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Liquid Biopsy and Circulating Tumor DNA for Monitoring Minimal Residual Disease in Hormone Receptor-Positive Breast Cancer: Current Evidence, Technologies, Clinical Outcomes, and Future Directions

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Abstract. *Background. Minimal residual disease (MRD) drives late relapse and mortality in hormone receptor-positive (HR+) breast cancer. Conventional surveillance modalities—serum tumor markers and cross-sectional imaging—lack sufficient sensitivity to detect MRD at a clinically actionable stage. Objective. This review synthesizes evidence on liquid biopsy and circulating tumor DNA (ctDNA) for MRD monitoring in HR+ breast cancer, covering assay technologies, clinical trial findings, methodological limitations, and future research priorities. Evidence Base. Evidence derives from systematic reviews, meta-analyses, prospective and retrospective cohorts, and interventional trials (PADA-1, SERENA-6) published between 2018 and 2026, as identified through the Scopus AI analytical platform. Key Findings. ctDNA-based MRD assays detect recurrence a median of 5–14 months before clinical or radiographic manifestation, with individual cases reporting a lead time of up to five years. Tumor-informed mutation-based assays achieve sensitivity of 50–79% with specificity approaching 100%. Methylation-based assays demonstrate sensitivity of 62.5% and specificity of 100%, outperforming mutation-based approaches in early-stage, low-shedding HR+ tumors. ctDNA dynamics correlate with progression-free and overall survival. The SERENA-6 trial demonstrated improved PFS and quality of life with ctDNA-guided early endocrine switching; the PADA-1 trial showed ctDNA dynamics predict PFS and OS, with ctDNA-based risk models outperforming clinical parameters. Conclusion. Despite robust prognostic evidence, assay standardization deficits, clonal hematopoiesis-related false positives, and cost barriers preclude routine clinical adoption. Large-scale, prospective, randomized trials are urgently required.*

Keywords: *liquid biopsy, circulating tumor DNA (ctDNA), minimal residual disease (MRD), hormone receptor-positive (HR+) breast cancer, tumor-informed assay, methylation-based assay, ESR1 mutation, clonal hematopoiesis, PADA-1, SERENA-6*

Introduction

Hormone receptor-positive (HR+) breast cancer is the most prevalent molecular subtype of breast malignancy. Despite substantial advances in adjuvant endocrine therapy, a significant proportion of patients experience disease relapse, often years or decades after the completion of primary treatment (Sears & Davis, 2023; Chen et al., 2025a). The primary biological driver of this late recurrence is minimal residual disease (MRD): the persistence of microscopic, dormant tumor cells following curative-intent therapy that remain entirely undetectable by conventional clinical and radiological surveillance (Chen et al., 2025a; Xu et al., 2025).

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The unique biology of HR+ tumors—characterized by comparatively low proliferative activity and correspondingly diminished ctDNA shedding into the bloodstream—imposes particular analytical demands on MRD detection platforms (Elliott et al., 2025b).

Traditional surveillance tools lack the sensitivity to identify residual disease at a stage when therapeutic intervention could be most effective, leaving the majority of patients undiagnosed until macroscopic metastatic disease is established (Xu et al., 2025; Abdo et al., 2026). Conventional surveillance methods, including imaging and serum tumor markers, lack the sensitivity to detect MRD at a stage when intervention could be most effective (Pfister et al., 2025b). This diagnostic gap has catalyzed intense scientific interest in non-invasive, molecularly sensitive tools for MRD monitoring in real time.

Liquid biopsy—the non-invasive analysis of tumor-derived biomarkers in peripheral blood—has emerged as a transformative paradigm in oncological diagnostics (Malik & Zaheer, 2025; Wu & Chu, 2022). Circulating tumor DNA (ctDNA) consists of small DNA fragments released into the bloodstream from apoptotic and necrotic tumor cells, carrying the somatic mutations and epigenetic alterations characteristic of the originating tumor (Sears & Davis, 2023). These features render ctDNA a real-time, dynamic reflection of tumor burden, treatment response, and molecular evolution.

In HR+ breast cancer, ctDNA-based MRD monitoring offers several clinically distinct advantages: early detection of residual disease months to years before clinical recurrence, enabling pre-emptive therapeutic intervention; dynamic risk stratification based on ctDNA kinetics rather than static clinical parameters; non-invasive, longitudinal assessment of tumor evolution and acquired resistance; and the potential to guide adaptive, personalized treatment strategies (Elliott et al., 2025a; Oliveira et al., 2026; Sears & Davis, 2023).

This review provides a critically appraised synthesis of ctDNA-mediated MRD monitoring in HR+ breast cancer. It addresses: (1) biological rationale for ctDNA as an MRD biomarker; (2) principal assay technologies and their comparative analytical performance; (3) clinical trial evidence from PADA-1 and SERENA-6; (4) the role of ESR1 mutations and resistance monitoring; (5) technological innovations including machine learning and multi-omic integration; (6) current methodological challenges; and (7) priority future research directions.

Theoretical and Biological Framework

Residual tumor cells following curative treatment in HR+ breast cancer may enter a state of prolonged biological dormancy—metabolically quiescent but genomically intact—shielded from immune surveillance and pharmacological pressure (Chen et al., 2025a; Sears & Davis, 2023). This dormancy underlies the characteristically protracted recurrence pattern of HR+ disease, with late relapses documented years to decades after initial diagnosis.

The low-shedding phenotype of dormant HR+ tumor cells means ctDNA concentrations in plasma may be extremely low—often below the detection threshold of standard sequencing pipelines (Elliott et al., 2025b). This demands assays of exceptional analytical sensitivity and has driven the development of methylation-based and multi-omic approaches as alternatives to mutation-based detection in early-stage and post-treatment surveillance settings.

ctDNA fragments are distinguished from non-tumor cell-free DNA (cfDNA) by somatic alterations: point mutations, copy number variations, structural variants, and cancer-specific epigenetic modifications (Sears & Davis, 2023; Widman et al., 2024). The fraction of ctDNA within total cfDNA—the variant allele fraction (VAF)—is proportional to tumor burden and provides a quantitative index of residual disease.

Beyond mutation detection, DNA methylation patterns and fragmentomic features (fragment length distribution and preferred end-sequence motifs) provide complementary or superior biomarker signals in low-shedding contexts (Elliott et al., 2025b; Janni et al., 2025). Cancer-specific methylation signatures are detectable at lower ctDNA concentrations than somatic mutations and are substantially less susceptible to confounding by clonal hematopoiesis—the primary source of false-positive results in mutation-based liquid biopsy.

A coherent clinical framework for ctDNA-based MRD monitoring integrates three dimensions. First, the detection modality: somatic mutation, DNA methylation, fragmentomics, or multi-omic integration. Second, the assay architecture: tumor-informed (personalized, patient-specific), tumor-agnostic (fixed panel or methylation signature, no tumor tissue required), or tumor-naive multi-omic (plasma-only). Third, the clinical application: early relapse prediction, risk stratification, therapy escalation or de-escalation, or resistance monitoring (Abdo et al., 2026; Sears & Davis, 2023).

Technologies and Methodologies

Next-generation sequencing (NGS) enables comprehensive mutational profiling across targeted gene panels or the entire tumor genome. Ultra-deep sequencing employing molecular barcoding and error-correction algorithms achieves the sensitivity required for low-frequency variant detection characteristic of MRD settings (Janni et al., 2025; Semenkovich et al., 2023). Tumor-informed NGS panels—designed around patient-specific somatic variants from primary tumor sequencing—represent the current analytical benchmark for ctDNA MRD detection (Panet et al., 2024; Qiu et al., 2025). Parsons et al. (2020) established the foundational proof-of-concept that tracking multiple patient-specific mutations simultaneously significantly enhances sensitivity compared to single-mutation approaches. The MAESTRO-Pool methodology further extended this principle to highly parallel cohort-level implementation (Blewett et al., 2024).

Digital droplet PCR (ddPCR) partitions the PCR reaction into thousands of individual droplets, enabling absolute quantification of target variants with high analytical sensitivity. It is particularly well-suited to longitudinal monitoring of known hotspot mutations, including *ESR1* ligand-binding domain variants associated with acquired endocrine resistance (Lu et al., 2025). Its principal limitation is the requirement for prior knowledge of the target mutation, restricting utility to hypothesis-directed monitoring.

Methylation-based ctDNA detection exploits cancer-specific DNA methylation signatures at loci differentially methylated in breast cancer relative to normal tissue (Elliott et al., 2025b). These assays require no prior knowledge of patient-specific mutations and are substantially less susceptible to clonal hematopoiesis interference. In HR+ breast cancer, methylation-based assays have demonstrated superior performance compared to tumor-agnostic mutation-based approaches in early-stage, low-shedding disease.

Single-cell sequencing platforms enable detailed molecular characterization of circulating tumor cells (CTCs), revealing intratumoral heterogeneity and clonal resistance mechanisms at single-cell resolution (Chen et al., 2023; Xu et al., 2021). Microfluidic technologies improve CTC isolation efficiency (Velpula & Buddolla, 2025; Li et al., 2021b). CTC and ctDNA analyses produce largely non-overlapping detection profiles, providing complementary biological information that—in combination—enhances MRD detection sensitivity, risk stratification, and resistance monitoring (Bortolini Silveira et al., 2021; Gerratana et al., 2021).

Tumor-informed assays employ whole-genome or whole-exome sequencing of primary tumor tissue to design a personalized tracking panel. Commercial platforms such as Signatera and NeXT Personal simultaneously interrogate dozens to hundreds of patient-specific loci (Chen & Zhou, 2023; Chen et

al., 2021). Tumor-agnostic assays apply fixed gene panels or methylation signatures across patients without tumor tissue, improving logistical feasibility at some cost to sensitivity in low-shedding tumors (Nguyen et al., 2025). Tumor-naive multi-omic assays integrate methylation and genomic features from plasma alone, entirely eliminating the tumor tissue requirement and reducing turnaround time (Janni et al., 2025). Overall, tumor-informed assays outperform tumor-agnostic alternatives, though the latter offer greater accessibility (Panet et al., 2024).

Critical Analysis of Evidence

The most clinically consequential attribute of ctDNA-based MRD monitoring is its capacity to detect impending relapse substantially in advance of conventional methods. ctDNA MRD detection precedes clinical or radiographic disease recurrence by a median of 5–14 months, with individual cases reporting a lead time of up to five years (Elliott et al., 2025a; Nader-Marta et al., 2024; Pfister et al., 2025a). This pre-clinical window provides a therapeutic opportunity for pre-emptive intervention before macroscopic metastatic disease is established.

ctDNA positivity in the post-adjuvant period is strongly associated with increased risk of recurrence and inferior survival outcomes in HR+ breast cancer (Lipsyc-Sharf et al., 2022; Ajjawi et al., 2025). Conversely, ctDNA clearance during or after treatment is strongly correlated with longer survival and prolonged time to treatment failure (Fuentes-Antras et al., 2025; Elliott et al., 2024). These bidirectional associations establish ctDNA dynamics as a robust real-time surrogate for treatment efficacy and residual disease burden.

The comparative analytical performance of principal ctDNA assay architectures is summarized in Table 1, based on data reported by Elliott et al. (2025b) and Nguyen et al. (2025). Tumor-informed mutation-based assays demonstrate sensitivity of 50–79% and specificity of approximately 100%, with a detection lead time of 5–14 months, but require primary tumor tissue and incur higher costs. Methylation-based assays achieve sensitivity of 62.5% and specificity of 100%, with a lead time exceeding five months; they outperform mutation-based tumor-agnostic assays in early-stage, low-shedding HR+ tumors and are less susceptible to clonal hematopoiesis interference (Elliott et al., 2025b). Tumor-agnostic multi-omic assays achieve sensitivity of approximately 54.5% and specificity of 98.8% but demonstrate lower sensitivity in early-stage disease (Nguyen et al., 2025).

Table 1
Comparative performance of ctDNA assay architectures in HR+ breast cancer MRD monitoring. Source: Elliott et al. (2025b); Nguyen et al. (2025).

Assay Architecture	Sensitivity	Specificity	Lead Time	Key Advantage	Primary Limitation
Tumor-informed (mutation-based)	50–79%	~100%	5–14 months	Highest per-patient sensitivity	Requires tumor tissue; higher cost
Methylation-based	62.5%	100%	>5 months	Superior in low-shedding tumors; resistant to clonal hematopoiesis	Requires further large-scale validation

Assay Architecture	Sensitivity	Specificity	Lead Time	Key Advantage	Primary Limitation
Tumor-agnostic (multi-omic)	~54.5%	~98.8%	Moderate	No tumor tissue required; broad applicability	Lower sensitivity in early-stage disease

The PADA-1 trial is a landmark investigation of ctDNA-guided therapeutic decision-making in advanced HR+/HER2− breast cancer. Longitudinal analysis by Mamann et al. (2025) demonstrated that early on-treatment evolution of ctDNA—including emergence of *ESR1* mutations and other resistance-associated alterations—was significantly associated with shorter progression-free survival (PFS) and overall survival (OS). Critically, ctDNA-based risk models outperformed conventional clinical parameters in prognostic discrimination (Mamann et al., 2025).

SERENA-6 evaluated ctDNA-guided early endocrine therapy switching in HR+/HER2− metastatic breast cancer. The trial demonstrated improved progression-free survival (PFS) and quality of life in patients managed with ctDNA-guided early therapy switching compared to imaging-based standard management (Oliveira et al., 2026). This demonstrates that ctDNA-based therapeutic decisions can be enacted weeks to months earlier than radiographic progression would otherwise mandate.

Oliveira et al. (2026) reviewed the combined evidence from both PADA-1 and SERENA-6 in the context of molecularly-driven early switch therapy, affirming that ctDNA-guided strategies represent an advance over conventional imaging-based monitoring. Fuentes-Antras et al. (2025) confirmed that ctDNA clearance during early treatment cycles was strongly correlated with prolonged time to treatment failure and longer overall survival in patients receiving endocrine therapy combined with CDK4/6 inhibitors. Elliott et al. (2024) further showed that ctDNA metrics of response and progression in the same therapeutic context provided clinically informative prognostic data. Collectively, these trials indicate that ctDNA-guided therapy adaptation is associated with improved outcomes, but definitive survival benefit requires further large-scale validation (Oliveira et al., 2026).

Mutations in the ligand-binding domain of the estrogen receptor gene (*ESR1*) represent the most clinically significant mechanism of acquired resistance in HR+ breast cancer patients receiving aromatase inhibitors. ctDNA-based longitudinal monitoring of *ESR1* mutational status enables real-time surveillance for emerging resistance before clinical progression, guiding timely therapy adaptation (Allouchery et al., 2018; Li et al., 2020; Maloberti et al., 2025). Different *ESR1* mutation subtypes confer heterogeneous resistance profiles and may be associated with distinct patterns of metastatic dissemination (Reinert et al., 2019). The systematic review by Najim et al. (2019) established that *ESR1* mutations arise predominantly under aromatase inhibitor pressure and associate with inferior clinical outcomes. CDK4/6 inhibitors have demonstrated capacity to partially overcome *ESR1* mutation-mediated resistance (Crucitta et al., 2023), reinforcing the value of ctDNA-based resistance monitoring for informing combination therapy decisions. The ctDNA profile and its clinical significance in patients with HR+/HER2− disease has been further characterized by Tang et al. (2022), confirming the importance of serial ctDNA monitoring for resistance management.

CTC and ctDNA analyses produce non-overlapping detection profiles. While ctDNA reflects the bulk genomic landscape through DNA shedding, CTCs represent intact disseminated tumor cells amenable to detailed phenotypic characterization (Bortolini Silveira et al., 2021). Their combination enhances early detection, risk stratification, and resistance monitoring beyond either modality alone (Gerratana et al., 2021; Park et al., 2024). Machine learning-driven integration of CTC and ctDNA data has been

shown to improve endocrine resistance profiling accuracy (Gerratana et al., 2025). Stergiopoulou et al. (2023) confirmed the clinical value of comprehensive multimodal liquid biopsy for early MRD detection in breast cancer.

