl gallate having a lower Ki value, indicating stronger overall inhibitory potential. This finding highlights the significance of functional modifications in shaping molecular interactions with protein targets.

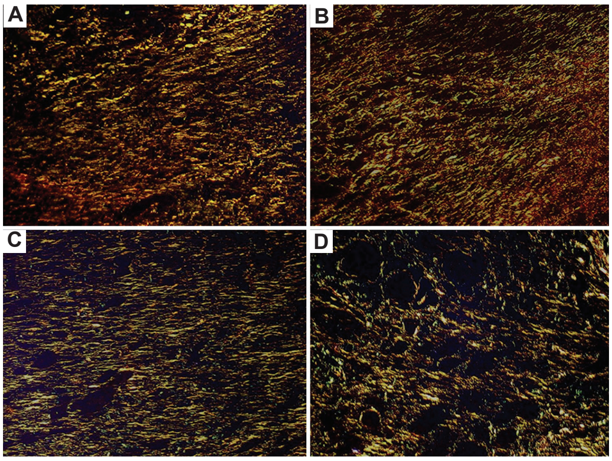

Figure 9. Collagen deposition under polarized light microscopy (scale bar: 100 µm; magnification: ×100). Groups treated with (A) GLf (188 mg/kg) and (B) Fbn (31 mg/kg) exhibited a predominance of type III collagen fibers (greenish-yellow birefringence), with evident deposition of type I collagen fibers (orange birefringence) in the deeper lamina, arranged predominantly in parallel bundles. (C) The InLf-treated group (182 mg/kg) and (D) the Con group exhibited exclusively type III collagen fibers, showing a parallel arrangement in the InLf group and a reticular pattern in the Con group.

Abbreviations: Con: Control; Fbn: Fibrinolysin; GcLf: Glycolic extract of *Libidibia ferrea*; GLf: Gel formulation; InLf: Infusion.

Docking analysis of gallic acid and ethyl gallate with the 3V2A protein revealed distinct interaction profiles reflecting their binding characteristics (Table 3). Gallic acid formed several conventional hydrogen bonds, notably with R: LYS286 (2.16 Å) and A: PHE47 (1.91 Å, 2.22 Å), along with an interaction with R: ASN253 (2.69 Å). It also exhibited carbon–hydrogen bonds with R: LYS286 (2.78 Å), and π-donor hydrogen bonding and π-sigma interactions with A: PHE47 (3.27 Å) and A: ILE46 (2.06 Å), respectively (Figure 10).

Table 2. Results of binding interactions of the compounds with the target protein (3V2A)

Bioactive compound	Binding energy (kcal/mol)	Ligand efficiency	Estimated inhibition constant (Ki µM)
Gallic acid	−5.5	−0.460	92.7
Ethyl gallate	−5.6	−0.400	83.9

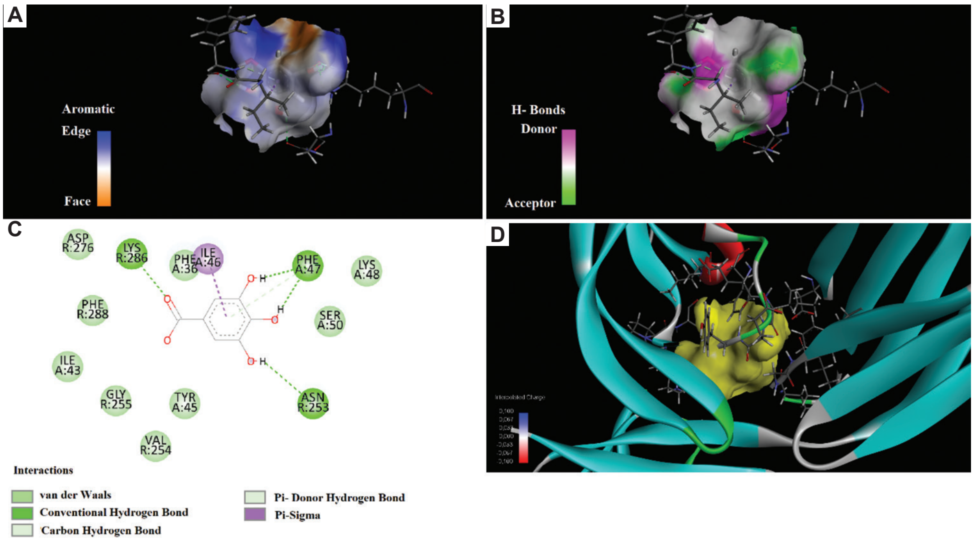

Figure 10. Molecular interactions of gallic acid with the target protein 3V2A: (A) Aromatic surface representation, (B) hydrogen bond distribution, (C) two-dimensional ligand-protein interaction, and (D) three-dimensional representation of the docked poses

Table 3. Predicted interactions of docked conformations of gallic acid and ethyl gallate with the 3V2A structure

Ligand	Amino acid	Interaction	Distance (Å)
Gallic acid	R: LYS286:HZ1 - :[001:O5	Conventional hydrogen bond	2.16
	:[001:H1 - A: PHE47:O	Conventional hydrogen bond	1.91
	:[001:H2 - A: PHE47:O	Conventional hydrogen bond	2.22
	:[001:H3 - R: ASN253:OD1	Conventional hydrogen bond	2.69
	R: LYS286:HE1 - :[001:O5	Carbon-hydrogen bond	2.78
	A: PHE47:HN - :[001	π-donor hydrogen bond	3.27
	A: ILE46:HB - :[001	π-sigma	2.06
Ethyl gallate	:[001:H7 - A: PHE47:O	Conventional hydrogen bond	1.79
	:[001:H8 - A: ASP34:OD1	Conventional hydrogen bond	1.81
	:[001:H9 - A: ASP34:OD1	Conventional hydrogen bond	2.58
	R: ASN253:HA - :[001:O2	Carbon-hydrogen bond	2.58
	:[001:H5 - A: TYR45:O	Carbon-hydrogen bond	2.93
	A: PHE36 - :[001	π-π T-shaped	4.29
	:[001:C3 - A: ILE46	Alkyl	4.67
	A: PHE36 - :[001:C3	π-alkyl	4.03

In contrast, ethyl gallate demonstrated a broader range of interactions, including hydrogen bonds with A: PHE47 (1.79 Å) and A: ASP34 (1.81 Å, 2.58 Å), as well as a carbon-hydrogen bond with R: ASN253 (2.58 Å). Furthermore, ethyl gallate displayed π–π T-shaped interactions with A: PHE36 (4.29 Å), alkyl interactions with A: ILE46 (4.67 Å), and π–alkyl bonding with A: PHE36 (4.03 Å) (Figure 11).

These findings suggest that ethyl gallate forms a more diverse set of non-covalent interactions due to its additional ethyl group, potentially contributing to its slightly enhanced binding affinity relative to gallic acid. This comparison highlights the impact of structural modifications on binding properties and potential biological activity.

3.6. Density functional theory analysis

The density functional theory analysis of gallic acid and ethyl gallate provides valuable insights into their electronic properties, which influence their reactivity and interaction potential (Table 4). Both compounds exhibited the same highest occupied molecular orbital energy (EHOMO) value of −0.2289 eV, indicating similar tendencies to donate electrons. However, their lowest unoccupied molecular orbital energy (ELUMO) values differ slightly, with gallic acid at −0.0535 eV and ethyl gallate at −0.0461 eV, suggesting that ethyl gallate has a marginally higher electron affinity. This is further supported by their ΔE(ELUMO-EHOMO) values, which are 0.1754 eV for gallic acid and 0.1828 eV for ethyl gallate, indicating a

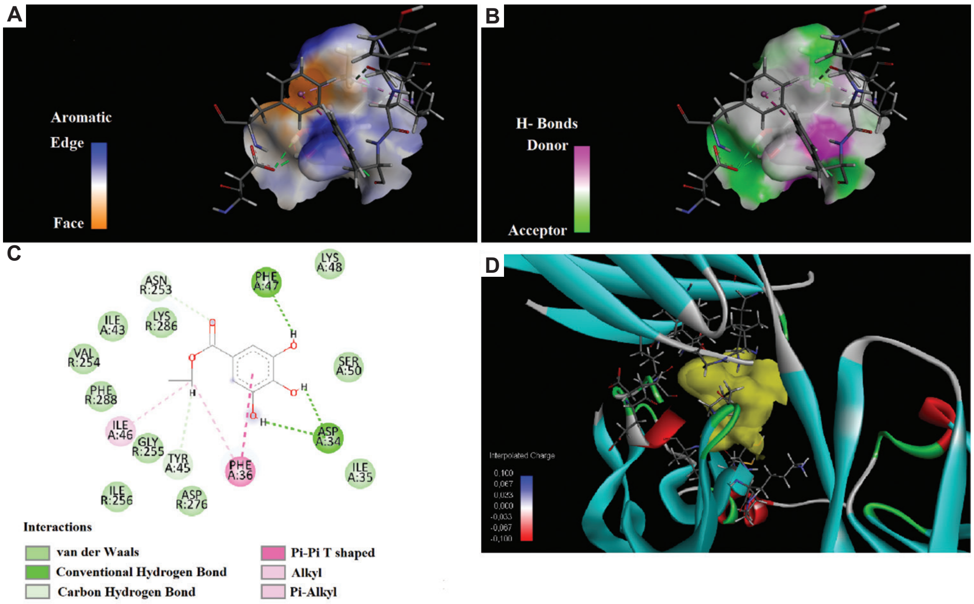

Figure 11. Molecular interactions of ethyl gallate with the target protein 3V2A: (A) Aromatic surface representation, (B) hydrogen bond distribution, (C) two-dimensional ligand–protein interaction, and (D) three-dimensional representation of the docked poses

slightly larger energy gap for ethyl gallate and potentially higher chemical stability (Figure 12).

The ionization energy (I) of both compounds is identical at 0.2289 eV, while their electron affinities (A) are 0.0535 eV and 0.0461 eV for gallic acid and ethyl gallate, respectively. The electronegativity index (χ) shows a slight difference, with gallic acid having a value of 0.1412 eV compared to 0.1375 eV for ethyl gallate. Additionally, their chemical potentials (μ) are −0.1412 eV and −0.1375 eV, respectively.

In terms of hardness (η), gallic acid exhibited a slightly lower value (0.0877 eV) compared to ethyl gallate (0.0914 eV), while their softness values, calculated as 1/η, are 11.40 eV^{-1} and 10.94 eV^{-1}, respectively. Finally, the electrophilicity index (ω), which assesses the compound's propensity to accept electrons, was marginally higher for gallic acid (0.1137 eV) compared to ethyl gallate (0.1034 eV).

Table 4. Key parameters in density functional theory analysis and their values

Parameters	Gallic acid	Ethyl gallate
EHOMO (eV)	−0.2289	−0.2289
ELUMO (eV)	−0.0535	−0.0461
ΔE (LUMO-HOMO) (eV)	0.1754	0.1828
Ionization energy (I) = −E (HOMO)(eV)	0.2289	0.2289
Electron affinity (A) = −E (LUMO) (eV)	0.0535	0.0461
Electronegativity index (χ) = (I+A)/2 (eV)	0.1412	0.1375
Chemical potential μ = −χ	−0.1412	−0.1375
Chemical hardness η = (I−A)/2 (eV)	0.0877	0.0914
Softness ($1/\eta\ eV^{-1}$)	11.40	10.94
Electrophilicity index ($\omega = \mu^2/2\ \eta$)	0.1137	0.1034

Abbreviations: EHOMO: Highest occupied molecular orbital energy; ELUMO: Lowest unoccupied molecular orbital energy. HOMO: Highest occupied molecular orbital; LUMO: Lowest unoccupied molecular orbital.

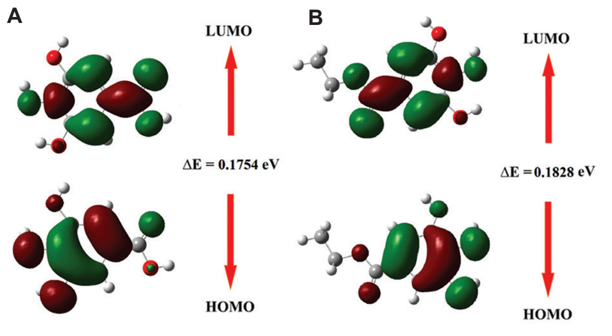

Figure 12. Frontier molecular orbital (HOMO–LUMO) distribution of (A) gallic acid and (B) ethyl gallate
Abbreviations: HOMO: Highest occupied molecular orbital; LUMO: Lowest unoccupied molecular orbital.

Overall, the subtle differences in electronic parameters highlight how the addition of an ethyl group to gallic acid affects its electronic properties, potentially altering its reactivity and interaction with biological targets.

4. Discussion

Wound healing is a complex process involving multiple phases, including inflammation, granulation tissue formation, and collagen remodeling. This study investigated the effects of topical application of products containing *L. ferrea* fruit derivatives on the healing process in cutaneous wounds, correlating the histopathological findings with the phytochemical composition of the formulations, specifically their tannin and flavonoid contents.

Phytochemical analyses revealed that the formulations had high tannin contents of 18–23% and flavonoid contents of 0.14–0.25%. Tannins and flavonoids are widely recognized for their antioxidant and anti-inflammatory properties, suggesting that formulations richer in these compounds may facilitate the healing process by modulating inflammation and protecting cells from oxidative stress.[3,6]

Topical *L. ferrea* formulations significantly reduced wound size and promoted lesion contraction in rats compared to the Con group. Wound contraction is a key process contributing to wound closure. It involves re-epithelialization, epithelial cell migration, fibroplasia, and angiogenesis, with granulation tissue occupying the injured tissue approximately 4 days after the injury.[22,23]

In the histopathological evaluation, on the 7th day, the inflammatory reaction was intense in all groups, as expected in wound healing by secondary intention, where inflammation is a necessary step for eliminating harmful agents and initiating the repair process.[1] However, distinct differences in the inflammatory pattern were observed between the groups. In the groups treated with *L. ferrea* (GLf and InLf) and Fbn, inflammation progressively decreased throughout the treatment, favoring wound closure. This suggests a rapid transition from acute to controlled inflammation and a decrease in inflammatory cells within the scar tissue, a known effect of *L. ferrea* formulations.[24] The high concentration of tannins in GLf and InLf modulated the inflammatory response, reduced the need for continuous neutrophil recruitment, and accelerated the transition to the proliferative phase, thereby promoting wound closure.[25]

During the proliferative phase, granulation tissue is characterized by the presence of fibroblasts and newly formed vessels. This phase was most pronounced in the groups treated with GLf, Fbn, and InLf, which presented higher rates of epithelialization and increased angiogenesis compared to the Con group. Angiogenesis is crucial for wound healing, as the newly formed blood vessels supply oxygen and nutrients necessary for tissue repair.[26]

The epithelialization process, which involves the proliferation and migration of epithelial cells to cover the injured area, was more advanced in the GLf, Fbn, and InLf groups than in the Con group. This suggests that formulations rich in tannins and flavonoids may accelerate epithelialization, possibly by modulating the inflammatory response and protecting epithelial cells from oxidative damage.[2,27] In addition, this pattern correlates with more advanced healing, as groups with higher ERs also exhibited increased numbers of tissue fibroblasts, indicating a greater tendency to transition from the proliferative phase to the remodeling phase.[28]

During the remodeling phase, wound tissue reorganizes and strengthens as collagen is produced and realigned, thereby increasing the wound's strength and elasticity.[25] The deposition of type I collagen, which confers greater resistance to scar tissue, was more prominent in the GLf and Fbn groups. In contrast, type III collagen, which predominates in the early phases of repair, was predominant in the Con group. The replacement of type III collagen by type I collagen is an important indicator of scar tissue maturation, suggesting that treatments with *L. ferrea*-based formulations contributed to and accelerated this process.[29,30]

The wound-healing effects of *L. ferrea* on skin injuries in Wistar rats were investigated, supported by molecular docking analyses of its bioactive compounds. Gallic acid and ethyl gallate, known for their antioxidant, anti-inflammatory,[31,32] and antimicrobial[33] properties, have been identified as key compounds that play a significant role in the wound healing process. These compounds demonstrated high binding affinity to the VEGFR-2/VEGF-A complex structure (PDB ID: 3V2A), with binding energies ranging from −5.5 to −5.6 kcal/mol.

It has been reported that topical application of gallic acid and ethyl gallate promotes healing by effectively modulating growth factors and cellular responses during the inflammation and proliferation phases of wound healing.[34] In this context, the VEGF, which promotes the formation of new blood vessels, plays a central role in wound healing.[35] VEGF activates three primary tyrosine kinase receptors—VEGFR-1, VEGFR-2, and VEGFR-3—to initiate angiogenic signaling, enhancing cellular angiogenic activity and supporting wound healing.[36]

Moreover, the upregulation of hypoxia-inducible factor-1α/VEGF/VEGFR-2 signaling has been reported to enhance angiogenic activity, thereby providing therapeutic effects in wound healing.[37] Binding ligands, such as gallic acid and ethyl gallate, trigger receptor dimerization, initiate transmembrane signaling, and activate receptor kinases, promoting angiogenesis and contributing to therapeutic effects in wound healing.[38]

5. Conclusion

The integration of phytochemical data with histopathological results demonstrates that formulations containing high levels of tannins and flavonoids from *L. ferrea* fruits exert beneficial effects on wound healing by secondary intention, modulating inflammation, promoting granulation tissue formation, and accelerating epithelialization and collagen remodeling. The *in silico* study demonstrated that the main bioactive compounds of this species, gallic acid and ethyl gallate, contribute to the observed pharmacological effects by modulating vascular proliferation and fibroblast activity. These findings highlight the therapeutic potential of *L. ferrea*-based pharmaceutical formulations in wound treatment.

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Conflict of interest

José Carlos Tavares Carvalho is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

Conceptualization: Ana Karina Lima Alves Cerdeira, Francineide Pereira da Silva Pena, Cecília Rafaela Salles Ferreira, Sérgio Gabriell Leite Brito, Luiza Pinon Nery de Oliveira
Data curation: Helison de Oliveira Carvalho
Formal analysis: Helison de Oliveira Carvalho, Ricardo Luiz Cavalcanti de Albuquerque Jr.
Funding acquisition: José Carlos Tavares Carvalho
Investigation: Ana Karina Lima Alves Cerdeira, Francineide Pereira da Silva Pena, Cecília Rafaela Salles Ferreira, Sérgio Gabriell Leite Brito, Luiza Pinon Nery de Oliveira
Methodology: Ana Karina Lima Alves Cerdeira, Francineide Pereira da Silva Pena, Cecília Rafaela Salles Ferreira, Sérgio Gabriell Leite Brito, Luiza Pinon Nery de Oliveira
Project administration: José Carlos Tavares Carvalho
Resources: Helison de Oliveira Carvalho, Ricardo Luiz Cavalcanti de Albuquerque Jr.
Supervision: José Carlos Tavares Carvalho
Validation: Helison de Oliveira Carvalho, Ricardo Luiz Cavalcanti de Albuquerque Jr.
Visualization: José Carlos Tavares Carvalho
Writing–original draft: José Carlos Tavares Carvalho
Writing–review & editing: José Carlos Tavares Carvalho

Ethics approval and consent to participate

The animal study protocol was approved by the Research Ethics Committee of Federal University of Amapá under protocol no. 008/2017 of 08/23/2017.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author on reasonable request.

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ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

Profiling the 8-nucleotide microRNA targets in genes involved in type 2 diabetes mellitus in association with oxidative and endoplasmic reticulum stress

Ayesha Jabeen[1]* and Umm E. Laila[2]

[1]Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova, Padova, Veneto, Italy

[2]Department of Biology, School of Life Sciences, Henan University, Kaifeng, Henan Province, China

Abstract

Type 2 diabetes mellitus (T2DM) is one of the top 10 global killers. The association between oxidative stress and T2DM has been reported, in which oxidative stress triggers the life-threatening consequences, including stroke, nephropathy, and myocardial infarction. MicroRNA (miRNA)-based therapies are thought to revolutionize early medical interventions, potentially enabling the treatment of T2DM. miRNAs are 20–22 nucleotide non-coding sequences used to silence genes at the post-transcriptional level. Gene testing registry, TargetScan, and other bioinformatic databases were used to identify the oxidative- and endoplasmic reticulum (ER) stress-linked genes and their targeted miRNAs (8-mers and species conserved) associated with T2DM. ShinyGO was used to establish links between T2DM and its associated genes. We identified putative common miRNAs, such as miR-26-5p/miR-124-3p.1/miR-124-3p.2/miR-98-5p/miR-17-5p/miR-519-3p/miR-20-5p/miR-93-5p/miR-106-5p, which may involve in the regulation of T2DM-associated genes. In addition, several common miRNAs were also considered in the regulation of pathological conditions. The miRNAs for T2DM associated with oxidative stress are as follows: miR-33-5p/miR-506-3p/miR-7-5p/miR-107/miR-93-5p, and for T2DM associated with ER stress are as follows: miR-195-5p/miR-181-5p/miR-101-3p.1/miR-424-5p/miR-145-5p/miR-519-3p/miR-16-5p. Overall, this study provides a framework for the rational selection of miRNAs for nucleic acid-based therapies and guides future studies in maximizing the potential of miRNAs against oxidative- and ER-stress associated with T2DM.

Keywords: miRNAs; Type 2 diabetes mellitus; Oxidative stress; Endoplasmic reticulum stress

***Corresponding author:**
Ayesha Jabeen
(ayesha.jabeen@studenti.unipd.it)

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1. Introduction

MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene activity after transcription and mediate RNA interference, leading to gene silencing or inhibition of translation to control gene expression.[1] In the human genome, around 2,600 miRNAs have been identified, which regulate thousands of protein-coding genes.[2] These miRNAs are associated with apoptosis, immunity, stress response, and angiogenesis, and they act as biomarkers in the diagnosis of several disorders.[3] Numerous studies have shown a link between miRNAs and various diseases.[4] Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by insulin resistance and progressive β-cell dysfunction, resulting in chronic hyperglycemia and dyslipidemia, triggering downstream oxidative and endoplasmic reticulum (ER) stress.[5] Oxidative stress is an imbalance between the production and clearance of reactive oxygen species (ROS), resulting in the accumulation of oxidants.[6] ER stress also contributes to oxidative stress by generating ROS.[7] Insulin resistance refers to a reduced responsiveness of insulin-target tissues—primarily the liver, skeletal muscle, and adipose tissues—to circulating insulin, whether at normal or elevated levels.[8] In the initial phase, this dysfunction is often compensated for by increased insulin production from pancreatic β-cells, leading to hyperinsulinemia. However, as the condition progresses, β-cell compensation frequently becomes insufficient, leading to elevated fasting and postprandial blood glucose levels and the transition from insulin resistance to overt T2DM.[9] Persistent hyperglycemia and lipid accumulation subsequently promote excessive mitochondrial ROS generation and disrupt ER proteinfolding homeostasis. Consequently, oxidative and ER stress develop as secondary insults that intensify insulin resistance, impair β-cell function, and contribute to diabetic complications.[10] Recent clinical and experimental findings therefore conceptualize T2DM as a disease arising from the interplay between systemic insulin resistance and inadequate β-cell adaptive capacity, rather than solely from oxidative stress.[11] Both classical metabolic studies and modern molecular signaling analyses reveal that individuals with T2DM display reduced insulin-driven glucose uptake in skeletal muscle, impaired hepatic glucose suppression, and defects in the insulin receptor (INSR), INSR substrate (IRS)1/2–phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) signaling cascade, these defects include diminished expression or activity of *IRS* and *AKT2*, altered association of PI3K subunits, and occasionally decreased INSR density or surface availability.[12] While receptor binding affinity is usually normal in typical T2DM, it can be altered in rare receptoropathies or specific receptor isoform variants. Within this mechanistic context, several genes highlighted in the current *in silico* analysis, such as *IRS1*, *IRS2*, *AKT2*, and *PIK3R1*, serve as key modulators of insulin signaling and sensitivity.[13] This suggests that the miRNAs identified here may influence not only oxidative and ER stress responses but also the core mechanisms driving primary insulin resistance and the subsequent diabetic cascade.

In T2DM, persistent high glucose and lipid levels create a self-reinforcing interaction between secondary parameters, such as oxidative stress and ER stress, based on the vicious cycle of insulin resistance and impaired INSR signaling in classic target tissues (liver, skeletal muscle, and adipose tissue), which collectively worsen β-cell dysfunction and increase insulin resistance.[14] Continuous metabolic overload increases the production of ROS from mitochondria and nicotinamide adenine dinucleotide phosphate oxidases, disrupting the ER's redox environment and impairing the formation of disulfide bonds necessary for proper proinsulin folding. This disturbance activates unfolded protein response (UPR) pathways through sensors such as protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6).[15] Prolonged engagement of these UPR mechanisms elevates pro-apoptotic factors, including CCAAT/enhancer-binding protein homologous protein and ER oxidoreductases like ER oxidoreductin 1, promoting additional ROS production and inducing calcium leakage from the ER to mitochondria. These events enhance mitochondrial damage, oxidative injury, and β-cell apoptosis.[16] Evidence from diabetic models and human studies indicates that, due to their limited antioxidant capacity, β-cells accumulate misfolded proinsulin and ER stress indicators together with increased lipid peroxidation and oxidative load. This supports the notion that oxidative stress not only arises from but also intensifies ER stress, ultimately driving β-cell dedifferentiation, muscle wasting, and diabetic complications.[17] Consequently, therapeutically targeting the ER-oxidative stress interplay using chemical chaperones, ER stress inhibitors, or antioxidant strategies has shown potential to re-establish redox equilibrium, reduce UPR-mediated apoptosis, and enhance metabolic regulation, making this interaction a major focus of current diabetes research.[18]

Beyond their established role in glucose regulation, miRNAs are now recognized as key modulators of adipose tissue function, coordinating processes such as adipocyte differentiation, lipid metabolism, inflammation, and insulin responsiveness.[19] Experimental evidence from 3T3-L1 cell models and animal studies indicates that miR-103, miR-107, and miR-143 are upregulated during adipogenesis, facilitating the maturation of preadipocytes into adipocytes by promoting adipogenic transcription factor expression and triglyceride deposition.[20] However, in the context of obesity, the expression of these lipid- and differentiation-related miRNAs becomes dysregulated within white adipose tissue; this alteration contributes to chronic low-grade inflammation, adipocyte enlargement, and disturbances in insulin signaling.[21] Mechanistic research has further shown that miR-103/107 target caveolin1 and other components of the INSR complex, meaning that their aberrant expression in obesity can exacerbate insulin resistance and disrupt downstream cascades, such as Akt/mechanistic target of rapamycin and ER stress-related pathways, processes that also play pivotal roles in T2DM pathogenesis.[22] Clinical and translational investigations increasingly suggest that obesity imprints a specific circulating miRNA profile that evolves further in the presence of diabetes. High-throughput sequencing studies in human cohorts reveal distinct circulating and exosomal miRNA signatures discriminating obese individuals with T2DM from those without, with progressive shifts observed from healthy controls to obese non-diabetic and diabetic subjects.[23] Growing evidence identifies adipose-derived and extracellular vesicle-associated miRNAs as critical mediators of inter-organ metabolic communication and as promising biomarkers for obesity-related disorders, including T2DM and cardiovascular disease.[24] Within the framework of the current *in silico* analysis, these observations suggest that obesity-induced remodeling of adipose and circulating miRNA patterns may intensify or modify the regulatory influence of stress-associated miRNAs on their T2DM, oxidative stress, and ER stress-related gene targets, thereby shaping both disease progression and the translational applicability of the predicted miRNA gene interaction networks.

For disease prevention and therapy, various bioinformatics tools have been used to identify novel molecular therapeutic interventions.[25] We intended to evaluate the importance of different miRNAs in controlling the expression of various biomarkers implicated in the development and progression of disease. The gene testing registry of the National Center for Biotechnology Information (NCBI) was used to obtain T2DM gene names associated with oxidative and ER stress. Potential miRNA targets for all genes were identified using Human TargetScan (7.2.2018, Whitehead Institute, United States of America). Several novel miRNA-gene pairs emerged from this prediction, guiding the discovery of new miRNA-based biomarkers for T2DM in association with oxidative and ER stress diagnosis. We aim to identify the most suitable miRNAs to design an effective therapeutic strategy against T2DM-linked oxidative and ER stress.

2. Materials and methods

2.1. Gene selection from the NCBI (gene testing registry) and Online Mendelian Inheritance in Man

The NCBI offers a comprehensive set of online biological information and data resources, including biomedical and biotechnology information. It collects data from a variety of databases, including NCBI epigenomics, GenBank nucleic acid sequence database, and PubMed. NCBI is the repository for information on related diseases and the underlying genes. Online Mendelian Inheritance in Man (OMIM) is a comprehensive, reliable, and up-to-date repository of human genes and genetic disorders, assembled to support clinical genetic investigations and human biological research. We used the NCBI (Figure 1A) and the OMIM website (Figure 1B) to obtain sequencing panels for T2DM- and stress-related genes.

2.2. TargetScan as an *in silico* tool for the prediction of miRNA-targeted genes

Various software uses different approaches to predict miRNA targets. Site accessibility, free energy, seed compatibility, and conservation are the four most commonly used characteristics for miRNA target prediction methods. miRTarBase, miRDB, miRWalk, and miRSystem[26] are the most extensively used software for predicting miRNA targets and facilitate statistically based comparison of results from various established tools. The most extensively used popular miRNA prediction tools are SVMicrO,[27] miRanda,[28] and TargetScan.[29]

TargetScan is a bioinformatics web server that identifies biological targets of miRNAs by checking for conserved 8mer, 7mer, and 6mer regions that are related to each miRNA's seed region (Figure 1C). We filtered candidates based on miRNA conservation across human, rat, and mouse, together with 8-nucleotide (8-nt) seed-site analysis (Figure 1D). This approach also provided percentile scores and context++ values, which helped to assess the reliability of the predicted interactions.

The TargetScan interface allows users to query the database by entering either gene or miRNA identifiers to retrieve predicted targets. We applied TargetScan to analyze all NCBI-documented genes associated with disease pathogenesis and stress-related pathways. We selected the species (human, rat, and mouse), entered the names of targeted genes, and obtained information on miRNA targets for different sites. We also adopted a complementary strategy by entering our miRNA candidates into the second search field, which returned a comprehensive list of genes sharing those miRNA targets. From this output, we identified our genes of interest and retrieved detailed information on conserved binding sites and miRNA positional context.

3. Results

3.1. Genes associated with T2DM and their interacting miRNAs

Our analysis included 13 genes involved in T2DM pathogenesis progression regulated by miRNAs with an 8-nt

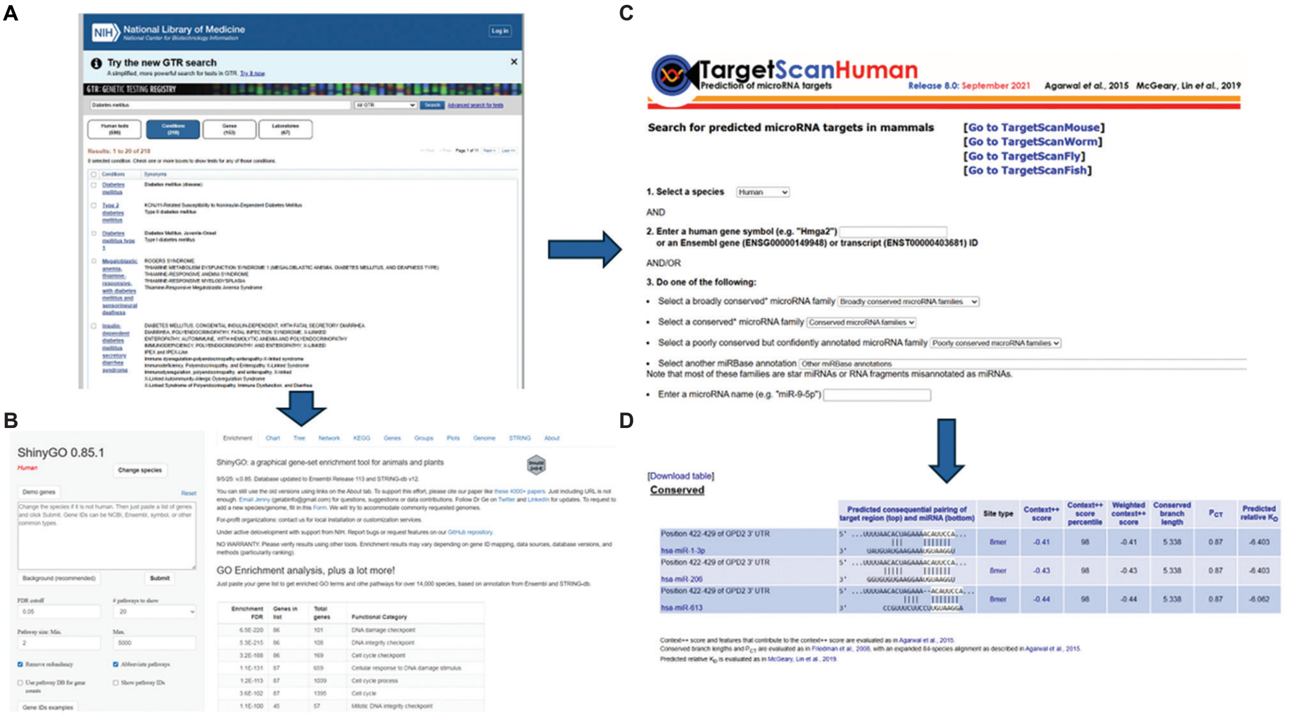

Figure 1. Bioinformatics analysis. (A) Disease-linked genes selection from the National Center for Biotechnology Information (gene testing registry). (B) Gene enrichment analysis using ShinyGO. (C) microRNA predicted by TargetScan. (D) Species conserved 8mer miRNA selection.

binding seed region, as shown in Table 1. *GPD2* expression was regulated by more than seven miRNAs, *HMGA1* was regulated by nine miRNAs, and *IRS2* was regulated by six miRNAs. All the main genes are represented graphically together with their corresponding 8-nt miRNAs, as shown in Figure 2. This distribution highlights the complex post-transcriptional regulation of key metabolic and signaling pathways implicated in T2DM. The higher number of 8-nt miRNA interactions for *HMGA1*, *GPD2*, and *IRS2* suggests that these genes may act as critical regulatory hubs in disease progression.

3.2. Genes associated with oxidative stress and their corresponding miRNA regulators

Our analysis focused on 18 genes involved in oxidative stress progression regulated by miRNAs with an 8-nt binding seed region, as shown in Table 2. *ERCC6*, *STK3*, *DHCR24*, and *ANK2* were regulated by three miRNAs, *AIFM1* was regulated by five distinct miRNAs, and *STC1* by 10 miRNAs. Figure 3 shows all the important genes together with their corresponding 8-nt miRNAs. This pattern suggests a layered regulatory network in which a subset of oxidative stress-related genes receives particularly dense miRNA input. The higher number of 8-nt miRNA interactions observed for *STC1* and *AIFM1* indicates

Table 1. List of microRNA targeting type 2 diabetes-associated genes

No.	Target genes	Species conserved microRNAs
1	*KCNJ11*	miR-98-5p, let-7-5p
2	*IRS1*	miR-145-5p, miR-183-3p. 1
3	*NEUROD1*	miR-30-5p, miR-101-3p. 2, miR-137, miR-153-5p, miR-101-3p. 1, miR-190-5p
4	*HMGA1*	miR-142-3p. 1, miR-15-5p, miR-16-5p, miR-195-5p, miR-424-5p, miR-497-5p, miR-26-5p, miR-103-5p, miR-107
5	*IGF2BP2*	miR-98-5p, miR-181-5p, let-7-5p, miR-9-5p
6	*WFS1*	miR-519-3p, miR-17-5p, miR-93-5p, miR-20-5p, miR-106-5p
7	*PTPN1*	miR-1-3p, miR-206, miR-124-3p. 2, miR-506-3p
8	*TCF7L2*	miR-155-5p, miR-1271-5p, miR-96-5p
9	*HNF1B*	miR-217, miR-375, miR-802, miR-24-3p, miR-101-3p. 2
10	*IRS2*	let-7-5p, miR-33-5p, miR-98-5p, miR-203a-3p. 1, miR-7-5p miR-181-5p
11	*GPD2*	miR-128-3p, miR-1-3p, miR-132-3p, miR-27-3p, miR-206, miR-181-5p, miR-212-3p
12	*AKT2*	miR-126-3p. 2, miR-137, miR-124-3p. 1, miR-194-5p
13	*PPARG*	miR-27-3p, miR-130-3p, miR-454-3p, miR-301-3p

Table 2. List of microRNA targeting oxidative stress-associated genes

No.	Putative target genes	Species conserved microRNAs
1	*MRPS25*	miR-33-5p
2	*FOXO4*	miR-142-3p. 1, miR-23-3p
3	*CTNNB1*	miR-142-3p. 2
4	*LRRK2*	miR-205-5p
5	*PARP1*	miR-7-5p, miR-129-3p
6	*CACNA1C*	miR-19-3p, miR-33-5p, miR-26-5p
7	*STC1*	miR-101-3p. 1, miR-101-3p. 2, miR-146-5p, miR-30-5p, miR-190-5p, miR-17-5p, miR-20-5p, miR-93-5p, miR-106-5p, miR-519-3p
8	*TP73*	miR-455-3p. 2, miR-200bc-3p, miR-429
9	*NFE2L2*	miR-142-5p, miR-153-3p, miR-140-5p. miR-144-3p
10	*G3BP2*	miR-19-3p, miR-7-5p, miR-130-3p, miR-301-3p, miR-454-3p, miR-199-3p, miR-26-5p
11	*OSR1*	miR-17-5p, miR-20-5p, miR-93-5p, miR-106-5p, miR-519-3p, miR-451
12	*STK4*	miR-124-3p. 2, miR-506-3p, miR-23-3p
13	*ANK2*	miR-9-5p, miR-139-5p, miR-142-3p. 2
14	*ITPR3*	miR-302c-3p. 2, miR-520-3p, miR-124-3p. 2, miR-506-3p
15	*DHCR24*	miR-96-5p, miR-1271-5p, miR-124-3p. 1
16	*KLHDC10*	miR-9-5p, miR-383-5p. 1
17	*AIFM1*	miR-103-3p, miR-107, let-7-5p, miR-98-5p, miR-125-5p
18	*ERCC6*	let-7-5p, miR-98-5p, miR-29-3p

that these genes may serve as key regulatory nodes in the cellular response to oxidative stress.

3.3. Genes associated with ER stress and their interacting miRNAs

We evaluated 21 genes involved in ER stress regulated by miRNAs with an 8-nt binding seed region, as shown in Table 3. *KLF11* expression was regulated by more than seven miRNAs, *GATA6* was regulated by four miRNAs, *PIK3R1* was regulated by seven miRNAs, and SEC16B and SERP1 were regulated by three miRNAs. Figure 4 shows all the important genes together with their corresponding 8-nt miRNAs. This distribution of miRNA-gene interactions underscores the complex regulatory architecture governing ER stress signaling. The higher density of 8-nt miRNA binding sites observed for *KLF11* and *PIK3R1* suggests that these genes may function as major regulatory hubs within the UPR.

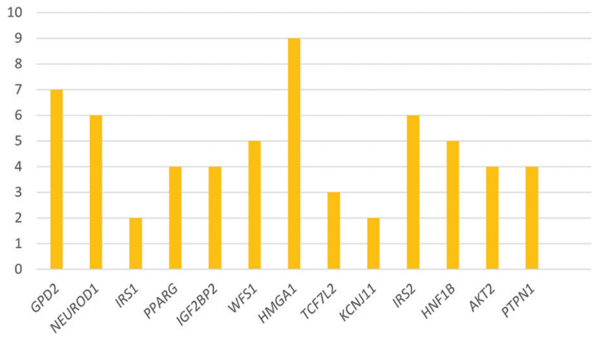

Figure 2. Number of 8-mer microRNAs predicted to target type 2 diabetes mellitus-associated genes

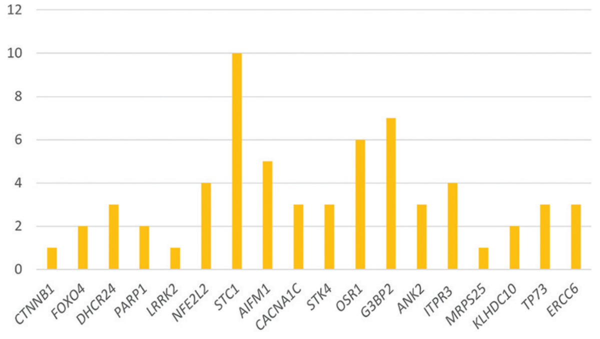

Figure 3. Number of 8-mer microRNAs predicted to target oxidative stress-associated genes

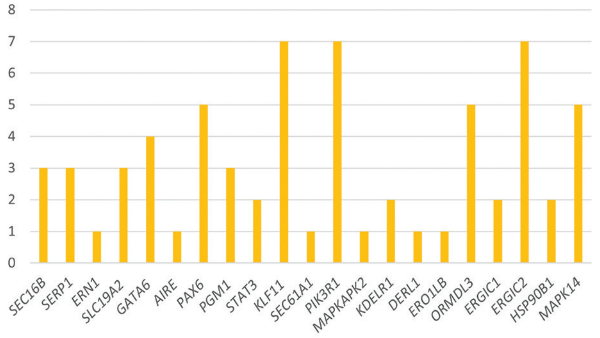

Figure 4. Number of 8-mer microRNAs predicted to target endoplasmic reticulum stress-associated genes

3.4. Commonly expressed miRNAs and their corresponding target genes in T2DM under oxidative stress

We evaluated 25 miRNAs involved in T2DM with oxidative stress. miR-98-5p is considered a key regulator of genes implicated in T2DM and oxidative stress. The *KCNJ11* gene is positioned at 11p15.1 with no introns.[30] The pancreatic beta-cell's Kir6.2 component of the ATP-sensitive potassium ion channels is encoded by the *KCNJ11*

Table 3. List of microRNAs targeting endoplasmic reticulum stress-associated genes

No.	Putative target genes	Species conserved microRNAs
1	*SEC16B*	miR-98-5p, miR-199-3p, let-7-5p
2	*HSP90B1*	miR-23-3p, miR-181-5p
3	*ERGIC2*	miR-25-3p, miR-32-5p, miR-92-3p, miR-363-3p, miR-367-3p, miR-194-5p, miR-135-5p
4	*SLC19A2*	miR-205-5p, miR-26-5p, miR-101-3p. 1
5	*KDELR1*	miR-182-5p, miR-142-3p. 2
6	*PIK3R1*	miR-153-3p, miR-218-5p, miR-15-5p, miR-16-5p, miR-195-5p, miR-424-5p, miR-497-5p
7	*AIRE*	miR-212-5p
8	*ERO1LB*	miR-153-3p
9	*STAT3*	miR-124-3p. 1, miR-125-5p
10	*KLF11*	miR-17-5p, miR-138-5p, miR-10-5p, miR-93-5p, miR-106-5p , miR-519-3p, miR-20-5p
11	*SEC61A1*	miR-218-5p.
12	*ORMDL3*	miR-17-5p, miR-20-5p, miR-93-5p, miR-106-5p, miR-519-3p
13	*MAPKAPK2*	miR-137
14	*GATA6*	miR-10-5p, miR-141-3p, miR-181-5p, miR-200a-3p
15	*DERL1*	miR-181-5p
16	*PGM1*	miR-34-5p, miR-449-5p, miR-30-5p
17	*PAX6*	miR-455-3p. 1, miR-129-5p, miR-190-5p, miR-383-5p. 1, miR-223-3p
18	*ERGIC1*	let-7-5p, miR-98-5p
19	*ERN1*	miR-145-5p
20	*SERP1*	miR-137, miR-183-5p. 1, miR-124-3p. 1
21	*MAPK14*	miR-128-3p, miR-124-3p. 2, miR-506-3p, miR-124-3p. 1, miR-19-3p

gene; defects in this gene may contribute to T2DM.[31] Its expression is regulated by miR-98-5p. The *IGF2BP2* and *IRS2* genes modulate the actions of insulin and insulin-like growth factor 1, and their cytoplasmic products function as T2DMrelated biomarkers under the regulatory control of miR-98-5p.[32] miR-9-5p targets *KLHDC10*, a regulator of ASK1, thereby modulating oxidative stress-induced cell death by inhibiting protein phosphatase 5.[33] miR-137 targets GPX7, which helps prevent hydrogen peroxide-induced oxidative stress in esophageal epithelia.[34] Various other miRNAs and their targets are listed in Figure 5.