Technological Innovations

Machine learning and advanced bioinformatics have substantially improved ctDNA analytical performance. Widman et al. (2024) developed an ultrasensitive plasma-based monitoring system employing machine learning-guided signal enrichment, achieving tumor burden quantification at variant allele fractions previously below the detection threshold of standard pipelines, with significant improvement in MRD detection sensitivity without compromising specificity. Machine learning further enhances variant calling accuracy, reduces technical noise, and integrates multi-omic data for improved performance in low-shedding contexts (Widman et al., 2024; Abdo et al., 2026).

Multi-omic approaches integrating genomic, epigenomic, and fragmentomic features demonstrate additive improvements in ctDNA detection (Janni et al., 2025; Semenkovich et al., 2023). By combining mutation-based signals with methylation signatures and fragment length information, these platforms extract richer biological information from the same plasma specimen. This is of particular value in HR+ breast cancer, where single-signal sensitivity is constrained by low ctDNA shedding.

Tumor-naive, plasma-only ctDNA assays integrating methylation profiling and genomic data require no prior tumor tissue sequencing, improving clinical feasibility and reducing turnaround time (Janni et al., 2025). Their performance is approaching, but has not yet equalled, that of tumor-informed personalized approaches (Nguyen et al., 2025). Ongoing methodological development and prospective validation of these platforms are active research priorities.

Structural variant-based ctDNA detection—targeting chromosomal rearrangements and large deletions nearly absent in normal cfDNA—offers inherently high specificity and is less susceptible to sequencing error and clonal hematopoiesis interference (Elliott et al., 2025a). Mohiuddin (2025) reviewed the landscape of ultrarapid sensitivity innovations, including nanomaterial-based sensors and AI-driven error suppression systems, as further tools for MRD detection in low-shedding tumor contexts.

Methodological Challenges and Limitations

The absence of standardized assay protocols, analytical thresholds, and reporting frameworks is the foremost barrier to clinical translation (Lin et al., 2026; Zhu et al., 2023). Heterogeneity in pre-analytical variables—blood collection tube type, plasma processing time and temperature, storage conditions, and DNA extraction methods—introduces substantial inter-laboratory variability impeding cross-study comparability and clinical interpretation (Lu et al., 2025). International consensus protocols are urgently needed. The definition of clinically appropriate MRD positivity thresholds is an equally pressing challenge. Longitudinal ctDNA trajectory monitoring, rather than single time-point results, may provide more robust evidence of true MRD (Chin et al., 2019; Klimova et al., 2025; Deveson et al., 2021).

Clonal hematopoiesis of indeterminate potential (CHIP)—the age-related accumulation of somatic mutations in hematopoietic stem cells—is a major source of false-positive ctDNA results in cancer surveillance (Janni et al., 2025; Chin et al., 2019). CHIP-derived mutations in plasma cfDNA can mimic low-burden ctDNA signals, potentially misclassifying patients as MRD-positive. Recommended mitigation strategies include paired white blood cell sequencing to subtract CHIP-derived variants, longitudinal monitoring to distinguish persistent from transient signals, and advanced bioinformatic filtering (Janni et al., 2025; Chin et al., 2019). Methylation-based assays offer

an intrinsic advantage, as methylation signatures are less susceptible to CHIP-related interference (Elliott et al., 2025b).

False-negative results—arising from ctDNA concentrations below current assay detection limits—are particularly acute in HR+ breast cancer. Patients with small residual tumor burden or predominantly dormant micrometastatic deposits may harbor biologically significant MRD undetectable by available platforms (Chen et al., 2025a; Lin et al., 2026). A ctDNA-negative result cannot be interpreted as confirmation of complete pathological remission in HR+ disease, underscoring the need for complementary multimodal approaches and continued assay sensitivity improvements.

The cost of tumor-informed ctDNA assays constitutes a prohibitive barrier in many healthcare systems (Abdo et al., 2026; Lin et al., 2026). Limited reimbursement and restricted assay availability in lower-resource settings raise important health equity concerns. Most evidence derives from high-resource clinical trial settings, limiting generalizability to global patient populations (Xu et al., 2025). Cost-effectiveness studies and simplified, accessible assay formats are critical prerequisites for equitable implementation.

Real-World Clinical Implications

The clinical implications of validated ctDNA-based MRD monitoring in HR+ breast cancer span several domains:

- *Personalized surveillance:* ctDNA monitoring enables individualized follow-up, reducing unnecessary imaging while allowing earlier intervention upon molecular evidence of relapse (Chen et al., 2025a; Sears & Davis, 2023).
- *Treatment adaptation:* Real-time ctDNA dynamics can inform decisions to escalate, de-escalate, or switch adjuvant therapy (Oliveira et al., 2026; Fuentes-Antras et al., 2025).
- *Resistance monitoring:* Detection of actionable mutations (e.g., *ESR1*, *PIK3CA*) via ctDNA supports timely therapy switching before overt clinical progression (Allouchery et al., 2018; Crucitta et al., 2023; Tang et al., 2022).
- *Multimodal integration:* Combining ctDNA with CTCs and advanced imaging may yield a composite MRD index with superior predictive accuracy (Bortolini Silveira et al., 2021; Stergiopoulou et al., 2023).
- *Implementation barriers:* Cost, assay accessibility, and standardization deficits currently limit widespread adoption (Abdo et al., 2026; Lin et al., 2026; Xu et al., 2025).

Future Research Directions

- *Large-scale randomized trials:* Prospective, adequately powered trials with survival endpoints are essential to definitively validate the clinical utility and survival benefit of ctDNA-guided therapeutic interventions (Abdo et al., 2026; Xu et al., 2025; Pfister et al., 2025b).
- *Consensus standardization:* International consensus on pre-analytical protocols, MRD positivity thresholds, and reporting standards is required (Klimova et al., 2025; Deveson et al., 2021; Lin et al., 2026).
- *Plasma-only multi-omic validation:* Tumor-naive plasma-only assays must be prospectively validated against tumor-informed standards (Janni et al., 2025; Nguyen et al., 2025).
- *Multi-modal biomarker integration:* Research combining ctDNA with CTCs, radiomics, and proteomic signatures may yield composite MRD indices with superior accuracy (Abdo et al., 2026; Stergiopoulou et al., 2023).

- *Implementation science and health economics:* Studies addressing workflow integration, cost-effectiveness, and implementation across diverse healthcare settings are critical (Abdo et al., 2026; Lin et al., 2026).

Conclusion

Liquid biopsy and ctDNA-based MRD monitoring represent a paradigm-shifting advance in the management of hormone receptor-positive breast cancer. The capacity to detect molecular evidence of residual disease months to years before clinical manifestation, and to dynamically monitor tumor evolutionary trajectories under therapeutic pressure, addresses fundamental limitations of conventional surveillance and opens substantive opportunities for pre-emptive, personalized intervention.

Current evidence demonstrates robust prognostic value for ctDNA positivity across early-stage and metastatic HR+ disease (Lipsyc-Sharf et al., 2022; Ajjawi et al., 2025). Tumor-informed and methylation-based assays lead in analytical performance (Elliott et al., 2025b). Clinical trial data from PADA-1 and SERENA-6 provide compelling evidence that ctDNA-guided therapeutic decisions can translate into measurable improvements in PFS and quality of life (Mamann et al., 2025; Oliveira et al., 2026), though definitive survival validation remains outstanding. Technological innovations in machine learning, multi-omic integration, and ultra-sensitive platforms are progressively expanding the sensitivity frontier (Widman et al., 2024; Elliott et al., 2025a).

The pathway to routine clinical adoption remains contingent on resolving outstanding challenges: pre-analytical standardization, validated MRD positivity thresholds, robust clonal hematopoiesis mitigation, and demonstrated cost-effectiveness across diverse healthcare systems (Lin et al., 2026; Xu et al., 2025; Abdo et al., 2026). The convergence of molecular diagnostics, computational analytics, and adaptive clinical trial design will determine the rate at which ctDNA-based MRD monitoring is translated into improved, equitably accessible care for the global population of patients with HR+ breast cancer.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Comparative Analysis of Retinoid Generations in the Management of Skin Photoaging

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Abstract. *Retinoids constitute the most thoroughly researched group of topical agents in dermatological anti-aging treatments due to their significant effects on epidermal cell proliferation, differentiation, and dermal extracellular matrix remodeling. This review comprehensively evaluates the pharmacological classification, mechanisms of action, clinical efficacy, safety profiles, and formulation-related issues of natural and synthetic retinoids used in the management of skin aging. Tretinoin, a first-generation retinoid, is still considered the gold standard, particularly in the improvement of photo-aged skin, thanks to strong clinical evidence; however, its use is often limited due to dose-dependent irritation. Although metabolic precursors such as retinol and retinaldehyde offer higher tolerability, their clinical success largely depends on enzymatic conversion capacity and formulation stability. Retinyl esters, frequently used in cosmetic products, show limited and variable clinical results and also raise additional concerns regarding photodegradation and potential pro-oxidant effects. While second-generation retinoids are not currently widely preferred in topical anti-aging applications, third-generation retinoids such as adapalene and tazarotene demonstrate similar efficacy to tretinoin but offer more selective receptor interaction and, in some cases, faster clinical responses. Although fourth-generation retinoids such as tripharotene and seletinoid G have shown promising results in preclinical studies, clinical data on their effects on photoaging are still insufficient. One of the key factors determining the effectiveness of retinoids is formulation technology; stability problems and limitations in skin penetration significantly affect treatment outcomes. While newly developed nanoformulation systems have the potential to increase efficacy and reduce irritation, clinical evidence in this area is still limited. Consequently, while tretinoin remains the reference standard, new generation retinoids and advanced delivery systems may offer more advantageous therapeutic profiles. However, more extensive controlled clinical trials are needed to clarify the long-term efficacy, safety, and comparative superiority of these agents.*

Keywords: *retinoids, skin aging, photoaging, topical anti-aging therapy, nanoformulations*

Introduction

Skin aging is the result of complex molecular and cellular changes caused by internal and external factors, manifesting with noticeable clinical findings such as wrinkle formation, thinning of the dermal layer, and loss of elasticity. These changes lead to progressive degeneration of the skin structure and a decrease in the functional components of the skin over time (Shin et al., 2019; Zhang & Duan, 2018).

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While chronological aging is considered part of the natural biological process, photoaging, caused by ultraviolet (UV) radiation, is considered the main external factor accelerating skin aging through the excessive production of reactive oxygen species (Ganguly et al., 2022).

In recent years, anti-aging dermatological applications have received intense scientific and clinical attention due to comprehensive approaches aimed at restoring skin structure, improving skin texture, and enhancing aesthetic appearance. Among these treatment options, topical retinoids are among the most researched and clinically proven agents. Current photoaging prevention and treatment strategies are mostly based on regular photoprotection, sunscreen use, and the combined application of topical preparations containing retinoids and antioxidants (Zasada & Budzisz, 2019).

Retinoids are a broad group of natural and synthetic compounds that show structural similarity to retinol (vitamin A). The anti-aging effects of topical retinoids are explained by various biological mechanisms such as increasing keratinocyte proliferation, supporting collagen synthesis, strengthening epidermal barrier function, reducing transepidermal water loss, suppressing collagen degradation, and inhibiting matrix metalloproteinase activity (Zasada & Budzisz, 2019).

The biological effects of retinoids occur via nuclear retinoic acid receptors (RAR-α, RAR-β, RAR-γ) and retinoid X receptors (RXR-α, RXR-β, RXR-γ) (Shin et al., 2019). Based on their molecular structure and receptor binding properties, retinoids are generally classified into four different generations, and this classification is summarized in Table 1.

Table 1
Classification of Retinoids According to Generation and Representative Compounds

Retinoid Generation	Structural Characteristics	Representative Compounds	Key Features
First-Generation Retinoids	Naturally occurring retinoids and closely related non-aromatic derivatives with flexible molecular structures	Tretinoin (all-trans-retinoic acid); Isotretinoin (13-cis-retinoic acid); Alitretinoin (9-cis-retinoic acid); Retinol (all-trans-retinol, vitamin A); Retinal (retinaldehyde); Retinyl palmitate; Retinyl propionate; Retinyl acetate; Retinyl retinoate; Retinyl N-formyl aspartamate	Exhibit broad receptor activity and are widely used in dermatological and cosmetic formulations
Second-Generation Retinoids	Monoaromatic retinoids produced through structural modification of the cyclic end group	Etretinate; Acitretin; Motretinate	Characterized by enhanced chemical stability and improved pharmacokinetic properties
Third-Generation Retinoids	Polyaromatic retinoids with greater receptor selectivity and reduced adverse effects	Tazarotene; Adapalene; Bexarotene	Designed to increase therapeutic specificity while minimizing irritation and systemic toxicity
Fourth-Generation Retinoids	Selective receptor-targeting retinoids developed using advanced molecular engineering approaches	Trifarotene; Seletinoid G	Demonstrate highly selective receptor affinity and improved tolerability profiles in topical therapy

Following topical application, retinyl esters are converted to retinol via enzymatic hydrolysis, and then retinol is converted to the biologically active metabolite retinoic acid (tretinoin) through a two-step oxidation process via retinaldehyde by dehydrogenase enzymes (Riahi et al., 2016).

Considering the metabolic conversion steps, the biological efficacy of first-generation retinoids increases gradually from retinyl esters to retinoic acid: retinyl esters << retinol < retinaldehyde < retinoic acid. Conversely, the tolerability profile progresses in the opposite direction; the highest tolerance is observed with retinyl esters, and the lowest with retinoic acid: retinyl esters > retinol = retinaldehyde >> retinoic acid.

Therefore, topical preparations containing retinol, retinaldehyde, and retinyl ester require metabolic conversion within the skin to form the active form, tretinoin. In contrast, some retinoids can exhibit biological activity directly in their applied form without requiring additional biochemical conversion.

Tretinoin, alitretinoin, adapalene, tazarotene, bexarotene, and tripharotene are included in approved topical pharmaceutical formulations used in the treatment of various dermatological diseases such as acne and psoriasis. Among these agents, tretinoin and tazarotene are the main retinoids approved for the adjunctive treatment of photo-aged skin.

In contrast, commercial cosmetic and cosmeceutical products mostly use retinol, retinaldehyde, and retinyl esters. The concept of cosmeceuticals defines cosmetic products containing biologically active ingredients intended to exert pharmacological effects on skin structure and function, forming an intermediate category between traditional cosmetics and prescription topical drugs. However, unlike pharmaceutical products, cosmeceuticals generally undergo more limited pre-marketing evaluations in terms of safety and clinical efficacy (Tetali et al., 2020).

Since retinoids in cosmeceutical products need to be converted to tretinoin in the skin to become biologically active, the effectiveness of these products remains a subject of scientific debate when compared to formulations containing directly active tretinoin (Resende et al., 2022).

Although retinoids are among the important anti-aging and depigmenting agents widely used in modern cosmetic formulations, they present various formulation challenges due to limited skin penetration, chemical stability problems, and irritation potential related to their physicochemical properties. Adverse effects associated with topical retinoid use are mostly dose-dependent and manifest as "retinoid dermatitis," characterized by erythema, dryness, desquamation, and irritation at the application site (Szymański et al., 2020).

One of the significant safety concerns regarding retinoid use is their potential teratogenic effects. Although the teratogenicity of oral retinoids has been clearly established, evidence regarding retinoid-induced embryopathy after topical use is largely based on limited case reports. However, despite the lack of definitive evidence of harm, the current clinical approach is cautious, and the use of topical retinoids during pregnancy is generally not recommended due to insufficient safety data.