3.5. Commonly expressed miRNAs and their target genes in T2DM under ER stress

We evaluated 24 miRNAs involved in T2DM with ER stress. High mobility group AT-hook 1 (HMGA1) protein is a key activator of *INSR* gene expression. Aberrant expression of the *HMGA1* gene and its protein product has been linked to increased risk of T2DM and insulin resistance.[35] During sepsis-related myocardial damage, miR-195-5p acts on *ATF6* to suppress inflammatory responses, apoptotic cell death, oxidative stress, and ER stress.[36] *PIK3R1* is a potential candidate gene involved in insulin signaling pathways in T2DM development.[37] These genes are modulated by miR-195-5p. miR-145-5p targets *ERN1*, a UPR sensor in the ER.[38] Numerous other miRNAs and their targets are listed in Figure 6.

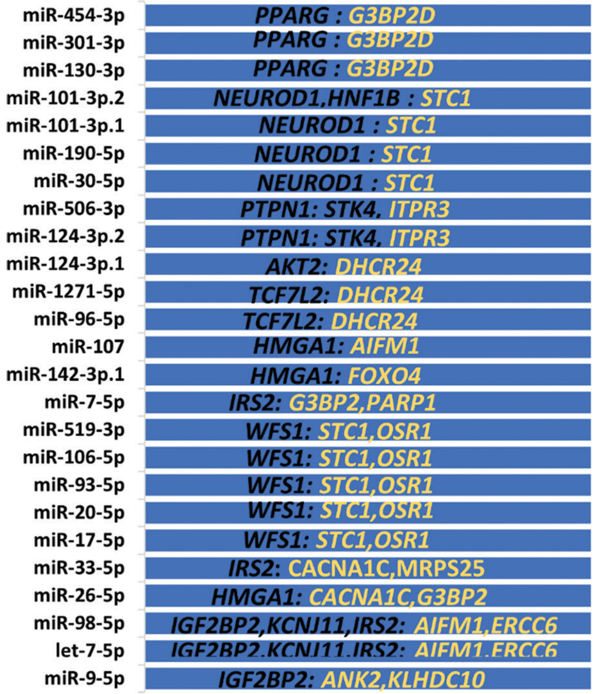

Figure 5. The most common microRNAs involved in type 2 diabetes mellitus (T2DM) and oxidative stress. Black color highlights the target genes involved in T2DM. The yellow color highlights the target genes involved in oxidative stress.

3.6. Co-expressed miRNAs and their targets in oxidative and ER stress associated with T2DM

We identified 14 miRNAs targeting common genes associated with T2DM, oxidative stress, and ER stress, as shown in Figure 7. It has been reported that individuals with T2DM are particularly susceptible to various forms of cellular stress, and the shared miRNAs identified here could inform the development of improved RNA-based therapies to reduce T2DM-associated oxidative and ER stress. In particular, miR-98-5p and let-7-5p may regulate seven putative genes closely linked to T2DM under oxidative and ER stress. All these miRNAs target more than one gene, as shown in Figure 7.

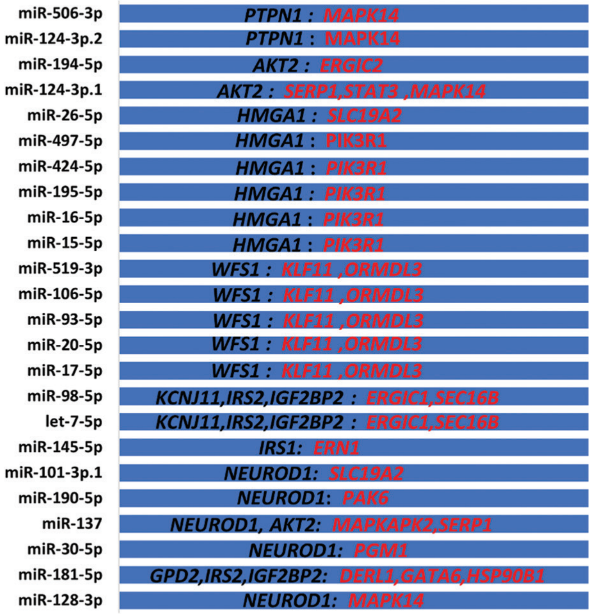

Figure 6. The most common microRNAs involved in type 2 diabetes mellitus (T2DM) and endoplasmic reticulum (ER) stress. Black color highlights the target genes involved in T2DM. Red color highlights the target genes involved in ER stress.

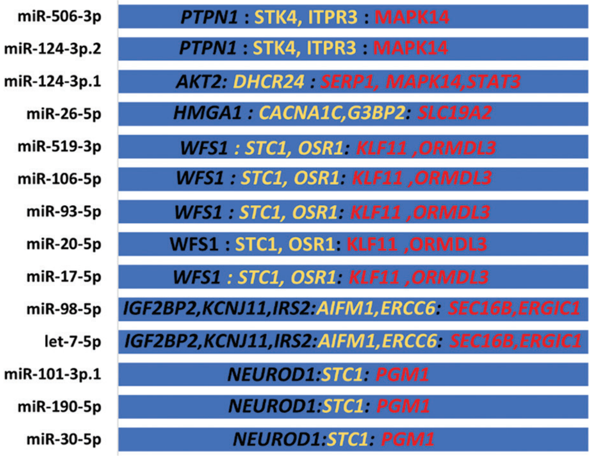

Figure 7. The most common microRNAs involved in type 2 diabetes mellitus (T2DM) associated with oxidative and endoplasmic reticulum (ER) stress. Black color highlights the target genes involved in T2DM. The yellow color highlights the target genes involved in oxidative stress. Red color highlights the target genes in ER stress.

3.7. Gene enrichment analysis for T2DM in relation to oxidative and ER stress

Figures 8-10 show the enriched biological function, pathways, domains, and networks associated with the upregulated genes in T2DM, oxidative stress, and ER stress, respectively, validated using the ShinyGO database. The associated pathways converge on protein folding, UPR, and ER-associated degradation, indicating that dysregulated proteostasis is a key component of the diabetic cellular environment.

Altogether, these enrichment analyses indicate that oxidative and ER stress pathways in T2DM largely represent downstream amplification loops of cellular injury arising from systemic insulin resistance and impaired insulin action, rather than initiating pathogenic events. Chronic hyperglycemia and lipotoxicity arising from impaired insulin signaling activate these stress networks, which, in turn, feed-back to further damage β-cells and insulin-responsive tissues, thereby exacerbating insulin resistance and accelerating disease progression.

4. Discussion

miRNAs have been extensively investigated to determine their functions and roles in disease. For numerous diseases, miRNAs have shown potential as early-detection biomarkers.[39] In the present study, we proposed a computational approach using bioinformatics to identify key miRNA regulators of gene sets implicated in T2DM related to oxidative and ER stress. Using stringent conservation criteria across human, rat, and mouse, we performed target prediction and selected conserved 8nt miRNAs that regulate multiple target genes involved in key cellular processes. The primary obstacle in clinical translation of miRNAs is Food and Drug Administration approval; a single miRNA may affect a large number of genes without discrimination.[40] However, it could be relevant for biomedical research.

T2DM is intricately linked to chronic cellular stress responses, particularly oxidative stress and ER stress that develop in response to persistent nutrient overload and metabolic imbalance. These stress pathways function as crucial molecular mediators connecting excess nutrient flux with cellular dysfunction and insulin resistance.[41] Sustained activation of oxidative and ER stress not only disrupts insulin signaling and β-cell integrity but also amplifies systemic inflammation and metabolic dysregulation, thereby accelerating disease progression and the onset of complications.[42] Insulin resistance, an early hallmark of T2DM, occurs when muscle, liver, and adipose tissues respond poorly to insulin, leading to compensatory hyperinsulinemia that eventually fails due to genetic, nutritional, and lipid-induced impairments in INSR and PI3K-Akt signaling, thereby reducing glucose uptake and glycogen synthesis while increasing hepatic glucose production.[43] Under these conditions, pancreatic β-cells experience sustained exposure to elevated glucose levels, which intensifies glucose metabolism and increases

Figure 8. Functional enrichment analysis of genes associated with type 2 diabetes mellitus

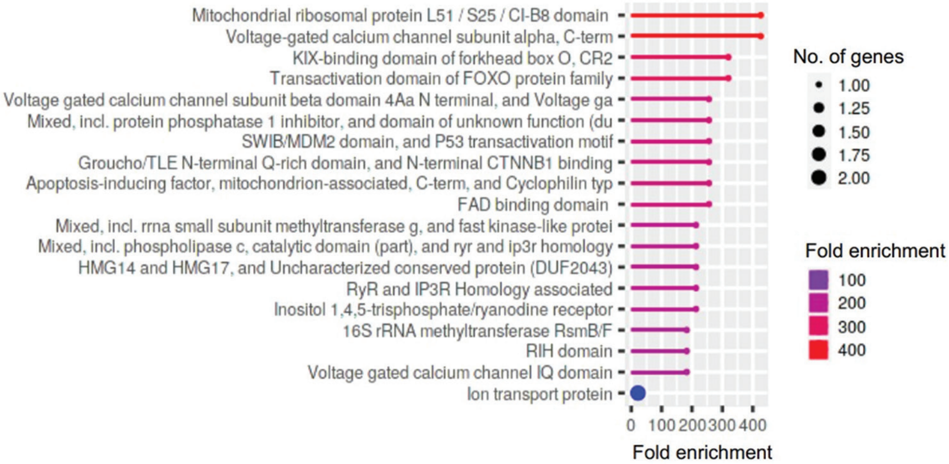

Figure 9. Functional enrichment analysis of genes associated with oxidative stress

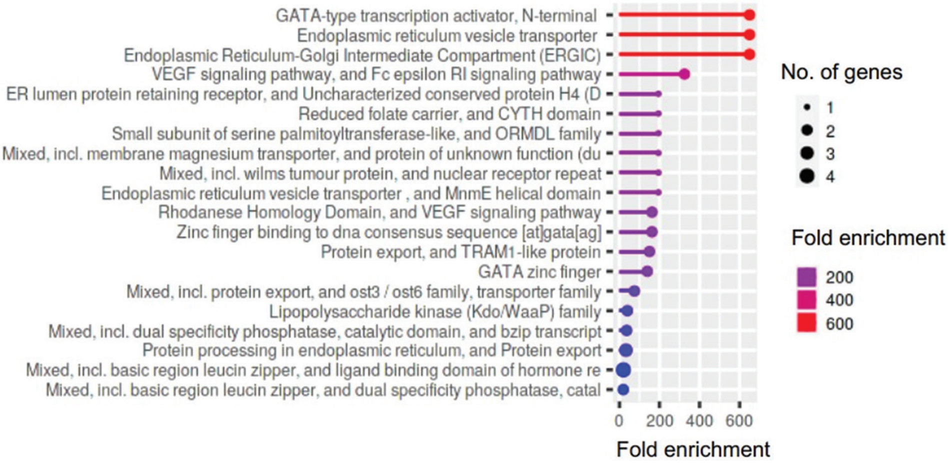

Figure 10. Functional enrichment analysis of genes associated with endoplasmic reticulum stress

electron flow through the mitochondrial electron transport chain (ETC). This heightened activity elevates mitochondrial ROS production.[44] Continuous nutrient oversupply and defective insulin signaling further burden the tricarboxylic acid cycle and ETC complexes, promoting electron leakage and the generation of superoxide and related reactive species. These reactive species progressively damage mitochondrial DNA, membrane lipids, and respiratory proteins.[45] Excess mitochondrial ROS amplifies ER stress, especially in β-cells, which face a persistent demand for proinsulin synthesis, folding, and secretion under hyperglycemic conditions.[41] ROS-mediated injury disrupts calcium homeostasis and impairs ER protein-folding capacity, activating the UPR. When this response becomes maladaptive, it engages pro-apoptotic signaling pathways, leading to β-cell loss and diminished insulin production.[46] Consequently, under these insulin-resistant conditions, persistent nutrient oversupply markedly enhances mitochondrial ROS production, and ER stress sustains a self-reinforcing cycle of oxidative stress that drives β-cell dysfunction, systemic insulin resistance, and the progression of diabetic complications.[47] Therefore, the vicious cycle between oxidative and ER stress should be viewed as a secondary amplifier of tissue damage, triggered by primary insulin resistance, which, in turn, feeds back to impair insulin signaling and β-cell failure.[48] Collectively, these mechanisms position oxidative and ER stress as both downstream consequences and reinforcing drivers of insulin dysfunction, promoting the transition from compensated dysglycemia to overt T2DM and its associated complications.

Recent evidence highlights that miRNAs act as crucial post-transcriptional regulators of the INSR signaling pathway, thereby influencing insulin sensitivity across skeletal muscle, liver, and adipose tissue.[49] Numerous studies have demonstrated that miRNAs can directly target key components, such as *INSR*, *IRS1/2*, *PIK3R1*, *AKT2*, and *GLUT4*, leading to impaired glucose uptake, reduced glycogen synthesis, and defective suppression of hepatic glucose output.[50] Consequently, insulin resistance in T2DM is driven not only by genetic and environmental factors but also by disrupted miRNA networks that dampen proximal insulin signaling. Recent *in silico* analysis identified several miRNAs previously linked to insulin resistance, including members of the miR-103/107, miR-143, miR-27, miR-29, and miR-144 families that modulate key nodes of the INSR-IRS-PI3K-Akt cascade and *GLUT4* expression. These alterations can initiate or exacerbate insulin resistance before overt oxidative and ER stress develops.[51] Notably, predicted miRNA-gene interactions involving canonical targets, such as *IRS1*, *IRS2*, *AKT2*, and *PIK3R1*, suggest that these miRNAs function not merely as stress markers but as early mechanistic drivers of insulin signaling defects. Collectively, the proposed miRNA-gene networks may contribute to a two-phase pathogenic process in T2DM, first by impairing insulin signaling and second by enhancing oxidative and ER stress during chronic metabolic overload.[52]

We identified various master regulators prevalent in T2DM with oxidative and ER stress. In addition, we searched for 8-nt-binding targeted genes related to T2DM, oxidative, and ER stress. Key genes identified in this study, including *KCNJ11*, *IRS2*, *IGF2BP2*, *AIFM1*, *ERCC6*, and *SEC16B*, are increasingly recognized as pivotal regulators connecting chronic hyperglycemia with oxidative and ER stress-driven β-cell dysfunction.[53] Among these, *KCNJ11* and *IRS2* play essential roles in insulin secretion and signaling, and their dysregulation heightens glucotoxicity-induced ROS production and stress kinase activation.[54] Meanwhile, *IGF2BP2* and *ERCC6* act as integrators of metabolic signals with RNA stability and DNA damage repair mechanisms under oxidative challenge.[55] The above genes were identified as the targets of miR-98 using TargetScan. As shown in the study by Yu *et al.*,[56] miR-98-5p exerts anti-apoptotic and antioxidative effects in mice, protecting against brain ischemia/reperfusion damage. Targeting *PPP1R15B* in keratinocytes dysregulates miR-98-5p levels in the blood of diabetic patients, thereby limiting proliferation and leading to apoptosis.[57] These results demonstrate that miR-98, which targets specific genes, is a promising miRNA for the treatment of T2DM and oxidative stress. In parallel, genes such as *NEUROD1*, *GPX7*, *AKT2*, *STK4*, *MAPK14*, *SERP1*, and *STAT3* are increasingly viewed as components of a dynamic stress-adaptation network in T2DM, coordinating transcriptional regulation of the insulin gene, antioxidant responses, the UPR, and inflammatory pathways.[58]

Through literature review, we discovered numerous genes associated with T2DM (Table 1), oxidative stress (Table 2), and ER stress (Table 3). Extensive research on miR-93-5p demonstrated its role in T2DM. By targeting *HGF*, this miRNA appears to reduce insulin resistance in HepG2 cells, thereby slowing progression toward T2DM.[59] *OSR1* involves a signaling cascade controlled by the PI3K-Akt signaling pathway, which increases sodium-chloride cotransporter phosphorylation in hyperinsulinemic mice.[60] These studies have demonstrated that miR-93-5p can be an effective target for various stress-mediated signaling related to T2DM. The decreased ER stress induced by miR-9 overexpression reduces ischemic damage and provides a target for ischemic stroke therapy.[61] miR-9 can regulate insulin secretion by suppressing the expression of *ONECUT2* and *SIRT1*. Reduced insulin production, which contributes to T2DM, has been shown to correlate with *IGF2BP2*.[62] miR-9 contributes to the regulation of these genes. miR-137 also regulates various genes, including *NEUROD1* and *GPX7*. The insulin gene's expression is regulated by the *NEUROD1* gene. T2DM is associated with mutations in the *NEUROD1* gene.[63] Maintaining redox homeostasis, glutathione peroxidase 7 (GPX7) has been shown to indirectly regulate signal transduction in the insulin and nuclear factor κB (NF-κB) signaling pathways.[64] One miRNA may bind to several targets, and miR-124 is anticipated to control the expression and silencing of genes, including *AKT2*, *DHCR24*, *STK4*, *MAPK14*, *SERP1*, and *STAT3*.[65]

Recent experimental research highlights that ongoing oxidative and ER stress in T2DM induces maladaptive activation of key signaling pathways, including c-Jun N-terminal kinase, NF-κB, and the PERK-IRE1α-ATF6 axis. These pathways place various implicated genes at critical junctions for β-cell failure and diabetes complications, such as cardiomyopathy and neuropathy.[66] Within this context, miRNA-mediated regulation is increasingly recognized as a mechanism that affects insulin signaling, antioxidant enzymes, such as GPX7, and ER stress-modulating genes, such as *SERP1* and *STAT3*, suggesting that subtle changes in miRNA expression can broadly impact stress responses in metabolic tissues.[67] In addition, contemporary investigation into diabetic complications, including retinopathy, cardiomyopathy, and slow-healing wounds, demonstrates that miRNAs linking persistent hyperglycemia to oxidative and ER stress are being explored as biomarkers of drug response and secondary therapeutic targets.[68] Clinical and translational studies have shown that glucagon-like peptide-1 receptor agonists, such as liraglutide, can regulate miRNAs, including miR-93-5p and miR-9-5p, in T2DM patients, with changes in these miRNA levels associated with better insulin signaling, reduced oxidative injury, and improved metabolic profiles, supporting the relevance of stress-related mechanisms.[69] Network analyses further reinforce the centrality of redox-sensitive miRNAs, such as miR-126, miR-146a, miR-98, miR-935p, miR-9, miR-124, and miR-137, in advanced glycation end-products (AGE)-receptor for AGE and inflammatory pathways, highlighting them as rational candidates for future T2DM therapeutics targeting oxidative and ER stress.[70]

Several miRNAs identified in our *in silico* analysis have been previously linked to adipose tissue biology and obesity-associated metabolic disturbances. Both experimental and clinical evidence indicate that miRNAs regulating adipogenesis exhibit opposing expression patterns in differentiated adipocytes compared with those in obese adipose tissue, implicating their dysregulation in local inflammation and insulin resistance.[71] Circulating miRNAs, such as miR-34a and miR-122, have also been correlated with obesity and obesity-related T2DM, underscoring the notion that excessive adiposity can reprogram systemic miRNA expression profiles. These observations imply that in obese individuals with T2DM, chronic nutrient surplus, adipose inflammation, and altered adipokine signaling may further influence the expression of stress-responsive miRNAs identified in our analysis as shared regulators of T2DM, oxidative stress, and ER stress.[72] While the current bioinformatic findings cannot quantify these effects, it is reasonable to propose that obesity may potentiate or modify the functional impact of specific miRNAs on their target genes, thereby shaping disease progression and therapeutic responsiveness. Future studies incorporating body mass index and adiposity stratification will be essential to clarify whether the miRNA networks described in our study are differentially regulated in lean and obese subjects with T2DM.