The mechanisms of retinoid-induced side effects at the molecular level have not yet been fully elucidated. In clinical practice, it is recommended to prefer low-concentration preparations and to discontinue treatment if skin intolerance develops. Furthermore, it has been reported that first-generation retinoids with non-selective receptor activity cause skin reactions more frequently than later-generation retinoids (Williams et al., 2020).

Tretinoin (all-trans-retinoic acid) is considered one of the most reliable topical retinoids for anti-aging, thanks to its potent therapeutic effect and extensive clinical study support. It is one of the most thoroughly researched retinoids in the field of dermatology. Topical formulations typically range from 0.01% to 0.1%, with the most commonly preferred concentrations being 0.025%, 0.05%, and 0.1%. These prescription preparations are widely used in the treatment of acne vulgaris, facial photoaging, and hyperpigmentation. Clinical studies have shown that tretinoin provides significant benefit in reducing signs of sun-induced skin aging (Darlenski et al., 2010).

In a two-year placebo-controlled study involving 204 individuals with moderate to severe facial photodamage, a moisturizing cream containing 0.05% tretinoin applied daily was found to be effective and safe with long-term use. Although a 0.05% concentration is often preferred in clinical practice, the appropriate dose and duration of treatment should be adjusted according to the patient's skin tolerance and the desired clinical outcome. General assessments report that 0.025% preparations offer more balanced results in terms of efficacy and tolerability (Darlenski et al., 2010).

The clinical success of tretinoin is directly related to the concentration used. Comparative studies show that lower doses provide significant clinical and histological improvement, albeit at a milder level, while also reducing the risk of side effects. Therefore, treatment is usually started with low doses, and the dose is gradually increased according to skin tolerance. Furthermore, its use in conjunction with moisturizing products is recommended to reduce irritation and support the skin barrier (Buchanan & Gilman, 2016).

In recent years, various nanoformulation technologies have been developed for tretinoin, such as liposomes, nanoemulsions, and lipid-based nanocarrier systems. While the majority of research focuses on controlled drug release and in vitro permeability, some studies have also evaluated irritation in rat and rabbit models (Liu et al., 2021). The findings indicate that these systems provide better stability compared to classical formulations, aid in more controlled drug release, and can reduce skin irritation (Pinto et al., 2019).

The main goal in developing nanoformulation-based tretinoin delivery systems is to increase product stability, improve safety profile, and enhance patient compliance (Lima et al., 2021). However, despite positive research results, there is currently no commercially available nanoformulated tretinoin product.

Isotretinoin, a cis-isomer of retinoic acid, is a retinoid used particularly in acne treatment in systemic capsule form and is associated with various side effects, primarily mucocutaneous dryness (Abdelmaksoud et al., 2020). Current studies show that topical isotretinoin application in skin with signs of aging due to sun damage; Studies show that it can significantly improve fine wrinkles, pigmentation irregularities, and skin texture.

Topical isotretinoin, despite its positive clinical effects, has some limitations in terms of use due to its potential to cause skin irritation and its low physicochemical stability. Although various nanoformulation approaches have been developed to mitigate these problems, the majority of current studies focus on acne treatment rather than photoaging (Gupta et al., 2020).

Alitretinoin, on the other hand, is a pan-agonist retinoid that can bind to all retinoic acid receptors and retinoid X receptors. A 0.1% topical alitretinoin gel has received approval for use in the treatment of cutaneous Kaposi's sarcoma. However, data on its effects on photoaged skin are quite limited and supported only by a 16-week open-label pilot study with 20 participants (Cheng et al., 2008). Although some improvement in skin parameters was observed in the study, the high rate of reported side effects limits the use of this agent in anti-aging treatments.

Retinol is a versatile retinoid that shows positive effects on photoaged skin. Hyaluronic acid contributes to improved skin structure by increasing collagen and elastin synthesis, as well as supporting epidermal proliferation and cellular differentiation (Li et al., 2017; Romana-Souza et al., 2019).

Among retinoids used in cosmetic products, retinol is one of the most thoroughly studied compounds in randomized, double-blind, and carrier-controlled trials. Clinical trials involving whole-face and

split-face designs have evaluated different concentrations ranging from 0.075% to 0.5%, but results have varied (Spierings, 2021).

The main factors that complicate the interpretation of these studies include differences in study designs, the variability in retinol concentrations used, and potential conflicts of interest in industry-sponsored research. Therefore, it is still uncertain to what extent the reported positive results reflect the true clinical efficacy of retinol-containing products.

To better assess the efficacy of retinol, direct comparisons with tretinoin have been made in many studies. The findings showed no statistically significant difference between retinol and tretinoin-based formulations in terms of the evaluated clinical parameters (Table 2).

In a more recent 12-week randomized, double-blind, controlled study, three different retinol serums were compared with equivalent tretinoin cream concentrations. The study employed a dose escalation protocol where application frequency and concentration were gradually increased to improve tolerability, and the use of moisturizers was also encouraged (Babcock et al., 2015). Overall, the results indicated that retinol-containing formulations showed similar, and even superior, clinical performance in some parameters compared to tretinoin-based products.

Table 2
Comparative Clinical Studies of Topical Retinoids in Facial Anti-Aging Treatment (Tretinoin vs. Other Retinoids)

Retinoid	Study design	Duration	Subjects	Comparison	Evaluated parameters	Main outcomes	References
Retinol	Randomized, double-blind, split-face study	12 weeks	65	Retinol 0.25%, 0.5%, 1.0% vs tretinoin 0.025%, 0.05%, 0.1%	Photodamage, fine/coarse wrinkles, skin tone, pigmentation, roughness	Significant improvement from baseline in all parameters; no significant differences between groups	(Babcock, 2015)
Retinol	Randomized, parallel, double-blind, whole-face controlled study	3 months	120	Retinol 0.2% + tetrahydrojasmonic acid 2% vs tretinoin 0.025%	Wrinkles, pores, pigmentation, global photodamage, 3D skin profiling	Both treatments improved skin parameters; no significant differences observed	(Bouloc, 2015)
Retinol derivatives	Double-blind, parallel-arm, whole-face study	24 weeks	24	Retinol (1%) + retinyl acetate (0.05%) + retinyl palmitate (0.05%) vs tretinoin 0.02%	Wrinkles, pigmentation, global photodamage (Griffiths scale)	Comparable efficacy; no significant differences	(Chien, 2022)
Retinaldehyde	Randomized, double-blind, vehicle-controlled study	44 weeks	125	Retinaldehyde 0.05% vs tretinoin 0.05% vs vehicle	Wrinkle depth, skin roughness (optical profilometry)	Both active treatments significantly improved skin; no differences between groups	(Kwon, 2018)
Retinaldehyde (nanoformulation)	Randomized, double-blind, split-face study	12 weeks	30	Retinaldehyde hydrogel (0.025%) vs tretinoin 0.025%	Wrinkles, fine lines, texture parameters (surface, entropy, contrast, etc.)	Nano-retinaldehyde showed superior anti-aging effects	(Pisetpackdeekul, 2016)

Adapalene	Multicenter, randomized, investigator-blinded study	24 weeks	128	Adapalene 0.3% gel vs tretinoin 0.05% cream	Global photodamage, wrinkles, pigmentation, actinic keratoses	Significant improvement in both groups; no significant differences	(Bagatin, 2018)
Tazarotene	Randomized, multicenter, vehicle-controlled study	24 weeks	349	Tazarotene 0.01–0.1% vs tretinoin 0.05% vs vehicle	Multiple photodamage signs (wrinkles, pigmentation, roughness, elastosis)	Both agents effective; 0.05% tazarotene ≈ tretinoin; 0.1% most effective	(Kang, 2001)
Tazarotene	Multicenter, double-blind, randomized study	24 weeks	173	Tazarotene 0.1% vs tretinoin 0.05%	Wrinkles, pigmentation, elastosis, actinic damage	Faster and stronger improvement with tazarotene 0.1%	(Liu, 2020)

In a separate 12-week clinical trial conducted on 41 healthy female volunteers, a formulation containing 0.1% retinol was shown to reduce the severity of facial wrinkles using imaging-based analysis methods. However, the absence of a control group in the study limits the scientific strength of the results obtained (Kong et al., 2016).

Retinol is frequently formulated with different bioactive components to enhance therapeutic efficacy and achieve potential synergistic effects. Bouloc et al. (2015) compared a commercial preparation containing 0.2% retinol and 2% tetrahydrojasmonic acid with a 0.025% tretinoin cream. The study results showed that both treatments demonstrated similar levels of efficacy, while the retinol-based formulation was reported to be better tolerated and more positively evaluated by users (see Table 2).

Nevertheless, clinical data supporting the efficacy of retinol still have some methodological limitations. In particular, small sample sizes, short follow-up periods, and the lack of carrier-controlled studies reduce the reliability of the current findings. This is important because the carrier systems themselves can improve skin appearance and thus affect the actual efficacy results (Spierings, 2021). However, the number of properly designed carrier-controlled studies in this area is quite limited.

One of the most significant problems with retinol is its low stability. Retinol is a highly sensitive compound that can easily degrade under the influence of light, oxygen, high temperatures, and trace metals. A recent evaluation of 12 commercial products containing retinoids revealed significant stability losses under both long-term and accelerated storage conditions, and showed degradation in most of the products studied. Under ideal production conditions — use of inert atmospheres, appropriate packaging such as aluminum tubes, and storage below 20 °C — retinol stability generally lasts less than six months. However, since these controlled conditions are often not achievable in daily use, questions arise regarding the consistency of results on the clinical effects of retinol. Furthermore, it is emphasized that each product should be evaluated individually, as the stability of retinoids largely depends on the formulation used (Temova Rakuša et al., 2021).

Quality control studies have also revealed significant discrepancies in retinol-containing cosmetic products on the market. In 35 products examined by researchers, serious discrepancies were found between the amount of retinoid stated on the label and the actual content; some products contained concentrations below therapeutic efficacy, while others contained concentrations above the recommended limits. Moreover, many cosmetic products do not explicitly state the amount of active ingredients. This situation highlights the need for stricter regulatory controls and improved quality control processes in retinol-containing cosmetics (Temova Rakuša et al., 2021).

To reduce retinol stability problems, intensive research is being conducted on nanotechnology-based carrier systems. In this context, various retinol-loaded nanoformulations have been developed. For example, solid lipid nanoparticles containing 0.5% retinol have been prepared using ultrasonication and integrated into gel systems. In vitro cytotoxicity and permeability analyses, as well as in vivo irritation tests performed on rats, have shown that these systems offer safe use and suitable application characteristics. The results support the idea that nanoformulations may be a promising approach for topical retinol applications (Boskabadi et al., 2021).

Despite these positive preclinical results, sufficiently robust and well-planned clinical trials evaluating the anti-aging effects of retinol-containing nanoformulations are still lacking.

Retinaldehyde

Retinaldehyde is a natural precursor form of retinoic acid and is considered one of the compounds with the best balance of efficacy and tolerability among retinoids used in cosmetic dermatology. Its conversion to retinoic acid depends on the state of cellular differentiation and occurs more efficiently, especially in mature keratinocytes.

Its low irritation potential is associated with the limited metabolic capacity of epidermal cells. After topical application, retinaldehyde does not bind directly to receptors; while the majority is converted to retinyl ester derivatives, only a small fraction is oxidized to retinoic acid, exhibiting biological activity. This controlled metabolism provides a significant advantage as it limits the irritation mechanism resulting from excessive receptor activation of retinoids, defined as "receptor overload." Thus, retinaldehyde, as a more stable precursor, reduces the risk of irritation.

In general, formulations containing 0.05% retinaldehyde are among the effective and well-tolerated options in anti-aging applications. Furthermore, the absence of a significant increase in systemic retinoid levels, even with long-term or higher doses, supports minimal systemic absorption (Kwon et al., 2018).

However, a significant disadvantage of retinaldehyde is its high sensitivity to both light and oxidation, meaning it has poor chemical stability (Pisetpackdeekul et al., 2016). Therefore, nanotechnology-based carrier systems have been developed to improve stability and ensure controlled release.

In vitro and in vivo studies show that proretinal nanoparticle systems can provide more effective and sustained release of retinaldehyde while maintaining a suitable safety profile (Limcharoen et al., 2020). However, despite these promising data, comprehensive clinical trials are still needed to definitively establish the efficacy and safety of retinaldehyde in different formulation systems.

Retinyl Palmitate

Retinyl palmitate (RP) is one of the most common retinyl esters naturally found in human skin. Due to its high thermal stability, it is preferred over retinol in many anti-aging cosmetic products. However, RP is more susceptible to photodegradation compared to retinol.

Under certain conditions, particularly when exposed to UV radiation, RP can exhibit pro-oxidant effects through processes such as photodegradation products, reactive oxygen species, and lipid peroxidation. Furthermore, some experimental studies suggest that topical products containing RP may enhance UV-related photocarcinogenesis (Farooq et al., 2018). However, the long-term clinical significance of these findings is not yet clear.

Based on current toxicological and regulatory data, RP is generally considered safe at recommended cosmetic concentrations. In terms of biological activity, 0.6% RP is estimated to have approximately half the efficacy of 0.25% retinol.

Despite its widespread use, there are not enough clinical studies conducted under standard conditions to evaluate the anti-aging effects of RP. Only one study comparing two commercially available RP-containing products exists in the literature, but the RP concentrations are not explicitly specified (Farooq et al., 2018). This indicates that existing studies are methodologically inadequate and lack standardization.

Despite this, RP continues to be widely used in cosmetic products. Current research focuses on improving stability and bioavailability through nanotechnology-based systems, microencapsulation approaches (Bradley et al., 2015), and combination formulations created with biopolymers such as pectin (Nandy et al., 2020; AlZahabi et al., 2019).

In general, nanoformulated RP systems show better stability, increased skin penetration, and reduced irritation potential. However, much of this data is based on preclinical studies, and a sufficient number of well-designed clinical trials are not available.

In conclusion, currently, there is no strong clinical evidence confirming the anti-aging efficacy of retinyl palmitate in either classical or advanced carrier systems. Despite its widespread use in cosmetics, RP is among the compounds whose anti-aging efficacy has not been sufficiently clinically proven.

Retinyl propionate is a retinyl ester derivative reported to exhibit higher retinoid activity in both in vitro and ex vivo settings compared to retinol and retinyl palmitate (Bjerke et al., 2021). Some research suggests that combination of this compound with other active substances may enhance anti-aging effects (Lam et al., 2021). However, there are currently no published studies examining the use of retinyl propionate in nanotechnology-based carrier systems. Given its potential biological activity, more comprehensive clinical and formulation research is needed to clarify its clinical value.

Retinyl acetate is another retinoid derivative widely used in cosmetic anti-aging products. Nevertheless, no clinical studies have yet evaluated its topical anti-aging efficacy alone. Although its potential cocarcinogenic effects in terms of photocarcinogenesis have been investigated, the current data are inconsistent, and its mechanistic effects are not fully elucidated. Retinyl retinoate is a hybrid retinoid structure obtained as a result of a condensation reaction between retinol and retinoic acid. This structure aims to increase light stability and reduce photodegradation. Compared to first-generation retinoid derivatives, it has been reported to have higher chemical stability and lower irritation potential, and can also support hyaluronan synthesis (Kim et al., 2010). Clinical data suggest that it may be effective in reducing fine wrinkles; however, due to the small sample size and methodological limitations of current studies, the results are not conclusive. Retinyl N-formyl aspartamate is a synthetic retinoid derivative that exhibits higher photostability and lower irritation potential compared to retinol. In a 24-week randomized, controlled, single-blind study with 24 participants, its topical use was reported to be well tolerated and resulted in significant improvement in clinical parameters compared to the carrier group (Lee et al., 2006). However, the small sample size limits the generalizability of the results. Overall, clinical evidence strongly supporting the anti-aging efficacy of retinyl esters such as retinyl palmitate, retinyl propionate, retinyl retinoate, and retinyl acetate is insufficient. Despite their widespread use in cosmetic products, high-quality studies confirming their therapeutic efficacy are still lacking.

Second-generation retinoids are not currently actively used in topical anti-aging treatments due to the development of more effective and better-tolerated alternatives (Motamedi et al., 2022). Therefore, their current clinical importance is quite limited.

Tazaroten
Tazaroten is a prodrug that is rapidly converted in the body to its active form, tazarotenic acid. It selectively exerts an agonist effect on retinoic acid receptors RAR-β and RAR-γ, while having no significant affinity for RXR receptors.

In clinical practice, tazaroten; Tazarotene is a prescription agent used in the treatment of acne and psoriasis at concentrations of 0.045%, 0.05%, and 0.1%. The 0.1% form is also approved as an adjunct in the treatment of fine wrinkles, pigmentation disorders, and lentigo associated with photoaging. Its efficacy and safety in sun-damaged skin are well-documented, and side effects are generally mild to moderate (Ogden et al., 2008).

Comparative studies with tretinoin have reported that tazarotene shows similar efficacy, but may cause a more frequent temporary burning sensation in the initial stages of treatment (Kang et al., 2001; Lowe et al., 2004). However, this effect is mostly short-lived.

In general, tazarotene offers comparable efficacy and tolerability profiles to tretinoin in the treatment of photoaging. Although theoretically it could be better tolerated due to its receptor selectivity, tretinoin is more widely used in clinical practice due to its longer history and cost advantage. Recent research on tazarotene has focused particularly on nanotechnology-based carrier systems and new dermatological applications (Liu et al., 2020).

Adapalene
Adapalene is a synthetic retinoid that selectively acts on retinoic acid receptors RAR-β and RAR-γ. Its 0.1% and 0.3% topical forms are used especially in acne treatment and are known for their good efficacy and tolerability profile. However, it also has off-label use in photoaging treatment (Rusu, 2020).

Bexarotene
Bexarotene is a retinoid that binds selectively to all subtypes of retinoid X receptors and has a complex mechanism of action. Clinically, it is approved in a 1% topical gel form for the treatment of stage IA and IB cutaneous T-cell lymphoma. To date, there are no clinical studies evaluating the use of bexarotene in anti-aging or photoaging treatment, and its potential in this area is limited. The study is still in the research phase (Schadt et al., 2013).

Trifarotene
Trifarotene is a fourth-generation retinoid and is currently an approved agent for acne treatment in a 0.005% topical cream form. Clinical studies are ongoing to evaluate its efficacy and safety profile in this indication (Blume-Peytavi et al., 2020).

Pharmacologically, trifarotene is a potent agonist showing high selectivity, particularly for RAR-γ, among retinoic acid receptors, and has more than 20 times greater affinity for RAR-γ compared to RAR-α and RAR-β. Preclinical studies have shown that it can exhibit both pigment-reducing and pigment-regulating effects in animal models (Aubert et al., 2018).

Due to the predominant retinoid receptor subtype in the dermis being RAR-γ, trifarotene is considered a promising candidate in the treatment of photoaging. Mechanistically, it is suggested that it may produce anti-aging effects by activating downstream signaling pathways similar to tretinoin (Cosio et al., 2021).

Preclinical data suggest that trifarotene may offer a more balanced efficacy-tolerability profile compared to previous generation retinoids. However, to date, no clinical studies have directly compared its efficacy and safety in terms of anti-aging use. Therefore, its role in the anti-aging field

is not yet definitively established and requires further clinical validation. Also, as it is a new retinoid, it is expected that studies on different formulations will increase in the future.

Seletinoid G

Seletinoid G is a synthetic retinoid derivative that exhibits selective agonist effects on the RAR-γ receptor. This compound has been reported to regulate the expression of extracellular matrix proteins and matrix-degrading enzymes in a manner similar to tretinoin. Thanks to these properties, it is suggested that it may contribute to the remodeling of aged dermal connective tissue and the reduction of UV-induced collagen degradation.

Seletinoid G has been reported to have significantly lower irritation potential compared to tretinoin. However, the fact that these findings are based on a limited sample size and short exposure time limits the generalizability of the results. In vitro preclinical data suggest that this compound can improve skin barrier function by increasing keratinocyte migration and normalizing dermal collagen regulation; this points to potential anti-aging activity with fewer side effects (Lee et al., 2020).

However, no clinical studies evaluating the anti-aging efficacy of seletinoid G under real-life conditions have yet been conducted. Therefore, its therapeutic potential is currently theoretical and needs to be confirmed by comprehensive clinical research.

Discussion

Retinoids remain the most extensively researched group of topical agents in anti-aging dermatological treatments, thanks to their ability to control epidermal cell proliferation, differentiation processes, and dermal structural remodeling. All retinoid generations primarily act via nuclear retinoic acid receptors, modulating gene expression pathways that regulate collagen synthesis, extracellular matrix turnover, and epidermal balance. However, despite shared mechanisms of action, different retinoid compounds exhibit significant differences in efficacy, receptor selectivity, tolerability, and level of clinical evidence. Tretinoin, a first-generation retinoid, is considered the reference standard in anti-aging treatments. Numerous long-term clinical studies have demonstrated its effectiveness in reducing signs of photoaging, reducing wrinkle depth, and improving dermal structure. However, this strong efficacy is often limited by dose-dependent irritation and poor tolerance. This has increased the need for the development of better-tolerated alternative retinoids. Although precursor molecules such as retinol and retinaldehyde provide better tolerability due to their stepwise conversion to retinoic acid, their clinical effects largely depend on formulation stability and intradermal enzymatic conversion capacity, resulting in variability in outcomes. Retinyl esters, despite their widespread use in cosmetic products, stand out as the retinoid group with the weakest level of clinical evidence. While generally considered safe, their bioavailability is low due to requiring multiple metabolic conversion steps, making their clinical efficacy uncertain. Safety concerns such as photodegradation under UV exposure and potential pro-oxidant effects are also reported. While retinyl retinoate offers relatively more positive data among ester derivatives, current studies are limited in both scale and methodological quality. Second-generation retinoids are no longer used in current topical anti-aging treatments with the development of better-tolerated and more selective molecules. In contrast, third-generation retinoids, such as adapalene and tazarotene, bind more selectively to RAR-β and RAR-γ receptors, producing more targeted biological effects. Clinical studies have shown that these agents can provide anti-aging results comparable to tretinoin; in some cases, they offer a faster onset of action or better tolerability. Nevertheless, their clinical use is mostly limited to acne and psoriasis indications, and the lack of long-term studies in the anti-aging field is noteworthy. Although adapalene was initially developed for the treatment of acne, controlled studies have shown that it improves pigmentation and tissue changes associated with photoaging. Similarly, tazarotene has demonstrated strong clinical efficacy, showing results equivalent to or superior to tretinoin in some studies. Despite this, tretinoin remains the most commonly preferred agent in clinical practice due to

its long-standing clinical experience and established usage protocols. Fourth-generation retinoids, such as tripharotene and seletinoid G, are notable for their high selectivity, particularly to the RAR-γ receptor, which is dominant in the dermis. Preclinical data suggest that these molecules may offer a better efficacy-tolerability balance and more targeted dermal restructuring potential. However, current clinical evidence is quite limited. While data on tripharotene are largely based on acne studies, seletinoid G is only supported by short-term experimental research. Therefore, their role in photoaging treatment remains at the theoretical level. Formulation properties play a critical role in the clinical success of retinoids. Retinol and retinaldehyde, in particular, experience stability problems due to their sensitivity to light, oxygen, and heat. Differences in ingredients, lack of standardization, and labeling inconsistencies in cosmetic products also complicate the interpretation of clinical results. While nanotechnology-based carrier systems are promising in terms of increasing stability and skin penetration, much of the evidence in this area is still at the preclinical level. Overall, the literature shows a clear hierarchy of evidence among retinoids: tretinoin maintains its gold standard status with strong clinical validation, while newer generation retinoids offer potential for better tolerability and selectivity but lack long-term comparative data. Cosmetic retinoids, particularly retinyl esters, are used with limited scientific support due to insufficient clinical evidence. Therefore, future studies should focus on standardized, large-scale, and carrier-controlled clinical designs; and also on different retinoids.

Conclusion

Research generally shows that the clinical success of topical retinoids depends not only on the active ingredient but also on formulation characteristics such as the adjuvants used and the manufacturing technologies. A properly developed formulation can increase the stability of the active ingredient, improve skin penetration, and enhance tolerability. This allows for the achievement of a clinically viable tretinoin-like effect while reducing the occurrence of side effects.

Increased tolerability is critical, especially in terms of long-term aging and improvements, as it directly extends patient compliance and treatment. Therefore, modern formulation solutions such as controlled-release systems and nanotechnology carriers hold significant potential for both reducing irritation and optimizing therapeutic effect.

Nevertheless, despite positive studies on different retinoid classes and new delivery systems, current products have some significant limitations. The small distribution of studies, short follow-up periods, high heterogeneity among formulations, and the lack of sufficient carrier-controlled or direct comparative studies reduce the generalizability of results. This situation makes it difficult to reach definitive conclusions regarding the efficacy of retinoid anti-aging trials.

Therefore, more comprehensive, well-designed, and standardized clinical trials are needed to establish evidence. Future studies should place great emphasis on direct comparison of different generations of retinoids, long-term efficacy and safety maintenance, and clinical development of advanced manufacturing technologies in real-life conditions.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Comparative Analysis of Cytokine Interactions in Herpes and Opportunistic Infections: The Role of Th1, Th2, and Regulatory Cytokines

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Abstract. *Herpes viruses are widespread pathogens that reactivate, particularly in immunosuppressive conditions, eliciting complex and variable immune responses. In this context, the role of cytokine networks is crucial for understanding inter-viral differences and clinical outcomes. In this article, I presented a comparative analysis of cytokine responses between herpes viruses (HSV-1, HSV-2, VZV) and opportunistic herpes viruses (EBV, CMV) in immunosuppressed patients. Based on scientific articles published between 2020 and 2026, I investigated the roles of Th1, Th2, and regulatory cytokines (IL-6, IL-10, IFN-γ, TNF-α). EBV induces a robust Th1/cytotoxic response (IFN-γ and TNF-α), while CMV is in most cases associated with an increase in IL-10 and exhibits a regulatory immunomodulatory profile. HSV-2 and VZV are characterized by a marked local increase in IFN-γ. However, there is insufficient data for IL-6, and information on systemic cytokine profiles in immunosuppressed populations for HSV/VZV is limited. The findings indicate that the pathogenesis of herpes viruses is closely linked not only to antiviral mechanisms but also to immunoregulatory processes, which requires new approaches to clinical management and lays the theoretical foundation for the future development of biomarker-based diagnostics and immunotherapeutics.*

Keywords: *Th1, Th2, cytokine, opportunistic herpes viruses, immune response*

Introduction

Herpesviruses are widespread latent pathogens that can lead to severe clinical courses, particularly upon reactivation in immunosuppressed individuals. Herpes viruses are divided into two main groups: standard herpes viruses (HSV-1, HSV-2, VZV) and opportunistic herpes viruses (EBV, CMV, HHV-6, KSHV). The immune responses to these viruses, particularly through cytokine networks, exhibit distinct characteristics (Ramos-Nino, M. E. 2026). The coordination of the immune response relies on a balance between Th1 cytokines (IFN-γ, TNF-α), which activate antiviral cell-mediated immunity; Th2 cytokines (IL-4, IL-5), which drive humoral responses; and regulatory cytokines (IL-10, TGF-β), which mitigate excessive inflammation. In this context, IL-6 exerts a pleiotropic effect, performing both pro-inflammatory and regulatory functions. The purpose of this work is to comparatively analyze the cytokine profiles among different groups of herpes viruses based on recent literature and to determine the functional role of key immune mediators.

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Regulation of the Immune Response Through T-Helper Cell Subsets and Cytokines

The immune response is complexly regulated by cytokines secreted by various T-helper (Th) cell subsets. These cytokines ensure both pathogen elimination and tissue homeostasis by balancing effector and regulatory mechanisms. A disruption of the immune balance between T-helper cell subsets plays a key role in the pathogenesis of pregnancy-induced hypertension.

In particular, Th1 and Th17-oriented pro-inflammatory cytokines (e.g., IFN-γ, IL-17) increase, which amplifies systemic inflammation and endothelial dysfunction, while a decrease in Th2 and Treg-derived cytokines (IL-4, IL-10) leads to a weakened immune tolerance (Zhou et al., 2023). The functional characteristics of follicular T-helper (Tfh) cells are differentially shaped depending on their cytokine profile. Cytokine-skewed Tfh subsets—particularly the populations producing IL-21, IL-4, and IFN-γ—selectively regulate B cell proliferation, isotype switching, and the generation of high-affinity antibodies. Thus, Tfh–cytokine serves as one of the key determinants of the specificity, strength, and durability of the humoral immune response (Olatunde et al., 2021). Th1-type cytokines primarily enhance cell-mediated immunity. Interferon-gamma (IFN-γ) plays a crucial role in the activation of macrophages and the formation of antiviral defense. Tumor necrosis factor-alpha (TNF-α) stimulates apoptosis and activates effector mechanisms against infection by enhancing the inflammatory response. Interleukin-2 (IL-2) supports the adaptive immune response by increasing the proliferation and cytotoxic activity of T lymphocytes. Th2-type cytokines are involved in the development of humoral immunity. Interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) stimulate B cell differentiation and antibody production, while also playing a key role in the development of allergic reactions. Regulatory cytokines prevent the overactivation of the immune response. Interleukin-10 (IL-10) has a potent anti-inflammatory effect, inhibiting the production of pro-inflammatory cytokines and reducing tissue damage. Interleukin-6 (IL-6) is pleiotropic, performing both pro-inflammatory (acute phase response) and, in some cases, regulatory functions. Transforming growth factor-beta (TGF-β) plays a key role in maintaining immune tolerance and tissue regeneration (Goetzke et al., 2025).

Cytokine Dynamics and Immune Regulation in HSV-1 Infections

Herpes viruses are characterized by their ability to establish latent infection and evade immune control; their reactivation occurs under immunosuppressive conditions, and the cytokine profile varies depending on the virus type, tissue tropism, and the host's immune status. In HSV and other alphaherpesviruses, immune surveillance during the latent infection period is primarily provided by weakly activated Th1 and resident T cell-mediated cytokine signals, which allows the virus to remain hidden long-term. During reactivation, an increase in IFN-γ and other pro-inflammatory cytokines enhances the antiviral response, limiting viral replication (Cao et al., 2024). In HSV-associated uveitis, the immune response is characterized by a predominantly Th1-oriented cytokine profile (particularly IFN-γ and TNF-α), which enhances cell-mediated recognition of viral antigens. Immuninformatics approaches aimed at multiepitope vaccine design, however, aim to optimize this Th1 dominant cytokine response while simultaneously balancing an excessive inflammatory reaction (Cao et al., 2025). During acute HSV oral mucosal infection, the immune response is characterized primarily by the activation of early Th1-type cytokines (particularly IFN-γ and TNF-α), which plays a key role in establishing local antiviral defense. At the same time, cytokines secreted from resident T cells effectively limit viral spread by ensuring the elimination of infected cells (Shannon et al., 2021). Viruses like HSV block interferon signals, weakening the host's antiviral defense and thereby shaping tissue tropism and the ability of the infection to spread. As a result of this interferon antagonism, the early Th1-mediated cytokine response (particularly IFN-α/β and IFN-γ signaling) is weakened, which allows the virus to evade the immune system (Streicher et al., 2025).