Oxidative stress plays a central role not only in cellular dysfunction and insulin resistance in T2DM but also in the development of atherosclerosis, where excess ROS drives endothelial injury, lipid peroxidation, and vascular inflammation.[73] Within the vascular system, miRNAs constitute complex regulatory networks that modulate redox homeostasis, endothelial activation, and smooth muscle cell proliferation, with several redox-responsive miRNAs already implicated in plaque initiation and progression.[74] Emerging evidence further suggests that circulating miRNAs, such as miR-122 and other oxidative stress-related variants, may serve as predictive biomarkers for coronary atherosclerosis and correlate closely with systemic indicators of oxidative injury.[75] Given that numerous miRNAs highlighted in our study target genes governing oxidative and ER stress responses, it is plausible that these same regulatory axes contribute to both T2DM pathogenesis and its macrovascular complications under chronic metabolic and oxidative stress.[76] Nonetheless, our *in silico* analysis did not include clinical vascular parameters; these proposed associations remain speculative and warrant validation in patient cohorts characterized for cardiovascular outcomes.

Additional *in vivo* analyses employing real-time quantitative polymerase chain reaction, next-generation sequencing, luciferase reporter assays, and western blotting are required to experimentally confirm the therapeutic miRNA targets proposed in this study. miRNA mimics and antagomirs are the two main categories for miRNA-based therapies. The miRNA mimics restore a specific miRNA's concentration that has decreased due to disease.

Antagomirs, on the other hand, silence disease-related miRNAs that are overexpressed.[77] Depending on the target gene's function, miRNA mimics or antagonists may be used to alter gene expression. Numerous biotechnology associations are developing miRNA-based therapeutics. In parallel, challenges related to delivery, off-target effects, and tissue specificity mean that rigorous preclinical testing in relevant T2DM models will be essential before clinical translation. However, exploration of miRNAs as therapeutic agents for T2DM involving oxidative and ER stress remains at an early stage. Early concept studies in diabetic animals suggest that modulating specific miRNAs can improve insulin sensitivity, glucose tolerance, and lipid metabolism, but large-scale clinical trials in humans are still lacking.

5. Conclusion

Various abnormalities, such as T2DM and related oxidative and ER stress, can be treated using miRNAs. We focused on TargetScan and *in silico* bioinformatic analysis using NCBI databases. Our study identifies potential therapeutic targets for treating diseases. A subset of these targets appears to be novel, and these newly identified candidates could support the design of new diagnostic biomarkers and treatment approaches for disease. Both *in vivo* and *in vitro* experiments are crucial to validate our findings.

Taken together, this study is limited by its purely *in silico* design, which relies exclusively on bioinformatic predictions and publicly available miRNA and gene databases without functional or experimental validation. The absence of individual patient information, such as body mass index, obesity status, or atherosclerotic disease, precludes assessing how these comorbidities, which are common in T2DM, might modulate the identified miRNA-gene interactions. Given that both obesity and atherosclerosis are characterized by distinct miRNA expression profiles and sustained oxidative stress, they could substantially influence the circulating and tissue miRNA signatures observed *in vivo*. Therefore, the predicted regulatory axes should be interpreted cautiously until validated in wellphenotyped T2DM cohorts with comprehensive clinical and metabolic data.

Moreover, our study simplified the complex network linking oxidative stress, ER stress, and inflammation by focusing on selected miRNAs and genes while excluding other critical regulators, such as long non-coding RNAs, transcription factors, and posttranslational modifications. The lack of tissue-specific or experimental validation further limits generalizability, as miRNA and gene expression patterns are highly context-dependent across pancreatic β-cells, liver, adipose tissue, and the endothelium. Finally, translational aspects, such as targeted delivery, off-target effects, immune activation, and pharmacokinetics, remain unexplored, leaving a significant gap between computational predictions and clinical application. As such, the present findings should be viewed as hypothesis-generating and warrant rigorous *in vitro*, *in vivo*, and clinical validation.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Ayesha Jabeen
Investigation: Ayesha Jabeen
Methodology: Ayesha Jabeen
Writing–original draft: Ayesha Jabeen
Writing–review & editing: All authors

Ethical approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

No datasets were generated or analyzed during the present study.

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ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

Heart rate variability and arrhythmic pattern among patients with goiter in southwest Nigeria

Adeola O. Ajibare[1,2]*, Rasaaq A. Adebayo[3], Babatope A. Kolawole[3], Michael O. Balogun[3], Oluwafemi T. Ojo[1,2], Adekunle Adeyemo[4], Akinola O. Dada[1,2], Ayoola S. Odeyemi[2], and Adebowale Olayinka Adekoya[1,2]

[1]Department of Medicine, Faculty of Clinical Sciences, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria

[2]Department of Medicine, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

[3]Department of Medicine, Faculty of Clinical Sciences, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun, Nigeria

[4]Department of Otorhinolaryngology, Faculty of Clinical Sciences, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun, Nigeria

***Corresponding author:**
Adeola O. Ajibare
(adeola.ajibare@lasucom.edu.ng)

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Abstract

Goiter remains prevalent in iodine-deficient communities of developing countries, such as Nigeria. It may present with cardiovascular abnormalities, including impaired heart rate variability (HRV) and arrhythmias. Impaired HRV has not been fully studied among patients in Nigeria despite the endemicity of goiter. We, therefore, aim to evaluate HRV and arrhythmic patterns among goiter patients in southwest Nigeria. A cross-sectional study involving 40 hyperthyroid, 40 euthyroid, and 20 hypothyroid participants, as well as 20 age- and gender-matched control participants, who all had cardiovascular evaluation, thyroid function tests, and 24-h Holter electrocardiogram monitoring. The mean ages among the hyperthyroid, euthyroid, hypothyroid, and control populations were 41.53 ± 14.90, 40.67 ± 12.29, 43.78 ± 11.49, and 46.92 ± 13.85 years, respectively. Cardiac arrhythmia was more prominent in the hyperthyroid and hypothyroid groups ($p<0.001$). The most common abnormalities of rhythm in the hyperthyroid group were sinus tachycardia and atrial fibrillation, while sinus bradycardia was the most common rhythm abnormality in the hypothyroid group. HRV indices (SD of all normal–normal intervals [SDNN], SD of the averages of normal–normal intervals [SDANN], and root mean squares of differences between adjacent normal–normal intervals [RMS-SD]) were all impaired in the hypothyroid and hyperthyroid goiter groups but normal in both the control and the euthyroid population ($p<0.01$). The duration of goiter, left atrial, and left ventricular dimensions were the predictors of cardiac arrhythmias and impaired HRV. In summary, cardiac arrhythmias and HRV abnormalities are relatively common in hypothyroid and hyperthyroid goiter patients. Early diagnosis and treatment of thyroid abnormalities will help prevent cardiac dysfunctions associated with thyroid diseases.

Keywords: Holter electrocardiogram; Thyroid diseases; Goiter; Arrhythmia; Heart rate variability

1. Introduction

Goiter is a common condition in iodine-deficient communities around the world, including the hilly terrains of southwest Nigeria, despite extensive iodination of various food items.[1,2] Thyroid disease is second to diabetes mellitus as the most common condition in most endocrinology outpatient clinics in Nigeria.[1] It primarily affects females, with a few male manifestations. The prevalence of thyroid diseases in sub-Saharan Africa has increased in recent times. This has been attributed to the rising prevalence of autoimmune disease, poor dietary intake of iodine, and malignancy, especially in men.[1]

The thyroid hormone has profound effects and manifestations on all the body organs and systems.[1] The heart and blood vessels are major targets due to the sensitivity of the cardiovascular system to either excess or deficiency of triiodothyronine (T3) and tetraiodothyronine (T4). Due to the interaction among these thyroid hormones (T3 and T4) in the cardiovascular system, as well as the autonomic nervous system, thyroid dysfunction is associated with arrhythmias and cardiovascular sequelae.[3] Thus, a deficiency or excess of thyroid hormones may cause abnormalities in both the parasympathetic and sympathetic pathways regulating the cardiovascular system due to the interplay between the thyroid axis, cardiovascular system, and autonomic system.[3-6] This interplay therefore affects cardiac function, cardiac rhythm, and heart rate variability (HRV).[7-10]

HRV is an evolving complex interplay among the multi-systemic regulatory organs that helps the body to adapt to stressful environmental, pathological, and psychological variations.[11] It is a useful non-invasive method of assessing the influence of the ANS on the heart. Variability in heart rate arises from the effect of the sympathovagal system on the sinus node, resulting in minimal fluctuations in the sinus rate. Impaired HRV reflects abnormalities in these interrelated systems, including the thyroid axis. An optimal HRV has been shown to predict adaptive response to various physiological challenges, while a reduced HRV has been reported as a predictor of cardiac morbidity and mortality in cardiac disease patients.[7,11] Structural cardiac abnormalities have also been observed in thyroid dysfunction and are shown to be a cause of cardiac dysfunction and a focus for cardiac arrhythmogenesis and impaired HRV.[7,12]

HRV can be assessed on an ultra-short, short, or long-term basis. A 24-h HRV recording using ambulatory recording is the ideal modality for long-term clinical HRV measurement because it achieves a greater level of predictability compared to a short-term HRV measurement.[13,14] Standard deviation of the averages of normal–normal (NN) intervals (SDANN) in the five-minute segments of the entire recording, standard deviation of all NN intervals (SDNN), and the root mean square of differences between adjacent NN intervals (RMS-SD) are common modalities in the time-domain measurements of 24-h ambulatory electrocardiogram (ECG) measurements.

Cardiac arrhythmias are observed among patients with thyroid dysfunction, potentially exerting attendant consequences on the HRV.[4-6,8,9,15] Thyrotoxicosis is a cause of atrial fibrillation (AF), which has been shown to increase HRV.[11,16] The presence of arrhythmias and cardiac conduction abnormalities on a background of structural heart disease is related to higher mortality risks, especially in the aged population.[17] Therefore, the study of arrhythmia and HRV among thyroid disease patients is essential, especially in goiter-prevalent areas, such as Nigeria. Despite the endemicity of goiter in this locality, HRV monitoring in thyroid dysfunction is yet to be well explored in the Nigerian setting. A few studies on goiter and cardiac arrhythmias have shown that some of the arrhythmias and cardiac morbidities are reversible with the initiation and commencement of appropriate treatment for thyroid dysfunction.

It is therefore important to study, characterize, and document various abnormalities of rhythm and HRV in thyroid dysfunction patients. This study aims to evaluate the HRV and arrhythmic pattern and to determine the associated factors of abnormal HRV and cardiac rhythm among goiter patients in southwest Nigeria. This will help deepen the knowledge of cardiac changes in thyroid dysfunction.

2. Methods

2.1. Study design

This cross-sectional study was conducted among goiter patients who attended either the surgery or the endocrinology outpatient specialty clinics of Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun, Nigeria. The hospital is a multicenter and multispecialty tertiary hospital that receives referrals from all six neighboring states. We consecutively recruited 100 goiter patients into the study, dividing them into hyperthyroid, euthyroid, and hypothyroid goiter groups. In addition, 20 age- and gender-matched healthy participants (without goiter) were recruited from the willing staff, students, and the patients' relatives within the hospital, serving as the control. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline was followed in reporting this study.

2.2. Sample size calculation

The minimum sample size for this study was calculated using Fisher's formula for a cross-sectional study.[18] The confidence level was set at 95% with a precision of 5%, and a standard normal deviation of 1.96 was used. An average proportion of 6.5% (2–20%) from a meta-analysis on the prevalence of arrhythmia among thyroid disorder patients was used.[19] An estimated sample size of 94 was obtained, which was rounded up to 100 participants to account for attrition (Figure 1).

2.3. Inclusion criteria

All consenting adult patients with goiter who attended the various specialty clinics within the study period and control participants were consecutively recruited into the study (Figure 1).

2.4. Exclusion criteria

All the post-thyroidectomy patients, patients with subclinical thyroid diseases, patients on thyroxine or anti-thyroid drugs, and patients with a comorbid history of hypertension, valvular heart disease, diabetes mellitus, and/or chronic kidney disease were excluded (Figure 1).

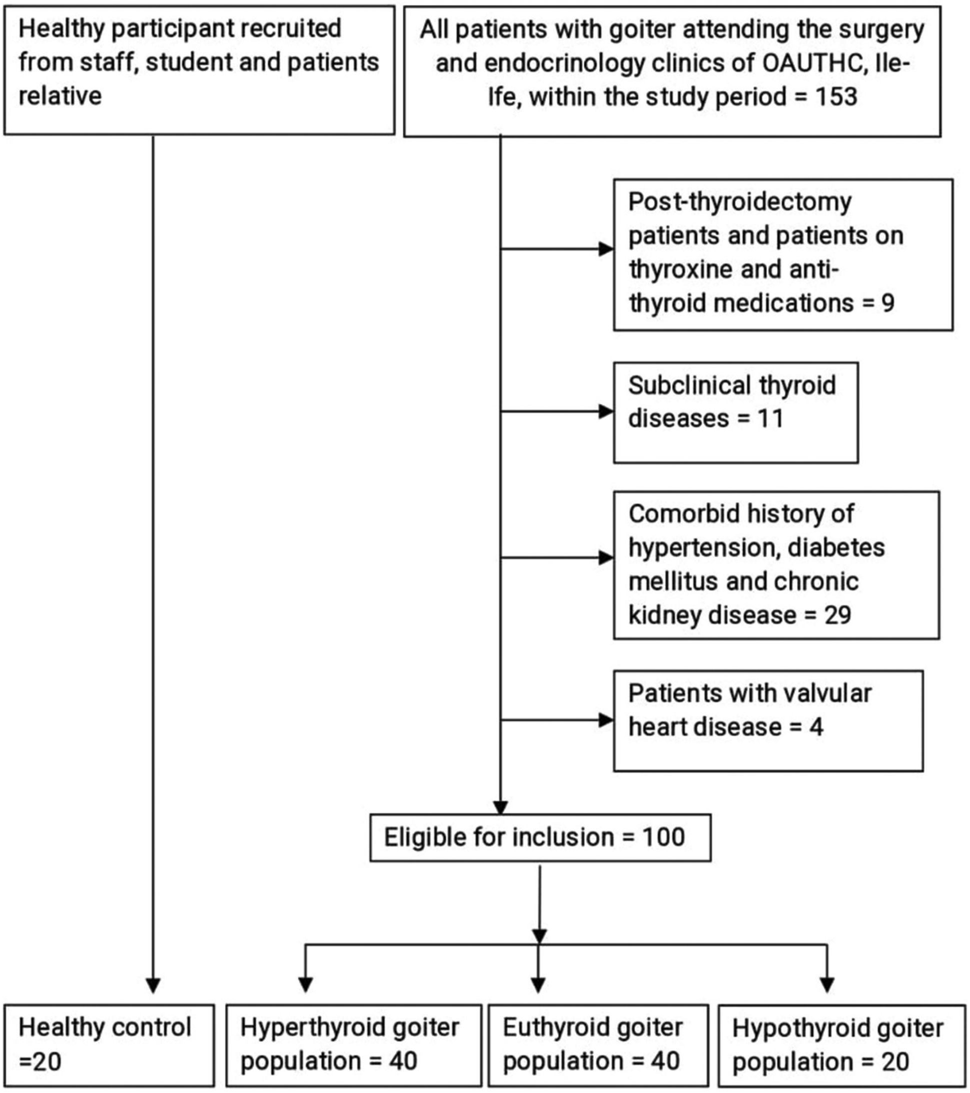

Figure 1. Eligibility flow chart of the participants
Note: Calculated sample size was 94.
Abbreviation: OAUTHC: Obafemi Awolowo University Teaching Hospitals Complex.

2.5. Sampling technique

A convenient sampling technique of consecutive goiter patients was used. The goiter group was divided into euthyroid, hyperthyroid, and hypothyroid subgroups based on their thyroid function test results.

2.6. Study procedure

All the participants had a full cardiovascular evaluation, serum chemistry, thyroid function tests (free triiodothyronine [fT3], tetraiodothyronine [fT4], and thyroid-stimulating hormone [TSH]), 24-h ambulatory (Holter) ECG, and echocardiography. These procedures were performed at the Cardiac Care Unit of the hospital.

The normal reference range for the enzyme immunoassay test for fT3 was 1.4–4.2 pg/mL, fT4 was 0.8–2.0 ng/dL, and TSH was 0.5–5.6 IU/mL. The hypothyroid group was defined as patients with goiter, low fT_3, low fT_4, and elevated TSH, while the hyperthyroid group was those with goiter, elevated fT_4, elevated fT_3, and low TSH. Goiter patients with normal fT_3, fT_4, and TSH were grouped into the euthyroid group, while the control participants had no goiter and had normal fT_3, fT_4, and TSH.

Transthoracic echocardiography was performed with a Vivid 7 Dimension echocardiography machine (GE Medical Systems [now GE HealthCare], United States [US]) using the 5S adult transthoracic phased array sector probe with simultaneous ECG tracing. All the measurements acquired during the study were performed according to the standard protocols of the American Society of Echocardiography, with an average of three consecutive cycles taken as the final measurements.[20] All the echocardiographic measurements were standardized and verified through the interpretation of at least two cardiologists.

The 24-h Holter ECG recording was performed with the Schiller Holter machine type (MT-101, SCHILLER AG, Switzerland) three-channel system. Participants were adequately counseled about the recorder, and the channel leads were placed conventionally.[13] The recording was performed for 24 h according to standard protocols, without impairment or alteration in the patient's daily activities. The machine was retrieved from the participants after recording, and the data were analyzed using the software CARDIOVIT CS-200 (SCHILLER AG, Switzerland) digital ECG on a personal computer. The heart rate, heart rhythm, arrhythmia burden, and HRV were recorded.

The time domain interval of HRV was analyzed according to the recommended guidelines.[13] Normal reference ranges for SDNN, SDANN, and RMS-SD were 141 ± 39 ms, 127 ± 39 ms, and 37 ± 15 ms, respectively.

The total heart rate, the minimum, the maximum, and the average heart rates over the 24-h recording were calculated and recorded. The heart rhythm within the study period was observed for sinus or ectopic atrial rhythms, while the observed arrhythmias were analyzed and divided into atrial and ventricular arrhythmias. Atrial arrhythmias were further subdivided into atrial premature complexes, atrial flutter, and AF, while ventricular arrhythmias were divided into isolated ventricular premature complexes, ventricular bigeminy, trigeminy, couplets, triplets, ventricular tachycardia, and fibrillation.[13] An arrhythmia burden, of either atrial or ventricular premature complexes, ≥10% of the total QRS, was termed a significant arrhythmia.

2.7. Statistical analysis

Data were input into a personal computer and analyzed using the International Business Machine SPSS version 20.0 software (IBM, US). All descriptive statistics were input. Continuous variables (e.g., age, laboratory parameters, echocardiographic parameters, and Holter-ECG measurements) were presented as means ± standard deviations. Categorical variables (e.g., gender, age groups, duration of goiter, social class, and arrhythmia burden) were presented as frequency and/or percentage. Chi-square ($\chi 2$) test was used to test the differences between groups of categorical variables, while the independent Student's *t*-test was used to determine the differences between two groups of continuous variables. The differences between three or more groups of continuous variables were tested using one-way analysis of variance with the Duncan *post hoc* test, while the Pearson coefficient test was used to determine the correlation between relevant variables. Logistic regression was performed to determine the predictors of HRV and arrhythmias among the statistically significant variables. Multivariate analysis, using statistical models, was performed to control for and adjust for confounding variables associated with arrhythmias and HRV, including age, gender, body weight, and body mass index. The alpha level was set at $p<0.05$ with a 95% confidence interval (CI).

3. Results

The study population consisted of 100 treatment-naïve goiter patients and 20 healthy controls. Both groups were age and sex-matched. The goiter patients were divided into hyperthyroid (40), euthyroid (40), and hypothyroid (20) groups.

The socio-demographic parameters of the participants are shown in Table 1. There was more female affectation. The mean age group was in the 40s. The majority of the patients had a goiter for at least 5 years, and many of the participants were in the low socioeconomic class.

The laboratory parameters of the participants are shown in Table 2. Thyroid hormone and TSH were significantly different across the groups, while other laboratory parameters were not significantly different.

The echocardiographic variables of the participants are shown in Table 3. The left atrial diameter index (LADi), left ventricular mass (LVM), LVM index (LVMi), relative wall thickness, left ventricular ejection fraction, and tricuspid annular plane systolic excursion were significantly different across the groups ($p<0.001$). *Post hoc* tests indicated that the echocardiographic variables of the control and the euthyroid groups were similar.

Table 4 shows the arrhythmic parameters of the participants. The heart rates of the hyperthyroid and hypothyroid groups were significantly higher and lower, respectively, compared to both the euthyroid and control groups ($p<0.05$). Sinus tachycardia was the most common arrhythmia observed in the hyperthyroid group, while the most common abnormality of rhythm seen in the hypothyroid group was sinus bradycardia. AF was only observed in the hyperthyroid group.

The HRV parameters of the participants are shown in Table 5. All the parameters were significantly reduced in both the hyperthyroid and hypothyroid groups ($p<0.05$) compared to both the control and euthyroid groups.

Table 6 shows the logistic regression of the significant parameters with arrhythmias and HRV. The predictors of arrhythmias and impaired HRV were duration of goiter, dilated left atrial diameter (LAD), and left ventricular diameter.

Table 1. Socio-demographic parameters of participants

Parameters	Hyperthyroid (*n*=40)	Euthyroid (*n*=40)	Hypothyroid (*n*=20)	Control (*n*=20)	*p*-value
Sex					0.75
Male	6	7	2	4	
Female	34	33	18	16	
Age (years; mean±SD)	41.53±14.90	40.67±12.29	43.78±11.49	46.92±13.85	0.85
Age group (years)					0.67
<20	4	4	3	1	
20–40	12	14	11	6	
40–60	18	15	4	10	
>60	6	7	2	3	
Duration of goiter (years)					0.04
<5	33	21	15	-	
5–10	5	6	3	-	
>10	2	13	2	-	

Table 2. Laboratory parameters of participants

Variables	Hyperthyroid (*n*=40)	Euthyroid (*n*=40)	Hypothyroid (*n*=20)	Control (*n*=20)	*p*-value
TSH (IU/mL)	0.30±0.10	1.44±0.85	11.62±5.03[b]	1.22±0.72	<0.0001
fT3 (pg/mL)	10.31±4.15[a]	3.11±0.73	0.88±0.21	3.15±0.77	<0.0001
fT4 (ng/dL)	4.25±1.34[a]	1.34±0.26	0.43±0.07	1.33±0.30	<0.0001
Serum sodium (mmol/L)	140.87±2.21	140.61±2.25	142.22±1.48	139.89±2.47	0.3000
Serum potassium (mmol/L)	3.80±0.27	3.87±0.21	3.93±0.27	3.87±0.22	0.2297
Serum chloride (mmol/L)	101.26±3.10	101.84±3.75	102.55±3.28	100.00±4.90	0.0525
Serum urea (mmol/L)	4.80±0.87	4.82±0.21	4.91±0.37	4.87±0.41	0.3286
Serum creatinine (Umol/L)	93.82±7.21	96.53±6.66	99.13±8.23	94.56±7.39	0.3100
Packed cell volume (%)	38.17±3.70	38.11±3.11	37.22±4.24	38.96±4.13	0.3597

Notes: Data are presented as mean±SD. [a]$p<0.05$ (*post hoc* test; hyperthyroid vs. euthyroid/control/hypothyroid); [b]$p<0.05$ (*post hoc* test; hypothyroid vs. euthyroid/control/hyperthyroid).

Abbreviations: fT3: Free triiodothyronine; fT4: Free thyroxine; TSH: Thyroid-stimulating hormone.

Table 3. Echocardiographic parameters of participants

Variables	Hyperthyroid (n=40)	Euthyroid (n=40)	Hypothyroid (n=20)	Control (n=20)	p-value
IVSD (cm)	1.20±0.13[a]	0.93±0.13	1.03±0.13	0.99±0.10	<0.001
LVPWD (cm)	1.18±0.11[a]	0.93±0.13	1.00±0.09	0.97±0.10	<0.001
LAD (cm)	3.66±0.47[b]	3.36±0.60	3.93±0.50[c]	3.37±0.53	0.005
LADi (cm/m^2)	2.13±0.32[b]	1.78±0.35	1.90±0.25[c]	1.87±0.33	<0.001
LVM (g)	174.51±33.66[b]	122.23±28.79	172.00±44.73[c]	129.06±23.57	<0.001
LVMi (g/m^2)	101.25±19.97[b]	64.89±16.29	82.98±22.41[c]	71.68±14.38	<0.001
RWT	0.27±0.05[a]	0.21±0.04	0.20±0.04	0.22±0.04	<0.001
SV (mL)	91.03±43.97	89.77±35.29	143.25±56.96[d]	88.90±37.14	0.002
SI (mL/m^2)	52.77±25.40	49.28±19.32	69.02±27.82[d]	49.43±21.45	0.078
CO (L/min)	9.62±4.69[a]	7.15±2.79	8.49±3.39	6.97±3.39	0.005
CI (L/min/m^2)	5.57±2.70[a]	3.80±1.54	4.09±1.66	3.87±1.92	<0.001
LVEF (%)	72.47±9.64[a]	65.21±8.09	48.44±7.73	65.35±8.70	<0.001
LVFS (%)	41.58±8.78[a]	35.89±6.02	24.67±4.53	36.05±6.36	<0.001
TAPSE (cm)	20.09±4.55[a]	18.59±2.37	13.67±3.21[d]	18.98±3.02	<0.001
LVIDD (cm)	4.40±0.65	4.45±0.59	5.13±0.78[d]	4.39±0.56	0.0059
LVDDi (cm/m^2)	2.56±0.40	2.36±0.36	2.47±0.39	2.44±0.38	0.1080
RVID (cm)	1.72±0.39	1.64±0.38	1.87±0.49	1.64±0.34	0.2282
RVIDi (cm/m^2)	1.00±0.26[a]	0.87±0.22	0.90±0.25	0.91±0.21	0.0369

Notes: Data are presented as mean±SD. [a]p<0.05 (*post hoc* test; hyperthyroid vs. euthyroid/control/hypothyroid); [b]p<0.05 (*post hoc* test; hyperthyroid vs. euthyroid/control); [c]p<0.05 (*post hoc* test; hypothyroid vs. euthyroid/control); [d]p<0.05 (*post hoc* test; hypothyroid vs. euthyroid/control/hyperthyroid). Abbreviations: CI: Cardiac index; CO: Cardiac output; IVSD: Interventricular septal thickness in diastole; LAD: Left atrial diameter; LADi: Left atrial diameter index; LVEF: Left ventricular ejection fraction; LVFS: Left ventricular fractional shortening; LVIDD: Left ventricular internal dimension in diastole; LVIDDi: Left ventricular internal dimension in diastole index; LVM: Left ventricular mass; LVMi: Left ventricular mass index; LVPWD: Left ventricular posterior wall thickness in diastole; RVID: Right ventricular internal dimension; RVIDi: Right ventricular internal dimension index; RWT: Relative wall thickness; SI: Stroke index; SV: Stroke volume; TAPSE: Tricuspid annular plane systolic excursion.

4. Discussion

This study evaluated the pattern of arrhythmias and abnormal HRV among patients with goiter in the goiter-prevalent communities of southwest Nigeria. It also identified the factors that predicted these abnormalities. The importance of evaluating the frequency and pattern of arrhythmias, as well as HRV abnormalities, in thyroid dysfunction within southwest Nigeria cannot be overemphasized, given the limited number of studies conducted in this region. Our study population was predominantly female, consistent with previous works in Nigeria where most of the participants with goiter were females aged between 40 and 60 years.[1,2,21]

4.1. Arrhythmias

In our study, sinus tachycardia and supraventricular arrhythmias were the most common rhythm abnormalities found among the hyperthyroid participants, whereas sinus bradycardia was predominant among hypothyroid participants. Both the euthyroid and the healthy control groups had no significant arrhythmias. These findings align with previous works reporting similar patterns.[10] The comparable outcomes between the euthyroid and healthy control groups may be explained by a previous study demonstrating that cardiac dysfunction in goiter patients is due to thyroid hormone derangements rather than the presence of goiter,[22] as both groups were euthyroid. Similarly, thyrotoxic heart disease has been documented to manifest commonly as sinus tachycardia and AF.[19,23] This concurs with the review by Klein and Danzi,[22] which identified tachycardia, AF, and atrial arrhythmias as frequently associated with thyroid dysfunction. In a community survey, Zhang *et al.*[10] reported tachycardia as the most common heart rhythm abnormality associated with increased serum T4 levels and noted that hypothyroidism was associated with bradycardia. They also described a positive linear relationship between heart rate and serum total T4 levels.

Approximately 50% of supraventricular arrhythmias observed in the hyperthyroid group were attributable to AF, consistent with previous works.[3-5,8,22] Cini *et al.*[3] attributed the increased rate of AF to abnormalities in

Table 4. Arrhythmic parameters among the participants

Parameters	Hyperthyroid (*n*=40)	Euthyroid (*n*=40)	Hypothyroid (*n*=20)	Control (*n*=20)	*p*-value
Total heart rate/24 h	153,955.80±11,723.83[a]	111,330.72±11,081.82	83,990.00±10,355.93[c]	112,935.10±11,899.38	<0.0001
Minimum heart rate/min	73.34±8.28[a]	52.21±5.28	41.78±4.55[c]	53.92±8.76	<0.0001
Maximum heart rate/min	132.28±10.15[a]	114.39±9.05	98.56±17.59[c]	116.76±12.55	<0.0001
Average heart rate/min	95.75±6.46[a]	74.71±6.89	57.00±7.38[c]	76.24±11.56	<0.0001
Heart rhythm analysis					<0.0001
Sinus rhythm	12 (30)	34 (85)	6 (30)	16 (80)	
Sinus bradycardia	0	0	14 (70)[d]	3 (15)	
Sinus tachycardia	18 (45)[b]	1 (2.5)	0	1 (5)	
Supraventricular arrhythmias	10 (25)	2 (5)	2 (10)	1 (5)	
Ventricular arrhythmias	7 (17.5)[a]	2 (5)	1 (5)[c]	1 (5)	
Supraventricular arrhythmias					<0.0001
Premature atrial complexes	4 (40)	2 (100)	2 (100)	1 (100)	
Atrial fibrillation	5 (50)[b]	0	0	0	
Atrial flutter	1 (10)[b]	0	0	0	
Ventricular arrhythmias					0.1990
Premature ventricular complex	5 (71.4)	2 (100)	0	1 (100)	
Couplets	1 (14.3)	0	1 (100)	0 (0)	
Triplets	1 (13.3)	0	0	0	

Notes: Data are presented as mean±SD or *n* (%). [a]$p<0.05$ (*post hoc* test; hyperthyroid vs. euthyroid/control); [b]$p<0.05$ (*post hoc* test; hyperthyroid vs. euthyroid/control/hypothyroid); [c]$p<0.05$ (*post hoc* test; hypothyroid vs. euthyroid/control); [d]$p<0.05$ (*post hoc* test; hypothyroid vs. euthyroid/control/hyperthyroid).

Table 5. 24-h heart rate variability parameters of participants

Parameters	Hyperthyroid (*n*=40)	Euthyroid (*n*=40)	Hypothyroid (*n*=20)	Control (*n*=20)	*p*-value
SDNN (ms)					
Day	107.40±21.76[a]	134.43±21.58	105.67±30.46[b]	139.08±16.69	<0.0001
Night	85.52±22.96[a]	130.52±22.97	87.78±51.22[b]	135.36±15.31	
All	95.27±21.62[a]	131.27±21.62	97.67±39.82[b]	136.04±15.89	
SDANN (ms)					<0.0001
Day	93.79±+18.09[a]	118.17±18.45	91.93±19.42[b]	120.27±16.63	
Night	68.48±19.45[a]	116.98±17.69	60.20±18.70[b]	118.92±15.16	
All	82.52±18.81[a]	117.34±18.14	80.74±19.00[b]	119.38±16.02	
RMS-SD (ms)					<0.0001
Day	20.05±15.09[a]	31.28±15.88	20.62±15.21[b]	30.58±14.67	
Night	17.48±11.68[a]	30.11±14.73	18.71±13.54[b]	30.23±14.30	
All	19.76±14.86[a]	30.20±14.88	19.34±13.98[b]	30.39±14.52	

Notes: [a]$p<0.05$ (*post hoc* test, hyperthyroid vs. euthyroid/control/hypothyroid); [b]$p<0.05$ (*post hoc* test, hypothyroid vs. euthyroid/control/hyperthyroid).
Abbreviations: RMS-SD: Root mean squares of differences between adjacent normal–normal intervals; SDANN: Standard deviation of the averages of all normal–normal intervals; SDNN: Standard deviation of all normal–normal intervals.

inotropic and chronotropic cardiac function. In addition, a higher prevalence of isolated ventricular pre-mature complexes was observed among the thyroid dysfunction participants in our study, exceeding rates reported in some previous works that documented a low prevalence of ventricular arrhythmias, including ventricular tachycardia, ventricular fibrillation, and other malignant arrhythmias.[23]

Table 6. Logistic regression analysis of significant parameters with arrhythmias and HRV

Parameters	Arrhythmias			HRV		
	Odds ratio	95% CI	*p*-value	Odds ratio	95% CI	*p*-value
Duration of goiter	0.720	0.529–0.981	0.038	0.361	0.133–0.967	0.041
LADi	0.159	0.037–0.679	0.013	0.273	0.085–0.936	0.024
LVIDD	5.286	1.664–16.792	0.005	2.588	1.031–7.954	0.034

Abbreviations: CI: Confidence interval; HRV: Heart rate variability; LADi: Left atrial diameter index; LVIDD: Left ventricular internal dimension in diastole.

Many participants had impaired left and right ventricular systolic function. Structural heart disease, along with impaired ventricular systolic and diastolic function, has been documented as a predictor of arrhythmia.[7,12,21,23,24] The presence of systolic dysfunction may be the reason for the higher prevalence of ventricular premature complexes among the hyperthyroid and hypothyroid groups in our study. Both groups also demonstrated significantly larger LAD, LADi, LVM, and LVMi, which are all documented predictors of arrhythmia.[7,12,23] Despite the higher prevalence of pre-mature ventricular complexes, no life-threatening arrhythmias (e.g., ventricular tachycardia and ventricular fibrillation) were observed. The small size of each study group in our index study may have limited the detection of such events.

4.2. HRV

This study showed that HRV was significantly impaired in thyroid dysfunction participants (hyperthyroid and hypothyroid groups) compared to the euthyroid and control groups. All HRV parameters studied (e.g., SDNN, SDANN, and RMS-SD) were reduced in the thyroid dysfunction groups. Similar findings have been reported.[3,25,26] This impairment is associated with sympathovagal imbalance, characterized by increased sympathetic variability in hyperthyroid participants and decreased sympathetic variability in hypothyroid participants.[11] In addition, reduced vagal tone and increased variability of ventricular recovery times have been implicated in these populations. Although our study did not directly measure vagal tone, Brusseau *et al.*[4] corroborated our findings in a meta-analysis examining HRV in hyperthyroid individuals. The deleterious effects of thyroid hormones and TSH have been associated with the decreased HRV observed in hyperthyroid participants. Similarly, reduced HRV in hypothyroid participants has been attributed to the effects of TSH, characterized by increased sympathetic and decreased parasympathetic activity.[27]

Previous studies investigating arrhythmias and HRV have reported a relationship between older age and increased prevalence of arrhythmias, attributed to progressive degeneration of intracardiac conduction pathways with aging.[24,28] However, age was not a predictor of arrhythmia or HRV in our study. This may be explained by the relatively younger mean age (46.92 ± 13.85 years) of our cohort compared to populations in other studies involving older thyroid patients. A previous study on ventricular repolarization abnormality, a precursor to ventricular arrhythmias, reported an increased arrhythmia risk in males.[24] This contrasts with our findings, possibly due to the smaller proportion of male participants in our sample. Other factors previously identified as predictors of arrhythmias and altered HRV include TSH levels, thyroid hormone levels, smoking, alcohol consumption, serum electrolytes, blood pressure, LVM, and LVMi.[28-31] Our participants reported minimal alcohol or tobacco use, and no significant electrolyte abnormalities were detected across the study groups.

Finally, factors associated with arrhythmias in our study included duration of goiter, left atrial dilatation, and left ventricular enlargement. Previous studies have shown that left atrial dimension and left ventricular size are associated with arrhythmias.[23,32-34] Moreover, late presentation in cardiac diseases has been associated with arrhythmias.[21]

4.3. Clinical implication

Impaired HRV and a high arrhythmia burden have been previously associated with increased cardiac morbidity and mortality, as well as a higher risk of sudden cardiac death,[35,36] particularly among patients with AF.[37,38] Individuals with overt hyperthyroidism are at a higher risk of developing AF compared to euthyroid controls.[22] Our findings indicate that hyperthyroid and hypothyroid patients with a prolonged duration of goiter, dilated left atrium, and enlarged left ventricle are at a higher risk for impaired HRV and arrhythmias. It is crucial to promptly identify these high-risk patients, perform Holter monitoring to quantify arrhythmia burden, and initiate early treatment to mitigate cardiovascular risk, prevent future morbidity, and reduce the incidence of sudden cardiac death.

4.4. Limitations

The cross-sectional nature of this study may make causality difficult to establish. In addition, as a single-center study, the findings may not be generalizable to broader populations. This study did not consider the potential influence of alcohol consumption, smoking, caffeine intake, and socioeconomic factors on arrhythmias and HRV. We recommend future research to explore these factors and suggest conducting larger, multicenter studies with increased sample size.

5. Conclusion

Thyroid dysfunction is associated with a higher prevalence of arrhythmias and impaired HRV, whereas euthyroid goiter patients have similar arrhythmic risk with the non-goitrous population. In addition, a long duration of goiter and the presence of left heart dilation in thyroid dysfunction patients were predictors of arrhythmias and abnormal HRV. We therefore advocate for early screening and intervention in patients with hypothyroid or hyperthyroid goiter to prevent arrhythmias and abnormal HRV, which may increase their cardiovascular risk, complicate surgical outcomes, and elevate the risk of sudden cardiac death.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Adeola O. Ajibare, Rasaaq A. Adebayo, Babatope A. Kolawole, Michael O. Balogun
Data curation: Adeola O. Ajibare, Oluwafemi T. Ojo, Adekunle Adeyemo
Formal analysis: Oluwafemi T. Ojo, Akinola O. Dada, Ayoola S. Odeyemi, Adebowale O. Adekoya
Investigations: All authors
Methodology: Adeola O. Ajibare, Adekunle Adeyemo, Adebowale O. Adekoya.
Writing – original draft: All authors
Writing – review & editing: All authors

Ethics approval and consent to participate

The protocol of this study was approved by the Obafemi Awolowo University Teaching Hospital Ethics and Research Committee (protocol number ERC/2013/04/01). The purpose and procedure of the study were explained to the participants, and their written and verbal informed consents were obtained. Patients were also notified of their rights to withdraw from the study at any time. Confidentiality of all the data obtained throughout and beyond the study period was also assured.

Consent for publication

All participants provided informed consent for the publication of the findings derived from this study. Where applicable, participants gave explicit permission for the publication of any data, images, or information that could potentially reveal their identity. The authors affirm that all the relevant forms have been obtained and are available on reasonable request.

Availability of data

Data can be obtained on reasonable request to the authors.

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ACCSCIENCE PUBLISHING

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MINI-REVIEW

Prevention and management of respiratory disease in cerebral palsy: Evidence-based strategies and future recommendations

Mohammed Tahayneh[1], **Rami Jamil Jaber**[2], **Barani Karikalan**[3], **Taha Sulayman**[4], **and Syeda Humayra**[5,6]*

[1]Department of Basic Medicine, Faculty of Medicine, Palestine Polytechnic University, Hebron, West Bank, Palestine

[2]Department of Physiotherapy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, West Bank, Palestine

[3]Department of Pathology, Faculty of Medicine, Bioscience and Nursing, Mahsa University, Saujana Putra, Selangor, Malaysia

[4]Department of Pharmacy Practice and Clinical Pharmacy, Faculty of Pharmacy, University Technology MARA, Bandar Puncak Alam, Selangor, Malaysia

[5]Department of Radiology, Faculty of Medicine, University Kebangsaan Malaysia, Kuala Lumpur, Malaysia

[6]Department of Public Health, Faculty of Allied Health Sciences, Daffodil International University, Dhaka, Bangladesh

***Corresponding author:**
Syeda Humayra
(humayra@ukm.edu.my)

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Abstract

Individuals with cerebral palsy (CP) are at an increased risk of developing respiratory diseases due to various factors, including motor impairments, postural abnormalities, and comorbidities, such as dysphagia and gastroesophageal reflux disease. This paper reviews evidence-based strategies and recommendations for the prevention and management of respiratory diseases in young adults with CP. It highlights the importance of early screening, vaccination, and infection control, as well as the role of pulmonary rehabilitation and physical therapy in improving respiratory function. Non-invasive ventilation and pharmacological management, including bronchodilators and mucolytics, are critical for individuals with compromised lung function. Multidisciplinary care, postural management, and swallowing therapy are key components of a comprehensive treatment approach. In addition, innovations in telemedicine and caregiver education are emerging as effective tools to enhance respiratory care and prevent complications. While challenges remain in accessibility and training, future directions, such as personalized medicine and rehabilitation technologies, offer promising solutions to further improve the respiratory health of individuals with CP. This review underscores the importance of early intervention, coordinated care, and ongoing support to optimize outcomes for individuals with CP and reduce the burden of respiratory diseases.