Cytokine Dynamics and Immune Regulation in HSV-2 Infections

The cytokine profile in herpes simplex virus type 2 infection is differential depending on the clinical phenotype of the disease. In HSV-2 meningitis, elevated pro-inflammatory cytokines and chemokines (particularly IL-6, IL-8, TNF-α) reflect a strong inflammatory response in the central nervous system. In contrast, during genital herpes, the cytokine response is more local, Th1-dominant (IFN-γ-mediated), and characterized by relatively limited immune activation, reflecting the localized course of the infection (Bjerhem et al., 2025). In HSV-2 infection, cytokines secreted by tissue-resident T cells form the basis of local antiviral defense. In particular, IFN-γ-mediated signaling limits viral replication and prevents the spread of infection by inducing an antiviral state in infected cells. This underscores the acute, local, and effective Th1-dominant character of the immune response against HSV-2 (Roychoudhury et al., 2020). HSV-2 is characterized by a very rapid local increase in IFN-γ. Studies in mucosal lesions show that the local increase of IFN-γ and granzyme B correlates with viral load and occurs before viral clearance (Souquette et al., 2026). This is consistent with an acute tissue-resident T cell cytokine alarm response and constitutes the primary mechanism of local antiviral defense against HSV-2. In HSV-2 infection, the prime/pull vaccine approach enhances the antiviral immune response by increasing the number of protective CD4+ and CD8+ T cells in the tissue. As a result, viral reactivation is better controlled and the likelihood of disease recurrence is reduced (Quadiri et al., 2024). The infection is accompanied by a higher pro-inflammatory cytokine profile, especially in the context of HIV-1 co-infection. In particular, an increase in IL-6, TNF-α, and other inflammatory mediators is associated with elevated levels of immune activation and persistent viral replication. These findings indicate that the cytokine response in HSV-2 infection is significantly dependent on the host's immune status and that it creates a more pronounced imbalance in the presence of HIV-1 (Aravantinou et al., 2022).

Cytokine Dynamics and Immune Regulation in EBV Infections

EBV is the most potent inducer of a Th1 response among opportunistic herpes viruses. Studies in lung transplant recipients show that EBV lytic antigen stimulation activates polyfunctional CD8+ T cells, which simultaneously express IFN-γ and TNF-α (Muruganandah et al., 2018). This indicates strong Th1/cytotoxic immunity against EBV antigens. Activation of CD150 (SLAM) signaling in EBV-transformed B cells induces the secretion of various cytokines. This regulates the differentiation of peripheral blood monocytes. The released cytokines cause reprogramming of the immune microenvironment by enhancing the interaction between the innate and adaptive immune responses. In conclusion, this demonstrates that in EBV infection, cytokines play a crucial role not only in the antiviral response but also in cell differentiation and the modulation of immune networks (Kim et al., 2024). The EBV virus enhances the local inflammatory microenvironment by increasing the production of pro-inflammatory cytokines (e.g., IL-6, IL-8) in gingival fibroblasts. At the same time, this cytokine environment contributes to tissue resorption and periodontal destruction processes by stimulating RANKL-mediated osteoclast differentiation. These findings indicate that EBV links the cytokine response not only to immune activation but also to tissue remodeling (Yokoe et al., 2022). The effect of EBV on the immune system is dependent on the viral state. Detection of latent EBV is associated with decreased cytokine levels, which has an immunomodulatory or tolerogenic effect. Conversely, when EBV enters lytic replication, systemic cytokinemia increases and the risk of graft rejection rises (Gabeleh et al., 2025). This dual nature reflects the complex immunological profile of EBV. TNF-α accompanies the EBV-specific IFN-γ response and enhances the role of Th1 inflammation in CD8 responses (Muruganandah et al., 2018). This underscores the importance of TNF-α in the formation of an effective cytotoxic response against EBV.

Cytokine Dynamics and Immune Regulation in CMV Infections

Cytomegalovirus stands out among opportunistic herpes viruses for its immunomodulatory properties. Detection of HCMV in transplant recipients is associated with an increase in IL-10, IL-4, and IL-8, and these changes correlate with the immune injury of the allograft (Saldan et al., 2023), indicating the formation of a Th2-like environment. During CMV infection, inflammasome activation mounts an intense innate immune response by increasing the secretion of key pro-inflammatory cytokines, including IL-1β and IL-18, and simultaneously induces pyroptosis-mediated cell death. Administration of inflammasome inhibitors significantly reduces cytokine secretion and pyropotic processes. These findings highlight the inflammasome–cytokine axis as a key regulatory mechanism of immune activation and tissue damage in CMV pathogenesis (Deng et al., 2024). The CC-type chemokines encoded by the CMV virus indirectly influence the formation of the local cytokine environment by modulating the migration of immune cells. Additionally, gH/gL-complex-mediated cell tropism acts as a separately regulated mechanism, independent of immune activation and viral spread. These findings indicate that in CMV infection, cell tropism is regulated by chemokine-mediated immune modulation in a partially independent manner from the cytokine response (Eletreby et al., 2023). Low IFN-γ production (IFN-γ +874 A>T) is associated with an increased risk of CMV disease (Vu et al., 2014; Ciccocioppo et al., 2025), which underscores the protective role of the Th1 response. However, CMV infection is often accompanied by an increase in regulatory cytokines. The lack of an effect of asymptomatic CMV shedding in early HIV on the systemic cytokine profile suggests that the immune effects of this virus are context-dependent (Vanpouille et al., 2022).

Cytokine Dynamics and Immune Regulation in VZV Infections

This prospective study conducted in the context of herpes zoster associated with the varicella-zoster virus shows a significant increase in serum pro-inflammatory cytokines (particularly IL-6, IL-8, and TNF-α). This increase correlates positively with the clinical severity of the disease. In particular, the elevation of IL-6 levels is associated with the development of postherpetic neuralgia (PHN), highlighting its role in the pathogenesis of persistent neuroinflammatory processes. This suggests that the cytokine response during VZV is not limited to antiviral effector mechanisms but also contributes to the development of chronic pain syndromes (Gu et al., 2023). In the context of COVID-19, activation of the IL-17/Th17 axis becomes a key component of the immune response. Enhanced IL-17 signaling promotes the recruitment of inflammatory cells and tissue damage by increasing the expression of cytokines and chemokines (e.g., IL-6, CXCL8) through NF-κB and other pro-inflammatory pathways. These findings indicate that VZV reactivation is closely associated not only with the classic Th1-mediated antiviral response but also with Th17-induced inflammatory networks, and that the virus–host interaction is particularly modulated at a systemic level (Yu et al., 2021). In rheumatoid arthritis patients receiving Janus kinase (JAK) inhibitors, the cellular-mediated immune response after herpes zoster vaccination is weakened. Accordingly, cytokine production, particularly the Th1 response characterized by IFN-γ, is significantly reduced. This may limit the effectiveness of antiviral defense, leading to inadequate immune control of VZV. The results indicate that inhibition of the JAK signaling pathway disrupts the mechanisms critical for the development of a cytokine-based immune response against herpes zoster (Källmark et al., 2026). This study, conducted in the context of the varicella-zoster virus-based live herpesvirus vaccine, shows that the strength of the innate immune response and its cytokine profile vary significantly depending on an individual's biological sex and prior viral exposure. In particular, the intensity of initial IFN-type I and pro-inflammatory cytokine responses (e.g., IL-6 and TNF-α) is modulated by prior immune memory and hormonal factors. Consequently, the innate immune response against herpesviruses is not static but is context-dependent and individualized (Cheung et al., 2023). VZV reactivation is associated with higher-frequency IFN-γ and IL-2-producing CD4+ memory T cells and increased cytotoxic markers during acute illness. The increase in IL-2 occurs during acute VZV reactivation and is accompanied

by polyfunctional CD4+ T cell responses associated with effector function. This indicates that VZV also elicits a strong Th1 response (Ma et., 2022).

Comparative Approach: Common and Distinguishing Features

Comparative Analysis of Th1-Type Immune Responses

All herpesviruses establish a core conserved mechanism of antiviral control early in infection by inducing a Th1-dominant response via IFN-γ, IL-2, and cytotoxic effector molecules (e.g., granzyme B). In HSV infection, the local IFN-γ-mediated antiviral effect of tissue-resident T cells plays a key role in virus control (Roychoudhury et al., 2020), whereas in HSV-2 meningitis, a more pronounced proinflammatory cytokine increase is observed (Bjerhem et al., 2025). Common herpes viruses (HSV-1/HSV-2) primarily induce a local Th1-dominant response during mucocutaneous infections, whereas Varicella-zoster virus manifests as varicella during primary infection and as herpes zoster upon reactivation. In CMV, the IFN-γ response acts as a protective factor, and its reduction is associated with an increased risk of infection (Vu et al., 2014; Ciccocioppo et al., 2025). In EBV, however, the Th1 response is phase-dependent: a strong IFN-γ/TNF-α response is observed in the lytic phase, while this activity weakens in the latent phase (Goetzke et al., 2025; García-Jiménez et al., 2026). Although a Th1 response is present in VZV infections, additional activation of the Th17 axis is noted, particularly during herpes zoster (Yu et al., 2021). Although the Th1 response is a common antiviral mechanism in all viruses, its intensity and duration vary depending on the virus type and clinical context.

Comparative Analysis of Regulatory Cytokines

Immunoregulatory cytokines in herpes viruses are a key component of mechanisms for chronic infection and immune evasion. In opportunistic herpes viruses (especially CMV and EBV), an increase in IL-10 is associated with the formation of an immunosuppressive microenvironment. Elevated IL-10/IL-4 levels in cases of CMV detection and EBV co-reactivation correlate with graft rejection (Saldan et al., 2023; Gabeleh et al., 2025). In EBV-associated inflammatory syndromes, TGF-β-mediated signaling plays a critical role in both immune suppression and the organization of pathogenic processes (Goetzke et al., 2025). In CMV, additional secretion of IL-1β and IL-18 is observed as a result of inflammasome activity, which is associated with pyroptosis and tissue damage (Deng et al., 2024). An increase in systemic pro-inflammatory cytokines (especially IL-6) in VZV correlates with postherpetic neuralgia (Gu et al., 2023). In HSV infections, the regulatory cytokine component operates primarily at a local level, and the systemic profile is relatively poorly characterized. While systemic immunoregulatory dominance is observed in EBV and CMV, in HSV and VZV, it is more local and episodic in nature.

Determinants Affecting the Immune Response

In herpes virus infections, the heterogeneity of cytokine responses is shaped not only by the intrinsic properties of the virus but also by the interplay of multifactorial host determinants. In Epstein-Barr virus infection, the viral phase is characterized by a sharp shift in the cytokine profile from the latent-to-lytic transition, from an immunoregulatory environment to a pro-inflammatory response (Goetzke et al., 2025). In transplant recipients, the immunosuppressive state significantly alters the cytokine balance; in this group, CMV and EBV infections are often characterized by the development of an immunomodulatory environment dominated by IL-10, and this change is associated in clinical practice with the risk of immune-mediated damage to the allograft (Saldan et al., 2023). In contrast, during the early HIV infection stage, the impact of CMV replication on the systemic cytokine network may be limited (Vanpouille et al., 2022), which underscores the influential role of the immune background. Among opportunistic herpes viruses, EBV remains latent in B lymphocytes, causing lymphoproliferative disorders in the context of transplantation, while CMV is associated with

systemic and highly morbid infections in cases of immunodeficiency (Lu et al., 2025). While HSV and VZV infections are characterized by a predominantly local (mucocutaneous or neurotropic) Th1-dominant response, EBV and CMV are more associated with the reprogramming of systemic cytokine networks (Lu et al., 2025). Host-genetic and immunological factors determine the amplitude of the cytokine response and clinical outcomes; particularly polymorphisms such as IFN-γ +874 A>T affect CMV susceptibility by modulating the effectiveness of the antiviral Th1 response, while prior exposure and individual immune history also contribute to the formation of innate and adaptive cytokine profiles (Vu et al., 2014; Cheung et al., 2023). Thus, in herpes virus infections, the cytokine response must be considered a multifactorial, dynamic, and context-dependent system; this approach provides the basis for a more precise understanding of clinical phenotypes and the personalization of therapeutic strategies.

Discussion

Confirms that cytokine responses in herpes virus infections are shaped not only by virus-specific characteristics but also by clinical context and host factors. Evaluation of viral nucleic acids in conjunction with cytokine markers (e.g., CXCL9, IL-8, IL-10), particularly in transplant and other immunosuppressive settings, allows for the simultaneous monitoring of both viral reactivation and allograft-related inflammation. The mRNA level of CXCL9, however, can be used as a useful biomarker indicating the link between Th1-type immune activation and graft injury. In CMV and EBV infections, however, it overlaps with the described IL-10-dominant immunoregulatory profile; this cytokine environment both facilitates viral persistence and is associated with immune-mediated damage of the allograft in the transplant context (Saldan et al., 2023). In contrast, infections caused by Herpes simplex virus and Varicella-zoster virus are characterized by a predominantly localized immune response with a Th1-type defense predominating, which is consistent with their primary involvement of the skin, mucosal surfaces, and neural tissues. However, the lack of direct comparative data with bacterial and fungal opportunistic infections also hinders the assessment of immune response specificity. The protective role of Th1 cytokines, particularly IFN-γ, is a common feature for all herpes viruses. In the context of CMV, a reduced ability to produce IFN-γ has been associated with susceptibility to infection, indicating that the effectiveness of the Th1 response is one of the key determinants of clinical outcomes (Vu et al., 2014; Ciccocioppo et al., 2025). In EBV, however, the cytokine profile changes depending on the virus's biological state. While an immunoregulatory environment predominates in the latent phase, pro-inflammatory cytokines increase in the lytic phase, leading to enhanced immune activation (Goetzke et al., 2025). Clinically, in the management of herpes virus infections, not only the viral load but also the cytokine signature (especially the IFN-γ/IL-10 balance) serves as a critical biomarker. Disruption of this balance determines the risk of disease by leading to either insufficient antiviral control (low IFN-γ) or excessive immunosuppression (high IL-10).

Conclusion

In herpes virus infections, the basis of the immune response is an early Th1-type antiviral response (IFN-γ, IL-2, and cytotoxic cells), which is a common defense mechanism for all herpes viruses. However, the clinical course is determined primarily by the degree and balance of this response. In opportunistic herpes viruses such as EBV and CMV, an increase in IL-10 (partially along with IL-6) creates an immunosuppressive environment, enhancing viral persistence and the likelihood of reactivation; this is particularly associated with clinical complications in immunosuppressive conditions such as transplantation. In contrast, the immune response in HSV and VZV infections is characterized by predominantly local and Th1-type control, and systemic immune alterations are less pronounced. Considering the existing gaps, future research should focus on the standardized and quantitative profiling of cytokines in HSV and VZV infections, as well as a more precise

determination of the virus-specific functional role of IL-6. At the same time, the joint assessment of genetic factors and cytokine markers can help to more accurately determine individual risks for patients. From a therapeutic perspective, it is important to test approaches targeting mechanisms related to IL-10, IFN-γ, and IL-6R in clinical trials. Finally, the joint analysis of different biological data and the comparison of different types of infection within the same groups could allow for a broader and more systematic understanding of the immune response. In conclusion, the optimal clinical approach should be based on the complex monitoring of immune markers and the enhancement of the Th1 response or the modulation of immunosuppressive cytokines. This approach lays the foundation for the future development of personalized immunotherapy strategies.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Comparative Analysis of Functional and Pathological Changes in the Cardiovascular and Respiratory Systems of Athletes During Heat Stroke

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Abstract. *In modern sports, athletes are often exposed to intense physical loads in order to achieve high performance. When these loads are carried out in conditions of high temperature. When these loads are carried out in conditions of high temperature and humidity, they can cause significant functional changes in the body. In particular, in high–intensity sports such as basketball and football, more heat is produced in the body. At the same time, the body's ability to regulate temperature becomes more difficult, which affects the functional state of the cardiovascular and respiratory systems. Therefore, studying the effects of heat stress on the body in different types of sports in a comparative way is of great scientific and practical importance. The aim of the study was to determine the characteristics of changes in the cardiovascular and respiratory systems of basketball and football players under heat conditions, as well as their pathophysiological mechanisms. A total of 60 athletes aged 18–25 were included in the study. Physical load was modeled under conditions of high temperature and humidity. During the observation, main hemodynamic and respiratory parameters, heat balance, and metabolic state were assessed. The analysis showed that in response to heat stress, adaptive mechanisms in the body are activated. This is mainly expressed by increased strain on the cardiovascular system and faster breathing. At the same time, in some cases these adaptive mechanisms are not sufficient, leading to pathological changes such as hypovolemia, hyperthermia, rhythm disturbances, and metabolic imbalance. Differences between types of sports were mainly related to the nature and duration of physical load, as well as environmental conditions. Thus, the results show that the effects of heat stress on athletes are complex and may include both adaptation and harmful changes. Therefore, proper planning of training in hot conditions and the use of preventive measures are essential.*

Keywords: *heat stroke, cardiovascular system, respiratory system, dehydration, basketball, football*

Introduction

In recent years, climate change and the increasing intensity of sports activities under high-temperature conditions have further heightened the risks posed by heat stress for athletes. Heat stroke ranks among the leading causes of death during sports activities and is characterized by multi-organ failure. Heat stress disrupts the integrity of the intestinal barrier, factilitating the translocation of bacterial products into the bloodstream (Garcia et al., 2022). The demage to the intestinal epithelium leading to the entry of lipopolysaccharides into the bloodstream plays a crucial role in the development of multi-otgan injuries (Lim & Mackinnon, 2006). During intense physical exertion, metabolic processes in skeletal muscles generate a large amount of heat, and its dissipation depends on the effective functioning of the body's thermoregulatory systems.

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However, under conditions of high temperature and humidity, the weakening of heat loss mechanisms can lead to hypertermia and its severe form, heat stroke. In response to heat stress, the cardiovascular system is the first to become activated. Y.Epstein and W.O.Roberts (2011) demonstrated that during hyperthermia, peripheral vasodilation leads to a reduction in the perfusion of internal organs. The pathogenesis is primarily associated with hyperthermia (>40 ^{0}C), cardiovascular system overload, and systemic inflammatory response syndrome (SIRS). Heat stress is one of the main factors leading to decreased performance and health complication in athletes. However, under conditions of high temperature and humidity, these mechanisms are insufficient, resulting in hyperthermia and peripheral vasodilation. L.W Rowell (1974) noted that cardiac output increases 2-3 times under heat conditions, playing a central role in thermoregulation. Additionally, an increase in ventilation is observed in the respiratory system, aimed at enhancing heat dissipation and optimizing qas exchange. However, under prolonged heat exposure, when these compensatory mechanisms prove insufficient, pathological conditions may arise, including hypovolemia, fluctuations in arterial blood pressure, cardiac arrythmias, as well as metabolic and respiratory imbalances. In thermoregulatory disorders, blood flow to the skin increases, resulting in hypoperfusion of internal organs. Sweating, dehydration, and a reduction in plasma volume occur. Heat stroke is characterized by a rise in body temperature above 40 ^{0}C and dysfunction of the central nervous system (Bouchama & Knochel, 2016). Heat and hypoxia contribute to damage of the intestinal barrier. Exercise-induced hyperthermia increases intestinal permeability, allowing endotoxins to enter the systemic circulation (Latiano, 2019). This leads to the development of a" leaky gut", where endotoxins pass into the bloodstream, resulting in endotoxemia, systemic inflammation, and shock. Activation of endothelial cells and a sharp increase in cytokine levels are closely associated with endotoxemia (Leon & Bouchama, 2015). In athletes experiencing heat stroke, this process is of particular significance, as blood flow to the intestines may decrease by up to 80%, leading to rapid disruption of the intestinal barrier. Endotoxemia further exacerbates cardiovascular collapse and aggravates respiratory failure. It is considered one of the key triggers of the systemic inflammatory response during heat stroke (Leon & Bouchama, 2015).

Literature date indicate that the effects of heat stress vary depending on the type of sport. A comparative analysis of the impact of heat stress on the cardiovascular and respiratory systems in basketball and football remains insufficiently investigated. In this context, the aim of the present study is to comparatively examine the functional and pathological changes occurring in the cardiovascular and respiratory systems of basketball and football players under heat conditions, as well as to evaluate the underlying pathophysiological mechanisms of these changes. G.P Lambert (2008) reported that intestinal hypoxia facilitates the translocation of endotoxins into the bloodstream and activates the systemic inflammatory response.

Materials and Methods

Comparative experimental study. A comparative experimental study was conducted to assess the effects of heat stress on cardiovascular and respiratory systems in athletes.
Participants. A total of 60 male athletes participated in the study:

Sport	Number	Age (years)
Basketball	30	21 ± 2
Football	30	22 ± 3

All participants were clinically healthy at baseline and had no diagnosed cardiovascular or respiratory diseases prior to the study. All athletes had at least 6 years of training experience.

Environmental conditions. The study was carried out under controlled environmental conditions:
- Temperature: 32 ^{0}C
- Relative humidity: 60%
- *Measured parameters*. The following physiological parameters were evaluated:
- Heart rate (HR)
- Systolic and diastolic blood pressure
- Respiratory rate
- Core body temperature
- Maximal oxygen uptake (VO_2 max)
- Body mass loss (as an indicator of dehydration)
- Blood lactate concentration

Exercise protocol. Participants performed a 60-minute high-intensity training session including:
- sprint intervals
- agility drills
- jumping exercises
- sport-specific movements

Measurements were recorded:
1. Before exercise
2. Immediately after exercise
3. 10 minutes after recovery

Statistical analysis. Statistical analysis was performed using SPSS version 26.0. The following statistical methods were applied:
- Student's t-test
- Analysis of variance (ANOVA)
- Pearson correlation analysis

A p-value of <0,05 was considered statistically significant.

Results

Cardiovascular responses

Parameter	Basketball	Football	p-value
HR (post-exercise)	178 ± 8 bmp	184 ± 10 bmp	0,03
Systolic BP	160 ± 12 mmHg	168 ± 14 mmHg	0.04
Core temperature	39.1 ^{0}C ± 0,4 ^{0}C	39.4 ^{0}C ± 0,5 ^{0}C	0.02
Dehydration	2.3%	3.5%	0.01

Football players demonstrated significantly higher cardiovascular strain and dehydration levels compared to basketball players.

Respiratory responses

Parameter	Basketball	Football	p-value
Respiratory rate	42 ± 6 breaths/min	36 ± 5 breaths/min	0.04
VO_2 max	52 ml/kg/min	55 ml/kg/min	0.05
Blood lactate	8.5 mmol/L	7.2 mmol/L	0.03

Basketball players exhibited greater ventilator responses and higher lactate accumulation.

A total of 60 athletes were evaluated in this study. Exposure to heat stress resulted in the development of functional (adaptive) responses in a majority of participants, while a subset of athletes progressed to clinically significant pathological conditions.

Functional changes. Functional (compensatory) responses were observed in 48 athletes (80%). This changes include:

- increased heart rate (physiological tachycardia)
- elevated respiratory rate
- increased body temperature
- enhanced sweating
- mild dehydration

These responses represent normal physiological adaptation mechanisms and were reversible.

Pathological changes. Pathological alteration was identified in 22 athletes (36.7%).

It is important to note that these athletes were part of the group exhibiting functional changes, indicating progression from adaptive responses to pathological conditions. The observed pathological findings included:

- sustained tachycardia (>180 bmp)
- cardiac arrhythmias
- episodes of arterial hypotension
- hyperthermia (>39–40 ^{0}C)
- significant dehydration (>3%)
- metabolic disturbances (elevated blood lactate levels)

Overall distribution

Category	Number	%
No significant changes	12	20%
Functional changes only	26	43.3%
Pathological changes	22	36.7%
Total	**60**	**100%**

Temporarily withdrawn athletes

A total of 14 athletes (23.3%) were temporarily withdrawn from training due to early signs of physiological overload and heat-related disturbances. Main reasons: excessive heart rate, hyperventilation, dizziness and weakness dehydration. These athletes recovered after appropriate rest and supportive measures.

Permanently withdrawn athletes. Three athletes (5%) were advised to discontinue intensive sports activity due to the development of clinically significant pathological conditions. *Reasons: Cardiovascular disorders:* persistent tachycardia, arrhythmias, arterial hypotension. *Severe hyperthermia:* core body temperature >40 ^{0}C, signs of exertional heat stroke. *Metabolic disturbances:* blood lactate >10 mmol/L, metabolic imbalance.

Discussion

The results of this study show that heat stress causes significant physical strain in athletes, expressed through adaptive responses of the body. However, in some participants these adaptive mechanisms were no sufficient, leading to pathological conditions. The transition from a functional to a

pathological state is mainly related to impaired thermoregulation and excessive strain on the cardiovascular system. Athletes exposed to prolonged or intense heat are at increased risk of dehydration, hyperthermia, and unstable blood circulation.

Practical implications. Based on the findings, the following preventive strategies are recommended: implementation of heat acclimatization programs, maintenance of adequate hydration, monitoring environmental conditions, adjustment of training intensity in hot environments, early identification of heat-related symptoms

Conclusion

Heat stress has a significant impact on cardiovascular and respiratory function in athletes. While the majority of athletes develop functional adaptive responses, a substantial proportion progresses to pathological conditions under excessive heat exposure. These finding underline the importance of preventive strategies and proper management of training in hot environments to reduce the risk of heat-related complications.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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COVID-19 and Anxiety: The Psychological Effects of the Pandemic

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Abstract. *According to the World Health Organization (WHO), a pandemic is a large-scale epidemic that affects millions of people in many countries and sometimes spreads worldwide. Pandemics pose a threat to health with an unknown nature, highly contagious characteristics, and symptoms. Restrictive measures taken to prevent the spread of the virus have both physical and mental effects on individuals.The COVID-19 pandemic has affected people's lives globally across many areas, including psychological, economic, and health aspects. In general, it is believed that the transition to a new lifestyle, the presence of restrictions, changes in routines, and uncertainties about health and the future can cause anxiety in individuals. In this context, a review of studies in the relevant literature shows that generalized anxiety disorder has increased during the pandemic. In addition, the study addresses a current problem and is considered important for future research, given the limited number of studies in this area. It is known that both the impact of the disease on human health and the limitations and consequences of pandemic measures have caused individuals to experience a difficult period in terms of mental health during the COVID-19 pandemic. This article presents the psychological symptoms of anxiety caused by the COVID-19 pandemic in humans and analyzes scientific studies. We hope that the article will explain the relationship between the COVID-19 pandemic and anxiety disorders and show how to deal with such situations.*

Keywords: *generalized anxiety disorder, fear of COVID-19, mental health, pandemic, health anxiety cognitive lexibility*

Introduction

A pandemic is the spread of an infectious disease from a specific region to multiple continents and poses a threat to many people (Merriam-Webster, 2020). Pandemics have been a global problem since the dawn of humanity. As a global crisis, pandemics affect international systems economically, culturally, socially, and politically. The virus known as "Covid-19," which has spread to many parts of the world, led the World Health Organization to declare a pandemic on March 11, 2020 (WHO, 2021). Taylor (2019) states that one of the vulnerability factors that makes individuals experience high levels of anxiety during a pandemic is health anxiety (Taylor, 2019). Supporting this, research has shown that health anxiety can increase the risk of generalized anxiety disorder during previous pandemics (Rubin et al., 2009). It is thought that cognitive flexibility may be a protective factor against generalized anxiety disorder during a pandemic.

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Cognitive flexibility encompasses characteristics such as adapting to different circumstances, adjusting information-processing strategies in the face of unexpected situations, and engaging in multifaceted thinking (Canas et al., 2003). Research has found that cognitive flexibility is important for health, well-being, and adaptation, and may benefit individuals facing stressors beyond their control (Arnau et al., 2007). In a pandemic, cognitive flexibility helps people reframe situations and reduce risks. Another possible predictor of generalized anxiety disorder during the COVID-19 pandemic is fear of COVID-19, defined as reactions triggered by intense fear of contracting the virus (Arora et al., 2020). Factors contributing to this fear include lack of knowledge about the disease, limited treatments and vaccines, the risk of death, and uncertainty over when the pandemic will end.

Methods

Based on the existing literature, this study will examine the relationships between generalized anxiety disorder, health anxiety, COVID-19 fear, and cognitive flexibility during the COVID-19 pandemic. In addition, we will examine the extent to which health anxiety predicts generalized anxiety disorder, COVID-19 fear, and cognitive flexibility. A study investigating the psychological distress caused by the pandemic found that areas with fewer restrictions and quarantines had lower prevalence of COVID-19 fear, stress, anxiety, and substance use than other regions (Gritsenko et al. 2020). Research also suggests that COVID-19 fear may be associated with various psychopathologies, such as anxiety and mood disorders (Rodríguez-Hidalgo et al., 2020).

Anxiety is a feeling of tension that arises from the thought that “something bad will happen” and is usually caused by the thought that something bad will happen. Anxiety is one of the symptoms that most people may experience at some point in their lives (Buelow, 2020). Semantically, the word “anxiety” does not fully correspond to the word “anxiety,” and it is suggested that the word “distress” be used instead. This is because it can more clearly indicate the severity of anxiety experienced clinically. In this article, the term “anxiety”, which is often used in the literature, is preferred. Anxiety has been a concept that has attracted the attention of many theorists. Anxiety is a part of human life and is present in the individual from birth to death. Neurotic anxiety arises as a result of the loss of meaning in life or its own fragmentation. Anxiety is defined as a concept that is future-oriented but arises in the present. The main factor driving a person to worry is uncertainty about the future. Anxiety can also be considered a kind of reaction. This reaction arises in response to threats that negatively affect the individual's internal balance. In this way, the individual seeks a new balance. According to the existentialist approach, anxiety is the individual's awareness of the reality of absence rather than of presence. Freud (1895) states that anxiety is the product of instinctual needs and that tension and anxiety arise in the individual because these instincts are unacceptable, and as a result, defense mechanisms such as repression are activated. He also drew attention to sexual desires and negative experiences in childhood in the formation of anxiety. Generalized anxiety disorder is a diagnosis characterized by chronic and persistent anxiety. It consists of a set of various psychological and physiological symptoms that accompany multidimensional anxiety. For this diagnosis, symptoms must persist for at least 6 months (Stein & Sareen, 2015). Generalized anxiety disorder is a disorder that is not specific to a specific object, situation, or thought, and presents itself with both psychological and physiological symptoms in the individual.

The impact of generalized anxiety disorder on a person can be observed in various areas, such as cognitive, physical, and functional. As with all anxiety disorders, the main point of generalized anxiety disorder is the presence of irrational and involuntary thoughts (Lee et al., 2010). These thoughts can cover a variety of topics, from family and financial situations to relationships, work, and health.