Keywords: Cerebral palsy; Clinical outcomes; Neurological disorder; Non-invasive ventilation; Respiratory disease

1. Introduction

Cerebral palsy (CP) is a group of neurological disorders resulting from non-progressive brain injury or malformation that occurs during early brain development. It is characterized by varying degrees of motor dysfunction, often accompanied by intellectual disabilities, sensory impairments, and musculoskeletal abnormalities. CP is one of the most common childhood disabilities.[1] Population-based data indicate that the estimated prevalence of CP ranges from 1 to almost 4/1,000 live births or children,[2] accounting for 8.1 million cases globally.[3] In addition, CP contributes to approximately 6.5% of all years lived with disability from adverse health conditions in children under 5 years.[3] As individuals with CP live into adulthood, they face numerous health challenges, and respiratory diseases have become a leading cause of morbidity and mortality within this population.[4] Respiratory complications in CP are often underappreciated in terms of their impact on both quality of life and life expectancy. This review aims to explore the prevention and management of respiratory diseases in young adults with CP, focusing on evidence-based strategies that can enhance health outcomes.

The respiratory complications in individuals with CP are multifactorial, arising from a combination of impaired muscle control, structural abnormalities, and neurological dysfunction. Muscular weakness is a hallmark feature of CP and significantly impairs respiratory muscle function.[5] Weakness in the diaphragm, intercostal muscles, and other muscles responsible for respiration weakens the ability to expel secretions from the airways, thereby increasing the risk of pneumonia.[6] The chronic inability to cough effectively and expel airway secretions can lead to the development of bronchiectasis, especially in individuals with CP. Bronchiectasis in CP is a serious but often overlooked consequence of chronic airway clearance dysfunction and recurrent aspiration, which necessitates early screening and intervention to improve respiratory outcomes.[7] Additionally, limited mobility and poor postural control, which are common in individuals with CP, contribute to ineffective ventilation and increased pulmonary congestion.[8] The combination of these impairments predisposes individuals to chronic respiratory conditions, such as recurrent chest infections, aspiration pneumonia, and respiratory failure.[4]

Individuals with CP may also suffer from structural abnormalities, such as scoliosis, which can further exacerbate respiratory issues.[9] Scoliosis alters the shape of the thoracic cavity, restricting lung expansion and reducing respiratory efficiency. This mechanical disadvantage is compounded by impaired cough reflexes, which are crucial for clearing mucus from the lungs and preventing infections.[10] Patients with CP, specifically those with severe impairments, tracheostomies, or chronic ventilation, are at higher risk of colonization with multidrug-resistant Gram-negative bacteria, notably *Pseudomonas aeruginosa*.[11] The respiratory vulnerabilities make individuals with CP particularly susceptible to a range of respiratory diseases, including asthma and obstructive sleep apnea.[12]

In addition to the physical health concerns, respiratory diseases in individuals with CP significantly impact their psychosocial well-being. Respiratory complications often limit physical activity, leading to a cycle of immobility and further muscle deconditioning.[13] This can hinder participation in social, educational, and recreational activities, negatively affecting the individual's quality of life. Frequent hospitalizations due to respiratory infections place an additional strain on families and caregivers, both emotionally and financially.[14] Furthermore, respiratory complications contribute to pre-mature mortality in individuals with CP, with respiratory failure cited as one of the most common causes of death in this population.[4]

Effective prevention and management strategies are essential to address the high burden of respiratory disease in individuals with CP. Early detection and intervention are critical to minimizing the risk of respiratory complications and improving long-term outcomes. Multidisciplinary care that includes respiratory therapy, physical therapy, and pharmacologic management has been shown to reduce the incidence of respiratory infections and improve pulmonary function.[6] Postural drainage techniques, respiratory muscle training, and mechanical ventilation have been explored as potential interventions for improving respiratory function in individuals with CP.[13] Pharmacological treatments, such as bronchodilators and mucolytics, can also play a role in managing airway clearance and reducing the frequency of respiratory exacerbations.[4,13]

Despite these advancements, much of the literature on respiratory management in CP is based on clinical observations and expert opinions, with limited high-quality, large-scale studies.[10] This highlights the need for robust evidence to identify the most effective strategies tailored to the diverse needs of individuals with CP. It is also essential to consider broader healthcare system implications, as healthcare providers must be equipped with the knowledge and tools to address the complex respiratory issues faced by individuals with CP. Our review examines the current evidence surrounding the prevention and management of respiratory diseases in individuals with CP. By analyzing both individual and systemic approaches, we seek to provide evidence-based recommendations to enhance respiratory health outcomes and improve the quality of life for individuals with CP.

1.1. Epidemiology of respiratory disease in CP

Young individuals with CP are at higher risk of developing respiratory diseases compared to their peers. Studies have shown that approximately 50–70% of individuals with CP experience chronic respiratory problems, with frequent hospitalizations due to respiratory infections, aspiration pneumonia, and respiratory failure.[4,10] Current literature exhibits gender bias, with CP frequently diagnosed in males, who also present with severe motor and health complications.[15] Black infants in the USA have a 29% higher CP risk than White infants, and Māori individuals in New Zealand with CP face significantly higher rates of respiratory-related hospitalization.[16] Finally, age-based gaps persist, as research predominantly focuses on children, overlooking adults with CP who may experience elevated respiratory morbidity.[16] The incidence of respiratory diseases increases as individuals with CP age, and the risk is further exacerbated by the severity of the motor impairment and the degree of cognitive involvement.[8]

Recent data also highlight that respiratory complications are one of the primary contributors to pre-mature mortality in individuals with CP, particularly those with severe conditions.[17] Respiratory issues are often overlooked during the transition from pediatric to adult care, resulting in delayed interventions and suboptimal management.[17]

1.2. Respiratory impairment in CP

The pathophysiology of respiratory impairment in CP is multifactorial. Respiratory muscle weakness, particularly in the diaphragm and intercostal muscles, significantly reduces the ability to clear mucus from the lungs. This predisposes individuals to chronic respiratory infections and atelectasis.[18] Additionally, numerous individuals with CP suffer from scoliosis, which restricts lung expansion and ventilation.[9] Gastroesophageal reflux disease (GERD) is also common in this population, leading to aspiration and recurrent respiratory infections.[19] Furthermore, impaired swallowing and ineffective cough reflexes can result in aspiration of food or liquids into the airway, leading to aspiration pneumonia.[19] The complex nature of these respiratory impairments requires comprehensive, individualized care strategies to prevent and manage respiratory disease.

1.3. Risk factors for respiratory diseases in CP

Individuals with CP face a heightened risk of respiratory diseases due to several interrelated factors that affect their respiratory function (Figure 1). The risk factors for respiratory disease in individuals with CP are multifaceted, involving motor, cognitive, anatomical, and comorbid conditions.[20] Understanding these risk factors is crucial for developing comprehensive care plans that address the unique respiratory needs of individuals with CP and reduce the incidence of respiratory complications.

1.3.1. Motor impairment

One of the primary risk factors is motor impairment, which directly impacts respiratory muscle strength and coordination. Individuals with severe forms of CP, such as quadriplegia, often experience weakness in the diaphragm and other respiratory muscles, making it difficult to clear mucus from the lungs effectively.[21] This impaired ability to clear secretions increases the susceptibility to respiratory infections, atelectasis, and chronic lung disease.[11] The severity of motor impairment correlates with an increased risk of respiratory complications, and this risk is pronounced in individuals who also experience cognitive impairments that hinder their ability to recognize or communicate respiratory distress.[22]

1.3.2. Impaired swallowing and aspiration

This is another significant risk factor for respiratory diseases associated with CP. Numerous individuals with CP experience dysphagia, or difficulty swallowing, increasing the likelihood of food or liquid being aspirated into the airway.[19] Aspiration can lead to aspiration pneumonia, a serious respiratory condition that often requires hospitalization and prolonged treatment. Additionally, the compromised airway clearance mechanisms in these individuals further complicate respiratory health.[17] Swallowing difficulties often require interventions, such as speech therapy or modifications in feeding techniques, to reduce the risk of aspiration.[19] Gastrostomy feeding in CP patients with chronic dysphagia improves nutritional status and can reduce aspiration during oral intake, but respiratory infections remain common due to ongoing aspiration of saliva or reflux. These individuals require comprehensive airway management and reflux control alongside enteral feeding to reduce morbidity.[23]

1.3.3. Postural abnormalities

Postural abnormalities, such as scoliosis, are another critical risk factor for respiratory disease in CP. Many individuals with CP develop scoliosis, which can lead to restricted lung expansion and reduced ventilation.[9] The curvature of the spine restricts the capacity of the lungs to fully expand, impairing the ability to breathe deeply and effectively. This can lead to hypoventilation, reduced oxygen levels, and chronic respiratory problems. The severity of scoliosis often correlates with the degree of respiratory dysfunction, and individuals with severe postural deformities often

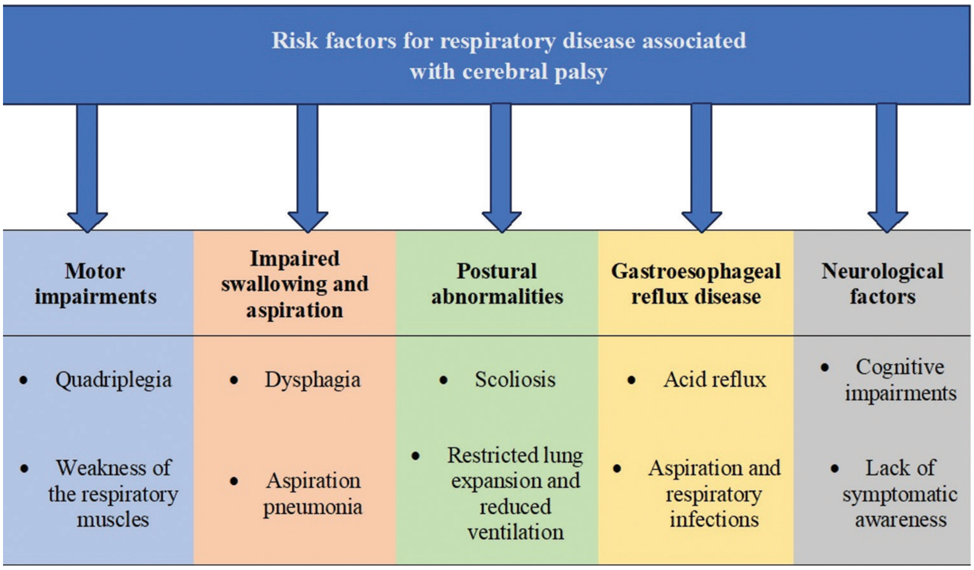

Figure 1. Schematic presentation of risk factors of respiratory disease associated with cerebral palsy. Image created by the authors.

require interventions, such as mechanical ventilation or surgical correction.[18,23]

1.3.4. GERD

GERD is another common comorbidity associated with CP that significantly increases the risk of respiratory complications. GERD can cause stomach contents to reflux into the esophagus and, in some cases, into the airway, leading to aspiration and respiratory infections.[19] The constant irritation and inflammation of the airway from acid reflux can lead to several conditions, such as asthma, chronic cough, or aspiration pneumonia, further complicating respiratory care.

1.3.5. Neurological factors

Cognitive impairments and lack of awareness contribute to the increased vulnerability to respiratory disease in individuals with CP. Most of them may not have the cognitive ability to recognize symptoms of respiratory distress, such as difficulty breathing or coughing, delaying timely medical intervention.[22,24] The inability to effectively communicate discomfort or respiratory symptoms can make it challenging for caregivers to provide appropriate care, leading to delayed responses to respiratory crises.

2. Evidence-based prevention strategies

2.1. Early screening and diagnosis

Early detection of respiratory dysfunction is crucial in preventing long-term complications. Regular screening for respiratory conditions, including pulmonary function tests and imaging studies, should be conducted to assess lung health and detect early signs of respiratory impairment.[11] Pulmonary function testing can help identify early abnormalities in lung function, enabling timely interventions.[25]

2.2. Vaccination and immunization

Vaccination plays a pivotal role in preventing respiratory infections in individuals with CP. Immunizations against influenza, pneumococcus, and other respiratory pathogens should be prioritized because individuals with CP are more vulnerable to severe outcomes from respiratory infections.[26] Therefore, ensuring that they are up-to-date on all recommended vaccinations is an essential preventive measure.[17]

2.3. Respiratory hygiene and infection control

Respiratory hygiene, including handwashing, mask use during illness, and avoidance of environmental pollutants, can reduce the risk of respiratory infections.[27] In care settings, strict infection control protocols are necessary to prevent cross-contamination and respiratory illness outbreaks.[17,28]

3. Management of respiratory disease: Therapeutic approaches

3.1. Pulmonary rehabilitation and physical therapy

Physical therapy aimed at strengthening respiratory muscles can improve lung function and overall respiratory health in individuals with CP. Regular exercise, including aerobic activities and chest physiotherapy, helps enhance lung capacity, improve mucus clearance, and reduce infection risks.[29] Breathing exercises, such as diaphragmatic and pursed-lip breathing, can also promote better ventilation.[30]

3.2. Non-invasive ventilation (NIV) and respiratory support

For individuals with significant respiratory compromise, NIV, such as bilevel positive airway pressure or continuous positive airway pressure, can improve oxygenation and reduce the work of breathing.[31] These devices help to maintain open airways and support breathing, particularly during sleep. NIV is effective in reducing the risk of respiratory failure and improving the quality of life for individuals with severe respiratory symptoms.[31] However, in individuals who develop severe chronic ventilatory insufficiency, invasive mechanical ventilation via tracheostomy is often required to maintain adequate respiratory function and manage airway secretions.[32] While tracheostomy can reduce upper airway obstruction and enhance secretion clearance, it significantly increases the risk of respiratory infections, particularly ventilator-associated pneumonia, due to frequent colonization by multidrug-resistant organisms.[11]

3.3. Pharmacological management

Pharmacological interventions, including bronchodilators, corticosteroids, and mucolytics, can be used to manage underlying respiratory conditions. Bronchodilators help open the airways, while corticosteroids can reduce lung inflammation.[8,28] Mucolytics aid in the clearance of thick mucus, making coughing easier and reducing the risk of infections.[33] Medications should be prescribed based on the individual's specific needs and the severity of respiratory disease.[33]

3.4. Therapeutic effect of magnetic nanomaterials

Magnetic nanoparticles have currently emerged as a promising tool in the treatment of pulmonary respiratory malignancies (such as lung cancer) due to their unique magnetic properties, biocompatibility, and ability to be functionalized for targeted therapy.[34] A recent study demonstrated that magnesium-doped ironoxide nanoparticles in combination with an alternating magnetic field can produce effective localized hyperthermia, significantly inhibiting lung tumor growth *in vivo*, while the broader literature supports their role in precision drug delivery, imaging, and combating drug resistance.[35] Another study showed that inhalable magnetite (iron [II, III] oxide)/poly-lactic-co-glycolic acid magnetic nanocomposite microparticles offered a powerful platform for targeted pulmonary delivery of cancer therapeutics, with strong safety, localization, and multi-modal capacity.[36]

3.5. Multidisciplinary approach in CP patients' respiratory care

Managing respiratory disease in patients with CP requires a multidisciplinary approach, involving healthcare professionals from various specialties, including pulmonology, physical therapy, speech therapy, nutrition, and nursing.[37] A coordinated care plan ensures comprehensive treatment, addressing the physical, nutritional, and psychological aspects of care. The involvement of a speech therapist is critical to address swallowing issues, while physical therapists can provide interventions to improve posture and respiratory muscle function.[30,37]

4. Challenges in implementing evidence-based strategies

While significant studies have been conducted in the prevention and management of respiratory disease in individuals with CP, several challenges persist in providing optimal care. One of the primary challenges is the lack of awareness and specialized training among healthcare providers to recognize and manage respiratory issues specific to individuals with CP. The transition from pediatric to adult care often leads to gaps in care due to the under-recognition of respiratory problems in this population, particularly in those with milder forms of CP who may not exhibit obvious signs of respiratory distress.[24] As a result, respiratory dysfunction may be undiagnosed or untreated, leading to severe complications.[8]

Another significant barrier is the limited access to specialized care, especially in rural or low-resource settings. Numerous individuals with CP face challenges in accessing high-quality respiratory therapies, such as pulmonary rehabilitation and NIV, due to cost, geographic location, or a lack of healthcare infrastructure.[38] In addition, the financial burden of advanced treatments, including NIV and chest physiotherapy, can be prohibitive for low-income families, limiting the availability of these interventions.[39]

Moreover, caregivers often lack sufficient training to manage the complex needs of individuals with CP, especially those with advanced respiratory diseases. Proper caregiver education and support are critical for the successful implementation of respiratory interventions at home.[17,40] Without adequate training, caregivers may struggle to perform daily respiratory exercises, monitor for signs of respiratory distress, or use ventilatory support devices effectively.[40]

5. Future directions in respiratory care for CP patients

Research in respiratory care for individuals with CP continues to evolve, with promising innovations on the horizon. Future directions include the use of telemedicine, which can improve access to care, enabling remote monitoring and consultations.[20,41] Advances in

Table 1. Key evidence-based strategies for managing respiratory diseases in cerebral palsy

Strategy/recommendation	Description	Outcome
Early screening and diagnosis	Regular screening for respiratory issues through pulmonary function tests and imaging studies	Early detection can prevent complications, allowing for timely interventions
Vaccination and immunization	Ensure up-to-date immunizations for influenza, pneumococcus, and other respiratory pathogens	Vaccination significantly reduces the risk of respiratory infections
Respiratory hygiene and infection control	Promote handwashing, mask-use during illness, and infection control measures in care settings	Helps prevent the spread of respiratory infections
Pulmonary rehabilitation and physical therapy	Implement physical therapy to strengthen respiratory muscles and improve lung capacity	Regular exercise improves lung function and reduces infection rates
NIV	Use devices such as BiPAP or CPAP for individuals with significant respiratory compromise to improve oxygenation	NIV is effective in preventing respiratory failure and improving the quality of life
Pharmacological management	Use bronchodilators, corticosteroids, and mucolytics to manage airway obstruction, inflammation, and mucus clearance	Medications reduce respiratory symptoms and improve lung function
Swallowing therapy	Provide speech therapy to address dysphagia and reduce the risk of aspiration	Reduces the risk of aspiration pneumonia
Multidisciplinary approach	Involve healthcare providers from various specialties, including pulmonologists, therapists, and nutritionists	A coordinated team approach improves overall management and outcomes
Postural management	Address postural abnormalities (e.g., scoliosis) with physical therapy, bracing, or surgery	Correcting postural deformities can improve lung function and reduce respiratory complications
Telemedicine and remote monitoring	Use telemedicine for remote consultations and monitoring of respiratory function	Telemedicine enhances accessibility to care, especially in rural areas
Early and ongoing caregiver training	Educate caregivers on proper respiratory care techniques and signs of distress	Caregiver education reduces the likelihood of complications and improves outcomes

Abbreviations: BiPAP: Bilevel positive airway pressure; CPAP: Continuous positive airway pressure; NIV: Non-invasive ventilation.

genomics and personalized medicine may provide more targeted therapies for respiratory diseases in CP.[28,42] The development of effective rehabilitation tools and technology, such as wearable devices for respiratory monitoring, could enhance the management of respiratory disease in CP patients.[43] In addition, advances in artificial intelligence can help shape future research directions and rehabilitation approaches, enhancing the functional outcomes in CP.[44] In summary, future research should focus on developing individualized airway clearance strategies and using mobile health technologies to monitor patient status, facilitating prompt intervention in cases of respiratory illness. Table 1 highlights the key evidence-based strategies for the prevention and management of respiratory diseases in CP.

6. Conclusion

Individuals with CP are at significant risk for respiratory diseases, and evidence-based prevention and management strategies are essential to optimizing their health outcomes. Early intervention, regular monitoring, and a multidisciplinary approach can significantly reduce the burden of respiratory disease, improving quality of life and longevity. With continued research and a focus on individualized care, young individuals with CP can achieve better respiratory health and quality of life. High-volume, multi-centric studies are crucial in CP research as they can enhance the generalizability of findings, improve statistical power, and reduce the risk of bias. By capturing diverse patient populations and healthcare settings, empirical studies are needed to evaluate interventions and facilitate evidence-based care in effective CP management.

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Author contributions

Conceptualization: Mohammed Tahayneh, Rami Jamil Jaber
Visualization: Mohammed Tahayneh, Rami Jamil Jaber
Writing – original draft: Mohammed Tahayneh

Writing – review & editing: Taha Sulayman, Syeda Humayra, Barani Karikalan

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ACCSCIENCE PUBLISHING

Global Translational Medicine

MINI-REVIEW

Renoprotective effect of traditional herbal medicine of ancient Chinese origin: A mini-review

Kazutomo Sawai*

Hanshin Medical Co-op Daisan Clinic, Amagasaki, Hyogo, Japan

Abstract

Prevention of chronic kidney disease (CKD) progression represents a critical challenge in global health care. However, the effectiveness of current pharmacologic interventions remains limited, requiring further therapeutic advancements. Traditional herbal medicine (THM) has been used worldwide in the treatment of CKD, but its effect remains uncertain. This review aims to evaluate the efficacy of THM in managing CKD and to identify appropriate methods for assessing its therapeutic effect. A literature search of six databases was conducted. The inclusion criteria comprised (i) ≥100 sample sizes, including retrospective studies, reviews, randomized controlled trials (RCTs), quasi-RCTs, crossover trials, and meta-analyses; (ii) CKD patients as the target; (iii) the use of oral THM as interventions; and (iv) reported primary and secondary outcomes. There were 18 RCTs with 3452 participants, 12 systematic reviews, 4 meta-analyses, and 4 retrospective studies. Although THM improved glomerular filtration rate, its therapeutic effect was uncertain due to major limitations, such as small sample sizes and short follow-up periods. THM formulas are prescribed based on THM diagnosis in a timely manner, meaning that patients sharing the same Western medical diagnosis may receive different formulas, and that prescriptions for a single patient may change during treatment. This approach may be a key reason for the small sample sizes and short follow-up periods of most THM studies against CKD. The use of quality-assured and ready-to-use Kampo extracts in a timely manner may be a promising method for the clinical application of THM in CKD treatment and the evaluation of its actual therapeutic effects.