Building on this, intolerance of uncertainty is the tendency to interpret uncertain situations as stressful and negative. This leads to chronic anxiety in individuals diagnosed with generalized anxiety disorder (Koerner & Dugas, 2006).
Towards the end of 2019, a rapid increase in pneumonia cases in Wuhan, China, attracted attention (Wu et al., 2020). Researchers identified a new coronavirus, which they called 2019-nCoV, as the cause of the increase in pneumonia cases. This new virus has also been identified as SARS-CoV-2 due to its similarity to SARS. The virus is named corona, which means crown in Latin, because the rod-shaped glycoprotein structures surrounding it resemble a crown (Lai and Cavanagh, 1997). Commonly referred to as COVID-19 (Co: corona, Vi: virus, D: disease), the most common symptoms include shortness of breath, fever, joint pain, fatigue, cough, and loss of smell or taste (WHO, 2020). The incubation period ranges from 2 to 14 days. The virus can be transmitted from infected individuals through droplets or contact with contaminated surfaces (Chagla et al., 2020). COVID-19, which has spread worldwide, was declared a pandemic by the World Health Organization on 11 March 2020. The WHO warns that hand hygiene, avoiding crowded environments, maintaining social distancing, and wearing masks are essential to prevent the spread of the virus (WHO, 2020). The increasing number of cases and deaths due to COVID-19 has prompted governments to take a number of precautionary measures to prevent the spread. In many countries, measures such as quarantine, mandatory mask wearing, school closures, work-from-home orders for private businesses and government agencies, and takeout services for restaurants and cafes have been implemented. Epidemics, beyond their biological and physiological effects, leave lasting economic, social, political, and psychological scars on society. Historical examples demonstrate that epidemics disrupt states and economic systems. The mental health impact of pandemics is often more persistent and widespread than their physical effects (Shigemura et al., 2020). The negative atmosphere caused by pandemics can contribute to various psychiatric illnesses. Research indicates that the negative mental health effects of pandemics often persist beyond the acute phase (Douglas et al., 2009). Anxiety disorders, OCD, mood, and sleep disorders may increase during pandemics. Mak et al. (2009) report that the most common psychiatric disorders that emerged in the long term after the SARS pandemic were PTSD, depression, sleep disorders, and anxiety disorders.
Similar to past pandemics, a study of individuals exposed to COVID-19 found that individuals experienced high levels of panic, fear, and anxiety (Smith et al., 2020). During the pandemic, individuals experienced fear and anxiety even when they had a cold or the flu, fearing that they would get COVID-19.

Furthermore, when examining people's reactions to the global crisis, the following were common fears: fear of contracting the virus and dying, fear of quarantine, feeling lonely and helpless, fear of being separated from loved ones due to quarantine, fear of experiencing economic hardship and losing their job due to the pandemic, and reluctance to seek medical attention.

The pandemic also negatively affects children and adolescents. School closures, limited socialization, isolation from peers, fear of illness, inadequate housing in crowded families, and the indirect impact of economic problems all contribute to the risk of negative emotions in both children and adolescents.

One of the most important measures in the fight against COVID-19 is social isolation. Those who test positive are isolated from their relatives and environment, and those suspected of infection are placed in a 14-day home quarantine. During this difficult process, the lack of social support, especially for the sick, can increase feelings of loneliness and cause anxiety. In addition, the closure of gathering places such as cinemas, theaters, and places of worship due to increased infection risk can limit individuals' socialization, leading to isolation and withdrawal from society.
It has been found that these factors are associated with greater anxiety and stress (Tzur et al., 2020).

Results

COVID-19 is causing two types of health problems. First, the physical health problems caused directly by the virus, and second, the increase in mental health disorders such as anxiety, panic, panic attacks, generalized anxiety disorder, and depression that have come with the pandemic. COVID-19 is not only a physical health crisis, but also a mental and psychological health emergency. Infectious diseases not only threaten people's physical health, but also harm the psychological health of the entire population, regardless of whether they are infected or not. In the early stages of the pandemic, more attention was paid to the physical illnesses caused by the virus, and the effects on mental and psychological health were not emphasized. However, this is likely to continue even after the pandemic is over, when the psychological effects of the disease will likely persist for months or even years after normal life returns. The COVID-19 pandemic, with the concept of a "new normal", may affect people's lives on a global scale and in many areas such as psychology, economics and health.

Many people may face problems while trying to adapt to the changing living conditions. With the advent of the new normal, people have to make radical changes in their lives, and this situation causes individuals to strive to find a "new balance". However, the existence of a life-threatening virus can cause people to question their values in life, and facing the reality of death can create tension in individuals. The impact of the global COVID-19 pandemic on people's psychology includes individual differences. The traumatic effects of stress during and after the COVID-19 pandemic, which threatens people's lives and is global in scope, may vary depending on people's socio-cultural characteristics, socio-economic status, mental health status and personality characteristics. In general, it is thought that situations such as new order, restrictions, changes in routines, and health-related uncertainties can cause anxiety in individuals. The main strategy implemented to prevent the spread of the Covid-19 virus was to physically separate people, i.e. isolate them. Although this measure has a protective effect against the pandemic, it can weaken social ties, posing a threat to mental health. Socialization is argued to be a vital biological need for psychological well-being, mental health, and survival. Therefore, the lack of social interaction during quarantine negatively impacts psychological and emotional well-being. In this context, when examining the relevant literature, it appears that widespread anxiety, especially during the pandemic, has increased.

Considering all these findings, it is believed that examining studies predicting generalized anxiety disorder in a pandemic atmosphere, in particular, determining the extent of the direct effect of COVID-19 fear on generalized anxiety disorder, and determining the content of interventions for increased anxiety during the pandemic, could contribute to the literature. In addition, the study addresses a current problem and, given the limited research on generalized anxiety disorder, health anxiety, cognitive flexibility, and COVID-19 fear, it is thought that it could contribute to the field and serve as a basis for future research.

Discussion

Major events such as the COVID-19 pandemic can erode people's sense of security. This erosion is primarily due to unanswered questions about when the pandemic will end and whether there will be a cure, as well as concerns about potential social and economic crises. Trauma arises as individuals are confronted with death and illness, often resulting in deteriorating mental health. Factors such as social isolation, prolonged stay-at-home orders, and constant exposure to information can amplify these negative effects. Additional stressors—including insufficient medical care, lack of or misleading information, infection, and financial losses—increase psychological impact. Increased time at home, new symptoms, exposure to more cases, and worsening illness can trigger mental health issues like depression, acute stress disorder, PTSD, generalized anxiety disorder, panic disorder, and psychosis. The number and severity of negative events vary by socioeconomic level, with main

challenges more closely tied to money (job and income loss) and basic needs (access to food and medicine) than to direct disease experience.

Given the psychological impact of the COVID-19 pandemic on people, negative moods and thoughts were widespread. Individuals feared the world as they knew it would change, and the possibility of a very bleak near future. In addition, those who were sick or in home quarantine due to exposure experienced intense thoughts about health and death. People were on high alert and avoided situations related to COVID-19, as authorities encouraged them to. However, despite the extraordinary scale of the pandemic's impact, it is recommended that behavioral change be guided by common sense. This may work in some cases, but it is not enough to educate the public about public health issues without scientists' involvement. The proven direct and indirect psychological and social impacts of the COVID-19 pandemic are widespread and may continue to impact mental health in the future. Innovative/creative research is needed to reduce mental health risks, achieve success, and inform interventions in pandemic settings.

Acceptance and Commitment Therapy (ACT) can be used to explore this. Acceptance and Commitment Therapy, a behavioral approach, is grounded in the psychological flexibility model. The model of psychological flexibility is informed by six interrelated processes: cognitive awareness, acceptance, present-moment engagement, contextual self-awareness, values, and value-oriented actions. One of these six processes, acceptance, is the ongoing process of actively accepting what is remembered and felt in the present moment, even if it causes psychological pain, without trying to change its frequency or form. When a person is unable to develop the ability to accept, they may exhibit avoidance behavior. Avoidance behavior refers to behaviors a person engages in to reduce and manage negative and painful emotions, as well as the thoughts, memories, and bodily sensations associated with them. Psychological flexibility is not a specific condition or set of symptoms. Instead, it is a trans-diagnostic process that supports mental health and well-being within a range of psychological endeavors. Thus, psychological flexibility can contribute to psychological resilience in difficult times. Furthermore, it can be said that a significant portion of the world's population has developed diagnostic psychopathology in response to this extraordinary event that the world is facing. Transdiagnostic processes are the most effective and widely used processes to support functional development and well-being and to prevent diagnosis. The impact of the COVID-19 pandemic can lead to unhealthy behaviors such as harmful alcohol use, staying home, and not following public health guidelines, such as vaccination. High levels of psychological resilience mediate the negative effects of COVID-19-related health anxiety, anxiety, and depression. In addition, two psychological resilience processes—cognitive dissociation and value-oriented actions—reduce mental health anxiety.

Conversely, acceptance (being open to internal turmoil), an element of the psychological flexibility process, mediates the increase in negative mental health outcomes. However, these processes reduced the negative effects of mental health anxiety during the lockdown. These results suggest that allowing oneself to experience internal turmoil, observing unhelpful thoughts, and engaging in value-oriented actions increases psychological resilience in the face of adversity. A review of the literature reveals the importance of the relationship between COVID-19 fear, perceived coping ability, perceived stress, and avoidance of the experience. No study in our country has examined these variables together. Based on the effects of the above-mentioned stress and coping mechanisms on general health and mental health, investigating the effects of the COVID-19 crisis on coping with stress and anxiety directly contributes to the field of mental health and, indirectly, to general health. A review of the literature suggests that avoidance of experience may be a determining factor in this effect. For these reasons, this study is expected to serve as a resource for primary, secondary, and tertiary prevention efforts aimed at protecting and maintaining individuals' mental health.

Conclusion

The COVID-19 pandemic presents a complex public health challenge that requires a multifaceted research approach. While medical advances are crucial, understanding and supporting society's psychological and social responses is equally vital to minimizing overall harm. Clearly identifying the public's psychological reactions—including fears, anxieties, and avoidance behaviors—and recognizing at-risk groups are essential to containing the pandemic's biopsychosocial impact. Accurate information must be provided by the state, healthcare professionals, and journalists, who carry significant responsibility in this effort. Guiding individuals toward effective psychological support and anxiety-management methods, such as supportive cognitive-behavioral therapy and relaxation techniques, will help strengthen mental resilience. Ultimately, integrating social and psychological insights with prevention strategies is key to effectively managing and ending the pandemic. Droplet and contact precautions should be taken to detect and isolate each case and prevent the spread of the disease. The use of masks as personal protection is a key part of these precautions. Physical distancing precautions are recommended to reduce the likelihood of virus transmission. Ventilation of indoor spaces is crucial to prevent transmission. General hygiene measures, such as frequent and proper handwashing, have been essential for reducing the risk of contamination. The health status of individuals should be comprehensively considered, and psychosocial problems should be identified and addressed early.

In order to more effectively diagnose mental disorders in individuals seeking primary care, primary care physicians should increase their knowledge and awareness of mental disorders through in-service training. Individuals should have easy access to psychological counseling and support services in crisis situations such as pandemics. Plans should be developed to provide online services as an alternative during times when face-to-face access to psychiatric diagnosis, treatment, and support services is limited. Supportive and guiding steps should be taken to increase psychological resilience, which is a protective factor in mental disorders. It is particularly important to equip individuals with the skills to cope with problems starting from childhood, a crucial period of development. Supportive attitudes should be instilled in family and school life from childhood, and stress-adaptation skills should be instilled. Primary care physicians should also be able to advise on factors that promote psychological resilience. Participation in sports and arts activities, which are known to enhance psychological resilience, should be encouraged from an early age. In light of current research findings, intervention programs could incorporate techniques that support cognitive flexibility, such as recognizing and reappraising crises, generating new solutions in context, and taking control. Alternatively, to reduce COVID-19 fear and health anxiety, individuals could receive psychoeducation about the structure of the COVID-19 virus, its transmission routes, and the coping and emotion regulation skills necessary to effectively manage the current situation.

Furthermore, teaching mindfulness techniques may have a protective effect on mental health by enabling individuals to remain calm in crisis situations and prevent loss of control. It is also very important to obtain accurate information about the virus during a pandemic. Continuous exposure to news about the pandemic from social media or television can increase both health anxiety and fear and anxiety levels in individuals. In this context, it is considered important for authorities to regularly inform the public to reduce anxiety levels in society. In the context of a pandemic, it is thought that psychoeducational training, which focuses on skills such as stress management and breathing exercises, may be useful for individuals to balance anxiety. The COVID-19 pandemic can affect many areas of human life, including family, work, school, friendships, the economy, and health. During this period, it may be important to encourage people to maintain their routines and seek social support. In addition, online mental health services can be supported to improve service delivery during a pandemic.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The Impact of Hashimoto's Thyroiditis on the Menstrual Cycle in Adolescent Girls: A Clinical-Retrospective Analysis

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Abstract. *Puberty represents a critical physiological window (ages 13–15) characterized by the maturation of the hypothalamic-pituitary-ovarian (HPO) axis. Hashimoto's Thyroiditis (HT), the most prevalent autoimmune endocrine disorder in this demographic, poses a significant threat to reproductive stability. This retrospective study evaluates the correlation between elevated thyroid autoantibodies (anti-TPO, anti-TG) and menstrual irregularities in 13–15-year-old girls. During this stage, the thyroid gland's functional state is essential for the harmonious operation of the neuroendocrine system. Our data analysis reveals that secondary hyperprolactinemia and impaired sex-hormone metabolism, often secondary to subclinical hypothyroidism, are the primary drivers of menstrual dysfunction. In this clinical-retrospective study, we analyzed the endocrine profiles and menstrual calendars of adolescents diagnosed with HT compared to a healthy control group. The findings indicate that oligomenorrhea was identified in 45% of the HT cohort, a stark contrast to the 10–12% observed in the control group. Furthermore, severe dysmenorrhea and menorrhagia were significantly more prevalent in patients with high anti-TPO titers. The results emphasize that the structural similarity between TSH and gonadotropins, along with the stimulatory effect of TRH on prolactin, creates a "hormonal storm" that disrupts ovulation. Early screening for autoimmune thyroid panels in adolescents with irregular cycles and timely initiation of hormone replacement therapy are paramount. This proactive approach holds significant prophylactic importance in preventing future reproductive sequelae, such as chronic anovulation and subfertility.*

Keywords: *hashimoto's thyroiditis, adolescent health, menstrual irregularities, pediatric endocrinology, autoimmune thyroiditis, anti-tPO, oligomenorrhea, hpo axis, hyperprolactinemia*

Introduction

The transition through puberty is governed by a complex orchestration of endocrine signals. For adolescent girls, the stabilization of the menstrual cycle is not merely a sign of fertility but a marker of systemic metabolic health. The thyroid gland plays a peripheral yet indispensable role in this process. Thyroid hormones (T3 and T4) exert a direct influence on ovarian follicular development and an indirect influence through the regulation of sex hormone-binding globulin (SHBG) (Selva et al., 2009). Reduced levels of SHBG in hypothyroid states increase the fraction of free testosterone, which can further mimic the clinical symptoms of hyperandrogenism. Timely identification of these shifts is critical, as autoimmune markers often precede clinical symptoms of reproductive dysfunction (Desai & Brinton, 2019).

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Hashimoto's Thyroiditis (HT), or chronic lymphocytic thyroiditis, involves the autoimmune-mediated destruction of thyroid parenchyma by T-cell infiltration and autoantibody production (Binay & Simsek, 2016). In the pediatric and adolescent population, HT is the most common cause of acquired hypothyroidism. The onset often coincides with the peak of pubertal development, making the HPO axis particularly susceptible to metabolic fluctuations. In adolescents, the clinical presentation of HT is frequently subtle, often presenting as fatigue or declining academic performance, which masks the underlying hormonal shift (Ozden & Doneray, 2024). The clinical complexity of HT in this age group is further compounded by the fact that thyroid autoantibodies can fluctuate significantly during different stages of Tanner's development. Consequently, the transition from euthyroid autoimmunity to overt clinical hypothyroidism is often non-linear, requiring serial monitoring of the thyroid status (D'Aurizio et al., 2022).

The persistence of autoimmune inflammation can lead to a disruption of the pulsatile secretion of Gonadotropin-Releasing Hormone (GnRH). This disruption is exacerbated by the cross-reactivity of high TSH levels with FSH receptors on the ovaries, leading to multicystic ovarian morphology in some severe cases. Consequently, conditions such as oligomenorrhea, secondary amenorrhea, and dysmenorrhea become clinical hallmarks of the disease in this age group (Sahay et al., 2025).

Methods

This clinical-retrospective study analyzed the medical records of female patients aged 13–15 years diagnosed with Hashimoto's Thyroiditis. The study group consisted of patients with confirmed HT (defined as TSH > 4.5 mIU/L and/or positive anti-TPO/anti-TG titers > 34 IU/mL), while the control group comprised age-matched healthy adolescents without thyroid or gynecological history.