Keywords: Traditional herbal medicine; Kampo extracts; Glomerular filtration rate; Chronic kidney disease

***Corresponding author:**
Kazutomo Sawai
(kazutomosawai@gmail.com)

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1. Introduction

Preservation of renal function represents a critical challenge in global healthcare: 1.2 million (95% uncertainty interval [UI] 1.2–1.3) people died from chronic kidney disease (CKD) globally in 2017, and the global all-age mortality rate of CKD increased 41.5% (95% UI 35.2–46.5) between 1990 and 2017.[1] CKD is not only a leading cause of end-stage renal disease (ESRD) but also associated with increased mortality and cardiovascular disease.[2] It is defined as abnormalities of kidney structure or function for a minimum of 3 months (decreased glomerular filtration rate [GFR] < 60 mL/min/1.73 m^2), or having at least one marker of kidney damage, including albuminuria (≥30 mg/gCr), urine sediment abnormalities, persistent hematuria, electrolyte and other abnormalities due to tubular disorders, abnormalities detected by histology, structural abnormalities detected by imaging, and a history of kidney transplantation.[2] Although current pharmacologic interventions, including sodium-glucose cotransporter 2 inhibitors, mineralocorticoid receptor antagonists, and renin–angiotensin system inhibitors, are commonly used to delay CKD progression, their effectiveness remains limited, requiring further therapeutic advancements.

Traditional herbal medicine (THM) has been used worldwide in the treatment of CKD. There are several randomized controlled trials (RCTs) of traditional Chinese medicine (TCM) against CKD. However, its therapeutic effect remains uncertain due to major limitations, such as small sample sizes and short follow-up periods.[3-5] THM, including Kampo formulas, is prescribed based not only on patients' complaints, signs, and symptoms but also on physical examinations, including abdominal, tongue, and pulse inspections. This means that patients sharing the same Western medical diagnosis (e.g., CKD due to diabetic nephropathy or systemic lupus erythematosus) may receive different formulas, and that prescriptions for a single patient may change during treatment based on traditional medical diagnosis (e.g., initially apply a formula to remove dampness, then change to another formula for invigorating qi). Hence, it is unusual to prescribe a single THM formula over a long period to all patients with CKD. It is therefore important to apply the correct formula to each patient in a timely fashion to determine the effectiveness of long-term administration of THM against CKD. This approach may be a key reason for the small sample sizes and short follow-up periods of most clinical studies of THM against CKD. This review aims to discuss recent reports on THM of ancient Chinese origin, against CKD, with a future perspective for the renoprotective effect of Kampo extract administration.

2. Methodology

Six databases (PubMed, the Cochrane Central Register, J-STAGE [https://www.jstage.jst.go.jp/browse/-char/ja], Web of Science, Evidence Reports of Kampo Treatment database [http://www.jsom.or.jp/medical/ebm/ere/index.html],[6] and the Japan Kampo Manufacturers Association database) were searched from inception to December 21, 2025. Missing studies were supplemented by manual retrieval of relevant reviews and studies. The search terms (subject headings, free terms, keywords, etc.) used included "chronic kidney disease, CKD," "chronic renal failure," "glomerular filtration rate, GFR," "creatinine," "cystatin C," "creatinine clearance," "eGFR slope," "proteinuria," "albuminuria," "urinary protein to creatinine ratio, UPCR," "urinary albumin to creatinine ratio, UACR," "traditional herbal medicine," "Kampo," "traditional Chinese medicine," and "randomized controlled trial, RCT." Different databases were searched using different strategies by combining various search terms.

The inclusion criteria comprised:
(i) Types of studies: Sample size of more than 100, including RCTs, quasi-RCTs, and crossover trials, as well as retrospective studies, reviews, and meta-analyses
(ii) Types of patients: CKD patients
(iii) Types of interventions: THM, orally combined with Western medicine in the treatment group versus Western medicine only in the control group, with unlimited courses of treatment.
(iv) Types of outcomes: Primary outcomes (e.g., serum creatinine, blood urea nitrogen, and cystatin C [cys]) and secondary outcomes (e.g., 24 h urine protein, urinary protein-to-creatinine ratio [UPCR], and urinary albumin-to-creatinine ratio).

The exclusion criteria comprised:
(i) Types of studies: Ongoing clinical trials, pre-clinical studies (cell and animal experiments), and non-comparative or uncontrolled clinical reports (e.g., self-controlled studies, case reports, etc.).
(ii) Types of patients: Acute kidney injury patients and CKD patients with ongoing renal replacement therapies.
(iii) Types of interventions: Non-oral traditional medicine, including acupuncture, intravenous injection, plaster, and foot bath, and the use of THM in the control group.
(iv) Types of outcomes: No primary and secondary outcomes.
(v) Others: Incomplete data and repeated publication.

3. Results

A total of 4742 records were identified, of which 4737 were retrieved through initial database searches. The remaining five studies were identified through the reference list screening of the 4742 records and subsequent database searches. After removal of duplicate records and exclusion of studies based on the exclusion criteria, 41 records remained for full-text review. There were 18 RCTs involving 3452 participants, with sample sizes ranging from 118 to 1,470. In addition, there were 12 systematic reviews, 4 meta-analyses, 4 retrospective studies, and 3 prospective studies.

4. Pathophysiology of CKD and limitations of clinical studies using THM

Pathologically, initial global glomerular sclerosis, or obstruction by inflammation or abnormal deposits, leads to the reduction of blood flow and glomerular filtration, and the tubules supplied by the efferent arteriole undergo ischemia and eventually atrophy with adjacent interstitial fibrosis.[7] There is also primary tubulointerstitial disease, which leads to tubular atrophy and interstitial fibrosis. Disease affecting glomeruli characteristically exhibits one or more patterns of injury, including histological signs of inflammation such as hypercellularity, infiltrating leukocytes, fibrinoid necrosis, segmental sclerosis, and glomerular basement membrane remodeling. Interstitial inflammation, tubular epithelial necrosis, and vascular sclerosis, hyalinosis, inflammation, necrosis, or thrombosis are characteristics of interstitial, tubular, and vascular diseases, respectively.[7] Glomerular diseases occur not only as diseases that primarily target the kidneys but also as components of systemic diseases, including systemic lupus erythematosus, diabetes mellitus, amyloidosis, monoclonal immunoglobulin deposition disease, hypertension, and systemic vasculitides such as anti-neutrophil cytoplasmic antibody vasculitis, immunoglobulin A vasculitis, and cryoglobulinemic vasculitis.[7] Nephrological treatments are mainly evaluated by the change in GFR and proteinuria, as decreased GFR and proteinuria are closely related to the development of advanced-stage CKD, which leads to further decline in GFR.[2]

Pathophysiology of CKD in traditional medicine is composed of qi deficiency, weakness of kidney$^{\text{traditional medicine module 1 (TM1)}}$, blood$^{\text{TM1}}$ deficiency, and abnormal blood$^{\text{TM1}}$ circulation, leading to dampness heat, blood$^{\text{TM1}}$ stasis, water stagnation, and turbid toxin.[4] Therefore, THM is thought to exert therapeutic effects against CKD through invigorating qi, tonifying kidney$^{\text{TM1}}$ and blood$^{\text{TM1}}$, promoting blood$^{\text{TM1}}$ circulation, and removing stasis, heat, dampness, turbidity, and edema. These effects are multitargeted and multifunctional.

THM exerts anti-inflammatory, antifibrotic, antioxidant, antiapoptotic, and immunomodulatory effects; the mechanisms of these renoprotective effects are recently reviewed in detail.[4] For example, transforming growth factor-β/Smad and Wnt/β-catenin signaling pathways are two antifibrotic mechanisms that have been validated by several TCM studies.[4] In addition to inhibiting the activation of advanced glycation end products, the anti-inflammatory effect of THM is also manifested in the inhibition of reactive oxygen species–extracellular signal-regulated kinase 1/2–nuclear factor kappa B-mediated NOD-, LRR-, and pyrin domain-containing protein 3 inflammasomes.[4]

THM is applied as formulas, which consist of several crude drugs. *Shanghanlun* and *Jinguiyaolue*, medical books considered to have been written by Zhang Zhongjing in the later Han dynasty, discuss the treatment of acute febrile illness and various chronic diseases, respectively.[8] These ancient medical books present precise prescriptions of hundreds of formulas and provide helpful suggestions to utilize each of them. There are also thousands of new formulas created after the Song and Yuan dynasties, and even now, new formulas are being introduced. New Kampo formulas have also emerged in Japan since THM was officially imported from China through the Korean peninsula in 552 AD and evolved for about 1500 years.[9] These formulas are prescribed to CKD patients under traditional medical diagnosis.[10,11]

Subsequent sections focus on the renoprotective effects of individual crude drugs and individual formulas, as well as on THM as a broad therapeutic category with pharmacological heterogeneity. Finally, this review proposes the use of Kampo extracts as an approach for the proper administration of THM in the treatment of CKD.

5. Renoprotective effects of individual crude drugs

Numerous reports have detailed the renoprotective effects of individual crude drugs, as extensively reviewed in recent literature.[3-5] Among these, astragalus root, which invigorates qi, is one of the most studied crude drugs for CKD treatment.[4] Multiple clinical studies have reported the renoprotective effects of astragalus root.[12-14] Astragalus root is thought to exert its effect through preserving the glomerular filtration barrier, enhancing tight junctions and glycocalyx, increasing nitric oxide (NO) production through the activation of endothelial NO synthase, scavenging reactive oxygen species, and modulating mitochondrial function.[12,15] Clinical studies have shown that astragalus root may improve proteinuria,[3-5] but others also reported that astragalus root improved GFR without affecting proteinuria.[14] *Rhei Radix et Rhizoma* for eliminating heat and dampness[16,17] and *Salviae Miltiorrhizae Radix et Rhizoma* (*Danshen*) for promoting blood circulation and removing stasis[16,18] are examples of crude drugs that are reported to have renoprotective effects.[3-5]

6. Renoprotective effects of individual formulas

There are numerous studies concerning the renoprotective effects of THM formulas, and these are described in detail in recent reviews.[3-5] *Shenqi* particle,[19] *Tangshen* formula,[20,21] *Jinshuibao*,[22] *Shenqi Yanshen* granule,[23] *Tongxinluo* capsule,[24] *Huangkui* capsule,[25,26] *Bupi Yishen* formula,[27] Niaoduqing particles,[28] and *saireito*[29] have been shown to exert renoprotective effects (e.g., improve GFR and reduce proteinuria) in clinical trials, but other formulas improve GFR without affecting proteinuria.[28] The longest intervention period for these clinical trials was 12 months,[19,25,27] which may indicate the difficulty of maintaining a single formula throughout the course of clinical trials involving THM. Table 1 presents representative clinical trials of THM formulas for CKD. Long-term RCTs with hard endpoints are required to evaluate the effects of THM in CKD management.

7. Renoprotective effects of THM as a broad therapeutic category with pharmacological heterogeneity

There are reports indicating that THM, as a broad therapeutic category, may provide renoprotective effects. A large-scale propensity score-matched analysis including 8195 pairs of newly diagnosed CKD patients utilizing the Taiwan National Health Insurance Research Database showed that prescription of THM, for a median of 22 days (interquartile range 7–62 days), resulted in 60% risk reduction of 6-year cumulative ESRD incidence compared with non-users.[31] The patients in that study consisted of stage 3–5 newly diagnosed CKD patients with a mean age of 54 years, 39% female, including 24% with diabetes mellitus, 33% with hypertension, and 6% with cardiovascular disease. More detailed analysis revealed that the use of harmonizing, wind-dampness-dispelling, blood-regulating, qi-regulating, and summer-heat-clearing formulas—comprising a total of 163 different formulas—was associated with a lower incidence of ESRD. However, the use of dampness-dispelling and purgative formulas, which included 78 different formulas, was associated with an increased ESRD risk. This increased ESRD risk may be due to the use of strong formulas that may lead to dehydration (e.g., *Shu Zao Yin Zi*, or *sosakuinshi*), but the precise dosage and treatment duration were not clearly defined.

Recently, a retrospective study evaluated the effectiveness of long-term administration of Kampo formula extracts on renal function.[32] Using a propensity score-matched analysis, 148 pairs of Kampo extract users and non-users with similar pre-treatment GFR slope, household income, educational level, and smoking status (64% female, mean ages 68.8 and 68.9 years, mean estimated GFR [eGFR] 68.3 and 68.2 mL/min/1.73 m^2, UPCR 0.12 ± 0.15 and 0.12 ± 0.18 g/gCr, respectively), were analyzed. The cohort included 40% CKD patients and 26% with diabetes mellitus. The analysis demonstrated that Kampo use may attenuate the decline in renal function. The mean difference in eGFR slope between Kampo users and non-users was 1.07 mL/min/1.73 m^2/year (95% confidence interval [CI] 0.68–1.46) over a mean follow-up of 8.7 ± 2.1 years in the overall cohort, and 1.48 mL/min/1.73 m^2/year (95% CI 0.75–2.22) over 9.4 ± 2.1 years among patients with CKD. These results meet the surrogate endpoint of

Table 1. Representative clinical trials of traditional herbal medicine for CKD

Intervention	Disease	*N*	Trial period (months)	Primary outcome	Outcome	Reference
Shenqi particle vs. prednisone/ cyclophosphamide	Idiopathic membranous nephropathy	190	12	CR+PR	46/63 (73.0%) vs. 54/69 (78.3%) ($p=0.5$)	19
Tangshen formula vs. placebo	Type 2 diabetic kidney disease	180	6	UAER+24 h UP	ΔUAER: −19.5 vs. −7.0 μg/min ($p=0.7$); Δ24 h UP: −0.2 vs. 0.4 g ($p<0.05$)	21
Huangkui capsule (*Abelmoschus manihot*) vs. losartan	Immunoglobulin A nephropathy	1,470	12	Pre vs. post 24 h UP	ΔUP: −230 vs. −253 mg ($p=0.6$)	25
Bupi Yishen formulation vs. Losartan	Non-diabetic CKD stage 4	567	12	eGFR slope	−2.3 vs. −4.5 mL/min/1.73 m^2/year ($p<0.05$)	27
Niaoduqing particles (UCG) vs. placebo	CKD stage 3b–4	300	6	Pre vs. post sCr and eGFR	ΔsCr: 1.1 vs. 11.7 μmol/L ($p<0.01$); ΔeGFR: −0.2 vs. −2.2 mL/min/1.73 m^2 ($p<0.05$)	28
Chinese herbal formulation granules vs. placebo	CKD stage 3	343	6	24 h UP+sCr+eGFR	24-h UP: 0.97±1.14 vs. 0.97±1.25 g/day ($p<0.05$); sCr: 130.8±32.6 vs. 149.1±41.3 μmol/L ($p<0.05$); eGFR: 55.74±50.82 vs. 44.46±12.60 mL/min/1.73 m^2 ($p<0.05$)	30

Abbreviations: CKD: Chronic kidney disease; CR: Complete remission; eGFR: Estimated glomerular filtration rate; PR: Partial remission; sCr: Serum creatinine; UAER: Urinary albumin excretion rate; UCG: Uremic clearance granules; UP: Urinary protein.

eGFR slope reduction of 0.5–1.0 mL/min/1.73 m^2/year, as proposed by an international workshop hosted by the National Kidney Foundation.[33] In that study, 107 Kampo extracts out of 148 Kampo extract prescription formulas approved by the Japanese insurance system (http://mpdb.nibiohn.go.jp/stork/) were administered.[32] Analysis of total Kampo extract prescriptions revealed six therapeutic categories—blood[TM1]-tonifying, kidney[TM1]-tonifying, yang-tonifying, qi- and yin-tonifying, qi-regulating, and wind-dampness-dispelling formulas—were more frequently prescribed to patients with preserved eGFR, although these differences were not statistically significant. Kampo formulas containing astragalus root were administered to 75% of Kampo users, with no significant difference in use between patients with preserved versus decreased eGFR. While Kampo users with preserved eGFR tended to have a higher intake of astragalus root compared to those with decreased eGFR (3,297 ± 2,266 g vs. 2,430 ± 2,001 g, respectively), this difference was not statistically significant. No individual Kampo formula or category was significantly associated with preservation or decline of eGFR. Furthermore, variations between eGFR calculated from serum creatinine (eGFRcr) and cystatin C (eGFRcys) were not significantly different between Kampo users and non-users at the study endpoint, suggesting that improvements in eGFRcr among Kampo users may reflect true renoprotection rather than compounding factors such as muscle mass or hemodynamic changes.

These findings indicate that Kampo extracts, administered as a broad therapeutic category with pharmacological heterogeneity, may exert renoprotective effects. However, despite adjustment for socioeconomic factors, there is a risk of healthy user bias, as patients who maintain long-term Kampo therapy may inherently represent a healthier or more adherent subpopulation, potentially contributing to preserved renal function.[32] Importantly, causal inferences cannot be drawn due to the observational and non-randomized nature of the study.

8. Future perspective on the renoprotective effects of THM: Use of Kampo extract granules as ready-to-use, quality-assured pharmaceuticals

To determine the efficacy of THM against CKD, treatment must be tailored to each patient's individual syndrome or pattern, with formulas adjusted accordingly as the patient's condition changes. Furthermore, long-term administration is essential, given that CKD is a chronic disorder that progresses over decades. Therefore, THM treatment must be easy and convenient for patients to adhere to.

Kampo extract granules are quality-assured pharmaceuticals listed in the Japanese pharmacopoeia (http://mpdb.nibiohn.go.jp/stork/),[34] approved and covered by the Japanese insurance system, and are orally administered. Utilizing ready-to-use Kampo extracts may facilitate the timely selection of the best-matched formula for each patient. Although Kampo medicine originated from ancient Chinese medicine, it has developed distinctly within Japan, where accumulated clinical experience has shaped its unique approach to understanding body responses and symptom management.[9,10,35]

In the Japanese medical system, Kampo extracts can be prescribed by Western-trained medical doctors without further certification or additional training in traditional medicine. It is reported that 86.7% of Japanese medical doctors are current users of Kampo formulas, with only 3.8% having no experience in their use.[36] Despite widespread clinical use, rigorous clinical studies evaluating Kampo formulas, especially for CKD, remain lacking, and no RCTs using Japanese Kampo formulas in CKD have been reported.

The *Evidence-based clinical practice guideline for CKD 2023*, published by the Japanese Society of Nephrology, highlights coffee intake as potentially suppressing the progression of CKD due to its antioxidants and anti-inflammatory substances, such as caffeine.[37] Kampo extracts similarly possess strong radical scavenging activity,[38,39] with reported antioxidant, anti-inflammatory, and antifibrotic effects across various diseases.[40,41]

Although some studies investigating THM in CKD failed to show significant improvements in proteinuria or albuminuria compared to controls, these negative results may be attributable to small sample sizes or relatively low baseline levels of proteinuria, limiting the ability to detect protective effects. Notably, 48-week administration of astragalus root has been reported to significantly improve eGFR slope without improving albuminuria in an RCT involving 118 diabetic patients with CKD stages G2–G3.[14] Similarly, *Niaoduqing* particles improved eGFR without improving proteinuria compared with controls.[28]. These findings suggest that the renoprotective effects of THM may occur independently of improvements in proteinuria.

9. Conclusion

Recent retrospective studies indicate that long-term administration of THM may slow eGFR decline in patients with CKD. The use of Kampo extracts represents a promising approach to rigorously evaluate the therapeutic potential of THM in CKD management.