Inclusion Criteria:

- Biological female, aged 13–15 years.
- Confirmed HT via serological markers and thyroid ultrasound (hypoechoic, heterogeneous texture).
- Documented menstrual history for at least 12 months post-menarche.
- Data Collection:

Medical histories were reviewed for BMI, thyroid panel (TSH, fT4, fT3), autoantibody titers, and serum prolactin. Menstrual parameters were categorized into cycle length, flow intensity, and pain scale (VAS). Statistical analysis followed the methods outlined in previous longitudinal studies to ensure the validity of the correlation between antibody titers and cycle delay (Shin et al., 2005).

Results

The data demonstrates a clear divergence in reproductive health between the HT cohort and the healthy control group. The most prevalent finding was oligomenorrhea, occurring in 45% of the HT group compared to only 12% in the control group.

Table 1
Comparative Endocrine and Menstrual Parameters

Parameter	Hashimoto Group (n=X)	Control Group (n=Y)	Statistical Significance
Oligomenorrhea (>35 days)	45%	12%	$p < 0.01$

Secondary Amenorrhea	8%	1.5%	$p < 0.05$
Mean TSH Level (mIU/L)	6.8 ± 2.1	1.9 ± 0.8	$p < 0.001$
Anti-TPO Positive (>34 IU/mL)	100%	0%	N/A
Severe Dysmenorrhea (VAS >7)	32%	14%	$p < 0.05$
Hyperprolactinemia (>25 ng/mL)	28%	5%	$p < 0.01$

A significant finding in our cohort was the high prevalence of menorrhagia (heavy menstrual bleeding) in patients with frank hypothyroidism (fT4 below normal). This is likely due to the decreased production of clotting factors and altered platelet aggregation associated with thyroid hormone deficiency (Ordookhani & Burman, 2017). Furthermore, the correlation between anti-TPO titers and the severity of dysmenorrhea suggests that the systemic inflammatory load contributes to uterine prostaglandin overproduction.

Notably, our analysis also identified a subset of patients within the HT group who exhibited subclinical hyperandrogenism despite normal adrenal function. This suggests that the metabolic disturbances triggered by chronic lymphocytic thyroiditis may indirectly alter the intraovarian androgen-estrogen balance, even before significant changes in TSH levels are detectable.

Discussion

The results of this study underscore a critical physiological link: the thyroid does not function in isolation. The high prevalence of menstrual dysfunction in the HT group suggests that autoimmune thyroiditis acts as a primary disruptor of the HPO axis during its most vulnerable developmental stage.

The TRH-Prolactin-GnRH Axis

The biochemical mechanism is primarily driven by the hypothalamus. In a hypothyroid state, the lack of negative feedback from T4 causes an overproduction of Thyrotropin-Releasing Hormone (TRH). TRH serves as a potent prolactin-releasing factor. Chronic elevation of prolactin (secondary hyperprolactinemia) interferes with the pulsatile release of GnRH from the hypothalamus (Tsutsumi & Webster, 2009). Without rhythmic GnRH pulses, the pituitary fails to produce the mid-cycle LH surge necessary for ovulation. This results in the anovulatory cycles and prolonged follicular phases observed in 45% of our patients.

Ovarian Microenvironment and Autoimmunity

Beyond hormonal levels, the "autoimmune milieu" itself plays a role. Anti-TPO and anti-TG antibodies may have cross-reactivity with ovarian tissues. Pro-inflammatory cytokines (IL-1, IL-6) released during the chronic thyroiditic process can penetrate the follicular fluid, impairing the granulosa cells' ability to convert androgens into estrogens (Martin et al., 2025). This -leads to a state of relative estrogen deficiency and progesterone withdrawal, contributing to the painful and irregular shedding of the endometrium. Furthermore, the chronic activation of the innate immune system in HT may lead to a localized inflammatory response within the pelvic environment. This systemic "low-grade inflammation" potentially alters the vascular permeability of the endometrium, which correlates with the increased pain scores and cycle irregularities observed in our study group

compared to healthy peers. Some research even suggests that high titers of thyroid antibodies are an early marker for decreased ovarian reserve (Morales-Martínez et al., 2021).

Clinical Trajectory and Future Fertility
The "fetal-like" sensitivity of the adolescent reproductive system means that even minor hormonal shifts can have long-term consequences. Untreated HT in adolescents may exacerbate the risk of developing Polycystic Ovary Syndrome (PCOS) phenotypes later in life, as both conditions share features of insulin resistance and anovulation (Nicolaides et al., 2020). Our findings indicate that patients treated with Levothyroxine (L-T4) demonstrated a significant improvement in cycle regularity. Prophylactic stabilization of TSH below 2.5 mIU/L in adolescent girls is essential not only for growth but for the "calibration" of the reproductive system.

Limitations and Future Research
As a retrospective study, the lack of standardized ultrasound monitoring of the ovaries in all patients limits our ability to confirm the prevalence of multicystic ovaries. Future prospective studies should focus on the impact of early L-T4 treatment on the anti-müllerian hormone (AMH) levels in adolescent HT patients to better understand the long-term fertility outlook.

Conclusion

Hashimoto's Thyroiditis is a major etiological factor in menstrual dysfunction among girls aged 13–15. The findings advocate for a mandatory thyroid screening protocol for any adolescent presenting with menstrual irregularities. Early detection of autoimmune markers (anti-TPO/anti-TG) and TSH stabilization can restore the HPO axis rhythm, mitigate the severity of dysmenorrhea, and preserve future reproductive health.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Epidemiological Analysis of Acute Intestinal Infections in Azerbaijan (2000-2024)

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Abstract. *Background. Acute intestinal infections (AII) remain a significant public health concern worldwide, especially in developing countries. This study aims to analyze the epidemiological trends of reported AII cases in Azerbaijan between 2000 and 2024. Methods. A retrospective descriptive study was conducted using national surveillance data on AII cases from the Ministry of Health of Azerbaijan. Incidence rates per 100,000 population were calculated for each year across four time intervals: 2000–2010, 2011–2015, 2016–2020, and 2021–2024. Results. The incidence of AII in Azerbaijan demonstrated fluctuations over the studied years. The highest rates were observed between 2000 and 2005, followed by a gradual decline until 2015. However, a moderate resurgence was noted during the 2021–2024 period. Factors contributing to these trends may include seasonal variations, food safety practices, access to clean water, and improvements in disease surveillance systems. Conclusion. Although overall AII incidence in Azerbaijan has decreased compared to early 2000s, periodic increases underline the need for continuous monitoring, strengthened hygiene policies, and targeted public health interventions to mitigate future outbreaks.*

Keywords: *Acute intestinal infections, epidemiology, Azerbaijan, public health, disease trends, incidence rate*

Introduction

Acute intestinal infections remain a significant global public health challenge, particularly in low- and middle-income countries, contributing substantially to morbidity (Kotloff et al., 2013) and mortality rates worldwide (Liu et al., 2015) These infections are primarily caused by various enteric pathogens, including Escherichia coli, Salmonella species, and Rotavirus, which are responsible for a wide spectrum of gastrointestinal diseases (Guerrant et al., 1992) ranging from mild diarrhea to severe dehydrating illness (Tate et al., 2010). Despite improvements in sanitation, vaccination, and healthcare access, acute intestinal infections continue to impose a heavy burden, especially among children under five years of age (Black et al., 2016). In Azerbaijan, limited comprehensive data exist regarding the long-term epidemiological trends of these infections. Monitoring incidence rates over extended periods is crucial for identifying outbreaks, assessing the effectiveness of public health interventions, and guiding resource allocation. Furthermore, the emergence of antibiotic resistance among enteric pathogens presents an ongoing threat to disease control efforts (Ochoa & Contreras, 2011).

This study aims to provide an epidemiological assessment of acute intestinal infections in Azerbaijan over a 25-year period (2000–2024), analyzing trends in reported cases and incidence rates.

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Materials and Methods

Study design
This is a retrospective descriptive study analyzing epidemiological data on acute intestinal infections in Azerbaijan from 2000 to 2024.

Setting
The study is based on national surveillance data collected by Azerbaijan's public health reporting system, which monitors infectious diseases across the country.

Participants
The study includes all reported cases of acute intestinal infections in the general population registered during the study period. No individual-level data or direct patient contact occurred.

Variables
Key variables include the number of reported cases, incidence rates per 100,000 population, and types of etiological agents such as Escherichia coli, Salmonella spp., and Rotavirus.

Data sources/measurement
Data were extracted from official national health surveillance databases and annual population statistics. Cases were confirmed according to standard diagnostic criteria reported by healthcare providers.

Bias
Potential reporting bias may exist due to underreporting or misclassification in surveillance data. Efforts were made to use official and consistent data sources to minimize bias.

Study size
The study covers all reported cases of acute intestinal infections nationwide between 2000 and 2024.

Quantitative variables
Incidence rates were calculated as cases per 100,000 population annually, based on official population estimates.

Statistical methods
Descriptive statistics were used to summarize data trends over time. Incidence rates were analyzed to identify temporal changes and disease patterns.

Ethical consideration
The study used anonymized, aggregated data without individual patient identifiers, thus ethical approval was not required.

Results

Participants
Between 2000 and 2024, the total number of reported acute intestinal infection cases varied annually across Azerbaijan’s population. The study analyzed data from national surveillance records covering all reported cases in the country.

Outcome data
The primary etiological agents throughout the period remained Escherichia coli, Salmonella spp., and Rotavirus.

Descriptive data
Between 2000 and 2010, the number of reported cases rose from 7,327 (92.1 per 100,000 population) to a peak of 13,762 cases (160.0 per 100,000) in 2007, followed by minor fluctuations through 2010 (Table 1). From 2011 to 2015, incidence rates remained relatively stable, ranging from 12,876 (142.2 per 100,000) to 14,496 (154.0 per 100,000) cases annually (Table 2). Between 2016 and 2020, the highest number of cases was recor.

An increasing trend in acute intestinal infection incidence was observed in the early 2000s, followed by a relatively stable mid-decade period, and fluctuations during recent years. The notable decrease in 2020 corresponds with the COVID-19 pandemic, suggesting potential impacts on disease transmission or reporting.

Other analyses
Annual variations in case numbers may reflect changes in healthcare accessibility, public health policies, surveillance sensitivity, and socio-environmental factors influencing infection dynamics.

Incidence of acute intestinal infections in Azerbaijan increased by 84.95% between 2000 and 2010, rising from 7,327 to 13,551 reported cases, with a peak incidence of 160.0 per 100,000 population in 2007. The resurgence in 2010 indicates a persistent public health challenge linked to diverse pathogens like E. coli and Salmonella. Data indicates that the rise in infections outpaced population growth over the decade.

Table 1
Incidence of Acute Intestinal Infections Among the Population in Azerbaijan (Number of Reported Cases). 2000–2010

2000–2010	2000	2005	2007	2008	2009	2010
Etiological agents: Common enteric pathogens (e.g., E. coli, Salmonella spp., Rotavirus).	7.327	10.520	13.762	12.769	11.737	13.551
	Number of registered acute intestinal infection cases per 100,000 population					
	92,1	125,5	160,0	146,5	133,0	151,7

Table 2 illustrates the epidemiological trends of acute intestinal infections (AII) caused by common enteric pathogens (such as *E. coli*, *Salmonella spp.*, and *Rotavirus*) in Azerbaijan over a five-year period from 2011 to 2015. The data presents both the absolute number of reported cases and the incidence rate per 100,000 population.

Table 2
Incidence of Acute Intestinal Infections Among the Population in Azerbaijan (Number of Reported Cases). 2011–2015

2011–2015	2011	2012	2013	2014	2015
Etiological agents: Common enteric pathogens (e.g., E. coli, Salmonella spp., Rotavirus).	12.876	13.923	13.476	14.496	14.306
	Number of registered acute intestinal infection cases per 100,000 population				
	142,2	151,7	145,0	154,0	150,1

Table 3 tracks the incidence of acute intestinal infections (AII) in Azerbaijan from 2016 to 2020. This period is characterized by an initial peak in reported cases followed by a dramatic decline, particularly in the final year of the study.

Table 3
Incidence of Acute Intestinal Infections Among the Population in Azerbaijan (Number of Reported Cases). 2016–2020

2016–2020	2016	2017	2018	2019	2020
Etiological agents: Common enteric pathogens (e.g., E. coli, Salmonella spp., Rotavirus).	15.880	16.017	13.182	13.504	7.601
	Number of registered acute intestinal infection cases per 100,000 population				
	164,8	164,5	134,2	136,0	76,0

Table 4 presents the data for acute intestinal infections (AII) in Azerbaijan from 2021 to 2024. This period reflects the "post-pandemic" epidemiological shift, showing how infection rates stabilized following the unique conditions of the 2020 lockdown year.

Table 4
Incidence of Acute Intestinal Infections Among the Population in Azerbaijan (Number of Reported Cases). 2020–2024

2020–2024	2021	2022	2023	2024
Etiological agents: Common enteric pathogens (e.g., E. coli, Salmonella spp., Rotavirus).	6.680	9.436	10.430	8.854
	Number of registered acute intestinal infection cases per 100,000 population			
	66,5	93,5	102,7	86,8

Discussion

The data presented in this study highlight the fluctuating incidence of acute intestinal infections in Azerbaijan from 2000 to 2024. The overall trend shows a peak in cases between 2016 and 2019, followed by a significant decline in 2020, likely related to the impact of the COVID-19 pandemic on healthcare access and public health measures such as lockdowns (Park et al., 2021). and improved hygiene practices (Baker et al., 2020). This pattern aligns with global observations where non-COVID infectious diseases temporarily decreased due to pandemic control efforts (Brueggemann et al., 2021).

The predominance of common enteric pathogens such as Escherichia coli, Salmonella spp., and rotavirus reflects the ongoing public health challenges in managing water quality, sanitation, and food safety in developing countries (Liu et al., 2015). These pathogens remain leading causes of morbidity and mortality, especially among children under five (Moe et al., 2011). Our findings underscore the urgent need for targeted interventions, including vaccination programs, improved sanitation infrastructure, and public education to reduce disease burden. Moreover, the decline in reported cases after 2019 might also indicate underreporting or reduced healthcare-seeking behavior during the pandemic, which warrants further investigation (Black et al., 2016). Continuous surveillance and robust epidemiological studies are essential to monitor trends and implement effective control measures.

In severe cases of acute intestinal infections requiring intensive care, secondary gastrointestinal complications such as gastric hemorrhage may arise, necessitating integrated management strategies for pathophysiology and treatment (Valizade, 2024).

Conclusion

This study highlights the persistent burden of acute intestinal infections in Azerbaijan over the last two decades, despite some fluctuations in incidence rates. The findings underscore the importance of sustained public health interventions targeting water quality, sanitation, and hygiene practices to reduce infection rates. Additionally, improvements in diagnostic capabilities and timely reporting systems are essential for effective disease monitoring and control. The impact of recent global events, including the COVID-19 pandemic, has further influenced infection dynamics, emphasizing the need for adaptable health strategies. Continued investment in vaccination programs, health education, and infrastructure development is critical to mitigate these infections and their associated morbidity. Policymakers should prioritize integrated approaches combining preventive and therapeutic measures to reduce the burden on healthcare systems and improve population health outcomes.

What is known:

- Acute intestinal infections remain a significant public health issue worldwide, especially in developing countries.
- Common causative agents include Escherichia coli, Salmonella spp., and Rotavirus.
- Disease incidence is influenced by sanitation, vaccination coverage, and healthcare access.
- *What this study adds:*
- Provides a 25-year epidemiological overview of acute intestinal infections in Azerbaijan from 2000 to 2024.
- Highlights fluctuations in incidence, including a sharp decline during the COVID-19 pandemic period.
- Identifies persistent main pathogens and suggests the need for strengthened surveillance and public health measures in Azerbaijan.

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Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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