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ACCSCIENCE PUBLISHING

Global Translational Medicine

BRIEF REPORT

Ex vivo thermographic analysis of the eyelid conjunctival surface during ultraviolet A-mediated photochemical crosslinking of tarsal collagen: A step in translational safety evaluation

Shuko Suzuki[1], **Nadja E. Pop**[2,3], **Alexandra I. Manta**[1,2,3,4], **and Traian V. Chirila**[1,2,3,5]*

[1]Queensland Eye Institute, Woolloongabba, Queensland, Australia

[2]Center for Advanced Medical and Pharmaceutical Research, George E. Palade University of Medicine, Pharmacy, Sciences and Technology, Târgu Mureş, Mureş, Romania

[3]Faculty of General Medicine, George E. Palade University of Medicine, Pharmacy, Sciences and Technology, Târgu Mureş, Mureş, Romania

[4]Faculty of Health and Behavioural Sciences, University of Queensland, St Lucia, Queensland, Australia

[5]Australian Institute of Bioengineering and Nanotechnology, University of Queensland, St Lucia, Queensland, Australia

***Corresponding author:**
Traian V. Chirila
(traian.chirila@qei.org.au)

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Abstract

Ultraviolet A (UVA)-induced collagen crosslinking can mechanically reinforce collagenous tissues and is being translated as a minimally invasive treatment for pathological eyelid laxity, yet the extent of irradiation-related heating at the conjunctival–tarsal interface remains unclear. In this study, we developed a novel procedure to treat pathological eyelid laxity. The methodology is based on the photocrosslinking of tarsal collagen, leading to mechanical reinforcement of the entire eyelid. In our previous studies, exposure to ultraviolet A (UVA) radiation at fluences below 20 J/cm^2 did not cause histopathological alterations to the eyelid tissues. Here, we further evaluated biological safety by quantifying conjunctival surface temperature increases during UVA irradiation to inform clinical translation. Using *ex vivo* sheep eyelids as a model, we used infrared thermography to measure the temperatures attained on the tarsal conjunctival surface during UVA irradiation (365 nm) at four irradiances for a maximum exposure time of 3 min, corresponding to fluences of 8.1, 13.5, 27, and 45 J/cm^2. Both the exposure time and irradiance caused an asymptotic increase in temperature, while a linear dependence was observed between fluence and temperature increase (ΔT). The recorded values for ΔT ranged from 3.3°C to 14.0°C. In summary, at clinically practicable fluences (<20 J/cm^2), such increments are not expected to raise the final temperature to the threshold for collagen denaturation and tissue damage.

Keywords: Eyelid laxity; Tarsal collagen; Photochemical crosslinking; Ultraviolet A radiation; Infrared thermography; Thermal damage

1. Introduction

Non-ionizing ultraviolet (UV) A (UVA) radiation is generally considered a non-thermal radiation. Compared to the other UV radiations (UVB and UVC), UVA has the longest wavelength (from 315 to 400 nm), corresponding to the lowest energy (3.94–3.10 eV). The effects of UVA exposure include photophysical and photochemical changes and are much less associated with the release of heat. The photophysical processes include photon absorption, localized excitation, and generation and migration of charge carriers (excited electrons and electron holes). These processes are always the same regardless of the nature of the materials. Importantly, these processes are the sources of photochemical changes, which are intricate and unfathomable, as they depend on the irradiated material's chemical structure. When absorbed by the target materials, UVA photons can excite molecules. This can trigger responses that damage biological matter, including chemical reactions such as oxidation and structural alterations to nucleic acids. Those excited electrons that do not contribute to such changes can recombine to release their energy through luminescence phenomena (fluorescence and phosphorescence) or through the production of heat.

At present, most studies have been dedicated to the complex effects of UVA and UVB radiations on the skin.[1-6] In brief, the main effects on the skin are photoaging, immunosuppression, and photocarcinogenesis. The pathways to such disorders involve radiation-induced processes, including lipid peroxidation, irreversible chemical damage to nucleic acids, chemical modification of proteins, altered intracellular signaling, and chronic inflammation. In general, these effects are mediated through UVA-photon absorption by the chromophores present in the skin and other tissues, acting as photosensitizers. Such biomolecular chromophores include lipophilic pigments, porphyrins, melanin and its precursors, flavins, pterins, aromatic amino acids, and vitamin B_6. Under certain conditions, they may protect against UV-induced damage. However, no evidence for a thermal effect of UVA on the skin has been provided or debated in the aforementioned literature.

The release of heat during UVA exposure, which may lead to an increase in the temperature of target materials, is relevant when connective tissues containing collagen are irradiated *in vivo* to induce the photochemical crosslinking of collagen. This exogenous process leads to a significant enhancement of the mechanical properties of tissues, specifically tensile strength and stiffness (Young's modulus), with remarkable therapeutic benefits in certain tissues, such as the cornea. Crosslinking of collagen has become an important method in tissue engineering and regenerative medicine, employed to generate scaffolds and implants by modifying the properties of collagenous substrates *in vitro*. The basics and practical relevance of collagen crosslinking have been comprehensively investigated,[7-19] although assigning the correct chemical structures to the resulting crosslinkages remains a challenge.[20-22]

At present, the most successful therapeutic application of UVA-induced collagen crosslinking is a treatment for keratoconus, an ectatic corneal disorder leading to severe impairment of vision. By irradiating the cornea with UVA rays in the presence of a photosensitizer, usually riboflavin (vitamin B_2), the progression of keratoconus is arrested as a consequence of the mechanical reinforcement generated through the photocrosslinking of corneal stromal collagen.[23-31] Inspired by this achievement, we have proposed[32-35] and are now developing a UVA/riboflavin strategy for treating eyelid laxity by photocrosslinking the collagen located inside the tarsal plates of the lower and upper lids. Eyelid laxity, also known as palpebral laxity, is one of the main causes for, or can be associated with, a range of pathological conditions such as floppy eyelid syndrome, involutional ptosis, ectropion, entropion, and blepharochalasis. Before validating our technique as a clinical procedure, it is crucial to assess its biological safety in terms of potential tissue damage caused by UVA radiation. Our previous investigations[32-34] have shown that at fluences (radiant exposures) lower than 20 J/cm^2, irradiation of the eyelid with UVA rays does not have any impact on the integrity of the tissues. Other investigators have confirmed our findings in *ex vivo* tarsal plate specimens of animal (rat) or human origin.[36-39]

The possibility of radiation-induced heat production at the interface with exposed eyelid tissues has never been explored. In the present study, the regional increase in temperature recorded on the surface of *ex vivo* ovine eyelids' tarsal conjunctiva resulting from its exposure to UVA (365 nm) radiation was specifically investigated at four different levels of radiant exposure. The results were compared to the temperature threshold reported for thermal denaturation of collagen. This is a significant phase in the translational cycle related to our proposed therapeutic strategy aimed at treating palpebral laxity. The study is intended to address a gap in the translational cycle associated with the proposed procedure, and the results will be of significant benefit for further clinical development.

2. Materials and methods

2.1. Materials

The eyelids were harvested on-site, postmortem, from sheep cadavers supplied by Brisbane Valley Meats Pty

Ltd., Australia, which is an abattoir unit operating under the rules of the Australian Code of Practice of the Animal Welfare Standards for Livestock Processing Establishments (2001) and the Australian Animal Welfare Standards and Guidelines for Sheep (2016). The animals were slaughtered exclusively for commercial purposes, and the eyelid tissues would have been discarded if not acquired for use in the present study.

Riboflavin 5'-phosphate (RF-5P) monosodium salt, supplied by Cayman Chemical Co. (United States of America [USA]), was dissolved in phosphate-buffered saline (PBS) to a concentration of 0.25% w/v. The solution was applied to the tissue specimens to replicate the conditions during an actual photocrosslinking procedure.

2.2. Methods

Twelve full-thickness eyelid specimens were excised postmortem from the ocular adnexa of six pairs of sheep eyes that were refrigerated for no longer than 24 h. For convenience, the eyelashes were trimmed. The specimens were soaked for 30 min at room temperature in a solution of RF-5P in PBS (0.25% w/v). After removal from the solution, each sample was placed on a Petri dish with the conjunctival side facing upward, and the excess liquid was gently blotted off with Kimwipe® paper tissues (KIMTECH Sciences, Australia).

A thermal camera model FLIR C5 (FLIR Systems Australia Pty Ltd., Australia) was used for the thermographic analysis, with a thermal sensitivity of <0.07°C. As a source of radiation, the UV curing system OmniCure® 1500 (Excelitas Technologies Corp., USA) was employed. To determine the level of irradiance at the exposure site, a radiometer (Dymax ACCU-CAL 50, Dymax Corp., USA) was used for the pre-irradiation adjustment of the distance between the UV source and the tissue surface. The experimental setting is shown in Figure 1. The samples were each exposed for 3 min to UVA radiation with a wavelength of 365 nm at irradiances of 45, 75, 150, or 250 mW/cm^2, corresponding to fluences of 8.1, 13.5, 27.0, or 45.0 J/cm^2. Three eyelid specimens were used for each irradiance level, and the data points are presented as mean values with 95% confidence intervals. Statistical analysis was performed using the Kruskal–Wallis test (n = 3 per irradiance, total n = 12) in GraphPad Prism® (Version 6; GraphPad Software, USA).

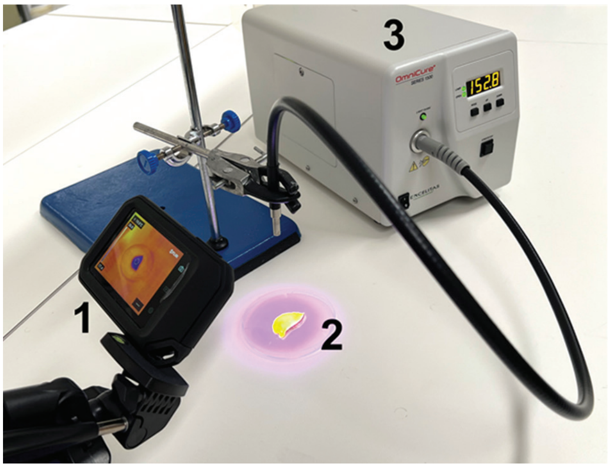

Figure 1. Experimental setting: (1) infrared camera, (2) tissue specimen in a Petri dish, and (3) ultraviolet A source

The infrared camera was mounted on a tripod and placed at a distance of ~ 20 cm from the sample. The measurement spot (the "spotmeter") on the camera screen was positioned on the surface of the tissue as centrally as possible. During each round of irradiation, images containing information regarding the temperature at the target spot were recorded every 30 s, along with baseline measurements taken before each irradiation episode. During the experiments, the ambient temperature in the laboratory was between 21 and 23°C, with a relative humidity of ~50%. The infrared camera was within its 12-month calibration interval at the time of the experiments.

3. Results

Figure 2A shows a photograph of a sheep eyelid tissue sample that was soaked for 30 min in the photosensitizer solution, ready to be exposed to UVA radiation. Figure 2B displays the thermographic image of a tissue specimen recorded before commencing the irradiation procedure. Figure 2C and 2D show representative thermographic images recorded at the end of an exposure protocol,

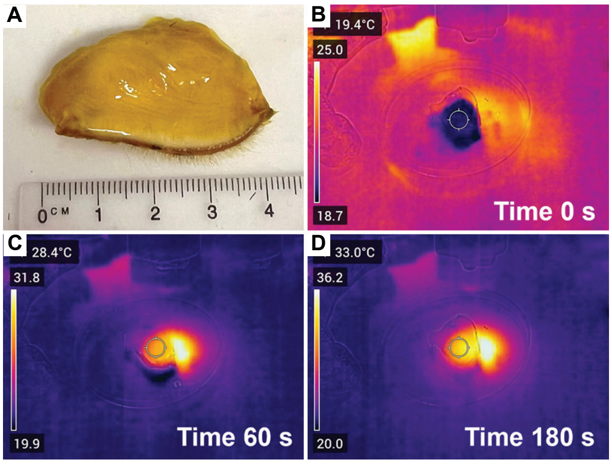

Figure 2. Photographic images of an eyelid tissue sample permeated with photosensitizer solution. (A) The eyelid tissue sample. (B–D) Thermographic images recorded (B) before and after exposure to ultraviolet A radiation (365 nm, 250 mW/cm^2) for (C) 60 s and (D) 180 s.

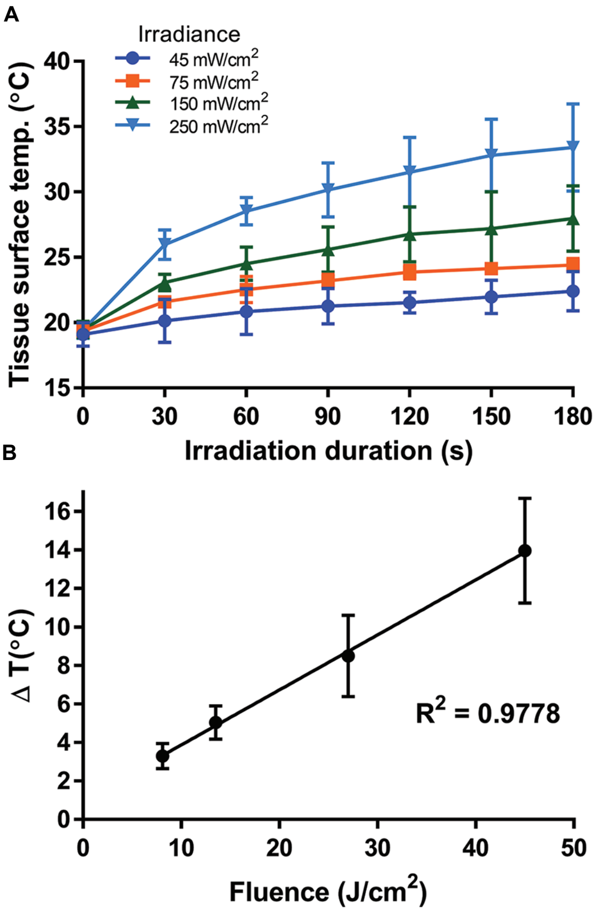

Figure 3. Thermal effects. (A) Plot of the temperature measured on the conjunctival surface as a function of exposure time at different irradiances. (B) Plot of the ΔT recorded on the conjunctival surface as a function of fluence. ΔT is defined as the difference between the temperature attained at the end of exposure time, i.e., after 3 min, and the baseline temperature recorded before commencing irradiation. Data points are presented with 95% confidence intervals.

exemplified here for an irradiance of 250 mW/cm^2 at two different exposure times.

As expected, the temperatures recorded on the surface of the tarsal conjunctiva increased both with the exposure time and with the irradiance (Figure 3A). Furthermore, there was a linear dependence between fluence and the temperature increase (ΔT) (Figure 3B). The baseline temperatures (before irradiation) were similar in all experiments, i.e., between 18.7 and 19.7°C, or 19.3 ± 0.3°C.

4. Discussion

When UV-induced crosslinking procedures are applied to collagenous tissues for therapeutic purposes, thermal effects associated with irradiation may induce a rise in temperature within the superficial layer of tissues or even deeper. However, as long as the temperature does not reach the threshold for thermal denaturation of collagen, which has been variably reported[40,41] between 50°C and 70°C, the procedure may be considered safe with respect to thermal tissue damage.

Reports on the thermal effect of UVA radiation on biological matter are sparse. For example, studies have been conducted[42,43] on cell cultures of human epithelial carcinoma cells (HeLa cell line), which were allowed to grow for 24 h before exposure to radiation while immersed in the growth medium. The rise in temperature recorded within the medium was highly dependent on the type of UV source. After 20 min of exposure, a BlackRay® lamp, delivering a fluence of 2.52 J/cm^2, produced an increase of 5.2°C, while a GreenSpot® super-pressure mercury lamp produced an increase of 1.4°C for a fluence of 7.6 J/cm^2. Despite apparent discrepancies, presumably due to technical variations related to the UV lamps, the recorded increases indicated minor thermal effects of UVA radiation. In a recent study[44] on UVA-based virus and bacteria inactivation, no thermal effects were mentioned for the remarkably high irradiance of 460 mW/cm^2, which allowed fluences of up to 800 J/cm^2.

More directly relevant to the present work, the effects of hyperthermia in *ex vivo* rabbit corneas, induced by ultrasonic energy delivered through the probe of a phacoemulsification machine, have been investigated using infrared thermography and histology.[45] No tissue injury was seen after 10 s of heating at 40°C. However, collagen disorganization, mild stromal edema, and cellular damage were noted at 50°C. With the same duration, massive stromal deterioration occurred at 60°C, characterized by the total disorganization of collagen fibers and endothelial detachment, which persisted after 1 week of follow-up. Another study has shown unequivocally that temperatures capable of promoting collagen denaturation can lead to significant tissue damage.[41] In a subsequent study,[46] the same research team performed thermographic analysis directly on the corneas of six patients during collagen crosslinking for treating keratoconus. During a 35-min treatment, instillations of riboflavin solution were carried out every 5 min. Irradiance and fluence were 3 mW/cm^2 and 5.4 J/cm^2, respectively. It was determined that the average temperature rise on the corneal surface during the procedure was 2.6°C. Such an increase was insufficient to raise the temperature to values characteristic of collagen thermal degradation. This possibility became even more remote due to supplementation with a solution of riboflavin, which had a temperature similar to room temperature or lower (if previously stored in a refrigerator).

We have already demonstrated histologically,[32-34] in both *ex vivo* animal and human eyelids, that UVA (365 nm) radiation does not cause degenerative changes in the anatomical components of the eyelid (conjunctiva, tarsus, meibomian glands, other accessory glands, muscles, and skin). However, less recognizable alteration of the tarsal network of fibrous proteins might occur if the temperature induced by irradiation exceeds the threshold levels attributed to denaturation of those proteins (including collagen) essential for mammalian tissue structure and function. The temperature increments recorded in the present study, in our opinion, were insufficient to reach those levels. At the irradiance values of 45 mW/cm^2 and 75 mW/cm^2, the corresponding increases in temperature were 3.3°C and 5°C, leading to final temperatures of the conjunctival surface of 22.4°C and 24.4°C, respectively. However, when the crosslinking procedure is performed in a clinical setting involving human patients, the baseline temperature may differ. For instance, a superficial temperature of the tarsal conjunctiva of 33.0±0.8°C has been reported in human subjects,[47] a value that falls well within the normal range measured for the ocular surface in general.[48] Such temperatures may drop upon adding the photosensitizer solution to the eyelid surface. Even with these higher baseline values, it is unlikely that the final conjunctival surface temperature will exceed 40°C, which can be considered safe for maintaining the thermal stability of biological molecules.[49-53] Similar considerations regarding collagen stability and tissue integrity have been discussed in a therapeutic context involving collagen scaffolds and growth factors, where histological evaluation emphasized the importance of avoiding structural denaturation to preserve biological function.[54]

The temperature rises of 8.5°C and 14°C that we have recorded at the higher irradiances of 150 mW/cm^2 and 250 mW/cm^2 (corresponding to fluences of 27 J/cm^2 and 45 J/cm^2), respectively, can indeed increase the final temperature of the tarsal conjunctiva (admittedly starting at ~ 33°C, see above) to values in the vicinity of 40°C or higher. However, such high radiant exposures are not relevant in this context because they are not recommended for therapeutic applications to any ocular tissue, and such levels are unlikely to be employed. In a previous study,[33] when an *ex vivo* tarsal plate was exposed directly to UVA radiation, i.e., after the conjunctiva was removed, a fluence of 45 J/cm^2 (generated by applying an irradiance of 250 mW/cm^2 for 3 min) caused substantial damage to the fibrous protein network, cells, and meibomian glands residing within the tarsus. Hence, from the current study, it can also be inferred that the tarsal conjunctiva plays a protective role during the exposure of the eyelid to UVA radiation.

5. Conclusion

The exposure to UVA radiation (with a wavelength of 365 nm) leads to an increase in temperature on the surface of the tarsal conjunctiva, as evidenced in this study using the eyelid of an ovine model. The post-exposure temperature increments are remarkably low, and there is an ideal linear dependence between them and the fluence of the incident radiation. Corroborated with previously reported data, thermal effects of this magnitude are not indicative of tissue damage occurring for the ranges of irradiance (intensity) and fluence (radiant exposure) applicable in the treatment of eyelid laxity. The results of this study are important for advancing to the next translational steps, including *in vivo* animal experimentation and human clinical trials, which are both currently in the preliminary preparation stages at the authors' facilities.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Traian V. Chirila, Shuko Suzuki
Formal analysis: Shuko Suzuki
Investigation: All authors
Methodology: All authors
Project administration: Traian V. Chirila
Writing–original draft: Traian V. Chirila
Writing–review & editing: Shuko Suzuki, Nadja E. Pop, Alexandra I. Manta

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All relevant data supporting this study are included in this article. Additional information is available from the corresponding author upon reasonable request.

Further disclosure

Australian Patent No. AU 2018201200 of February 20, 2018, and U.S. Patent No. 11,240,073 (B2) of August 23, 2022, have been granted for a method of treatment related to this research and were assigned to the Queensland Eye Institute Foundation by the authors Shuko Suzuki and Traian V. Chirila.

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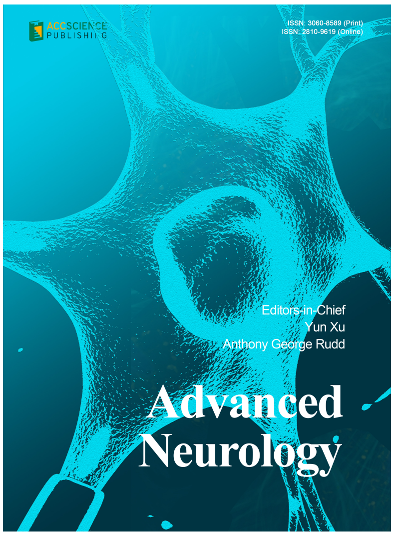
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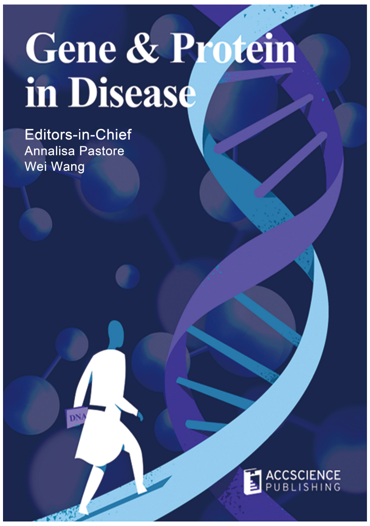
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